Jean-Marie Saudubray · Matthias R. Baumgartner
Ángeles García-Cazorla · John H. Walter *Eds.*

Inborn Metabolic Diseases

Diagnosis and Treatment

7th Edition

Springer

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Editors

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Seventh Edition

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Preface

This 7th edition is a milestone in the series of Inborn Metabolic Diseases (IMD): Diagnosis and Treatment, first published in 1990.

Within the last 6 years a Copernican revolution in our understanding of IMD has changed the definition, concepts, paradigms, and classification. This new edition confirms the ubiquity of biochemical reactions in cellular biological processes, disturbances of which cause the large majority of human genetic disorders, many not classically labelled as metabolic diseases.

While previous editions remained largely focused on disorders of intermediary metabolism and organelles, mostly diagnosed with metabolic markers, this edition extends the concept of IMD to include disturbances of molecular machinery, diagnosed by molecular techniques but which currently may not have measurable metabolic markers.

About 600 new disorders are described in this 7th edition. Many are included in two new chapters: ► Chap. 39, *Disorders of nucleic acid metabolism, tRNA metabolism and ribosomal biogenesis*, and ► Chap. 44, *Disorders of Cellular Trafficking*. Defects in complex molecule synthesis and remodelling, many disorders of cellular or organelles transporters involving small or complex molecules, and new disorders involving energetic processes have also been considerably expanded.

Up to 85% of IMDs impact on neurodevelopment or are responsible for neurodegeneration. Acute, chronic or progressive neurological syndromes, psychiatric presentations, developmental delay, intellectual disability, neurodevelopment disturbances and neurodegeneration at any age deserve special attention. A new editor with a special expertise in neurology has joined us for the purpose of describing these disorders. The description of neurological presentations, with about 35 tables and algorithms, now accounts for about 50% of ► Chap. 1. The new concept of synaptic vesicle disorders is developed in ► Chap. 30.

While the most recent international classification of inborn errors of metabolism (IEM) encompasses >1400 disorders, from a clinical point of view, all IEM can be maintained in a simplified classification that mixes elements from a clinical diagnostic perspective and a pathophysiological approach based on three large groups. Our general clinical approach and algorithms are based upon this simplified classification and presented in ► Chap. 1 which should be read first.

In this era of genome sequencing, powerful computerized programs, and emerging artificial intelligence, we remain convinced that metabolic physicians must have a sound clinical training. As biochemical and molecular investigations grow in complexity, there is a risk that these effective but complex, time consuming, and expensive methods will be used in an uncontrolled and uncritical way. In view of the major improvements in treatment, it is crucial that clinicians do not miss IMD for which specific and effective treatment may be available. Physicians should be able to initiate a simple method of clinical screening, particularly in the emergency room, not only for children but also for adults.

While this new edition highlights recent findings, it continues to provide a comprehensive review of all IEM, with a particular focus on the clinical and biochemical approach to recognition, diagnosis and treatment at all ages. The clinical algorithms of ► Chaps. 1 and 2 incorporate both 'old' and 'new' disorders, and there are now many more algorithms detailing neurological presentations. An updated listing of metabolic markers and profiles and a section on molecular techniques including next generation sequencing and gene panels are included in ► Chap. 3.

As before, we continue to advocate referral to specialist centres for the diagnosis and treatment of inherited metabolic disorders. For countries in the Europe a list of such centres is compiled by the Society for the Study of Inborn Errors of Metabolism (SSIEM), while for the United States and Canada, Japan, Australia, South American

and Middle East countries, comparable lists are compiled by the American (SIMD), Japanese (JIMD), Australian (AIMD) South Latin America (SLEIMPN) and Middle East societies for the study of inherited metabolic diseases, respectively.

We welcome new authors and thank those previous authors who, while not involved with this edition, have helped to lay the foundation for this book.

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Diagnosis and Treatment: General Principles

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Clinical Approach to Inborn Errors of Metabolism in Paediatrics

Jean-Marie Saudubray and Ángeles García-Cazorla

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1

Introduction

Inborn errors of metabolism (IEM) are individually rare, but collectively numerous. The application of tandem mass spectrometry (tandem MS) to newborn screening and prenatal diagnosis has enabled presymptomatic diagnosis for some IEM. However, for most, neonatal screening tests are either too slow, expensive or unreliable and, as a consequence, a simple method of clinical screening is mandatory before initiating sophisticated biochemical or molecular investigations. The clinical diagnosis of IEM relies upon a limited number of principles:

- In the appropriate clinical context consider IEM in parallel with other more common conditions.
- Be aware of symptoms that persist and remain unexplained after the initial treatment and usual investigations have been performed for more common disorders, may be due to an IEM.
- Suspect that any neonatal death may possibly be due to an IEM, particularly those that have been attributed to sepsis. Additionaly, true sepsis can trigger acute decompensation when there is an underlying IEM. Carefully review all autopsy findings.
- Do not confuse a symptom or a syndrome with aetiology- the underlying cause may be an IEM yet to be defined.
- Remember that IEM can present at any age, from fetal life to old age.
- Be aware that because most IEM have a recessive inheritance (although some have dominant, X-linked, or maternal inheritance), the majority of individual cases may appear sporadic.
- In the acute emergency situation first consider those IEM that are most amenable to treatment.
- Get help from specialized centers.

Until recently IEM were considered as a speciality of paediatricians. Indeed, the term 'inborn' in the mind of clinicians has for a long time meant a disease which starts in the newborn period or at least in childhood. Although paediatricians have learned with time that in addition to severe neonatal forms most IEM can have mild forms with first clinical signs starting in adolescence or very late in adulthood, this concept of 'adult onset IEM' only recently reached the adult medical community (▶Chap. 2). Since these late onset forms are often unrecognized, their exact prevalence is unknown. Based mainly upon personal experience over 40 years, the content of chapters in this book, and on the literature analysis, this chapter gives an overview of clinical clues to the diagnosis of IEM in paediatrics. In the following pages, inborn errors amenable to treatment are printed in **bold**.

Do not miss a treatable disorder!

1.1 Simplified Classification of IEM in 3 Groups

Metabolism involves thousands of proteins mostly enzymes, receptors and transporters, deficits of which cause IEM that effect small or complex molecules. No matter their size, metabolites involved in IEM can behave as signalling molecules, structural components and fuels, and many metabolites have more than one role. According to Morava et al., the classification of a disorder as an IEM requires only that "impairment of specific enzymes or biochemical pathways is intrinsic to the pathomechanism" [1]. DNA testing has recently revolutionized the diagnostic approach of IEM [2]. Using this extended definition and this new diagnostic approach, the more recent international classification of IEM currently encompasses more than 1400 disorders, provisionally divided into 23 groups [3]. However, from a clinical point of view, all IEM can be maintained in a simplified classification that mixes elements from clinical diagnostic perspective and a pathophysiological approach based on three large groups [4]. We highlight the increasing importance of complex molecule metabolism, and their connection with cell biology processes and intracellular trafficking [5]. Transporters, such as those of SLC25A mitochondrial family, play a crucial role in transporting molecules and ions across membrane to link cytosolic and mitochondrial metabolism and to provide compounds for building and maintenance of the mitochondrion and the cell [6].

1.1.1 Group 1. Small Molecule Disorders (▶ Chaps. 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, and 34)

Almost all these IEM have plasma and urines metabolic marker(s) (small diffusible water-soluble molecules) that can be easily and rapidly measured in an emergency in a single chromatographic run (amino acids, organic acids, acylcarnitines…), or by using specific methods (metals or galactose metabolites.).

There are two subcategories in small molecule disorders defined by whether the phenotype primarily results from an acute or progressive "intoxication" caused by accumulation of toxic compounds proximal to the metabolic block or a deficiency where symptoms are primarily due to the defective synthesis of compounds distal from the block or from the defective transportation of an essential molecule through membranes. The "deficiency" subgroup shares many characteristics with defects in the complex molecule disorders group 2 (see below).

1.1.1.1 Accumulation of Small Molecules

This includes inborn errors of intermediary metabolism (IEIM) that lead to an acute or progressive intoxication from the accumulation of small molecules proximal to the metabolic block. In this group are the inborn errors of amino acid (AA) catabolism (phenylketonuria, maple syrup urine disease, homocystinuria, tyrosinemia etc.), most organic acidurias (OA) (methylmalonic, propionic, isovaleric etc.), congenital urea cycle defects (UCD), sugar intolerances (galactosaemia: ▶Chap. 14) hereditary fructose intolerance (▶Chap. 15), metal intoxication (Wilson, Menkes, haemochromatosis…) (▶Chap. 34), and porphyrias (▶Chap. 33). Many purine and a few pyrimidine disorders may be classified in this group. Indeed, most that involve either nucleotide synthesis, catabolism or salvage pathways may be screened by the plasma/urine purines and pyrimidines profile (▶Chap. 32). Some metabolite repair defects like D/L-2-OH-glutaric (▶Chap. 22) and NAXE deficiency (▶Sect. 11.14) are also included in this group. Hyperglycinaemias (▶Chap. 23) behave more as neurotransmitters disorders (▶Chap. 30) than as an intoxication (see later ▶Sect. 1.2). Vitamins interfere with many different metabolic pathways (transport, and intracellular processing) where they act as enzymatic cofactor, chaperone, or signalling molecules. Therefore, IEM of vitamins may manifest as a treatable intoxication disorder or a complex severe congenital encephalopathy (▶Chaps. 27, 28, and 29).

All the conditions in this group share clinical similarities: they do not interfere with the embryo-fetal development; they present with a symptom-free interval and clinical signs of intoxication, which may be acute (vomiting, coma, liver failure, thromboembolic complications etc.) or chronic (failure to thrive, developmental delay, ectopia lentis, cardiomyopathy etc.). Circumstances that can provoke acute metabolic attacks include catabolism, fever, intercurrent illness and food intake. Clinical expression is often both late in onset and intermittent. The diagnosis is straightforward and most commonly relies on plasma and urine AA, OA and acylcarnitine chromatography. Most of these disorders are treatable and require urgent removal of the toxin by special diets, extra-corporeal procedures, or 'cleansing' drugs (carnitine, sodium benzoate, penicillamine, etc.) chaperons and vitamins.

1.1.1.2 Deficiency of Small Molecules

Symptoms result primarily from the defective synthesis of compounds that are distal from the block or from the defective transportation of an essential molecule through intestinal epithelium, blood-brain barrier, and cytoplasmic or organelle membranes. Clinical signs are, at least in theory, treatable by providing the missing compound. Most of these defects cause a neurodevelopmental disruption, have a congenital presentation (antenatal), and may present as birth defects (see ▶Sect. 1.2, ◘Table 1.1). They share many characteristics with disorders in the complex molecules group (see later).

This group encompasses all carrier defects of essential molecules (AA, FA, Metals, Vitamins) that must be transported through cellular membranes, inborn errors in the synthesis of nonessential amino acids (▶Chap. 24) and fatty acids, (▶Chap. 42) and several pyrimidine disorders that affects the synthesis of cytidine, uridine and thymidine nucleosides and are treatable, such as CAD deficiency, or the congenital orotic aciduria (▶Chap. 32). Severe defects affect early developmental stages and behave as brain malformations whereas mild forms may present as 'synaptopathies' [7].

In addition to amino acid and fatty acid metabolism defects, the small molecules group defects also encompass the IEM of neurotransmitters. Hyperglycinaemias and dopamine transporter defect behave more as neurotransmitter disorders and signalling molecule defects than as intoxication disorders (▶Chap. 30).

In summary, most small molecule defect disorders produce major neurodevelopmental disruptions, thereby leading to severe global encephalopathies where almost all neurological functions are chronically altered. These defects mimic early 'non-metabolic' genetic encephalopathies.

1.1.2 Group 2. Complex Molecule Disorders (▶Chaps. 35, 36, 37, 38, 39, 40, 41, 42, 43, and 44)

This expanding group encompasses diseases that disturb the metabolism of complex molecules that are neither water-soluble nor diffusible. The main chemical categories of such complex molecules are glycogen, sphingolipids (SPL), triglycerides (TG) phospholipids (PL), complex long chain fatty acids (LCFA), cholesterol and bile acids, glycosaminoglycans (GAGs), oligosaccharides (OLS), glycoproteins, glycolipids and nucleic acids. These complex metabolic processes of synthesis and recycling take place in organelles (mitochondria, lysosomes, peroxisomes, endoplasmic reticulum and Golgi apparatus) and most pathways involve several organelles and require transporters.

Congenital disorders of glycosylation (CDG) (▶Chap. 43) and trafficking, processing and quality control disorders, belong also to this category (▶Chap. 44). Clinical symptoms are permanent, very often progressive, independent of intercurrent events, and unrelated to food intake. Most disorders do not present with acute crises.

Similar to the small molecule disorders there are also two subcategories in complex molecule disorders, defined by whether the phenotype primarily results from an accumulation or a deficiency.

1.1.2.1 Accumulation of Complex Molecules

Catabolism defects lead to storage of compounds visible in the cytoplasm or in lysosomes causing classical lysosomal storage disorders (LSD) (►Chaps. 40 and 41) or glycogenosis (GSD) (►Chap. 5). The neurological presentations consist of progressive disorders with late onset neurodegeneration with or without obvious 'storage' signs. Intracellular cytoplasmic TG disorders display non-cerebral presentations including hepatic steatosis with hypertriglyceridemia, neutral lipid storage disorders, congenital lipodystrophy with insulin resistance and diabetes and several other tissue specific disorders (►Chap. 35).

1.1.2.2 Deficiency of Complex Molecules

TG, PL, SPL, cholesterol and bile acids, GAGs, and OLS synthesis/remodeling disorders comprise a new rapidly expanding group of IEM without storage signs. Defects of non mitochondrial fatty acid metabolism including peroxisomal disorders, congenital disorders of glycosylation, nucleic acid metabolism, trafficking, processing and quality control disorders also belong to this category.

PL (►Chap. 35) and SPL (►Chap. 40) synthesis and remodeling defects display a variety of neurodegenerative symptoms, orthopaedic signs (bone and chondrodysplasia, malformation), syndromic ichthyosis, and retinal dystrophy. Phosphatidylinositides (P-ins) metabolism mutations are responsible for neurodevelopment and neurodegenerative disorders (►Chap. 35). Overall, there are not readily measurable metabolic markers for these diseases and diagnosis relies on NGS.

Most peroxisomal disorders involve complex lipids. They should be reclassified as non-mitochondrial fatty acid metabolism disorders in a vast complex lipid category (►Chap. 42). Many present at birth with a polymalformative syndrome, severe neurologic signs and hepatic involvement such as the Zellweger syndrome and chondrodysplasia punctata. Others present later between the first and second decade of life or in adulthood with neurodegenerative disorders.

Inborn errors of cholesterol (►Chap. 37) and bile acid synthesis (►Chap. 38) present either with polymalformative syndromes (such as Smith-Lemli-Opitz syndrome), neonatal cholestasis, or with late onset neurodegenerative disorders.

GAGs and oligosaccharides synthesis disorders do not present with preponderant neurological symptoms but rather should be suspected in patients with a combination of characteristic clinical features in more than one connective tissue compartment: bone and cartilage, ligaments, and subepithelial (skin, sclerae). Some produce distinct clinical syndromes with bone dysplasias (►Chap. 41). Many are usually classified in CDG syndromes (►Chap. 43).

1.1.2.3 Cellular Trafficking and Processing Disorders (►Chap. 44)

This is a new and growing category that encompasses CDG syndromes and many other defects affecting systems involved in intracellular vesiculation, trafficking, processing of complex molecules, and quality control processes (such as protein folding and autophagy). They should be considered with any unexplained clinical condition, particularly in multiorgan disease with neurological involvement but also with non-specific developmental disability (►Chap. 44).

The concept of 'synaptic metabolism' has been recently introduced; it can be defined as the specific chemical composition and metabolic functions occurring at the synapse [8]. Mutations coding for different proteins that regulate the synaptic vesicle (SV) exocytic-endocytic pathway have been described as responsible of a variety of disorders (►Chap. 30).

This oversimplified classification does not take into account the complexity of cellular biology and the more recent trafficking breakthroughs like the membrane contact sites and the membraneless organelles (►Chap. 44). The newly described metabolic disorders affecting cytoplasmic and mitochondrial tRNA synthetases, nucleic acid metabolism (►Chap. 39) and other factors related to cytoplasmic protein synthesis, transporters, channels and enzymes implicated in the signaling, logistics and regulation of the cell, challenge our current classification based on organelles. The same is true for nuclear factors related to gene expression and splicing. All these "new IEM" form a bridge between "classic" metabolic diseases with metabolic markers and those caused by structural proteins mutations without such markers and which are most often diagnosed by molecular techniques.

1.1.3 Group 3: Disorders Involving Energy Metabolism (►Chaps. 5, 6, 7, 8, 9, 10, 11, 12, and 13)

These consist of IEM with symptoms due, at least in part, to a deficiency in energy production or utilisation within the liver, myocardium, muscle, brain, and other tissues. Common symptoms in this group include hypoglycaemia, hyperlactataemia, hepatomegaly, severe generalized hypotonia, myopathy, cardiomyopathy, failure to thrive, cardiac failure, circulatory collapse, sudden unexpected death in infancy, and brain involvement. These diseases present an overlapping clinical spectrum, and manifestations sometimes result from accumulation of toxic compounds as well as from the deficiency in energy production or abnormal signaling.

Group 3 disorders can be divided in 3 sub-categories: energetic molecule transporter, cytoplasmic and mitochondrial defects. Diagnosis is difficult and relies on functional tests, enzymatic analyses requiring biopsies or cell culture, and increasingly on molecular analyses. Mitochondrial diseases are the most common group of IEM and are among the most common forms of inherited neurological disorders (▶Chap. 10).

1.1.3.1 Membrane Carriers of Energetic Molecules

Membrane carriers of energetic molecules include many tissue specific isozymes. The solute carrier (SLC) *SLC2* and *SLC5* gene families encode for the glucose carriers GLUT and GLT respectively while the *SLC16* gene family encodes for FA, ketone bodies, and monocarboxylic acid (MCT) carriers (▶Chap. 8).

1.1.3.2 Mitochondrial Defects

Mitochondrial defects encompass aerobic glucose oxidation defects presenting with congenital lactic acidemias (pyruvate transporter, pyruvate carboxylase, pyruvate dehydrogenase system, Krebs cycle and malate-aspartate shuttle defects) (▶Chap. 11), mitochondrial respiratory-chain disorders, mitochondrial transporters of energetic and other indispensable molecules (mostly belonging to the SLC25A family [6]), coenzyme Q biosynthesis (▶Chap. 10), β-oxidation, (▶Chap. 12) and ketone body defects (▶Chap. 13). A large and growing group of disorders, already more than 110, involve mitochondrial machinery (▶Chap. 10). Some of the mitochondrial disorders and pentose phosphate pathway (PPP) defects can interfere with the embryo-fetal development and give rise to dysmorphism, dysplasia and malformations. NAXE deficiency causes a severe neonatal encephalopathy (▶Sect. 11.14).

1.1.3.3 Cytoplasmic Energy Defects

Cytoplasmic energy defects are generally less severe. They include: disorders of glycolysis (▶Chap. 7), glycogen metabolism (▶Chap. 5), gluconeogenesis (▶Sects. 11.2 and 15.3) and hyperinsulinism (▶Chap. 6), all of which are treatable; disorders of creatine metabolism (▶Chap. 9), which are partially treatable; and inborn errors of the pentose phosphate pathways, with a phenotype mostly linked to defective NADP/NADPH production and which are largely untreatable [9] (▶Chap. 7).

1.1.4 Clinical Approach

The careful grouping of patients in well-defined clinical entities may provide algorithms for orientating metabolic (e.g., metabolomics and lipidomic approaches) and genetic (e.g., next generation sequencing) investigations.

Besides newborn screening in the general population or in at-risk families, the clinical diagnostic circumstances observed in IEM are divided in this chapter into five categories numbered 1.2–1.6:

- 1.2 Antenatal and congenital presentations
- 1.3 Neonatal presentations
- 1.4 Later-onset emergencies (from early childhood to adulthood) with acute (and recurrent) manifestations such as coma, ataxia, acidosis, exercise intolerance, or visceral failure…
- 1.5 Chronic and progressive neurological presentation (from early childhood to adulthood) (developmental delay, intellectual disability, epilepsy, neurological deterioration, psychiatric signs).
- 1.6 Specific and permanent organ/system presentations that may concern all medical specialities (cardiology, dermatology, endocrinology, gastroenterology, hematology … etc.).

1.2 Antenatal and Congenital Presentations

Dysmorphism is often combined with a neurodevelopmental disorder and other manifestations. About 30%–40% of genetic disorders manifest craniofacial abnormalities but only a few IEM present with antenatal symptoms. Metabolic disorders change the cellular environment and impact on gene expression and regulation. Those alterations affecting morphogenesis (during the embryonic period lasting the first 6–8 weeks) result in malformations, while those affecting growth and cellular differentiation of a tissue (during the fetal period starting after 8–9 weeks) result in dysplasias.

1.2.1 Classification of Antenatal Manifestations in Three Major Clinical Categories

1. True malformations (such as skeletal malformations, congenital heart disease, visceral aplasias and neural tube defects),
2. Dysplasias (such as brain malformations, polycystic kidneys, liver cysts),
3. Functional signs (such as intrauterine growth retardation, hydrops fetalis, hepatosplenomegaly, microcephaly).

Neurodevelopment may be disrupted in IEM at different stages producing a wide repertoire of clinical manifestations that range from severe brain malformations

with early complex encephalopathies to mild neurological signs such as learning difficulties [10].

True irreversible central nervous system (CNS) anomalies are observed in disorders of cellular trafficking (▶Chap. 44) O-glycosylation disorders (▶Chap. 43), cholesterol synthesis defects (▶Chap. 37), amino acid synthesis (▶Chap. 24) and transport defects (▶Chap. 25), NKH (▶Chap. 23), MFSD2A defect (DHA transporter) (▶Sect. 42.4.13), congenital Amish microcephaly due to mutations in *SLC25A19* (▶Sect. 29.1.3), congenital lipofuscinosis (brain shrinking at birth) (▶Chap. 40), receptor neurotransmitter defects (in particular ionotropic receptors) (▶Chap. 30), rarely in glutaric aciduria type II and in respiratory chain disorders (Table 1.1). Congenital NAD deficiency disorders have also been recently identified as an important potential cause of major congenital malformations that could be potentially prevented by niacin supplementation (▶Sect. 24.1.2). Of note the congenital microcephaly observed in serine synthesis defects is partially reversible with early treatment (▶Sect. 24.2). Lysosomal, peroxisomal and N-glycosylation defects are responsible for dysplasia and in the less severe cases partially reversible functional abnormalities. Ciliopathies (such

Table 1.1 Clinical, imaging, macroscopic and microscopic features in fetuses and neonates with IEM

Clinical feature		Disorder
Bones	Stippled epiphyses chondrodysplasia punctata)	Lysosomal disorders Peroxisomal disorders Cholesterol synthesis defects ALG3-CDG
	Shortening of the limbs	**Small molecules defects** Glutamine synthetase deficiency, *PAICS* mutation (purine defect), *SLC39A8* mutations (Mn transporter)
		Complex molecules defects ML II; RCDP; cholesterol synthesis defects Phospholipids synthesis defects (▶Chap. 35) *PLCB3* mutations leading to phosphoinositide signalling defect CDG syndromes: ALG3-CDG, ALG12-CDG, **SLC39A8**-CDG
		Other: Hypophosphatasia (alkaline phosphatase)
	Shortening of the extremities and malformations	Cholesterol synthesis defects CDG syndromes: ALG12-CDG, **SLC39A8**-CDG, COG7-CDG (▶Chap. 43) Phosphoinositide synthesis defects (▶Sect. 35.5) Other disorders: Arylsulfatase E deficiency, Refsum disease, *PAICS* mutation
	Joint contractures, arthrogryposis	Lysosomal storage diseases (LSD) and related disorders Gaucher type II, Mucopolysaccharidosis and Prosaposin deficiency Multiple sulfatase deficiency, Neutral sphingomyelinase-3 deficiency
		Complex molecules synthesis and trafficking defects: RCDP types I-III, Cholesterol synthesis defects, COASY deficiency Cellular trafficking disorders such as SLC35A3-CDG, ERG1C1, KIAA1109, Biallelic deletion of SOX 10 (associated with blond hair)
		Other disorders: Glycogenosis type IV, respiratory chain defects, Hyperekplexia type 4, glycine transporter 1 deficiency (▶Chap. 30), Glutamate decarboxylase (*GAD1*) (with cleft palate and omphalocele), severe fetal neuromuscular diseases and congenital myopathies
	Dysostosis multiplex: LSD (see ▶Chap. 41)	MPS types I, II, IV, VI, VII, multiple sulfatase deficiency, ML types I, II (with fractures), III, Pycnodysostosis, ISSD Alpha mannosidosis
	Vertebral defects Vertebral defects in VACTERL association Thoraco lumbar kyphosis	Congenital NAD synthesis defects (▶Sect. 24.1.2) *PAICS* mutation (purine defect) 3-hydroxyisobutyrylCoA deacylase deficiency MPS IH (at birth)

Table 1.1 (continued)

Clinical feature		Disorder
Head /face	Coarse facies Coarse facies appears later in life in many progressive storage disorders	At birth: Galactosialidosis, I-cell disease, GM1 gangliosidosis, Sialidosis type 2, MPS VII. In many other LSDs facial coarsening starts later in infancy as it is also observed in *AIFM1* mutations (see below)
		Onset in infancy/early childhood: Multiple sulfatase deficiency, Fucosidosis type I, Mannosidosis, MPS type IH, V, VII), Salla disease, Sialidosis type II, AIFM1 mutation (with Spondylo-epimetaphyseal dysplasia)
		Onset in childhood: Aspartylglucosaminuria, MPSs types I, III, & X Pseudo-Hurler polydystrophy
	Facial dysmorphism can be found in a large number of diseases. Some may have characteristic metabolic markers, such as Zelwegger syndrome, Smith Lemli Opitz (SLO) syndrome, PMM2-CDG or PDH deficiency	Maternal metabolic disturbances: PKU, Foetal alcohol syndrome, diabetes, drugs, riboflavin deficiency
		Defects of small molecules synthesis and transport: Amino acids synthesis defects; serine (including Neu Laxova syndrome), glutamine, asparagine synthesis defects Large neutral amino acid transporter (SLC7A5) and branched chain dehydrogenase kinase (BCDK) overactivity syndrome Purines (*PAICS* mutations, AICA ribosiduria) pyrimidines (***CAD*** and *DHODH* mutations causing Miller syndrome) **Molybdenum cofactor** / Sulfite oxidase deficiency, metal transporter defects
		Complex molecules synthesis, transport and processing: Lysophosphatidylcholine LPC symporter 1: (*MFSD2A*) Cholesterol synthesis defects (mostly SLO) Phospholipids synthesis defects (several types including plasmalogens and polyphosphoinositides defects) CDG (many types including PMM2-CDG, **SLC39A8** and Phosphoglucomutase defects (with cleft uvula and palate) Peroxisomal disorders (many types including Zellweger and RCDP)
		Defects in trafficking (see ▶Chap. 44, ▶Table 44.1)
		Defects in energy availability: mtDNA depletion, respiratory chain defects, PPP defects, MADD, CPT2 deficiency, PDH deficiency, Malate-aspartate shuttle defects (▶Chap. 11)
		Defects of post translational modifications and signalling Ralopathies (*RALGAPA1*) *PPP1R21* mutations (PP1 regulatory proteins)
		Many new syndromes without markers have been found with the use of NEGS as diagnostic tool …
	Craniosynostosis	I-cell disease, Antley-Bixler, *SLC39A8, KAT6A* mutations
	Cleft palate, Bifida uvula	*GAD* (cleft palate), PGM1-CDG, Ethanolaminephosphotransferase 1 Congenital NAD synthesis defects (▶Sect. 24.1.2)
	Macrocephaly	See below ▶Sect. 1.5.5
	Cephalhematomas	See below ▶Sect. 1.5.5
	Microcephaly	See below ▶Sect. 1.5.5
	Midface hypoplasia	Sly disease (MPS type VII) Peroxisomal biogenesis disorders

(continued)

Table 1.1 (continued)

Clinical feature		Disorder
Growth	Overgrowth	*PIK3CA*-related segmental overgrowth disorders (with lipomatosis) Child syndrome (congenital hemidysplasia) Congenital hyperinsulinism and INSR/PI3K/AKT signalling pathway *AIFM1* mutation (with Spondyloepimetaphyseal dysplasia and severe neurodegeneration)
	Intra uterine growth restriction (IUGR)	Fetal alcohol syndrome, infants born to mothers with untreated PKU
		Complex molecules defects: Cholesterol biosynthesis defects (mostly with malformations) CDG (several types) and N-Glycanase defect Lysosomal storage disorders, peroxisomal disorders **Neutral sphingomyelinase-3 deficiency** Cytosolic isoleucyl-tRNA synthetase (IARS) *ATAD3* locus duplication (with cardiac failure) (see ▶ Sect. 1.3.7)
		Others: Respiratory chain disorders (isolated or with mild signs) *TMEM70* Transaldolase deficiency (with hydrops and pseudo cutis laxa) Many polymalformative syndromes Microcephalic primordial dwarfism
	Ascites and hydrops fetalis polyhydramnios NIHF (defined by the presence of fetal ascites, pleural or pericardial effusions, skin oedema, cystic hygroma, increased nuchal translucency, or a combination of these conditions)	Storage disorders **Niemann Pick type C** disease (with hepatosplenomegaly), Sphingosine-1-phosphate lyase (SGPL1) deficiency MLP type 2 (with multiple dysostosis), Wolman disease (with adrenal calcifications). MPS type VII, sialidosis, galactosialidosis Glycogenosis type IV (with fetal akinesia and arthrogryposis)
		Complex molecules synthesis and trafficking Cholesterol (Greenberg skeletal dysplasia: sterol C14 reductase) CDG: PMM2-CDG, ALG1-CDG, ALG8-CDG, and ALG12-CDG Ichthyosis prematurity syndrome (fatty acid transport protein 4) (▶ Sect. 42.4.2) Plasmalemma vesicle-associated protein (*PLVAP*) hydrops with facial dysmorphic features, and cardiac and renal abnormalities RAS–MAPK cell-signalling pathway (RASopathies)
		Miscellaneous TALDO deficiency, Pearson (with anemia), Barth (with myocardiopathy) Fumarase deficiency S-adenosylhomocysteine hydrolase deficiency (SAHH) (▶ Chap. 20) De novo purine synthesis: PAICS deficiency (with malformations) Thrombospondin-1 domain containing protein 1 defects (see ▶ Sect. 1.3.5.2) INSR/PI3K/AKT signalling pathway defects (with severe hypoglycaemia, megalencephaly and multiple malformations) (▶ Chap. 6) Ornithine decarboxylase overactivity Growth and differentiation factor 2 (GDF2) mutations
Liver	Hepatomegaly/splenomegaly	See later ▶ Sect. 1.3.5
	Hemosiderosis	Neonatal hemochromatosis, Respiratory chain disorders, TALDO, Zellweger
	Steatosis	See later ▶ Sect. 1.3.5
	Bile ducts anomalies	Zellweger, PMM2-CDG, ALG3-CDG Respiratory chain disorders

Table 1.1 (continued)

Clinical feature		Disorder
Kidneys	Cysts	Zellweger, MADD, CPTII, promotor mutation in phosphomannomutase II (with hyperinsulinaemic hypoglycaemia: HIPCKD) Ciliopathies (*TMEM67*)
Heart	Hypertrophic cardiomyopathy	See ▶ Sects. 1.3.7 and 1.4.8
	Malformations (only few examples are cited)	Baby born to mother with untreated PKU
		Several trafficking disorders (▶ Chap. 44): Vesicular trafficking (*RAB23, STRADA, VPS13B, WFS1*) Organelles and interorganelles trafficking (*COG1, PACS1, SLC35A1, ARCN1*) Cytoskeleton (*DYNC2H1, FLNA*)
		Miscellaneous *KAT6A* mutations *CNNM2* homozygous mutations Congenital NAD synthesis defects *PLVAP* mutations
Digestive system	Oesophageal atresia, tracheoesophageal fistula Choanal stenosis/atresia	*PAICS* mutation (purine defect) Cobalamin C and F disorders
	Omphalocele	Glutamate decarboxylase (GAD) deficiency
	Hyperechogenic colon (cysteine crystals)	Cystinuria
Skin	Ichthyosis See later ▶ Sect. 1.6.2	
Nail	Nail hypoplasia	Glycosylphosphatidylinositol (GPI) deficiency
Genitalia	Hypospadias, sexual ambiguity	Smith Lemli Opitz, Antley Bixler, Desmosterolosis, Respiratory chain Disorders of adrenal steroid metabolism *RAB18* related Warburg micro syndrome *PAICS* mutations (small penis with hypospadias, cryptorchidism)
Brain (only a few disorders are cited) See ▶ Sect. 1.5.6	Hydrocephalus, Ventriculomegaly	Brain essential fatty acid transporter (*MFSD2A*) (▶ Sect. 42.4.13) INSR/PI3K/AKT signalling pathway defects (Arnold Chiari malformation) *WDR45B* mutations
	Gyration anomalies	Peroxisome defects (Zellweger spectrum), Amino acid synthesis defects, Congenital Amish microcephaly, O-CDG, tubulin defects, Congenital lipofuscinosis, Glutaric aciduria type II, Respiratory chain disorders, *SCHIP1/IQCJ-SCHIP1* mutations, Several cell trafficking disorders in particular Golgipathies GRINpathies Lissencephaly: Tubulin disorders, MAPS (microtubule associated proteins), ACTB genes (actin related) Severe pachygyria: Glutathione peroxidase 4 deficiency
	Corpus callosum agenesis/hypoplasia	Many disorders: NKH, PDH, Respiratory chain, Complex molecules synthesis defects, most Trafficking disorders (▶ Chap. 44). Corpus callosum thickening: Neurofibromatosis, syndromes with hyperactivation of PI3K/AKT3/mT MAST1 mutations
	Posterior fossa anomalies	CDG, peroxisomal disorders, pontocerebellar hypoplasia (ribosomopathies, tRNA synthetase deficiencies, Golgipathies), (▶ Chap. 39) Respiratory chain disorders.
	Neural tube defects	Folate disorders
	Calcifications	See later ▶ Sect. 1.5.6

(continued)

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Table 1.1 (continued)

Clinical feature		Disorder
Cells	Overloaded cells	Most lysosomal disorders Neutral lipid storage disorders (Jordan's anomaly)

Adapted from Collardeau and Guibaud [14]
ARCI autosomal recessive congenital ichthyosis, *CDG* congenital disorder of glycosylation, *CPT* carnitine palmityl transferase, *FA* fatty acid, *HIPCKD* hyperinsulinism polycystic kidney disease syndrome, *ISSD* infantile sialic acid storage disease, *LSD* lysosome storage disorder, *MADD* multiple acyl-CoA dehydrogenase, *MLII* mucolipidosis II, *MPS* mucopolysaccharidosis, *NBIA* neurodegeneration with brain iron accumulation, *NIHF* non immune hydrops fetalis, *NKH* non ketotic hyperglycinaemia, *PAICS* bifunctional purine enzyme (▶Chap. 36). *PDH* pyruvate deshydrogenase, *PPP* pentose phosphate pathway, *RCDP* rhizomelic chondrodysplasia punctata. *TALDO* transaldolase

as *TMEM67* mutations) and cytoskeleton disorders are cell trafficking defects that may display a wide range of presentations from lethal phenotypes to specific organ involvement only (▶Chap. 44). *SLC35A2* (encoding a UDP-galactose transporter) brain mosaicism has been very recently described in mild malformation of cortical development with oligodendroglial hyperplasia in epilepsy (MOGHE) [11].

The vast majority of "true intoxication" disorders (amino acid and organic acid catabolism disorders) do not interfere with the embryo-fetal development and do not give rise to dysmorphism and antenatal manifestations despite the presence of some toxic compounds prenatally. Coarse facies is seen in relatively few lysosomal disorders at birth but when present is an important diagnostic feature. Untreated maternal PKU (and possible tyrosinemia type II) can cause fetal dysplasia with intrauterine growth retardation (IUGR), mimicking foetal alcoholic syndrome (Table 1.1) (▶Sects. 16.3.1 and 17.3). Non immune hydrops fetalis and severe IUGR are frequent findings in a large number of inborn errors that disturb the synthesis or the catabolism of complex molecules, including some LSDs, peroxisomal disorders and cholesterol biosynthesis defects and in trafficking disorders such as those affecting the RAS–MAPK cell-signalling pathway (rasopathies) [12]. In most of these cases, many other significant clinical symptoms, such as facial dysmorphism, malformations, visceral or neurologic manifestations, lactic acidosis, and liver hemosiderosis, are present. IUGR is also a very frequent finding in a number of polymalformative syndromes of genetic and non-genetic origin. It is a preponderant symptom in respiratory chain disorders such as TMEM79 deficiency [13] and ATAD3 locus mutations (see below ▶Sect. 1.3.7).

1.2.2 Clinical Circumstances of Presentations

Antenatal manifestations of IEM can be suggested and should be investigated when facing some prenatal imaging findings, especially in cases of consanguinity and/or recurrence of symptoms, after exclusion of the most frequent non-metabolic aetiologies. Most imaging findings suggestive of IEM in the prenatal period are non-specific. They comprise ascites and hydrops fetalis, IUGR, CNS anomalies, echogenic kidneys, visceromegaly and a wide spectrum of dysostosis [14]. These anomalies can be isolated, but a combination of findings can be more suggestive of an IEM. An autopsy is essential when an IEM is suspected after an abortion [15]. It also allows the collection of fluid and tissue samples required for biochemical investigations to confirm the diagnosis [16]. The concept of a metabolic autopsy'has been recently developed [17].

A clinical examination in the delivery room often remains the difficult circumstance in which an IEM is suspected. The most important clinical, imaging, macroscopic and microscopic pathological findings reported in fetuses and neonates with IEM are listed in Table 1.1.

1.3 Presentation in Neonates and Infants (<1 Year)

The neonate has a limited repertoire of responses to severe illness. IEM may present with non-specific symptoms such as respiratory distress, hypotonia, poor sucking reflex, vomiting, diarrhoea, dehydration, lethargy, seizures; all problems that can easily be attributed to sepsis or some other common cause. Where a previously

affected sibling has died, this may have been wrongly attributed to sepsis, heart failure, or intraventricular haemorrhage, and it is important to critically review clinical records and autopsy reports when they are available.

In Group 1 disorders (small molecule accumulation that gives rise to intoxication), an extremely suggestive clinical picture is that of a baby, born at full-term after a normal pregnancy and delivery, who, after an initial symptom-free period, relentlessly deteriorates for no apparent reason and does not respond to symptomatic therapy. The interval between birth and clinical symptoms may range from hours to weeks, depending on the nature of the metabolic block and the environment. Investigations routinely performed in sick neonates, including a chest X-ray, CSF examination, septic screen, and cerebral ultrasound, yield normal results. This unexpected and 'mysterious' deterioration after a normal initial period is the most important indication for this group of an IEM. Careful re-evaluation of the child's condition is then warranted. In this context signs previously interpreted as non-specific manifestations of neonatal hypoxia, infection, or other common diagnoses take on a new significance. In energy deficiencies (group 3 disorders), the clinical presentation is often less evocative and displays variable severity. A general clinical algorithm for screening for treatable IEM in neonates is presented in Fig. 1.1. Not all treatable disorders are cited. See text and Table 1.2 for full list. A careful reappraisal of the child is warranted for the following three schematical presentations.

1.3.1 Neurological Deterioration (Coma, Lethargy): Metabolic Encephalopathy

Most IEM that result in intoxication or energy deficiency are brought to a doctor's attention because of neurological deterioration. With intoxication, the initial symptom-free interval varies in duration depending on the condition. Typically, the first reported sign is poor sucking and feeding, after which the child sinks into an unexplained coma despite supportive measures. At a more advanced state, neurovegetative problems with respiratory abnormalities, hiccups, apneas, bradycardia, and hypothermia can appear. In the comatose state, characteristic changes in muscle tone and involuntary movements appear. In **maple syrup urine disease (MSUD)** generalized hypertonic episodes with opisthotonus, and slow boxing or pedalling movements are observed. Of note most non-metabolic causes of coma are associated with hypotonia, so that the presence of 'normal' peripheral muscle tone in a comatose child reflects a relative hypertonia. Another neurological pattern observed in **OA** is axial hypotonia and limb hypertonia with fast large amplitude tremors and myoclonic jerks which are often mistaken for convulsions. An abnormal urine and body odour is present in some diseases in which volatile metabolites accumulate as in the maple syrup odour in **MSUD** and the sweaty feet odour in **isovaleric acidaemia** (IVA) and **type II glutaric acidaemia (GAII)**. If any of the preceding signs or symptoms are present, treatable metabolic disorders should be given a high diagnostic priority.

In energy deficiencies, the clinical presentation is less evocative and displays a more variable severity. In many conditions, there is no symptom-free interval. The most frequent findings are a severe generalized hypotonia, rapidly progressive neurological deterioration, and possible dysmorphism, or malformations. However, in contrast to the intoxication group, lethargy and coma are rarely initial signs. Hyperlactataemia with or without metabolic acidosis is very frequent. Cardiac and hepatic involvement are commonly associated (see below).

A few LSDs present in the neonatal period with neurological deterioration, hydrops fetalis or ichthyosis, such as Gaucher type II (collodion baby) and multiple steroid sulfatase deficiency. Most severe peroxisomal biogenesis disorder (PBD) present at birth with dysmorphism (Zellweger phenotype) and severe neurological dysfunction (neonatal adrenoleukodystrophy phenotype) (▶ Sect. 42.2.9). Severe forms of CDG involving N and O-glycosylation, glycosylphosphatidylinositol anchor and dolichol phosphate biosynthesis may also present with acute congenital neurological dysfunction although they more often present with hypotonia, seizures, dysmorphism, malformations and diverse visceral involvement (▶ Chap. 43). Cellular trafficking disorders often present as congenital microcephaly with diverse brain malformations. Brain MRI is an important biomarker in the differential diagnosis (▶ Chap. 44).

1.3.2 Seizures

Always consider the possibility of an IEM in a neonate with unexplained and refractory epilepsy [18]. Neonatal metabolic seizures are often a mixture of partial, erratic myoclonus of the face and extremities, or tonic seizures.

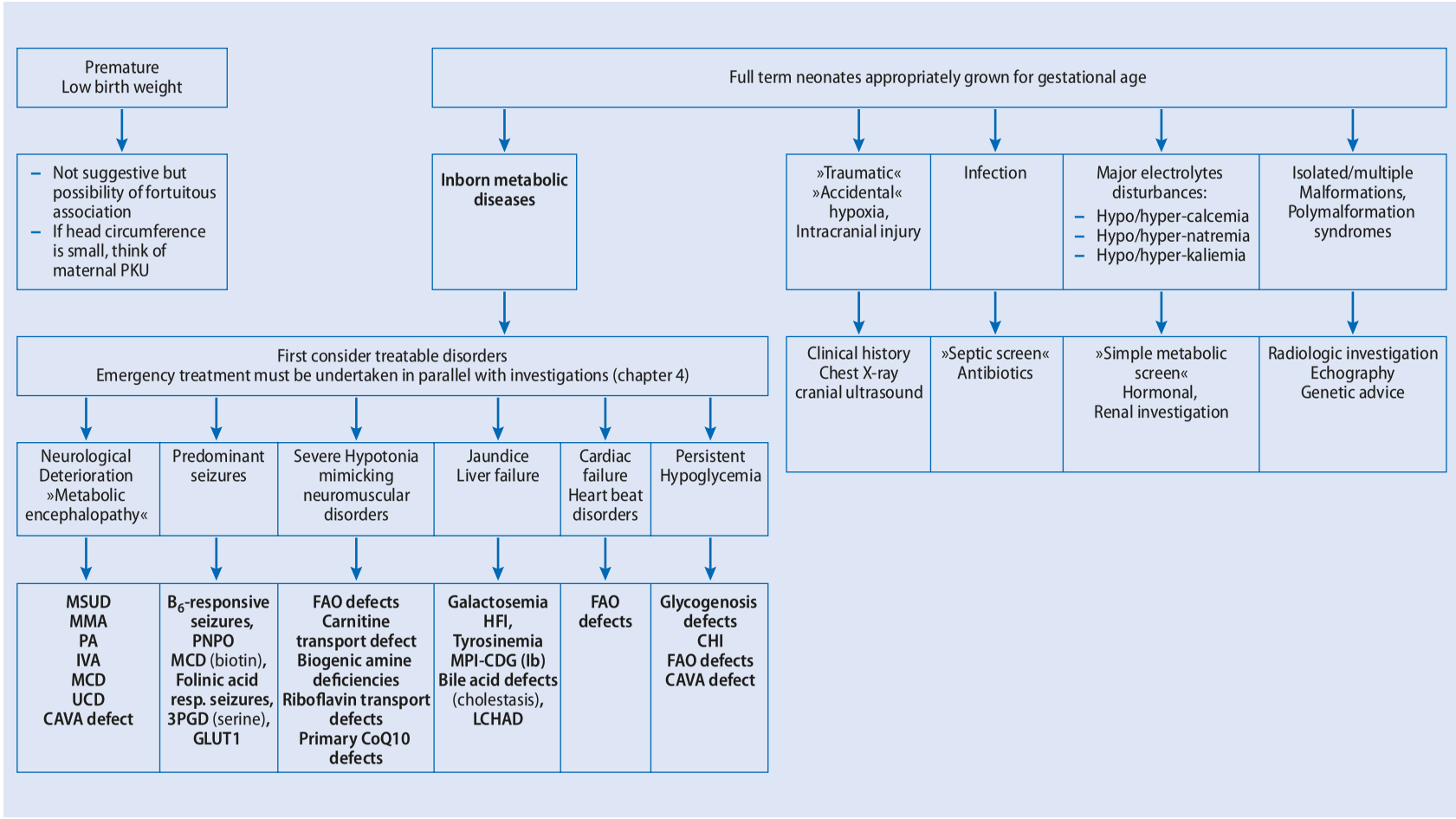

Fig. 1.1 The 'Sick' neonate: an algorithm for screening for treatable inborn errors of metabolism. CAVA carbonic anhydrase VA deficiency, CDG congenital disorders of glycosylation, FAO fatty acid oxidation disorders, CoQ10 coenzyme Q 10, HFI hereditary fructose intolerance, IVA isovaleric acidemia, LCHAD 3-hydroxy long chain acyl-CoA dehydrogenase, MCD multiple carboxylase deficiency, MMA methylmalonic aciduria, MSUD maple syrup urine disease, PA propionic acidemia, CHI congenital hyperinsulinism, PKU phenylketonuria, UCD urea cycle defects, PNPO pyridox(am)ine-5′-phosphate oxidase, 3PGD 3-phosphoglycerate dehydrogenase

Classically the term **'early myoclonic encephalopathy'** has been used if myoclonic seizures dominate the clinical pattern. The EEG often shows a burst-suppression pattern; however, myoclonic jerks may occur without EEG abnormalities.

1.3.2.1 Treatable and Potentially Treatable Disorders

- Several **treatable metabolic disorders** can present in the neonatal period or early in infancy predominantly with intractable seizures: **Congenital magnesium malabsorption** (in which seizures are linked to severe hypomagnesemia and hypocalcemia), **pyridoxine responsive seizures**, **folinic acid–responsive epilepsy** (both allelic to antiquitin deficiency)**, pyridoxal phosphate** (PLP) **responsive pyridox(am)ine-5′-phosphate oxidase deficiency**, the newly described pyridoxine responsive **PLPBP** (PROSC) deficiency (▶ Sect. 29.2.6), **3-phosphoglycerate dehydrogenase deficiency and other serine synthesis defects** responsive to serine supplementation, and **congenital hyperinsulinism** in which seizures are linked to hypoglycaemia. Of note **antiquitin deficiency** may present with secondary hypoglycaemia (and sometimes with hyperlactataemia) that may mislead the clinician towards other (untreatable) IEM. Interestingly, **GOT2 deficiency** (a PLP-dependent enzyme) is partly B_6 and serine responsive as may be all other defects disturbing the malate aspartate shuttle system (*MDH1, MDH2, AGC1* mutations) (▶ Sect. 11.11).
- Biotin responsive **holocarboxylase synthetase deficiency** can also rarely present predominantly with neonatal seizures. **GLUT1 deficiency syndrome (GLUT1DS)**, which is responsive to hyperketotic diet, and biotin responsive **biotinidase deficiency** can also rarely present in the first months of life as an epileptic encephalopathy but in these two disorders the early neonatal period is normally free of symptoms. Mutations in the *SLC5A6* a rare disease mimicking biotinidase deficiency has been described in few cases beyond the neonatal period (▶ Sect. 27.1.3). **Dihydropteridine reductase (DHPR)** deficiency is a treatable BH_4 synthesis disorder that may present as an early epileptic disorder associated with truncal hypotonia and abnormal movements.
- There are other recently described disorders presenting with severe early seizures in which a potential treatment has been suggested. These disorders are: (i) manganese deficiency due to *SLC39A8* mutations (potentially treated by galactose, uridine and manganese (▶ Sects. 34.4.3 and 43.4.8); (ii) SLC35A2-CDG that improves with D-galactose supplementation (▶ Table 43.4) (iii) **CAD** deficiency, involved in first steps of de novo pyrimidine biosynthesis, is responsive to uridine but most often starts in the second year of life (▶ Sect. 32.1.10). Several channelopathies (*SCNA*, ***KCNQ2*** mutations) present with severe neonatal epilepsy dramatically responsive to carbamazepine. Some electro-clinical features are pathognomonic of specific aetiologies and could lead to the early treatment with carbamazepine or oxcarbazepine [19]. Some patients with ***KCNQ2*** mutations are B_6 responsive [20]. Mutations in *SLC13A5*, a sodium and citrate cotransporter, causes neonatal epilepsy. Some patients may respond to acetazolamide [21].

Table 1.2 Neurological presentations with metabolic markers

Predominant clinical symptom	Main clinical signs	Metabolic marker /other suggestive abnormalities	Most likely diagnoses (disorder/enzyme deficiency)
NEUROLOGICAL DETERIORATION Metabolic encephalopathy **Mostly metabolic** and treatable	Lethargy, coma, hiccups Poor sucking hypothermia Hypotonia, hypertonia Abnormal movements Large amplitude tremor Myoclonic jerks "Burst suppression" Abnormal odour	Ketosis, acidosis	**MSUD** (odour)
		Ketoacidosis, bone marrow suppression	**OA: MMA, PA, IVA** (odour)
		Hyperlactataemia	**MCD**, mitochondrial disorders
		Hyperammonaemia	**Urea cycle defects, OAs CAVA deficiency**
		Characteristic changes of AAC or OAC (all disorders)	GA type II (odour)
		Hyperglycaemia (neonatal diabetes)	Ketolysis defects (mimicking neonatal diabetes) IER3IP1, PTF1A, WFS1 mutations (associate diabetes microcephaly and brain malformations)

Table 1.2 (continued)

Predominant clinical symptom	Main clinical signs	Metabolic marker /other suggestive abnormalities	Most likely diagnoses (disorder/enzyme deficiency)
SEIZURES Some metabolic, Some treatable	Mostly repetitive seizures unresponsive to antiepileptic drugs, and only responsive to specific treatments	Metabolic ketoacidosis, abnormal organic acids	**MCD**
		Elevated lactate, glycerol-3-P, in blood. Krebs cycle intermediates in urines	Mitochondrial, Krebs cycle and **MAS defects** **B_6/serine responsive**
		Elevated citrate (CSF, plasma)	*SLC13A5* (sodium and citrate cotransporter)
		Elevated pipecolic acid (CSF, P, U) and alpha-aminoadipic semialdehyde Hyperphosphatasia	**Pyridoxine responsive** **Folinic acid responsive** **GPI defects**
		Elevated CSF glycine, threonine, 3-orthomethyldopa, lactate, low dopamine and serotonin	**Pyridoxal phosphate responsive**
		Hypocalcemia Hypomagnesiemia	**Congenital magnesium Malabsorption**
		Severe hypoglycaemia	**PHHI**, adenosine kinase
		Low serine (P / CSF)	3PGD and other serine synthesis defects
		Low copper (P)	Menkes disease
		Hyperglycinemia	NKH
		Sulphite test (U) low uric acid (P, U) S-sulfocysteine (U)	**Molybdenum cofactor disease**/SO deficiency
		GABA in the CSF	GABA transaminase Glutamate decarboxylase?
		Low glutamine (P, U, CSF)	Glutamine synthetase deficiency
		Low asparagine	Asparagine synthase deficiency
		High glutamine	Glutaminase deficiency
		Hyperglycaemia (neonatal diabetes)	IER3IP1 (microcephaly, severe early epileptic encephalopathy)
		Glutamate oxidation in fibroblasts	Glutamate transporter deficiency
		High proline (P), low glutamate (CSF)	Hyperprolinaemia due to *SLC25A22* defect
		Diverse complex lipid and fatty acid profile abnormalities (P)	GM3 synthetase deficiency, mutations in FAH2, ELOVL4, GPI anchor defects, PBD
		Glycosylated transferrin (P)	CDG syndrome
		Purines (U)	Adenylosuccinate lyase deficiency *ITPA* mutations
		Abnormal pterins and biogenic amines in the CSF	**DHPR deficiency** Consider other BH_4 and monoamine defects such as AADC

Table 1.2 (continued)

Predominant clinical symptom	Main clinical signs	Metabolic marker /other suggestive abnormalities	Most likely diagnoses (disorder/enzyme deficiency)
	Facial dysmorphia Malformations Severe hypotonia	Abnormal VLCFA, phytanic, Plasmalogen (P and fibroblasts)	Peroxisomal defects
		Glycosylated transferrin (P)	CDG syndrome
		Low plasma manganese	**SLC39A8 (Mn transporter)**
		None/Hyperphosphatasia (P)	GPI anchor synthesis defects
		Sterols (P)	Cholesterol biosynthesis
		No biomarkers detected	*GAD1* variants
		High homocysteine and MMA	HCFC1
		Brain malformations	Cell trafficking disorders: CNPNY3, ATP6AP2, HCFC1, KIF5A
	Distinctive electro-clinical features	No biomarkers detected	**Several channelopathies (SCNA, KCNQ2 mutations**) responsive to carbamazepine
	Synaptopathies: epilepsy is associated to DD and other signs such as movement and autistic features	No biomarkers detected	Mostly post synaptic diseases related to mutations in GABA and glutamate receptors beyond the first month
SEVERE HYPOTONIA Rarely treatable	Isolated mimicking neuromuscular diseases	None	Prader Willi syndrome PFK deficiency (severe form)
		Glycosylated transferrin	IEM of the dolichols, CDG
		VLCFA, Phytanic acid	Peroxisome biogenesis defects
		Massive cystinuria	Hypotonia/cystinuria syndrome
		Hyperlactataemia COX deficiency related to some mt-tRNAGlu mutations	Mitochondrial disorders Reversible myopathy Aminoacyl-tRNA synthetases defects
		Abnormal CSF biogenic amines/pterines, glycine	**Neurotransmitter disorders**
		Acylcarnitines (P) organic acids, (U) low carnitine (P)	**Riboflavin transporter defects, FAO, carnitine transporter**
		COQ10 fibroblasts, muscle	**Primary COQ10 defects**

(continued)

1

Table 1.2 (continued)

Predominant clinical symptom	Main clinical signs	Metabolic marker /other suggestive abnormalities	Most likely diagnoses (disorder/enzyme deficiency)
	Fetal distress Hydramnios Arthrogryposis Respiratory failure	None	Severe fetal neuromuscular diseases Steinert, myasthenia (***SLC5A7***, ***CHAT***) Congenital myopathy Sensory-motor neuropathy AA-tRNA synthetase defects *RALGAPA1* variants
		Hyperlactataemia	Mitochondrial disorders
		Hyperglycinaemia, high BCAA, (P) ketoglutarate (U)	Lipoilation defects
	Predominant dysmorphia Malformations	VLCFA, phytanic, plasmalogen (P and fibro)	Peroxisomal defects
		Sterols (P)	Cholesterol defects
		Tubulopathy	Lowe
		Glycosylated transferrin (P)	N glycosylation defects
		APO-B glycosylation (P)	O glycosylation defects
		None/hyperphosphatasia (P)	GPI anchor synthesis defects
		Chromosome analyses	Chromosomal abnormalities
	Cataract, Tubulopathy	Hyperaminoaciduria Enzyme / DNA analyses	Lowe syndrome Respiratory chain disorders
	Cardiomyopathy Macroglossia	Vacuolated lymphocytes	Pompe, *PRKAG2*
		Hyperlactataemia	Respiratory chain disorders
		Acylcarnitine (P)	Trifunctional enzyme deficiency
	Nystagmus, hypomyelination, demyelination	Abnormal brain MRI findings that may be suggestive of each particular disease	Neonatal VWM (demyelination) Congenital hypomyelination due to: TMEM106B, X-linked MCT8 Congenital Pelizaeus-Merbacher Other genes: *MPZ, PMP22, ERG2, MTMR2, SOX10, CNTNAP1*

AAC amino acid chromatography, *CDG* congenital disorders of glycosylation, *CSF* cerebrospinal fluid, *COQ* coenzyme Q, *DD* developmental delay, *GAII* glutaric aciduria type II, *GPI* glycosylphosphatidylinositol, *HVA* homovanillic acid, *IVA* isovaleric acidaemia, *MAS* malate aspartate shuttle, *MCD* multiple carboxylase deficiency, *MMA* methylmalonic aciduria, *MSUD* maple syrup urine disease, *NKH* non ketotic hyperglycinaemia, *OA* organic acidurias, *OAC* organic acid chromatography, *P* plasma, *PA* propionic acidaemia, *PBD* peroxisomal biogenesis, *PFK* phosphofructokinase, *PHHI* primary hyperinsulinaemic hypoglycaemia of infancy, *PNPO* pyridox(am)ine-5′-phosphate oxidase, *SO* sulfite oxidase, *U* urine, *VLCFA* very long chain fatty acids, *3PGD* 3-phosphoglycerate dehydrogenase, *5HIAA* hydroxyindole acetic acid, *VWM* vanishing white matter

1.3.2.2 Disorders without Specific Treatment

- Many other non-treatable inherited disorders can present in the neonatal period or early in infancy with severe epilepsy. Severe forms of non ketotic hyperglycinaemia (NKH) and the new disease related to *SLC6A9* mutations encoding a glycine transporter (▶ Sect. 30.3) and sulfite oxidase deficiency (SO) are the most frequent. NKH displays a very typical, but not pathognomonic presentation with a burst-suppression pattern on EEG (▶ Chap. 23). The very rare D-glyceric acidaemia shares most of the signs of NKH. The clinical spectrum of SO and molybdenum cofactor (MoCo) deficiencies includes hypotonia, seizures, myoclonic jerks, and microcephaly. These disorders are probably underdiagnosed, because the clinical pattern shares many similarities with common acute fetal distress. Early diagnosis of MoCo type A deficiency could enable effective treatment with cPMP (▶ Sect. 20.10). Many other very rare untreatable disorders that may mimick NKH or present with prominent epilepsy are listed in ◘ Table 1.2. Most of these disorders have obvious or subtle metabolic markers in blood, urines and CSF.
- Many other rare variants without any metabolic makers have been described, including (i) mutations in the glutamodecarboxylase gene frequently associated with cardiomyopathy, cataracts and a brain MRI pattern that mimics Krabbe disease (▶ Sect. 30.1.3), (ii) Synaptic vesicle related defects, in particular those involved with docking and release such as *STXBP1, SPTAN1*, *TBC1D24, SIK1* (▶ Chap. 30), (iii) some other diseases related to these pathways such as vesicular traffic disorders *CNPNY3, ATP6AP2, HCFC1*. Early infantile spasms with hypsarhythmia were observed beyond the neonatal period in untreated PKU and have been recently described in a growing number of trafficking and related disorders, as in mutations of *NEUROD2* encoding for the transcription factor neuronal differentiation factor 2 [22] or *CNPY3*, encoding an Endoplasmic Reticulum Chaperone (▶ Chap. 44).
- Beside these well characterized IEM there are many early infantile monogenic epilepsy disorders that involve biochemical mechanisms. These were recently reviewed and elegantly classified into three general mechanisms: N- methyl D aspartate (NMDA)-pathies, phasic GABA-pathies and tonic GABA-pathies, that provide diagnostic clues and help to guide treatment strategy [23].

1.3.3 Hypotonia

Some IEM present with isolated hypotonia in the neonatal period but only very few are treatable. The most severe acute metabolic hypotonias are observed in pyruvate /lactate oxidation and respiratory chain disorders (with hyperlactataemia), **urea cycle defects** (with hyperammonaemia), NKH (with plasma/CSF glycine elevation), sulphite oxidase (SO) **/MoCo deficiency**, (with positive sulfi-test and sulfocysteine and low uric acid), peroxisomal disorders (with elevation of VLCFA and low plasmalogens). Severe global hypotonia and hypomotility mimicking neuromuscular diseases can appear in some treatable IEM such as **biogenic amine defects** [in particular **aromatic L-amino acid decarboxylase deficiency (AADC)**] with paroxysmal movements of limbs that can mimic those observed in MSUD and organic acidurias, and very suggestive oculogyric crises (▶ Sect. 30.5.2), **trifunctional enzyme deficiency, carnitine** (not strictly in the neonatal period) and **riboflavin transport defects** (with suggestive acylcarnitine and organic acid profile) (▶ Chap. 12) and **primary CoQ10 defect** (▶ Chap. 10). A reversible myopathy due to some particular mt-tRNAGlu mutations causes severe neonatal hypotonia (starting from 0 to 2 months of life) frequently requiring intensive care and nutritional support. Floppy infants with suspected mitochondrial myopathy should be screened for this mutation [24].

A diagnostic approach of the main metabolic causes of hypotonia is presented in ◘ Table 1.2.

Congenital mutations encoding for very early hypomyelinating disorders, such as TMEM106B involved in lysosomal trafficking are characterized by severe hypotonia with nystagmus and a distinctive brain MRI pattern [25]. Transcriptional co-repressor atrophin-1, encoded by *ATN1,* may present with early infantile hypotonia and severe cognitive impairment (CHEDDA syndrome) [26].

RALGAPA1 variants (encoding Ral GTPase activating protein) present at birth with nonspecific respiratory distress, muscular hypotonia, feeding abnormalities, recurrent fever episodes, and infantile spasms [27]. RNA polymerase II complex (POLR2A)

has recently been described as a cause of profound infantile onset hypotonia [28]. PURA (purine-rich element binding protein A) haploinsufficiency is responsible for a neurodevelopmental disorder with constant neonatal hypotonia; hypoglycorrhachias has been described in some cases [29].

1.3.4 Other Severe Motor Dysfunctions

Neonatal hypertonia is very common in SO deficiency and in hyperekplexia due to abnormal glycinergic transmission [30] (▶Sect. 30.3). Severe pontocerebellar hypoplasia, regardless of the specific genetic cause, early neonatal forms of Krabbe disease and gangliosidosis can also produce severe hypertonia with hyperexcitability signs. Fluctuating muscle strength, switching rapidly from normal state to hypertonus of the extremities and trunk, can be the equivalent of dystonic movements in the immature newborn brain. In such a clinical scenario, disorders with prominent basal ganglia, cerebellum and brainstem dysfunction should be considered (e.g., mitochondrial disorders and **neurotransmitter defects**). Additionally, oculogyric crisis and other abnormal ocular movements can be associated to GRIN1 defects, channelopathies (CACNA1), biogenic amine deficiencies and **GLUT1DS**. However, more than oculogyric crisis, GLUT1DS has a specific pattern of abnormal ocular movements (rapid, multidirectional, and often accompanied by a head movement in the same direction) that appears normally during the first months of life (▶Sect. 8.3.1) [31].

In general, IEM exhibit diverse patterns of abnormal movements in the neonatal period with specific features in particular disorders and pathophysiological groups.

These three neurological presentations are summarized in ◘Table 1.2.

1.3.5 Hepatic Presentations

Six clinical groups of hepatic presentations can be identified:

1.3.5.1 Hepatomegaly with Hypoglycaemia

- In a first group of disorders, hepatomegaly with hypoglycaemic seizures are often the presenting sign. The main diseases in this group with significant to severe hypoglycaemia are glucose 6-phosphatase deficiency (GSD type I) and fructose 1,6-biphosphatase (FBP) deficiency both with fasting lactic acidosis, and GSD type III with ketosis and postprandial hyperlactataemia. All 3 disorders improve dramatically with IV glucose administration delivered at physiological rate (<6–8 mg/kg/mn). Marked hepatocellular dysfunction is uncommon but may occur, especially in FBP deficiency. **Severe hyperinsulinism** may also present with a moderate hepatomegaly (▶Chap. 6).

1.3.5.2 Liver Failure

- Liver failure (jaundice, coagulopathy, hepatocellular necrosis with elevated transaminases, hypoglycaemia, ascites and oedema) with vomiting, poor feeding and failure to thrive suggests, **galactosaemia** (check neonatal screening) **fructosaemia** (now very rare since infant formulas are fructose/sucrose free), **tyrosinaemia type I** (with obvious clinical signs usually only after 3 weeks), all 3 disorders with tubulopathy, neonatal hemochromatosis (with normal or disproportionately low serum transaminases), respiratory chain disorders (mostly mitochondrial DNA stability and depletion (▶Chap. 10) and transaldolase deficiency which can present with hydrops fetalis and pseudo cutis laxa (▶Chap. 7). GRACILE syndrome (▶Chap. 10, ◘Table 10.2), alpha-1-antitrypsin deficiency, Wilson disease, and LCHAD deficiency can rarely present as liver failure early in the first month of life. One must emphasize that there are frequent difficulties in investigating patients with severe hepatic failure. At an advanced stage, many non-specific abnormalities secondary to liver damage can be present. Mellituria (galactosuria, glycosuria, fructosuria), hyperammonaemia, hyperlactataemia, hypoglycaemia after a short fast, hypertyrosinaemia (>200 µmol/l), hypermethioninaemia (sometimes higher than 500 µmol/l), and high excretion of phenolic acid derivatives (▶Chap. 3) are encountered in many cases of advanced hepatocellular disease. *IARS, LARS*, (▶Chap. 39) *SCYL1* and *NBAS* (▶Chap. 44) mutations cause recurrent acute liver failure triggered by fever in early infancy but which often does not start strictly within the neonatal period. The autosomal recessive *THSD1* mutations associated disorder (coding for the thrombospondin 1 domain containing protein 1) presents with fetal hydrops and polyhydramnios with ascites sometimes associated with haemangiomas and intracranial aneurysms [32].

1.3.5.3 Cholestatic Jaundice

- Cholestatic jaundice with failure to thrive is a predominant finding in alpha-1-antitrypsin deficiency, Byler disease (with intractable pruritus and normal serum gamma-GT), **inborn errors of bile acid metabolism**, peroxisomal disorders (including ACOX2), **Niemann-Pick type C** disease, CDG syndromes, hepatocerebral syndrome due to mitochondrial DNA depletion (▶Chap. 10), and *IARS* mutations associated with severe IUGR. Cerebrotendinous xanthomatosis (▶Sect. 38.3), citrin deficiency mostly but not only in Asia (▶Sect. 19.3.2), arginase deficiency and Niemann Pick type C disease can present as a transient asymptomatic jaundice before neurological signs appear later in life. Two new complex lipid synthesis disorders, the MEGDHEL syndrome (*SERAC* mutation) that can mimic Niemann Pick C with a positive filippin test (▶Sect. 35.3.7), and the spastic paraparesis type 5 due to oxysterol 7-hydroxylase deficiency (▶Sect. 38.4) may also present with transient cholestatic liver disease.

1.3.5.4 Liver Steatosis

- Liver steatosis: Hepatic presentations of inherited FAO disorders and urea cycle defects consist of acute steatosis or Reye syndrome with normal bilirubin rather than true liver failure. LCHAD deficiency is an exception that may present early in infancy (but not strictly in the neonatal period) as cholestatic jaundice, liver failure and hepatic fibrosis. Chanarin Dorfman syndrome presents early in infancy with liver steatosis, cataract, deafness and myopathy (▶Sect. 35.2.3). The newly described cytoplasmic Glycerol 3 phosphate dehydrogenase 1 deficiency presents as an asymptomatic early infantile hepatomegaly and steatosis with transient hypertriglyceridaemia (▶Sect. 35.1.1).

1.3.5.5 Hepatosplenomegaly with Storage Signs

- Hepatosplenomegaly (HSM) with storage signs is observed in LSD (GM1 gangliosidosis, sialidosis type II, I-cell disease, Niemann Pick type A disease, MPS type VII, Galactosialidosis, Prosaposin deficiency (with severe neurovisceral storage disease), Wolman disease (▶Sect. 36.4), ALG1-CDG and congenital erythropoietic porphyria (▶Sect. 33.2.3). Cherry red spot on fundoscopy (see below ▶Sect. 1.5.7) and vacuolated lymphocytes are suggestive findings (see below ▶Sect. 1.6.5). In some patients, Pearson syndrome [33] or CDG (*PMM2, ALG1, 8, 9-CDG, MGAT2 and COG 6*) can present as hydrops fetalis and simulate storage disorders [34]. HSM with liver failure is a presenting sign of the very rare PMP 70 deficiency (*ABCD3* mutations) (▶Sect. 42.2.6).

1.3.5.6 Hepatosplenomegaly with Inflammatory Syndrome

- HSM with inflammatory syndrome, haematological or immunologic features can be seen in many patients with SCID (such as caused by ADA or *RIPK1* mutations) (▶Sect. 32.3), lysinuric protein intolerance (macrophage activating syndrome, leucopenia), (▶Sect. 25.3), mevalonic aciduria (inflammatory syndrome, lymphadenopathy and recurrent severe anemia) (▶Sect. 37.1), and transaldolase deficiency (hydrops fetalis with severe anemia) (▶Sect. 7.10).

1.3.6 Congenital Diarrhoeal Disorders (CDD)

Affected genes include those related to disaccharidase deficiency, ion or nutrient transport defects, pancreatic insufficiency, lipid trafficking defects or PMI-CDG and ALG8-CDG. A new disorder presenting with CDD linked to *DGAT1* mutations has been recently described (▶Sect. 35.2.1). Mutations in *PLVAP* (encoding for a plasmalemma vesicle-associated protein) lead to a lethal form of protein-losing enteropathy (PLE) [35] as in another patient with lethal PLE associated with facial, cardiac, ocular, and renal anomalies [36]. Recently there has been growing interest in the genetic causes of severe intestinal phenotypes [37].

1.3.7 Cardiac and Vascular Presentations (See Also Later Presentations 1.4.8)

1.3.7.1 Cardiomyopathies

Some metabolic disorders can present predominantly with cardiac disease. Cardiac failure and a dilated hypertrophic cardiomyopathy (HCMNO) (pure dilated cardiomyopathy is very rare), most often associated with hypotonia, muscle weakness, and failure to thrive, suggests FAO disorders (with hypoglycaemia) (▶Chap. 12), respiratory chain disorders (with severe lactic acidosis) (▶Chap. 10), Pompe disease (with vacuolated lymphocytes) or fatal congenital heart glycogenosis due to mutation in *PRKAG* both with suggestive EKG (▶Chap. 5). Methylglutaconic aciduria (MGA) is found in Barth syndrome (▶Sect. 35.3.8) and ketoglutarate excretion in ketoglutarate dehydrogenase deficiency (▶Sect. 11.5). Respiratory chain disorders are a frequent cause of neonatal HCMNO. Some disorders are tissue specific while many others are ubiquitous such as the *TSFM*, *MRPS22*, *MRPL44,* the recently described MRPS*14* [38] or *TMEM70* mutations that present with severe lactic acidosis, hyperammonaemia and

hypercitrullinaemia [12] (▶Chap. 10). *ATAD3* locus de novo duplications present with severe neonatal multivisceral distress, HCMNO, leading to fatal cardiac failure, IUGR, corneal clouding, and hyperlactataemia with MGA [39]. Mutations of *AARS2* cause perinatal or infantile cardiomyopathy with near-total combined mitochondrial respiratory chain deficiency (▶Chap. 10). *SHMT2* mutations cause congenital microcephaly in neonates, however, hypertrophic cardiomyopathy appears later (▶Sect. 28.3.10). PMM-CDG syndrome (type Ia) can sometimes present in infancy with cardiac failure due to pericardial effusions, cardiac tamponade, and cardiomyopathy. Early progressive dilated cardiomyopathy resulting in death within 1 year is a presenting sign in dolichol kinase 1 deficiency (▶Chap. 43, ◘Table 43.4), recessive mutations in *ITPA,* (▶Sect. 32.1.9) and mutations in *PPCS* with severe lactic acidosis (▶Sect. 34.2.3.5).

1.3.7.2 Arrhythmias and Conduction Defects

Many defects of long-chain FAO can cause neonatal cardiomyopathy and/or arrhythmias and conduction defects (auriculoventricular block, bundle branch blocks, ventricular tachycardia) that may lead to cardiac arrest and sudden death (▶Sect. 12.1). Short PR interval and high QRS complexes are features in cardiac glycogenosis. Cardiac conduction defects are part of the lethal form of spondylometaphyseal dysplasia due to glutathione peroxidase 4 deficiency (▶Sect. 31.3.7).

1.3.7.3 Widespread Arterial Calcification

Infants with disorders of ectonucleotide metabolism (▶Sect. 39.1.1) can present with widespread arterial calcification (generalized arterial calcification of Infancy, leading to early demise in a significant number of cases. Arterial stenosis is common.

1.3.8 Neonatal Onset Multisystemic, Chronic Inflammation Disease (NOMID)

A few inherited multisystemic chronic inflammation disease (see ▶Sect. 1.6.7) may start in the early infantile period, either 'primary' such as mutations of *NLRP3,* encoding cryopyrin or secondary to various IEM like mevalonate kinase, some early severe lysosomal disorders such as Gaucher type 2 [40] and several trafficking disorders involving vesicular, interorganelle trafficking, autophagy or others (▶Chap. 44).

1.3.9 Initial Approach and Protocol for Investigation at the Bedside

As soon as there is clinical suspicion of an IEM, general supportive measures and laboratory investigations should be undertaken concurrently (◘Table 1.3). Although serum ketone bodies reach 0.5–1 mmol/l in early neonatal life, acetonuria, if observed in a newborn, is always abnormal and an important sign of a metabolic disease. Hypocalcaemia and elevated or reduced blood glucose are frequently present in metabolic diseases and the physician should be wary of attributing marked neurological dysfunction purely to these findings. Diagnostic approach of metabolic acidosis, ketosis, hyperlactataemia, hyperammonaemia and hypoglycaemia are discussed later in ▶Sects. 1.4.11–1.4.16.

The metabolic acidosis in OA is usually accompanied by an elevated anion gap. Urine pH should be below 5; otherwise, renal acidosis is a consideration. Metabolic acidosis resulting from IEM may develop as result of accumulation of fixed anion (lactate, ketone bodies, organic acid or a combination of both) or loss of bicarbonate, which is usually due to tubular dysfunction. In metabolic acidosis resulting from fixed anion, the plasma chloride concentration is normal and the anion gap, a reflection of the concentration of unmeasured anions, is increased. In patients with metabolic acidosis caused by loss of bicarbonate, the plasma chloride is elevated and the anion gap (the difference between the plasma sodium and the sum of the chloride and bicarbonate) is generally normal (ie, 10–15mmol/L). In metabolic acidosis with high anion gap the presence or absence of ketonuria is the major clinical clue to the diagnosis (▶Sect. 1.4.11). A normal blood pH does not exclude hyperlactataemia, as neutrality is usually maintained until serum levels reach 6 mmol/l (as long as bicarbonate levels remain >18 mmol/). Elevated lactic acid levels in the absence of infection or tissue hypoxia are a significant finding (▶Sect. 1.4.13). An elevated ammonia level in itself can induce respiratory alkalosis (▶Sect. 1.4.16). PA, MMA and IVA may induce granulocytopenia and thrombocytopenia (bone marrow suppression), which may be mistaken for sepsis. Transaldolase deficiency and early onset forms of mevalonate kinase deficiency present with severe recurrent hemolytic anemia.

Table 1.3 Protocol for emergency investigations

	Immediate investigations	Storage of samples
Urine	Smell (special odour) Look (special colour) Ketones (Acetest, ketostick, Ames) Reducing substances (multisticks pH: pHstix Merck) Sulfitest (Merck) Electrolytes (Na, K), urea, creatinine Uric acid	Urine collection: collect fresh samples before and after treatment and freeze at −20 °C. Do not use the samples without expert metabolic advice. Specialist metabolic investigation include: OAC, AAC, orotic acid, porphyrins
Blood	Blood cell count Electrolytes (search for anion gap) Glucose, calcium Blood gases (pH, pCO_2, HCO_3, pO_2) Uric acid Prothrombin time Transaminases (and other liver tests) Ammonia Lactic acid 3-hydroxybutyrate[a] Free fatty acids (FFA)[a]	Plasma (5 ml) heparinized at −20 °C Blood on filter paper: 2 spots (as 'Guthrie' test) Whole blood (1–5 ml) collected on EDTA and frozen (for molecular biology studies) Specialist metabolic investigations include: Total homocysteine, AAC (P) Acylcarnitine (tandem MS) (P, blood spot) Purines, pyrimidines Neurotransmitters (P, CSF, U) (HPLC, tandem MS) Peroxisome investigations (VLCFA, plasmalogen, phytanic acid CDG screening tests Specific markers (eg galactose markers, metals …)
Miscellaneous	Lumbar puncture Chest X-ray Cardiac echography, ECG Cerebral ultrasound, EEG	Skin biopsy (fibroblast culture) CSF (1 ml), frozen (neurotransmitters, AA) Postmortem: liver, muscle biopsies (▶Chap. 3)

AA amino acid, *AAC* amino acid chromatography, *CSF* cerebrospinal fluid, *DNPH* dinitrophenylhydrazine; the dinitrophenylhydrasine (DNPH) test screens for the presence of alpha-keto acids as occur in MSUD (however, it has now largely been abandoned.), *ECG* electrocardiogram, *EDTA* ethylenediaminetetra-acetic acid, *EEG* electroencephalogram, *MS* mass spectrometry, *HPLC* high performance liquid chromatography, *OAC* organic acid chromatography, *P* plasma, *U* urine, *VLCFA* very long chain fatty acids

[a]Blood ketone analysis is available using a bedside meter. 3-hydroxybutyrate and FFA data are generally not obtained in an emergency but are useful for interpreting the metabolic profile. Similarly, pyruvate and acetoacetate are not included in this emergency protocol

The storage of adequate amounts of plasma, urine, blood on filter paper, and CSF, is an important element in reaching a diagnosis. The utilization of these precious samples should be carefully planned after taking advice from specialists in IEM.

1.3.10 According to this Bedside Protocol Most Patients with Neurological Findings Can Be Classified into One of Six Metabolic Types (Table 1.4)

Once the above clinical and laboratory data have been collected, first line therapeutic recommendations can be made. This process is completed within 2–4 h and often precludes waiting long periods for the results of sophisticated diagnostic investigations. On the basis of this evaluation, most patients can be classified into one of six types that may overlap (Table 1.4).

1.3.10.1 With First Line Metabolic Disturbances

The experienced clinician will, of course, have to carefully interpret the metabolic data, particularly in relation to time of collection and ongoing treatment. At the same time, it is important to collect all the first line data listed in Table 1.3. Some very significant symptoms (such as metabolic acidosis and especially ketosis) can be moderate and transient, largely depending on the symptomatic therapy. Conversely, at an advanced state, many non-specific abnormalities (such as respiratory acidosis, severe hyperlactataemia, secondary hyperammonaemia) can disturb the original metabolic profile. This applies particularly to IEM with a rapidly fatal course such as severe urea cycle defects, in which the initial characteristic presentation of hyperammonaemia with respiratory alkalosis shifts rapidly to a rather non-specific picture of acidosis and hyperlactataemia.

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Table 1.4 Classification of inborn errors with neurological deterioration presenting in the neonatal period and in early infancy (according to preponderant first line metabolic disturbance)

	Clinical types	Acidosis/Ketosis	Other signs	Most likely diagnosis (disorder/enzyme deficiency)	Metabolic test
I	**With ketosis** "Intoxication" type, 4–8 days of "well" period Slow abnormal movements Hypertonia	Acidosis 0/± Ketone urine test 0/±	NH_3 N or ↑ ± Lactate N Blood count N Glucose N Calcium N	**MSUD** (▶Chap. 18) (abnormal odour) Mild forms of OA (see below)	AAC (P, U) Blood spot for tandem MS-MS OAC (U)
II	**With ketoacidosis** "Intoxication" type 1–3 days of 'well' period Fast abnormal movements Dehydration	Acidosis ++ Ketone urine test ++ Ketoacidosis	NH_3 ↑ +/++ Lactate N or ↑ ± Blood count: leucopenia, Thrombopenia (BMS) Glucose N or ↑ + Calcium N or ↓ +	OA (▶Chap. 18) Ketolysis defect (▶Chap. 13)	OAC (U, P) Carnitine (P) AcylCarnitin (U, P, DBS) By tandem MS-MS
	With non (hypo) ketotic acidosis "Energy deficiency" type, with liver or cardiac signs	Acidosis ++/± Ketone urine test 0 **(no ketosis)**	NH_3 ↑ ±/++ Lactate ↑ ±/++ Blood count N Glucose ↓ +/++ **(hypoketotic hypoglycaemia)**	**FAO and ketogenesis defects** (▶Chap. 12)	Idem above Metabolic profile FAO studies Lymphocytes fibroblasts, DNA tests
III	**With hyperlactataemia** "Energy deficiency" type, Polypnea, Hypotonia Lactic acidosis may be sometimes well tolerated	Acidosis +++/+ Ketone urine test++/0 Lactate ++	NH_3 N or ↑ ± Blood count: Anemia /N Glucose N or ↓ ± Calcium N	Pyruvate oxidation and TCA cycle defects (▶Chap. 11) Respiratory chain, (▶Chap. 14) Lipoylation defects (▶Sect. 23.2.3)	(L:P 3OHB: AA) OAC (U), AAC (P) PolarographEnzyme test DNA tests
IV	**With Hyperammonaemia**				
	Hyperammonaemia, without ketoacidosis "Intoxication" type, 0–3 days "well" period Moderate liver findings High blood pressure	**Alkalosis** Ketone urine test 0/+	NH_3 ↑ +/+++ lactate N↑/ + Blood count N Glucose N (low in CAVAS) Calcium N	UCD, CAVA, HHH (▶Chap. 19), LPI (▶Chap. 25), GS deficiency (▶Chap. 24)	AAC, OAC Orotic acid Carbaglu test DNA tests
	Hyperammonaemia with ketoacidosis "Intoxication" type, 0–3 days "well" period, dehydration.	Acidosis +/+++ Ketone urine test +/+++	NH_3 ↑ +/+++ **Lactate +/ ++** **Blood count: BMS**	OA (▶Chap. 18), PC (▶Chap. 11), MCD (▶Chap. 27)	AAC, OAC Carbaglu test + (OA) Biotin test + (MCD)
	Hyperammonaemia with hypoketotic hypoglycaemia Moderate liver findings Cardiac failure, cardiac arrythmias, muscle involvement	Acidosis 0/++ Ketone urine test 0/+/–	NH_3 ↑ +/+++ **Lactate N or ↑ +** Blood count N Glucose ↓ +/+++	FAO defects HI/HH syndrome (▶Chap. 12)	OAC carnitine Acylcarnitine FAO studies DNA tests Insulin
	Hyperammonaemia with elevated lactate Cardiomyopathy (TMEM 70)	Acidosis 0/++ Ketone urine test ++/0	NH_3 ↑ +/++ Lactate ↑ +/+++ Citrulline↑ +	PC, MCD, MAS TMEM70, (with high citrulline PDH, Krebs cycle defects	L/Pyr (P) OAC (U) Enzyme test DNA tests

Table 1.4 (continued)

	Clinical types	Acidosis/Ketosis	Other signs	Most likely diagnosis (disorder/enzyme deficiency)	Metabolic test
V	**With severe hypoglycaemia**				
	Recurrent seizures, hypotonia, and lethargy are mostly linked to hypoglycaemia and improve quickly after blood glucose comes back to normal (but in FAO)			**CHI and CHI like** (▶ Chap. 9) **FAO** **GSD I and III** FBPase deficiency **HFI, galactosemia, Tyr I,** TALDO, hemochromatosis, Mito DNA depletion	See later ▶ Chap. 3
VI	**With no first line metabolic disturbances** (see Table 1.5)				

N normal (normal values = NH_3 <80 μM; lactate <1.5 mM; glucose 3.5–5.5 mM), ± slightly modified, + moderate, ++ marked, +++ significant/massive, ↑ elevated, ↓ decreased, 0 absent (acidosis) or negative (acetest, dinitrophenylhydrazine, DNPH)

AA acetoacetate, *AASA* α-aminoadipic acid semialdehyde, *AAC* amino acid chromatography, *AGSD* acetylglutamate dehydrogenase deficiency, *BMS* bone marrow suppression, *CAT* carnitine acylcarnitine translocase, *CAVA* carbonic anhydrase Va, *CHI* congenital hyperinsulinisms, *CPS* carbamyl phosphate synthetase, *CPT II* carnitine palmitoyltransferase II, *DBS* dried bloodspots, *FAO* fatty acid oxidation, *GA II* glutaric aciduria type II, *GLCMS* gas liquid chromatography mass spectrometry, *GS* glutamine synthetase, *HFI* hereditary fructose intolerance, *HMGCoA* 3-OH-3-methylglutaryl coenzyme A, *ISSD* infantile sialic acid storage disease, *IVA* isovaleric acidemia, *L* lactate, *LARS* encodes a cytoplasmic leucyl-tRNA synthetase enzyme, *LCHAD* 3-OH long-chain acyl CoA dehydrogenase, *LPI* lysinuric protein in tolerance, *MAS* malate aspartate shuttle, *MCD* multiple carboxylase, *MCKAT* medium-chain 3-ketoacylCoA A thiolase, *MMA* methylmalonic acidemia, *MPS VII* mucopolysaccharidosis type VII, *MS* mass spectrometry, *MSUD* maple syrup urine disease, *NKH* nonketotic hyperglycinemia, *OAC* organic acid chromatography, *OTC* ornithine transcarbamylase, *P* plasma, *PA* propionic acidemia, *PC* pyruvate carboxylase, *PDH* pyruvate dehydrogenase, *PNPO* pyridox(am)ine-5′-phosphate oxidase, *Pyr* pyruvate, *PZO* peroxisomal disorders, *SO* sulfite oxidase, *SCOT* succinyl CoA trasferase, *TALDO* transaldolase, *U* urine, *UCD* Urea cycle defects, *TRMU* involved in thio-modified mitochondrial tRNAs, *VLCAD* very-long-chain acyl CoA dehydrogenase, *XO* xanthine oxidase, *3OHB* 3-hydroxybutyrate, *3PGD* 3-phosphoglycerate dehydrogenase

1.3.10.2 Without First Line Metabolic Disturbance (Type VI; Table 1.5)

These disorders cannot be screened on the basis of emergency protocol (Table 1.3). They present with complex encephalopathy with variable combination of hypotonia, seizures, motor disturbances +/− dysmorphism and various specific associated signs: neurosensorial, cutaneous, endocrine, immune, hematologic, orthopedic or visceral. They are summarized below in four categories following the simplified classification (see above ▶ Sect. 1.2). For many of treatable disorders diagnosis in emergency can be easily suspected based on second line metabolic tests (such as AAC, OAC … and more specific tests) quickly available in metabolic centres (▶ Chap. 3). Untargeted metabolomic, lipidomic and proteomic investigation is accessible in only few specialized centres and not in as an emergency. DNA testing is increasingly a first line diagnostic approach and is the only approach for those IEM without available metabolic markers (such as most trafficking disorders) (▶ Chap. 44).

1.4 Later Onset Emergencies: Acute and Recurrent Attacks (Early Childhood and Beyond)

Clinical Presentations

Consider the possibility of an IEM in a child or adult at any age with an acute unexplained, recurrent, or refractory attack. The symptom-free period is often longer than 1 year and may extend into late childhood, adolescence, or even late adulthood. Each attack can follow a rapid course ending either in spontaneous improvement or unexplained death, despite (or because of!) supportive measures in the intensive care unit. Between attacks the patient may appear well. Onset of acute disease may be precipitated by an intercurrent event or may occur without overt cause. Fever, excessive protein intake, prolonged fasting, prolonged exercise, and all conditions that enhance protein catabolism, may exacerbate such decompensations. Some interventions, such as general anaesthesia (performed for brain MRI) or corticoids prescription (for suspected encephalitis), made too rapidly before a comprehensive clinical evaluation, can have devastating consequences.

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Table 1.5 Classification of inborn errors with neurological deterioration presenting in the neonatal period and in early infancy without first line significant metabolic disturbances (type VI)

Disorders	Diagnostic tests
1. Small molecule defects accumulation or deficiency: most have plasma/urine/CSF metabolic markers	
NKH, SO plus XO, **MOCS**	AAC P, CSF high levels of specific AA, uric acid
Asparagine, glutamine, serine synthesis defects, glutaminase deficiency (► Chap. 24)	AAC P, CSF low levels of specific AA High glutamine Enzyme and DNA tests
Specific cerebral transporters defects of essential molecules: BCAA amino acids: SLC7A5 (►Sect. 25.6) Fatty acids: MFSD2A (►Sect. 42.4.13) Energetic molecules: Glucose and monocarboxylic acids, vitamins (see below ►Sect. 1.4)	AAC, OAC, glucose, lactate, fatty acids, in plasma, and CSF. DNA tests
BCKDH kinase deficiency (►Sect. 18.1.2)	Low plasma/CSF BCAA DNA tests
Metal disorders:	
Manganese transport (*SLC39A8* mutations ►Sect. 34.4.3)	Abnormal transferrin, low Mn DNA tests
Copper: ►Sect. 34.1	
Menkes disease (*ATP7A*) MEDNIK syndrome (*AP1S1* copper regulator) *SLC33A1* (acetylCoA transporter)	Low serum copper and ceruloplasmin in all 3 defects DNA tests
B_6-dependent seizures	OAC (AASA, pipecolic) B_6 response test DNA tests
Neurotransmitters and **PNPO defects, Biopterin defects**	OAC, neurotransmitters (P, U, CSF) therapeutic tests (L-DOPA, pyridoxal-Ph). DNA tests
A few purine and pyrimidine defects	Purines /pyrimidines P/U profile. Enzyme and DNA tests
2. Complex molecule disorders catabolism and synthesis defects with P/U metabolic markers	
A few lysosomal storage disorders- most with storage signs See ►Sect. 1.3.5	Oligosaccharides, sulfatides, sialic acid Enzyme and DNA tests
Peroxisomal defects (biogenesis defects, and bifunctional enzyme and ACO defects) Other non-mitochondrial fatty acid metabolism (ELOV, FALDH) (► Chap. 42)	VLCFA, phytanic acid, Plasmalogen, pipecolic acid, prostaglandins, leukotriens in plasma, urines DNA analysis
Trifunctional enzyme (also an energy disorder)	Acylcarnitine (P) OAC (U)
Respiratory chain (also energy disorder) see below	Lactate (P), OAC (U)
CDG syndrome type 1 (N-glycosylation) Some GPI defects GALNT2 (O-glycosylation)	Glycosylated transferrin (P) DNA analysis High alkaline phosphatases (in some GPI defects) Low HDL cholesterol in GALNT2 (Zilmer)
Cholesterol biosynthesis defects	Plasma sterols (P) DNA analysis
3. Complex molecule and trafficking disorders without metabolic markers	

Table 1.5 (continued)

Disorders	Diagnostic tests
Non systemic lysosomal disorders Krabbe's, ceroid lipofuscinosis	Enzyme and DNA analysis
Many CDG (O-glycosylation including the newly described GALNT2), GPI anchor and others…)	See also above with metabolic markers DNA analysis, Glycomics
Complex lipid and fatty acid synthesis and recycling defects (phospholipids, sphingolipids, complex fatty acids) (▶Chaps. 35, 40, and 42 respectively)	Lipidomics: P/CSF/fibroblasts DNA analysis
Dolichol synthesis defects (▶Sect. 43.3 and Fig. 43.2)	DNA analysis
Many inborn errors of trafficking, vesiculation, quality control and autophagy such as Vici syndrome (▶Sect. 44.3.2) Many are related to complex lipid metabolism Some causing leukodystrophy, demyelination/hypomyelination…)	DNA analysis
Synaptic vesicle cycle disorders	DNA analysis
Nucleic acid disorders (▶Chap. 39) t-RNA synthetases deficiency (KARS…) (▶Sect. 39.2.3) Ribosomopathies, (▶Sect. 39.3) DNA/RNA damage reparation defects, DNA methylation defects	Aminoacyl-tRNA Synthetases analysis DNA analysis
Tubulin subunits defects (TUBA1A, TUBB2B, TUBG1) and molecular motor proteins (KIF2A, KIF5C, DYNC1H1) (▶Sect. 44.2)	DNA analysis
4. Disorders involving primarily energy metabolism	
GLUT1DS	Low CSF/plasma glucose ratio
Mitochondrial disorders (many types) of them: Mitochondrial DNA depletion syndromes: Encephalomyopathic (*RRM2B*-related) Hepatocerebral (*DGUOK*-related, *C10orf2*-related) (▶Sect. 10.3.2)	Mitochondrial and nuclear DNA analysis

1.4.1 Coma, Strokes and Attacks of Vomiting with Lethargy (Table 1.6)

Acute encephalopathy is a common problem in infants and children with IEM. All types of coma can be indicative of an IEM, including those presenting with focal neurological signs. Neither the age at onset, the accompanying clinical signs (hepatic, gastrointestinal, neurological, psychiatric etc.), the mode of evolution (improvement, sequelae, death), nor the routine laboratory data, allow an IEM to be ruled out a priori. Most metabolic comas are linked to intoxication disorders and energy deficiency and many are treatable. Two categories can be distinguished. For adult presentations see ▶Chap. 2.

1.4.1.1 Metabolic Coma without Focal Neurological Signs

The main varieties of metabolic comas that may be observed in these late-onset, acute diseases are: coma with predominant metabolic acidosis, coma with predominant hyperammonaemia, coma with predominant hypoglycaemia, and combinations of these. A rather confusing finding in some OA and ketolytic defects is ketoacidosis with hyperglycaemia and glycosuria that mimics diabetic coma. In some severe cases with acute myolysis, as can occur in FAO and TANGO defects, clinical muscular symptoms can be missed if the patient is in a comatose state. An important rule in such conditions is to measure serum CK and test for myoglobinuria (see ▶Sect. 1.4.6). The diagnostic approach to these metabolic derangements is developed below (see ▶Sects. 1.4.11–1.4.18).

1.4.1.2 Neurological Coma with Focal Signs, Seizures, Severe Intracranial Hypertension, Strokes or Stroke-Like Episodes

Although most recurrent metabolic comas are not accompanied by neurological signs other than encephalopathy, some patients with **OA** and **UCD** present with focal neurological signs or cerebral oedema. These patients can be mistakenly diagnosed as having a cerebrovascular accident or cerebral tumour. In fact, IEM

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Table 1.6 Diagnostic approach to recurrent attacks of coma and vomiting with lethargy

Clinical Presentation	Metabolic derangements or other important abnormalities		Most frequent diagnosis (disorder/enzyme deficiency)	Differential diagnosis
Metabolic Coma (without focal neurological signs)	Acidosis (metabolic) pH <7.20 HCO_3^- <10 mmol/l PCO_2 <25 mmHg	Ketosis + (acetest ++)	**BCAA catabolism defects** (▶Chap. 18) **GAI** **Ketolysis defects, MCT1** **Gluconeogenesis defects** **MCD**, SLC5A6, PC Mitochondrial disorder **Sulfide:quinone oxidoreductase deficiency**	Diabetes Intoxication Encephalitis
		Ketosis -	**PDH, Ketogenesis defects** **FAO, FBP, EPEMA**	
	Hyperammonaemia NH_3 >100 µmol/l Resp alkalosis pH >7.45 pCO_2 <25 mmHg	Normal glucose	**Urea cycle defects**[a] **HHH syndrome** **LPI**	Reye syndrome Encephalitis Intoxication
		Hypoglycaemia	**FAO (MCAD**[a]**)** TANGO2 defect **HMGCoA lyase** **CAVA deficiency**	
	Hypoglycaemia (<2 mmol/l)	Acidosis +	**Gluconeogenesis defects** **MSUD** **HMGCoA lyase** **FAO, MCT1 (rare)**	Drugs and toxin Ketotic hypoglycaemia Adrenal insufficiency GH deficiency Hypopituitarism
	Hyperlactataemia (>4 mmol/l)	Normal glucose	**MCD**, PC, Krebs cycle Respiratory chain[a], **PDH**[a] (without ketosis) EPEMA syndrome	Hypoxia Sepsis
		Hypoglycaemia	**Gluconeogenesis defects** (ketosis variable) **FAO** (moderate hyperlactataemia, no ketosis)	

Table 1.6 (continued)

Clinical Presentation	Metabolic derangements or other important abnormalities		Most frequent diagnosis (disorder/enzyme deficiency)	Differential diagnosis
Neurological coma (with focal signs, seizures, or intracranial hypertension))	Biological signs are very variable, can be absent or moderate; see above 'metabolic coma'	Cerebral oedema Focal signs e.g. hemiplegia hemianopsia)	**MSUD, OTC** **MSUD, OTC, MMA, PA,** PGK	Cerebral tumour Migraine Encephalitis
		Basal ganglia involvement/ extrapyramidal signs	**TBBGD,** Leigh syndrome of diverse aetiologies, **MMA, GA1, Wilson, homocystinurias[a], Manganese transporter defect**	Acute encephalitis (infectious, immuno-mediated), metal intoxication
		Cranial nerve involvement, bulbar palsy	**Riboflavin transporter defects**	Brainstem encephalitis
		'Stroke-like' attacks Recurrent spontaneous vasospasm of internal carotid artery	**UCD, MMA, PA, IVA**[a] Energy metabolism defects[a] (mitochondrial DNA mutations such as MELAS, *POLG* mutations) Homocystinurias[a] CDG syndrome ***THTR1* mutations** **Fabry**[a] Acid maltase deficiency[a] (rare) Racemase deficiency (rare) ACOX 3	Moya Moya syndrome Vascular hemiplegia Cerebral thrombophlebitis, cerebral tumor
		Acute necrotizing encephalopathy triggered by febrile infection	Nuclear pore protein ran binding protein 2 (*RANBP2*)	
	Abnormal coagulation, (not constant alterations) Haemolytic anaemia	'Classic strokes' thromboembolic events	AT III, protein C, S deficiencies **Homocystinurias**[a] CDG, PGK Menkes and **GAI** can produce subarachnoid haemorrhage	Sickle cell anaemia

(continued)

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Table 1.6 (continued)

Clinical Presentation	Metabolic derangements or other important abnormalities		Most frequent diagnosis (disorder/enzyme deficiency)	Differential diagnosis
Hepatic coma (hepatomegaly, cytolysis or liver failure) Reye syndrome	Normal bilirubin Slight elevation of transaminases	Steatosis and fibrosis	**FAO, UCD**	
	Very high transaminases Severe coagulopathy	Recurrent acute liver failure (RALF) triggered by fever	*NBAS* mutations	
	Hypoalbuminemia, anaemia, seizures and encephalopathic crisis	Recurrent acute liver failure (RALF)	*LARS* (coding for cytoplasmic leucyl-tRNA synthetase)	
	Hyperlactataemia	Liver failure	Respiratory chain defects	Reye syndrome Hepatitis, intoxication
	Haemolytic jaundice	Cirrhosis Chronic hepatic dysfunction	**Wilson[a], Mn carrier defect** (SLC30A10)	
	Hypoglycaemia	Exudative enteropathy	Hepatic fibrosis with enteropathy **(MPI-CDG)**	

Bold face: treatable disorders

AT III antithrombin III, *CAVA* carbonic anhydrase Va, *CDG* carbohydrate-deficient glycoprotein syndrome, *EPEMA* encephalopathy, petechiae, ethylmalonic aciduria syndrome, *FAO* fatty acid oxidation, *FBP* fructose 1–6 biphosphatase, *GA* glutaric aciduria, *GH* growth hormone, *HMG-CoA* 3-hydroxy-3-methylglutaryl coenzyme A, *IVA* isovaleric acidaemia, *LPI* lysinuric protein intolerance, *MCD* multiple carboxylase deficiency, *MCT1* monocarboxylate transporter, *MELAS* mitochondrial encephalopathy lactic acidosis stroke-like episodes, *MMA* methylmalonic acidemia, *MSUD* maple syrup urine disease, *Mn* Manganese, *OTC* ornithine transcarbamylase, *PA* propionic acidaemia, *PC* pyruvate carboxylase, *PDH* pyruvate dehydrogenase, *PGK* phosphoglycerate kinase, *RALF* recurrent acute liver failure, *TBBGD* thiamine-biotin-responsive basal ganglia disease, *THTR1* thiamine transporter (thiamine responsive megaloblastic anaemia), *UCD* urea cycle disorders

[a]Cases reported in adults as presenting or predominant symptom

can lead to 'classic strokes' (either ischemic or haemorrhagic; they follow a well-defined anatomic vascular territory), and 'stroke-like' episodes, where the areas involved do not follow these precise anatomic vascular territories and mostly produce high intensity images in the basal ganglia and the cortex.

In most of these disorders, stopping the protein intake, delivering high glucose infusion rate and giving 'cleansing drugs' can be lifesaving. Some disorders may be dramatically vitamin responsive, for example **thiamine-biotin-responsive basal ganglia disease (TBBGD)** with Leigh-like changes involving the caudate, putamen and the medial thalami (▶ Sect. 29.1.2). **PDH** (thiamine), **biotinidase deficiency** (biotin), **SLC5A6** (biotin, pantothenic acid and lipoate (▶ Sect. 27.1.3), **riboflavin transporter defects** (riboflavin) (▶ Sect. 12.2), are other treatable causes that may present in a similar manner. All severe forms of **homocystinuria** (total homocysteine >100 μM) may cause an acute cerebrovascular accident from late childhood to adulthood and which can be the presenting sign. These include **cystathionine-β-synthase deficiency** (usually B_6-responsive in the late onset presentations), the severe **MTHFR** defects (folate/betaine responsive) and **CblC, CblD** defects (hydroxocobalamin responsive) (▶ Chaps. 20 and 28). Patients with **MMA** may, after first presenting with metabolic decompensation, have acute extrapyramidal and corticospinal tract involvement as a result of irreversible bilateral destruction of the globus pallidus (▶ Sect. 18.1). EPEMA syndrome (ethylmalonic encephalopathy) starts early in infancy with recurrent attacks of metabolic decompensation with lactic acidosis and bilateral lesions in the striatum, relapsing petechiae and orthostatic acrocyanosis (▶ Sect. 20.9). Late onset forms of **PDH** can present in childhood with recurrent attacks of ataxia, sometimes described by the patient as recurrent episodes of pain or muscular weakness (due to dystonia or to peripheral neuropathy) (▶ Sect. 11.3). Many other disorders with

abnormal organic acidurias including lactate may present with an acute encephalopathic episode, such as **GA1** mimicking encephalitis, 3-hydroxyisobutyric aciduria, malonic decarboxylase deficiency, **monocarboxylate transporter type 1 deficiency (MCT1)** (▶ Sect. 13.2), *SLC13A3* or metabolite repair defects (▶ Chap. 18 and ▶ Sect. 22.7.2).

Patients with mitochondrial DNA mutations have presented with cyclical vomiting associated with intermittent lactic acidosis. MELAS syndrome is another important diagnostic consideration in such late-onset and recurrent comas (▶ Chap. 10). Early episodic central nervous system problems, possibly associated with liver insufficiency or cardiac failure, have been the initial findings in some cases of CDG syndrome and **Fabry disease**. **Wilson disease** can rarely present with an acute episode of encephalopathy with extrapyramidal signs. Aicardi Goutiéres syndrome may present with strokes, inflammatory syndrome, chronic arthropathy and chilblains (▶ Sects. 1.6.15 and 39.1.2). Arterial tortuosity syndrome (*GLUT10* mutations) characterized by generalized tortuosity and elongation of all major arteries may result in acute infarction due to ischaemic strokes or an increased risk of thrombosis (▶ Sect. 8.6).

In summary, all these disorders should be considered in the differential diagnosis of acute cerebral injury and clinical pictures mimicking infectious/inflammatory encephalitis, strokes or stroke-like episodes. Vaguely defined and/or undocumented diagnoses such as encephalitis, basilar migraine, intoxication, poisoning, or cerebral thrombophlebitis should therefore be questioned, particularly when even moderate ketoacidosis, hyperlactataemia, or hyperammonaemia is present. In fact, these apparent initial acute manifestations are frequently preceded by other premonitory symptoms, which may be unrecognized or misinterpreted. Such symptoms include acute ataxia, persistent anorexia, chronic vomiting, failure to thrive, hypotonia, and progressive developmental delay – symptoms that are often observed in **UCD**, respiratory chain defects, and **OA**.

Certain features or symptoms are characteristic of particular disorders. For example, macrocephaly in **GAI**; angiokeratomas in **Fabry disease**; bone marrow suppression and recurrent infections or unexplained episodes of dehydration in **OA**; Macrocytic anaemia may be an important clue indicating a **cobalamin or folate disorder**.

When coma is associated with hepatic dysfunction, Reye syndrome secondary to disorders of **FAO or UCD** should be considered. In adults both conditions may mimic an alcoholic hepatic encephalopathy. Hepatic coma with liver failure and hyperlactataemia can be the presenting sign of respiratory chain disorders. Finally, hepatic coma with cirrhosis, chronic hepatic dysfunction, haemolytic jaundice, and various neurological signs (psychiatric, extrapyramidal) is a classic, but underdiagnosed manifestation of **Wilson disease.** A similar clinical scenario can be found at advanced stages of **manganese transporter deficiency** (▶ Sect. 34.4).

1.4.2 Recurrent Attacks of Ataxia (Table 1.7) for Adult Presentations See ▶ Chap. 2

Intermittent acute ataxia and disturbed behaviour can be the presenting signs of late-onset intermittent **MSUD** and **OA**, where they are associated with ketoacidosis and sometimes with hyperglycaemia which can mimic diabetic ketoacidosis (▶ Chap. 18). Late onset **OTC** and **ASS** deficiency can present with recurrent attacks of ataxia. Energy defects such as **GLUT1DS** may present with intermittent ataxia (or gait dyspraxia) as the only finding. In these cases, ataxia is usually worse before meals (▶ Sect. 8.2). Mild forms of **biotinidase deficiency and Hartnup disease** (▶ Sect. 25.4) may also cause intermittent ataxia. Acute ataxia associated with peripheral neuropathy is a frequent presenting sign of **PDH** deficiency; moderate hyperlactataemia with a normal L/P ratio supports this diagnosis (▶ Sect. 11.3). In general, most episodic ataxias belong to the channelopathies, which are not classical IEM but as other channel disorders in the nervous system, contribute to modulate brain neurotransmission. They have no metabolic marker (▶ Chap. 30).

1.4.3 Acute Psychiatric Symptoms (Table 1.8) for Adult Presentations See ▶ Chap. 2

Late-onset forms of **UCD,** mainly partial **OTC** deficiency, can present late in childhood or in adolescence with psychiatric symptoms. Because hyperammonaemia and liver dysfunction can be mild even at the time of acute attacks, these intermittent late-onset forms of **UCD** can easily be misdiagnosed as hysteria, schizophrenia, or alcohol or drug intoxication (▶ Chap. 19). **Acute intermittent porphyria and hereditary coproporphyria** present classically with recurrent attacks of vomiting, abdominal pain, neuropathy, and psychiatric symptoms (▶ Chap. 33). Finally, patients with **homocysteine remethylation defects** may present with schizophrenia-like, betaine and sometimes folate-responsive episodes. In view of **these possible diagnoses, it is justified to systematically measure**

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Table 1.7 Acute ataxias (often with disturbed behaviour and triggered by fever and infection)

Metabolic derangements or other important signs	Additional symptoms	Most frequent diagnosis (disorder/enzyme deficiency)	Differential diagnosis
Ketoacidosis Characteristic AAC (P) and OAC (U) profiles	Special odour Neutropenia Thrombopenia Hyperglycaemia	**Late onset MSUD** **MMA, PA, IVA**	Diabetes
Hyperammonaemia (sometimes slight elevation) AAC (P), orotic acid (U)	Respiratory alkalosis Hepatomegaly	**Urea cycle defects** (OTC, ASS)	Intoxication Encephalitis Brain tumour
Hyperlactataemia (sometimes very moderate and only in post-prandial state)	Normal L/P ratio No ketosis Peripheral neuropathy	**PDH**	Migraine Cerebellitis (varicella) Polymyoclonia Acetazolamide responsive polymyoclonia
	High L/P ratio Ketosis Cutaneous signs	**MCD**, Respiratory chain defects	Ataxia Acute exacerbation in chronic ataxias Channelopathies
AAC (U) (neutral AA in urines)	Skin rashes, pellagra, sun intolerance	**Hartnup and *CLTRN*** (▶ Sects. 25.4 and 25.5)	
OAC (U) (2 ketoglutarate and N-acetyl aspartate)	Reversible leukoencephalopathy	*SLC13A3* (encoding Na+/dicarboxylate cotransporter 3)	Encephalitis
Cytotoxic oedema of the basal ganglia	Skin rashes, pellagra-like vesiculobullous lesions Neurodegeneration Early death	NAXE, NAXD: NAD/NADP metabolite repair defects (▶ Sect. 11.14)	Encephalitis, meningitis, or autoimmune-mediated inflammation
Low glycorrachia (CSF) compared to blood glucose	Worse with fasting, may be associated with abnormal movements	**GLUT1DS**	
Low plasma biotinidase		**Biotinidase deficiency**	
No marker	Interictally myokymia	*KCNA1*	
	Nystagmus Down beat and gaze-evoked nystagmus Horizontal gaze-evoked nystagmus	*CACNA1A* *CACNB4* *SLC1A3*	
	Attack of dystonia/chorea	*PRRT2*	
	Areflexia, pes cavus, optic atrophy and deafness	*ATP1A*	

Bold face: treatable disorders
AAC amino acid chromatography, *ASA* argininosuccinic aciduria, *IVA* isovaleric academia, *L* lactate, *LPI* lysinuric protein intolerance, *MMA* methylmalonic acidaemia, *MCD* multiple carboxylase deficiency, *MSUD* maple syrup urine disease, *OAC* organic acid chromatography, *OTC* ornithine transcarbamylase, *P* pyruvate, *PA* propionic acidaemia, *PDH* pyruvate dehydrogenase

ammonia, porphyrins and plasma homocysteine in every patient presenting with unexplained acute psychiatric symptoms. Episodes of acute psychosis mostly in adulthood also occur in **cerebrotendinous xanthomatosis**, in the autosomal dominant disorder neuroferritinopathy which is associated with low serum ferritin, and in several late-onset complex molecule disorders (such as LSD and NBIA syndromes) (▶ Chap. 2). The newly described GALNT2-CDG exhibits a syndrome characterized by autistic features, behavioural abnormalities associated with global developmental delay, intellectual disability with language deficit, epilepsy, chronic insomnia, and white matter changes on brain MRI [41].

Table 1.8 Diagnostic approach to recurrent attacks of psychiatric symptoms

Clinical presentation	Metabolic derangements or other important signs	Additional symptoms	Most frequent diagnosis (disorder/enzyme deficiency)
Psychiatric symptoms, particularly if atypical (hallucinations, delirium, dizziness, aggressivity, anxiety, schizophrenic-like behaviour, agitation)	Hyperammonaemia (sometimes moderate), AAC, orotic acid	Slight liver dysfunction Vomiting Failure to thrive	**Urea cycle defects (OTC, ASA, ARG1)** **LPI, Citrin deficiency**
	Ketoacidosis AAC, OAC	Ataxia, neutropenia	**Organic acid defects, MSUD**
	Port-wine urine P and U porphyrins	Abdominal pain, vomiting, all forms of neuropathy	**AIP, hereditary coproporphyria**
	Homocystinuria (total homocysteine >100 µM)	Stroke, seizures, Myelopathy	**Methylene tetrahydrofolate reductase deficiency**
	AAC (neutral AA or neutral and charged AA in urines)	Skin rashes, pellagra	**Hartnup disease, CLTRN** (▶ Sect. 25.5)
	Low copper, ceruloplasmin Low serum ferritin	Dystonia, parkinsonism, Kayser–Fleischer rings, Pallidal necrosis	**Wilson** Neuroferritinopathy PKAN 2
	Foam cells in bone marrow, Cholestanol (plasma sterols)	Vertical ophthalmoplegia, Dentate nuclei hyperintensity	**Niemann-Pick type C** **Cerebrotendinous xanthomatosis**
	Low HDL cholesterol Loss of O-glycosylation of apolipoprotein C-III	ID, epilepsy Insomnia	**GALNT2-CDG**
	None	Epilepsy, retinitis pigmentosa hereditary spastic paraplegia (SPG26)	Ceroid Lipofuscinosis GM2/GD2 synthase deficiency

Bold face: treatable disorders
AAC amino acid chromatography, *AIP* acute intermittent porphyria, *ASA* arginosuccinic aciduria, *ID* intellectual disability, *MSUD* maple syrup urine disease, *LPI* lysinuric protein intolerance, *PKAN* panthothenate kinase associated degeneration, *OAC* organic acid chromatography, *OTC* ornithine transcarbamylase, *P* plasma, *U* urine

1.4.4 Dehydration (Table 1.9)

In children, dehydration is a common consequence of diarrhoea caused by a variety of enteral or parenteral acute infections. However, these common infectious diseases can occasionally trigger acute decompensation of an IEM. Moreover, aside from dehydration due to gastrointestinal losses, some IEM can present as recurrent attacks of dehydration secondary to polyuria (in **OA**), hyperventilation (in severe acidosis or alkalosis) or excessive sweating (cystic fibrosis). The main accompanying findings (severe diarrhoea, salt wasting, ketoacidosis, failure to thrive, Fanconi syndrome) can be used to classify dehydration due to IEM. Carbonic anhydrase 12 deficiency presents with hyperchlorhydrosis whose only symptoms were visible salt precipitates after sweating, hyponatremic dehydration, poor feeding and slow weight gain in infancy [42].

1.4.5 Reye Syndrome, Sudden Unexpected Death in Infancy (SUDI) and Near-Miss

A large number of IEM has been described that produce episodes fitting the criteria originally used to define Reye syndrome. There is now considerable evidence that many of the disorders responsible for Reye syndrome were misdiagnosed in the past because of inadequate investigations for IEM. Another important reason for this underestimation is the necessity of collecting blood and urine specimens for metabolic investigations at an appropriate time in relation to the illness since most disorders affecting the mitochondrial pathway, **UCD, HHH** syndrome and **FAO disorders** may produce only intermittent abnormalities. In addition, in contrast to the usual belief, a normal or nonspecific urinary OA and acylcarnitine pattern, even at the time of an acute attack, does not exclude an inherited FAO disorder. True SUDI

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Table 1.9 Attacks of dehydration

Leading symptoms	Other signs	Age at onset	Diagnosis (disorder/enzyme deficiency)
Severe diarrhoea: 'gastrointestinal causes'	Severe watery acidic diarrhoea Glycosuria	Neonatal	**Glucose galactose malabsorption** **Lactase deficiency**
	Hydramnios, no meconium Severe watery nonacidic diarrhoea Metabolic alkalosis Low K^+, Cl^-	Congenital	**Congenital chloride diarrhoea**
	Severe watery diarrhoea	Neonatal	Diacylglycerol acyl transferase Plasmalemma vesicle associated protein (*PVAP*)
		After weaning or when sucrose or starch dextrins are added to the diet	**Sucrase isomaltase**
	Anorexia, failure to thrive Weight loss (before cutaneous lesions and alopecia)	2–4 weeks or after weaning	**Acrodermatitis enteropathica**
Ketoacidosis: 'organic acidurias'	Polyuria Polypnea Hyperglycaemia Glycosuria	Infancy to early childhood	**Diabetic coma** **MMA, PA, IVA** **3-Ketothiolase** Hydroxyisobutyric aciduria
Failure to thrive, anorexia, poor feeding, polydipsia, polyuria: 'renal tubular dysfunction'	Photophobia, renal Fanconi syndrome	Infancy (3–6 months)	**Cystinosis**
	Hypernatremia, vomiting Spasticity, DD	Neonatal to first month	**Nephrogenesis diabetes insipidus** (X-linked)
	Hyperchloremia Metabolic acidosis Alkaline urine pH	Early in infancy	**RTA type I (distal)** **RTA type II (proximal)** **RTA type IV**
	Hypoglycaemia Hepatic glycogenosis Fanconi syndrome	Early in infancy	**Fanconi Bickel syndrome** (GLUT II deficiency)
	Pulmonary infections Chronic diarrhoea Salted sweet	Infancy to early childhood	**Cystic fibrosis** **Carbonic anhydrase (CA) 12 (salted sweet and hyponatraemic dehydration)**
Salt-losing syndrome: 'adrenal dysfunctions'	Severe hyponatraemia Ambiguous genitalia	End of first week of life	**Congenital adrenal hyperplasia**
	Unambiguous genitalia	End of first week	**Hypoaldosteronism**
	Ambiguous genitalia	Infancy to early childhood	**Congenital adrenal hypoplasia** **Congenital adrenal hyperplasia,** late-onset forms
	Unambiguous genitalia		Hypo & pseudohypoaldosteronism
	Hypoglycaemia		**FAO (CPT I and II) hypoketotic** **X-ALD (ketotic)**

Bold face: treatable disorders

CPT carnitine palmitoyl transferase, *DD* developmental delay, *FAO* fatty acid oxidation, *GLUT* glucose transporter, *IVA* isovaleric acidaemia, *MMA* methylmalonic aciduria, *PA* propionic acidaemia, *RAT* renal tubular acidosis

due to an IEM is, however, a rare event despite the large number of publications on the topic and despite the fact that >30 metabolic defects are possible causes [43]. This assertion is not true in the first week of life in which unexpected death (SIDS or near-miss) **is a priori due to a FAO disorder** until proven otherwise. The recently described *LARS* and *NBAS* mutations (the latter triggered by fever) presenting with recurrent episodes of acute liver failure (RALF) may have been mistaken with Reye syndrome (▶Chap. 44). Cardiac arrhythmias cause sudden loss of conscious and sudden death. (see below Cardiac failure and rhythm disorders). SUD and SIDS occurred in 15% of 808 patients with mitochondrial diseases either in a suggestive clinical context (such as cardiomyopathy or Leigh syndrome) or sometimes as a presenting sign [44].

1.4.6 Exercise Intolerance and Rhabdomyolysis (Recurrent Myoglobinuria) (See Also ▶Sect. 2.3.10 Adult Presentations)

Exercise intolerance and recurrent myoglobinuria syndrome is defined as myalgias, cramping, and/or limb weakness associated with elevated serum levels of creatine phosphokinase (CK) and other sarcoplasmic enzymes (usually >100 times upper limit of normal), recurrent pigmenturia, and sometimes acute renal failure. In the last instance, or when the patient is in a comatose state, clinical muscular symptoms can be missed. An important rule is to check serum CK and for myoglobinuria in such conditions. Rhabdomyolysis may be also observed in acute intermittent porphyrias. The spectrum of genetic susceptibility for rhabdomyolysis has not yet been completely clarified and the following criteria have been listed to trigger an extensive genetic investigation [45]: RHABDO.

- **R** – Recurrent episodes of exertional rhabdomyolysis
- **H** – HyperCKaemia more than 8 weeks after event
- **A** – Accustomed to exercise
- **B** – Blood creatine kinase (CK) >50 × upper limit of normal
- **D** – Drug ingestion insufficient to explain exertional rhabdomyolysis
- **O** – Other family members affected or other exertional symptoms (e.g., cramps or myalgia)

The disorders of muscle energy metabolism present in two ways:

1.4.6.1 Glycolytic Disorders

In the glycolytic disorders, exercising muscle is most vulnerable during the initial stages of exercise and during intense exercise. A 'second-wind' phenomenon sometimes develops. Clinically, the glycolytic disorders are mostly observed in late childhood, adolescence, or adulthood. The CK levels remain elevated in most patients. The most frequent and typical disorder in this group is McArdle disease (▶Sect. 5.2.1).

1.4.6.2 FAO Disorders

In the FAO disorders, attacks of myoglobinuria occur typically after mild to moderate prolonged exercise and are particularly likely when patients are additionally stressed by fasting, cold, or infection. This group is largely dominated by muscle **CPT II, VLCAD, LCHAD and trifunctional (TF)** deficiencies, which may occur in childhood, in adolescence, or later. Recurrent rhabdomyolysis has been described in a child with **GAI** (▶Chap. 22) and recently in one patient with an **FAD transporter defect** (▶Sect. 12.2). **Deficiencies of VLCAD** and SCHAD may also present with a myopathy.

1.4.6.3 Complex Molecules Disorders

Mutations in *TANGO2* (▶Sect. 44.3.1) present in infants and children with episodic rhabdomyolysis, hypoglycaemia, hyperammonaemia, and susceptibility to life-threatening cardiac tachyarrhythmias mimicking a FAO.

Similarly, mutations in *TRAPPC2L* may present with the combination of febrile illness-induced encephalopathy and rhabdomyolysis [46] (▶Sect. 44.3.1).

Mutations in RYR 1 encoding the ryanodine receptor present with muscle rigidity and rhabdomyolysis when affected individuals are exposed to general anaesthesia from infancy (recessive mutations) to adults (dominant mutations) [47].

LPIN1 gene mutations should be regarded as a major cause of severe fever induced myoglobinuria in infancy and rarely in adults (▶Sect. 35.1.4). Recurrent rhabdomyolysis and stroke like episodes has been described in a single adult patient with racemase deficiency (*AMACR*) (▶Sect. 42.2.4).

1.4.6.4 Other Causes

Adenylate deaminase deficiency has been suspected to cause exercise intolerance and cramps in a few patients, but the relationship between clinical symptoms and the enzyme defect is uncertain (▶Sect. 32.4.1). Respiratory chain disorders (RCD) can present with recurrent muscle pain and myoglobinuria from neonatal period to adolescence often associated with cardiomyopathy or diverse neurologic signs (encephalomyopathy) (▶Chap. 10). A case of lipoamide dehydrogenase deficiency presenting with recurrent myoglobinuria has been described in an adult. It is not clear whether normo- and hyperkalaemic paralysis due to sodium channel gene mutations may present with attacks of exercise intolerance. Attention has been directed toward myoglobinuria associated with Xp21-linked myopathies. (Becker syndrome). Dystonic

crises occurring in **GLUT1DS** and PDH deficiency may clinically mimic cramping. **Cramp fasciculations syndrome** is a rare muscular hyperexcitability disorder characterized by spontaneous, painful muscle cramps and diffuse fasciculations, predominantly in the lower extremities. This syndrome has been recently shown to be linked to mutations in Transient receptor potential ankyrin 1 (TRPA1) a plasmalemmal cation channel and is carbamazepine responsive [48]. Chvostek and Trousseau's signs, spasticity and tetany, cramps, paraesthesia or even cardiac arrest are common presenting signs in **congenital hypomagnesemias** (▶Sect. 34.3).

1.4.7 Abdominal Pain (Recurrent Attacks)

Table 1.10 contains all pertinent information on recurrent attacks of abdominal pain. Mevalonic aciduria due to mevalonate kinase deficiency can present as recurrent attacks of abdominal pain with fever, skin rashes, arthralgias, and inflammatory syndrome and hyper IgD mimicking Mediterranean fever (▶Sect. 37.1).

Table 1.10 Abdominal pain

With flatulence, diarrhoea, loose stools	Diacylglycerol acyltransferase 1 deficiency **Lactose malabsorption** **Congenital sucrase isomaltase deficiency**
With vomiting, lethargy, ketoacidosis	**Urea cycle defects (OTC, ASA)** **Organic acidurias** (MMA, PA, IVA) **Ketolysis defects** Respiratory chain disorders **Diabetes**
With neuropathy, psychiatric symptoms	**Porphyrias** **Tyrosinemia type I acute crisis** **OTC** (late onset) MNGIE syndrome (intestinal obstruction)
With fatigue, weakness	Hemochromatosis
With hepatomegaly and splenomegaly	Cholesterol ester storage disease Lipoprotein lipase deficiency **Lysinuric protein intolerance** **MPI-CDG (protein losing enteropathy)** Hemochromatosis Mevalonate kinase deficiency
With pain in extremities	**Fabry disease** **δ-aminolevulinate dehydratase deficiency** **Sickle cell anaemia**
With hemolytic anemia	**Acute intermittent porphyria, Coproporphyria** Hereditary spherocytosis **Sickle cell anemia** Nocturnal paroxysmal hemoglobinuria
With Crohn/Pseudo Crohn disease	Trifunctional enzyme deficiency(?) **Carnitine transporter** deficiency (?) **Glycogenosis type Ib**
With inflammatory syndrome (fever rash, IC reactive protein)	Hyper IgD syndrome (mevalonate kinase deficiency)

1.4.8 Cardiac Failure, Cardiac Arrhythmias and Cardiomyopathy (Tables 1.11 and 1.12)

Acute cardiac failure, arrythmia and cardiomyopathy non obstructive (CMNO) can be the first signs in many IEM.

1.4.8.1 Hypertrophic Cardiomyopathy

Hypertrophic cardiomyopathy is a preponderant presenting sign in (1) several glycogen disorders (**Pompe** and Danon disease, AMP activated protein kinase, GSD III, GSD 0b, *RBCK1* and *GYG1* (Glycogenin) mutations (▶Sect. 5.2) (2) all **long-chain FAO disorders** except CPT I deficiency, and MADD (▶Chap. 12), (3) some RCD (▶Chap. 10), and Barth and Sengers syndromes (▶Sects. 35.3.6 and 35.3.8). Fatal congenital heart glycogenosis due to mutation in *PRKAG2* can initially mimic RCD, or trifunctional enzyme deficiency when generalized hypotonia is associated with cardiomyopathy. In adults *PRKAG2*/*AMPK* clinical phenotype displays a hypertrophic cardiomyopathy and preexcitation syndrome (Wolf Parkinson White and atrioventricular block) (▶Sect. 5.2). Many other IEM cause syndromic CMNO including lysosomal storage diseases and trafficking disorders (▶Chap. 44) (Table 1.12).

1.4.8.2 Dilated Cardiomyopathy

Dilated cardiomyopathy is a major sign in several dolichol synthesis/recycling defects and in COG7-CDG (▶Chap. 43). PMM2-CDG may at times present in infancy as tamponade with pericardial effusion, multiorgan failure, and characteristic cutaneous and neurologic features. Pericardial effusion associated with severe fatty liver has been observed in late onset type II glutaric aciduria. Isolated isobutyryl CoA dehydrogenase deficiency presenting with dilated cardiomyopathy has been recently described (▶Sect. 18.6).

1.4.8.3 Arrhytmias and Conduction

Alternatively, heart failure may result from disturbed cardiac rhythm. In congenital hypoparathyroidism and pseudohypoparathyroidism, cardiac failure can be the

Table 1.11 Arrhythmias, conduction defects

Primary or preponderant dysrhythmias	**Secondary to ion disturbances** **Adrenal dysfunction** (hyperkalemia) **Hypoparathyroidism** (hypocalcemia): prolonged QT interval **Congenital hypomagnesemias:** prolonged QT interval (especially with concomitant hypokalemia) Cardiac sodium and potassium channels genes mutations *SCN5A, KVLQT1, and HERG*
	Cardiac glycogenosis (▶Sect. 5.2)
	Pompe, Danon disease (short PR interval, high QRS complexes) AMP activated protein kinase (*PRKAG2*: WPW, supraventricular arrhythmias
	Primary and secondary energy deficiency+/– intoxication process
	FAO (▶Chap. 12), *TANGO2* mutations (▶Sect. 44.3.1) **Carnitine transporter defect** (only in adults) Phytanyl CoA hydroxylase deficiency (Adult Refsum disease) (▶Sect. 42.3) Triose phosphate isomerase deficiency (▶Sect. 7.3) Kearns-Sayre syndrome (▶Sect. 10.1, ■Table 10.2) **Propionic acidaemia** (prolonged QTc) (▶Sect. 18.1.1) **Thiamine deficiency/dependent states** (▶Sect. 29.1) D2-hydroxyglutaric aciduria (AV block) (▶Sect. 22.8)
With cardiac/multiorgan failure	PMM2-CDG (Ia) (with tam ▶Sect. 43.1.1) Timothy syndrome (de novo CaV1.2 missense mutation *G406R*.)

WPW Wolf Parkinson White, *AV* auriculoventricular, *CPT* carnitine palmitoyl transferase, *LCAD*/*LCHAD*/*VLCAD* long/3hydroxy long/very long chain acyl-CoA dehydrogenase, *TF* trifunctional enzyme, *CDG* congenital disorders of glycosylation

Table 1.12 Cardiomyopathies

Complex molecules accumulation disorders (accumulated compounds in parenthesis)	
Glycogenosis (▶Chap. 5) (glycogen)	AMP activated protein kinase (presenting sign)
	Glycogenosis type III, and IV (can be presenting sign) Glycogenin 1 and *RBCK1* **(adult presenting sign)**
	Muscular glycogen synthetase (GSD0b) (presenting sign) NO glycogen accumulation
	Pompe disease, Danon disease (with eye fundus abnormality) (presenting sign in both)
LSD (▶Chaps. 40 and 41) (sphingolipids, GAGs) Lipopigments	**Fabry disease** (may be presenting sign with myocardial infarction) **or conduction defects**
	GM1 gangliosidosis
	MPS cardiac valve disease with thickening of valves is common in MPS with accumulation of dermatan sulfate and MLIII Infantile free sialic acid storage disease (ISSD), MLII, MLIII
	CLN3 (adolescents to adults)
Complex lipids (▶Chap. 35) (phospholipids, neutral lipids)	Neutral lipid storage myopathy (Chanarin Dorfman and PNPLA2)
	Lipin1
Disorders involving energy metabolism	

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Table 1.12 (continued)

Mitochondrial disorders (▶ Chap. 10)	Primary respiratory chain defects
	Barth (3 methylglutaconic aciduria)
	Sengers syndrome
	Friedreich ataxia (presenting sign)
	Mitochondrial DNA depletion syndrome
	Dilated cardiomyopathy with ataxia syndrome (*DNAJC19* mutations)
	BOLA3 deficiency (with severe neurological dysfunction in infancy)
	TMEM70 mutations (complex V)
CoA synthesis defect (▶ Sect. 34.2.3)	***PPCS*** mutations
FAO disorders (presenting sign) (▶Chap. 12)	Carnitine transport defect and all FAO disorders except CPT1
Thiamine metabolism defect (▶Chap. 29)	**Thiamine responsive anaemia** **Thiamine deficiency states (BeriBeri)**
TCA Krebs cycle deficiency	Succinate dehydrogenase deficiency (▶Sect. 11.7)
Small molecules catabolism defects (secondary energy deficiency, +/– intoxication)	
Branched chain amino acid (▶Chap. 18)	**MMA (Cbl C) and rarely MMCoA mutase,** malonic aciduria
	Propionic aciduria
	3-Ketothiolase deficiency
	Isobutyryl-CoA dehydrogenase
	Short-chain enoyl-CoA hydratase deficiency (with Leigh like syndrome)
Disorders of intracellular trafficking	
CDG syndromes (▶Chap. 43)	PMM2-CDG (with pericardial effusion, can be the presenting sign)
	(COG7-CDG) defects (CDG IIe: dilated cardiomyopathy)
	Dolichol synthesis/recycling disorders (SRD5A3, DOLK, and DPM3-CDG): dilated cardiomyopathy
Vesicular, organelles and cytoskeleton trafficking disorders (▶Chap. 44)	Familial hypertrophic cardiomyopathy (*CAV3*) Martsolf syndrome (*RAB3GAP2*) Vici syndrome (*EPG5*) Wolfram syndrome (*WFS1*) *ATAD3A* mutations (hypertrophic CMNO with neurodegeneration)
Miscellaneous	Congenital muscle dystrophies
	Steinert disease myotonic dystrophy
	Selenium deficiency
	Taurine transporter deficiency (SLC6A6) (▶Sect. 25.8) [49]
	Tyrosinemia type 1 (long term complication)

consequence of severe hypocalcemia with a prolonged QT interval on ECG. Several arrhythmia susceptibility genes which encode cardiac sodium and potassium channels, such as *SCN5A, KVLQT1, and HERG* mutations, have been described. In the vast majority of such arrhythmia syndromes, individuals appear normal except for subtle electrocardiographic abnormalities. Timothy syndrome is due to mutations in *CACNA1C* encoding CaV1.2, the cardiac L-type calcium channel causing nearly complete loss of voltage-dependent channel inactivation. It is a multiorgan disorder including lethal arrhythmias, webbing of fingers and toes, congenital heart disease, immune deficiency, intermittent hypoglycaemia, cognitive abnormalities, and autism [50].

In Kearns-Sayre syndrome (KSS) (▶ Sect. 10.2), as in *PRKAG2*/*AMPK* (▶ Sect. 5.2), atrioventricular block with syncope is a classic sign. In the rare disorder triosephosphate isomerase deficiency, which presents early in infancy as hemolytic anemia and progressive neurologic dysfunction, arrhythmia may cause sudden cardiac death (▶ Sect. 7.3). A hyperkinetic hemodynamic state with sinus tachycardia, a classic finding in hyperthyroidism is also an early presenting sign in **thiamine-deficient and dependent states** associated with lactic acidosis which can be dramatically relieved by thiamine administration (▶ Sect. 29.1). Finally, all **long-chain FAO disorders** except CPT I deficiency can present in early infancy, even in the neonatal period, with cardiac arrest or hypotension, which is readily misdiagnosed as toxic shock or malignant hyperthermia. Disorders of heart rhythm (premature ventricular complexes, atrioventricular block, and ventricular tachycardia) are frequent features (▶ Chap. 12). Mechanisms of cardiac conduction defects observed in Adult Refsum disease are not fully elucidated (▶ Sect. 42.3).

Extreme sinus tachycardia with acute hypertension are hallmarks of an acute intermittent porphyria crisis (▶ Sect. 33.2.2).

1.4.9 Liver Failure, Ascites, Oedema

Table 1.13 presents the disorders with liver failure according to age of onset. Recent literature highlights the disorders that involve the mitochondrial tRNA translation (*TRMU*, *LARS*, *HARS*) and *MARS* or *KARS* mutations (with severe progressive leukodystrophy) (▶ Chaps. 10 and 39) and proteins involved in the retrograde trafficking at the endoplasmic reticulum (ER) membrane like *NBAS* and *SCYL1* mutations causing recurrent acute infantile liver failure (RALF) triggered by fever with neurodegeneration (CALFAN syndrome) [51] (▶ Chap. 44).

Table 1.13 Liver failure (ascites, oedema)

Age at onset	Disorder
Congenital, neonatal <1 year	See Table 1.1 and ▶ Sect. 1.3.5
Infancy (mostly recurrent crises triggered by fever and infections)	Complex molecules disorders: Alpha-1 antitrypsin deficiency CDG (of them familial hepatic fibrosis with exudative enteropathy **(MPI-CDG)** and ALG8-CDG), NGLY1-CDG. Cholesterol ester storage disease
	Trafficking disorders (▶ Chap. 44) Vesicular and organelle/interorganelle trafficking: *ATP7B, BCAP31, HYOU1, NBAS, PRF1, UNC13D COG4, STX11, SCYL1* mutations Other trafficking disorders: *RINT1* (part of the Syntaxin 18 complex at the ER; interacts with NBAS) *SCYL1* variants (Calfan syndrome) Cystic fibrosis
	Disorders involving energy metabolism Alpers-Huttenlocher (▶ Chap. 10) Mitochondrial DNA depletion syndromes (▶ Chap. 10) *TRMU* mutations (transient acute liver failure) Pearson syndrome Pyruvate carboxylase deficiency *ACAD9* mutations **Ketogenesis defects** Mitochondrial tRNA translation disorders (*LARS, MARS, HARS*)
	Miscellaneous **S-adenosylhomocystine hydrolase deficiency** **Urea cycle defects** Wolman disease
Childhood to adolescence	**Wilson disease**

1.4.10 Pain in Extremities and Bone Crisis

Pain in extremities can be due to bone, vascular, neurologic, autonomic or complex mechanisms. Painful crisis can be observed as presenting or major symptom in four groups of metabolic/genetic disorders. Bone pain is a frequent symptom in vitamin D deficiency and hereditary hypophosphataemic rickets where it is associated with bone changes (rickets).

Bone pain can occur in sickle-cell anaemia and various porphyrias in association with other signs. One particularly important form of painful crisis that occurs early in infancy in sickle-cell anaemia is the hand-foot syndrome which is a dactylitis characterized by sudden onset of painful swelling of the dorsum of the hands and feet. In erythropoietic protoporphyria and X-Linked protoporphyria pain can affect sun-exposed areas within minutes of exposure, and if exposure is prolonged be followed by diffuse oedema that may resemble angioneurotic oedema. In various porphyrias, bone crisis is associated with abdominal pain, constipation, vomiting, and neuropathy. These crises may also occur in tyrosinemia type I.

Bone pain can occur in three progressive neurologic disorders:

- In the infantile form of Krabbe disease, bone crises are expressed by irritability and incessant crying, which may precede by a few weeks the characteristic psychomotor deterioration with peripheral neuropathy.
- Similar crises have been observed in defects of biopterin synthesis and in aromatic amino acid decarboxyase deficiency.
- In late infantile metachromatic leukodystrophy, bone crises are associated with limb hypertonia, muscle weakness, and progressive neurologic deterioration. In Gaucher disease type III, bone crises can precede, accompany, or follow a large variety of neurologic symptoms; they appear in late childhood or adolescence and are associated with splenomegaly (▶Chap. 40).

Finally, bone pain may occur as an isolated symptom. Bone crisis is frequently the presenting symptom in hemizygotic Fabry disease, non neuronopathic Gaucher disease (type I) (▶Chap. 40) and in hereditary sensory autonomic neuropathy (HSAN) (▶Sect. 40.1.1). The 'Fabry crisis' can last from minutes to several days. It consists of agonizing burning pain commencing in the palms and soles and radiating to the proximal extremities. It is often associated with fever and elevated erythrocyte sedimentation and may be confused with rheumatic fever, neurosis, or erythromelalgia. Another item in the differential diagnosis may be metabolic crisis in mevalonic aciduria which causes arthralgias, morbilliform rash, fever, lymphadenopathy, and hepatosplenomegaly (▶Sect. 37.1) (see also below ▶Sect. 1.6.7). A 'Fabry crisis' is rarely observed in female heterozygotes for Fabry disease.

Chronic recurrent multifocal osteomyelitis (CRMO) is an inflammatory disorder that primarily affects children. Its hallmark is recurring episodes of sterile osteomyelitis. The clinical presentation is with an insidious onset of bone pain with or without fever. Two genetic syndromes have CRMO as a prominent phenotype - Majeed syndrome (▶Sect. 35.1.4) and deficiency of the interleukin-1 receptor antagonist [52].

Many patients with **Gaucher** disease type I (chronic nonneuronopathic) experience episodic pain lasting for days to months in the hips, legs, back, and shoulders. Rarely, bone crisis precedes hepatosplenomegaly and is the presenting sign of the disease (▶Sect. 40.2.1).

1.4.11 Metabolic Derangements and Diagnostic Tests

The initial approach to the late-onset acute forms of IEM, as with the approach to acute neonatal distress, is based on the proper use of a few screening tests. As with neonates, the laboratory data listed in ◘Table 1.3 must be collected at the same time during the acute attack and both before and after treatment.

1.4.12 Metabolic Acidosis (◘Fig. 1.2)

Metabolic acidosis is a very common finding in paediatrics and is defined by a plasma bicarbonate level <18 mmol/l while the pH is <7.38. It can be observed in a large variety of acquired conditions, including infections, severe catabolic states, tissue anoxia, severe dehydration, and intoxication, all of which should be ruled out. However, these can also trigger an acute decompensation of an unrecognized IEM. The vast majority of metabolic acidosis due to IEM are associated with an anion gap (▶Sect. 1.3.9). The presence or absence of ketonuria (see below ▶Sect. 1.4.12) associated with metabolic acidosis is the major clinical clue to the diagnosis. If metabolic acidosis is NOT associated with ketosis (or only very mild) in the absence of glucose infusion, **PDH** deficiency in a neurological context, **FAO**, gluconeogenesis defects (**GSD1** and FBP) and **HMG-CoA lyase**, in the context of hypoglycaemia, should be considered. In both lactate levels are elevated and there is an anion gap. When metabolic acidosis occurs with a "normal" anion gap, and without hypoglycaemia nor hyperlactataemia the most frequent cause is renal tubular acidosis with loss of bicarbonate and hyperchloremia (RTA). Early severe forms of pyroglutamic acidurias can be mistaken for proximal RTA.

A number of IEM cause a metabolic acidosis with an associated ketosis. The range of serum ketone body concentration varies with age and nutritional state. The main groups of metabolic disorders are **insulin-dependent diabetes**, inborn errors of **branched-chain amino acid metabolism** (▶Chap. 18) congenital lactic acidoses such as **multiple carboxylases** (▶Chap. 27) and pyruvate carboxylase deficiencies (▶Chap. 11), **glucose-6-phosphatase** (▶Sect. 5.1.2) and **fructose bisphosphatase deficiencies** (▶Sect. 15.3) and **ketolytic defects** (▶Sect. 13.2). Heterozygous **Monocarboxylate Transporter 1** (MCT1, *SLC16A1*) mutations may present as recurrent attacks of ketoacidosis (▶Sect. 8.7).

The glucose level which can be high, normal, or low is the first parameter to be considered in order to classify these disorders.

In the case of hyperglycaemia, the classic diagnosis is diabetic ketoacidosis. However, **OA** such as **PA, MMA, or IVA and ketolytic defects** can also be associated with hyperglycaemia and glycosuria, mimicking diabetes. Hyperglycaemia resolves easily within few hours after insulin infusion. The distinction between OA and ketolytic defects is based on ammonia and lactate levels

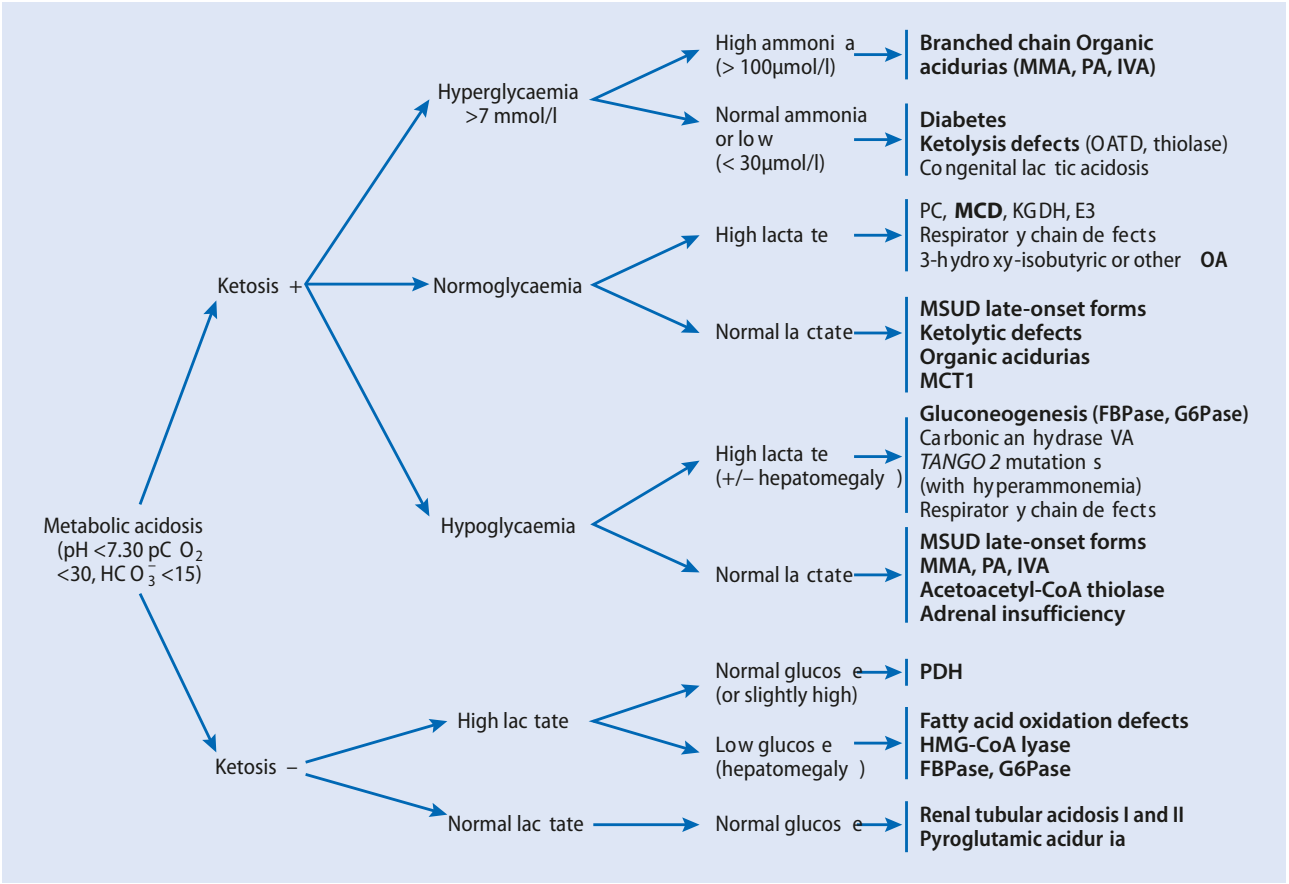

Fig. 1.2 Metabolic acidosis. E3 lipoamido oxido reductase, FBP fructose bisphosphatase, G6P glucose-6-phosphatase, HMG-CoA 3-hydroxy-3-methylglutaryl coenzyme A, IVA isovaleric acidemia, KGDH alpha-ketoglutarate dehydrogenase, MCD multiple carboxylase deficiency, MCT1 monocarboxylate transporter1, MMA methylmalonic aciduria, MSUD maple syrup urine disease, OATD oxoacid CoA transferase, PA propionic acidemia, PC pyruvate carboxylase, PDH pyruvate dehydrogenase, SCAD short chain acyl-CoA dehydrogenase, bold face treatable disorders

(which are generally increased in OA and normal or low in ketolytic defects) and on the organic acid and acylcarnitine profile.

In the case of hypoglycaemia, the first group of disorders to be considered is the **gluconeogenesis** defects and **GSD type Ia and b**. The main findings suggestive of this group are hepatomegaly and hyperlactataemia, although they are not constant. When there is no significant hepatomegaly, late-onset forms of **MSUD** and **OA** deficiency should be considered. A classic differential diagnosis is **adrenal insufficiency**, which can cause a ketoacidotic attack with hypoglycaemia. **GSD III and glycogen synthetase deficiency** do not present with severe ketoacidosis but rather with recurrent ketotic hypoglycaemia (see below).

If the glucose level is normal, congenital lactic acidosis must be considered in addition to the disorders discussed above. Severe ketoacidosis recurrent crises with normal glucose and lactate is very suggestive of ketolytic defects and MCT1 deficiency. According to this schematic approach to inherited ketoacidotic states, a simplistic diagnosis of fasting ketoacidosis or idiopathic ketotic hypoglycaemia should be questioned when there is a concomitant severe metabolic acidosis.

1.4.13 Ketosis (Fig. 1.3)

While ketonuria should always be considered abnormal in neonates, it is a physiological result of catabolism in late infancy, childhood, and even adolescence. However, as a general rule, hyperketosis >6 mmol/l of total plasma ketone bodies that produces metabolic acidosis (serum bicarbonate <18 mmol/l) is not physiological. Ketosis which is not associated with acidosis, hyperlactataemia, or hypoglycaemia, is likely to be a normal physiological reflection of the nutritional state (fasting, catabolism, vomiting, medium-chain triglyceride-enriched or other ketogenic diets). Of interest, severe forms of ketolytic defects (**succinyl-CoA transferase** and **3-ketothiolase**

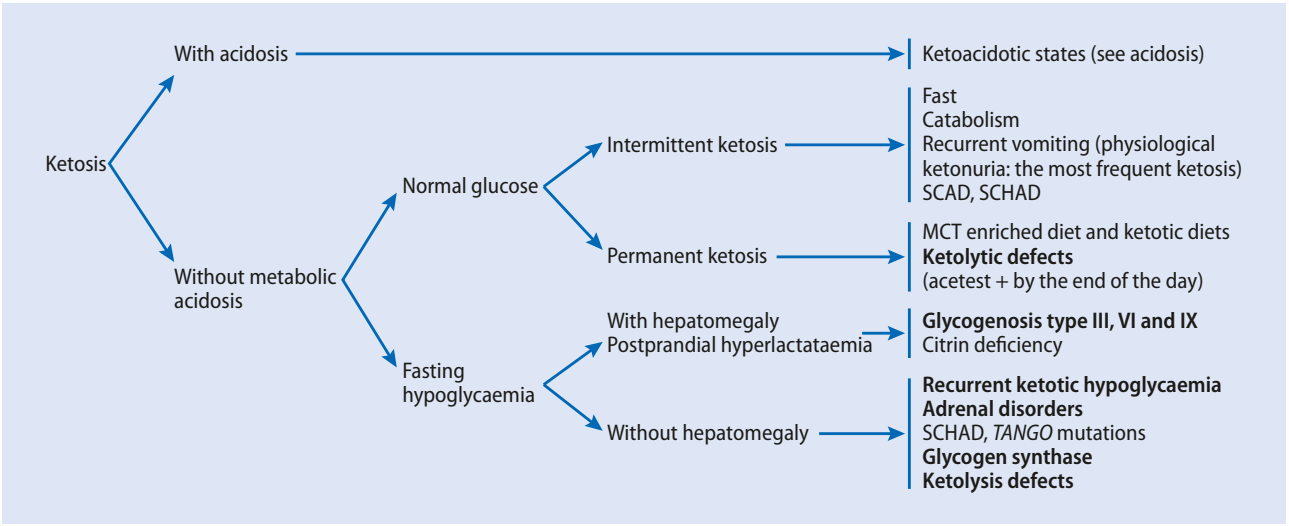

Fig. 1.3 Ketosis. (see also **Fig. 1.2**) MCAD medium chain acyl coenzyme A dehydrogenase, MCT medium chain triglycerides, SCAD short-chain acyl coenzyme A dehydrogenase, SCHAD hydroxy short-chain acyl coenzyme A dehydrogenase. **Bold face** treatable disorders

deficiencies) can present with persistent moderate ketonuria occurring mainly in the fed state at the end of the day (▶Chap. 13). Paradoxical post prandial ketosis suggests a deficient anaplerosis of the Krebs cycle that is actually observed in pyruvate carboxylase deficiency (▶Sect. 11.1). Urine ketone test (eg Acetest) is a very sensitive test that becomes positive as soon as plasma acetoacetate level reach 0.1 mmol/l (corresponding to total plasma ketones >0.2) while the normal total plasma ketones level at fed state is <0.1.

Significant fasting ketonuria without acidosis is often observed in **GSD type III** in childhood (with marked hepatomegaly) and in **glycogen synthase** defect in infancy (with normal liver size). In both disorders there is a characteristic metabolic profile with fasting ketotic hypoglycaemia and normal lactate, and postprandial hyperlactataemia and hyperglycemia (▶Sect. 5.1.3).

Ketosis without acidosis is observed in "idiopathic" ketotic hypoglycaemia of childhood (a frequent condition) and in association with hypoglycaemia s due to **adrenal insufficiency**. Absence of ketonuria in hypoglycaemic states, as well as in fasting and catabolic circumstances (such as vomiting, anorexia, or intercurrent infections), is an important observation, suggesting a **FAO or ketogenesis disorder** (▶Chaps. 12 and 13) and has been recently observed in citrin deficiency (▶Sect. 19.3.2). It can also be observed in hyperinsulinaemic states at any age and in growth hormone deficiency in infancy. However, **SCHAD**, **SCAD**, and **MCAD** deficiencies can present as recurrent attacks of ketotic hypoglycemia as these enzymes are both sufficiently far down the β-oxidation pathway to be able to generate some ketones from long chain fatty acids (▶Chap. 12).

1.4.14 Hyperlactataemia

Lactate and pyruvate are normal metabolites. Their plasma levels reflect the equilibrium between their cytoplasmic production from glycolysis and their mitochondrial consumption by different tissues. The reversible cytoplasmic enzyme lactate dehydrogenase catalyses lactate synthesis from pyruvate by using NADH as a hydrogen donor (Pyruvate + NADH + H = Lactate + NAD or Lactate = Pyruvate + NADH/NAD + H). Accordingly, the blood lactate and pyruvate levels and the L/P ratio reflects the redox state of the cells (NADH/NAD ratio). Hyperlactataemia can be due to an elevation of the pyruvate, the NADH/NAD ratio, H+ (acidosis) or both.

Blood lactate accumulates due to elevation of NADH/NAD ratio in circulatory collapse, in hypoxia, and in other conditions involving failure of cellular respiration and all severe acidotic states. These conditions must be excluded before an inborn error of lactate-pyruvate oxidation is sought. Persistent hyperlactataemia s can also result from many acquired conditions, such as diarrhoea, persistent infections (mainly of the urinary tract), hyperventilation, and hepatic failure. Ketosis is absent in most hyperlactataemia s secondary to tissue hypoxia, while it is a nearly constant finding in most IEM (except in **PDH deficiency**, many **GSD type I** and **FAO disorders**. On the other hand, the level of lactate is not discriminating; some acquired disorders are associated with very high levels, whereas it is only moderately raised in some inborn errors of lactate-pyruvate metabolism. Nutritional state also influences the levels of lactate and pyruvate.

Once the **OA**, **UCD** (mainly **citrullinemia**), and **FAO defects** that can cause secondary moderate hyperlactataemia have been excluded as possible diagnoses, four types of IEM remain to be considered:

The cytoplasmic defects present in a context of hypoglycaemia:

- disorders of liver glycogen metabolism (▶ Chap. 5),
- disorders of liver gluconeogenesis (▶ Chaps. 11 and 15).

The mitochondrial defects present in a context of neurological disturbances

- Lactate-pyruvate oxidation defects (mitochondrial pyruvate transporter (MPC), PDH, PC, Krebs cycle defects and Malate-aspartate shuttle defects) (▶ Chap. 11),
- Thiamine metabolism dysfunction syndromes (▶ Chap. 29),
- Deficient activity of oxidative phosphorylation (▶ Chap. 10).

The diagnosis of hyperlactataemia s is further based upon two metabolic criteria: (▶ Chap. 3)

- *Time of occurrence of hyperlactataemia relative to feeding:* in disorders of gluconeogenesis (**PEPCK C**, **fructose bisphosphatase** and **glucose-6-phosphatase deficiencies**), hyperlactataemia reaches its maximum level (up to 15 mmol/l) when the patient is fasting, acidotic and hypoglycemic. By contrast in **GSD types III and VI** and in **glycogen synthetase deficiency**, hyperlactataemia is mild and observed mostly (only) in the postprandial period in patients on a carbohydrate-rich diet. Hyperlactataemia never exceeds 6 mmol/l and therefore there is no acidosis (bicarbonates >18 mmol/l). In PC deficiency severe hyperlactataemia (>7 mmol/l) is present in both the fed and the fasted state but tends to decrease in post prandial period. In disorders of MPC, **PDH**, alpha-ketoglutarate dehydrogenase, and respiratory chain function (RCD), maximum lactate levels are observed in the fed state (although all hyperlactataemias exceeding 7 mmol/l appear more or less persistent). In these disorders, there is a real risk of missing a moderate (although significant) hyperlactataemia if the level is checked only before breakfast after an overnight fast (as it is usual for laboratory determinations). In thiamine metabolism dysfunction syndromes elevation of lactate is mostly observed in acute episodes.
- *Determinations of L/P and ketone bodies ratios before and after meals.* These ratios are useful only in 'mitochondrial' hyperlactataemia s in a neurologic context. They indirectly reflect cytoplasmic (L/P) and mitochondrial (3OHB/AA) redox potential states. They must be measured in carefully collected blood samples. Three abnormal hyperlactataemia /hyperpyruvicemia profiles are nearly pathognomonic of an inborn error of lactate-pyruvate metabolism:
  - When hyperpyruvicemia (>0.3 mmol/l) is associated with a normal or low L/P ratio (<12) without hyperketonemia, **PDH deficiency** or MPC are highly probable, regardless of the lactate level.
  - When the L/P ratio is very high (>30) and is associated with a paradoxical postprandial hyperketonemia and with a normal or low 3OHB/AA ratio (<1.5), a diagnosis of PC deficiency (isolated or secondary to **biotinidase** or **holocarboxylase synthetase deficiency**) or alpha-ketoglutarate dehydrogenase deficiency is virtually certain. In PC deficiency, there is also a very characteristic AA profile with hyperammonaemia, high citrulline and low glutamine.
  - When both L/P and 3OHB/AA ratios are elevated and associated with a significant postprandial hyperketonaemia, RCD should be suspected.
- All other situations, especially when the L/P ratio is high without hyperketonaemia, are compatible with RCD, but acquired anoxic conditions should also be ruled out (see above).
- The association to other biomarkers can be useful. Hyperglycinaemia and abnormal concentration of plasma branched chain AA (not constant) can be found in lipoylation defects (▶ Chap. 23, ▶ Fig. 23.2). Low serine has been recently described in GOT2 deficiency. High plasma level of Glycerol-3-phosphate is observed in MDH1 deficiency (▶ Sect. 11.11). Certain OA profiles are also very helpful such as in the case of 3-methylglutaconic acid for the '3-methylglutaconic acidurias' (▶ Sect. 18.3), and Krebs cycle metabolites (as examples high excretion of alpha-ketoglutarate can point towards, alpha-ketoglutarate dehydrogenase deficiency but it is also found in lipoilation and thiamine transport defects; high malate and fumarate can point to MDH2 deficiency (▶ Sect. 11.11) and PEPCKC (▶ Sect. 11.2). Low thiamine concentration in the CSF is also a marker for thiamine transporter defects (▶ Sect. 29.1).

1.4.15 Hypoglycaemia

In children the preponderant glucose sources are [53]

- food in the postprandial insulinemic period (<2.5 h after a meal),
- hepatic glycogenolysis during a short fast (>2.5 h to 8 h after meal),
- hepatic glycogenolysis (progressively decreasing) and gluconeogenesis (progressively increasing) during a short to moderate fast (>8 h to 12–15 h), and
- hepatic gluconeogenesis during a long fast (>15 h).

This timing may vary with age and nutritional state (▶ Chap. 3).

The clinical approach to hypoglycaemia is based on four major clinical criteria:

- the liver size,
- the characteristic timing of hypoglycaemia,
- the association with lactic acidosis (suggesting impairment of gluconeogenesis), and
- the association with hyperketosis or hypoketosis (the latter suggesting FAO or ketogenesis disorders, hyperinsulinism and INSR/PI3K/AKT signalling pathway defects) (▶Chap. 6).

Crucial information comes from the timing of hypoglycaemia which can be:

- unpredictable and only postprandial: <2.5 h after meal (suggests hyperinsulinism or Munchausen by proxy),
- only after a short fast >2.5 h to 8 hours (suggests glycogenosis type I, III or 0),
- after a moderate to long fast >8 h to 24 h (suggests **gluconeogenesis defects**: 'enzymatic' causes such as **FBPase and PEPCKC** deficiency or of 'energetic' causes, mostly **FAO and ketogenesis** defects and RCD). Other clinical findings of interest are hepatic failure, vascular hypotension, dehydration, short stature, neonatal body size (head circumference, weight and height), and evidence of encephalopathy, myopathy, or cardiomyopathy.

Based on the liver size, hypoglycaemia s can be classified into two major groups:

- *Hypoglycaemia with permanent hepatomegaly*. Hypoglycaemia associated with permanent hepatomegaly is usually due to an IEM. When hepatomegaly is the most prominent feature without liver failure, **GSD type I** and **type III** are the most likely diagnoses. **FBPase deficiency and mitochondrial FAO defects** may present with a major to moderate hepatomegaly during hypoglycaemic attacks. Disorders presenting with hepatic fibrosis and cirrhosis, such as hereditary **tyrosinemia type I**, also can give rise to hypoglycaemia. The late-onset form of **HFI** rarely, if ever, presents with isolated postprandial fructose induced hypoglycemic attacks (▶Sect. 15.2). S-adenosyl homocysteine hydrolase deficiency presents with fasting hypoglycaemia and liver failure, often triggered by high protein or methionine ingestion, and is associated with marked hypermethioninemia (▶ Sect. 20.4). RCD (complex III deficiency *UQCRB* mutation) can present with liver failure, hypoglycaemia and fasting lactic acidosis which can mimic FBPase deficiency (▶Chap. 10). PMM2 and **MPI-CDG** (**phosphomannose isomerase** deficiency) with hepatic fibrosis and exudative enteropathy can cause hypoglycaemia early in infancy (▶Sect. 43.1.2)
- *Hypoglycaemia without permanent hepatomegaly*. It is important to determine the timing of hypoglycaemia and to look for metabolic acidosis and ketosis when the patient is hypoglycaemic. Unpredictable hypoglycaemic attacks occurring postprandial or after a very short fast and without ketosis are mostly due to **hyperinsulinism (congenital or Munchausen by proxy)** (▶Chap. 6) at any age, or to **growth hormone deficiency** or related disorders in early infancy. Adenosine kinase deficiency may also present early in infancy with hyperinsulinaemic hypoglycaemia (▶ Sect. 32.5.1).

Most episodes of hypoglycaemia, due to IEM that are not accompanied by permanent hepatomegaly, appear after at least 8 h of fasting. This is particularly true for inherited **FAO disorders** except in the neonatal period. Severe fasting Hypoglycaemia without ketosis, strongly suggests **FAO disorders** (without severe acidosis) (▶ Chap. 12), **HMG-CoA lyase deficiency**, or **HMG-CoA synthetase deficiency** (with acidosis) (▶Sect. 13.1) and PEPCKC (▶Sect. 11.2). When ketoacidosis is present at the time of hypoglycaemia, **ketolytic defects** (▶Chap. 13), **OA**, **late-onset MSUD** (▶Sect. 18.1), and glycerol kinase deficiencies (▶Sect. 7.8) should be considered but hypoglycaemia is very rarely the presenting metabolic abnormality in these disorders. **Adrenal insufficiencies (including those presenting undiagnosed X-ALD)** should be systematically considered in the differential diagnosis, especially when vascular hypotension, dehydration, and hyponatremia are present. Fasting Hypoglycaemia with ketosis occurring mainly in the morning and in the absence of metabolic acidosis suggests recurrent "idiopathic" ketotic hypoglycaemia, which presents mostly in late infancy or childhood in those who were small for gestational age or with macrocephaly. This pattern is rarely associated with IEM but **glycogen synthetase deficiency with intermittent glycosuria that is under-recognized** (see below ▶Sect. 1.4.19) (▶Sect. 5.1.1). All types of **adrenal insufficiencies** (peripheral or central) can share this presentation. SCHAD and **MCAD** deficiency can rarely present as recurrent attacks of ketotic Hypoglycaemia (▶Chap. 12) as can **glycogen synthetase deficiency**. ◘ Figure 1.4 summarizes the simplified diagnostic approach to hypoglycaemia. Although not a constant finding, some neurotransmitter defects (amino-acid decarboxylase deficiency (AADC) and dopamine beta-hydroxylase deficiency) can also present with hypoketotic hypoglycaemia, especially in stressful situations (▶Sect. 30.5). Additionally, pyridoxine-dependent epilepsy can present with profound hypoglycaemia associated with hyperlactataemia (▶Sect. 29.2.1).

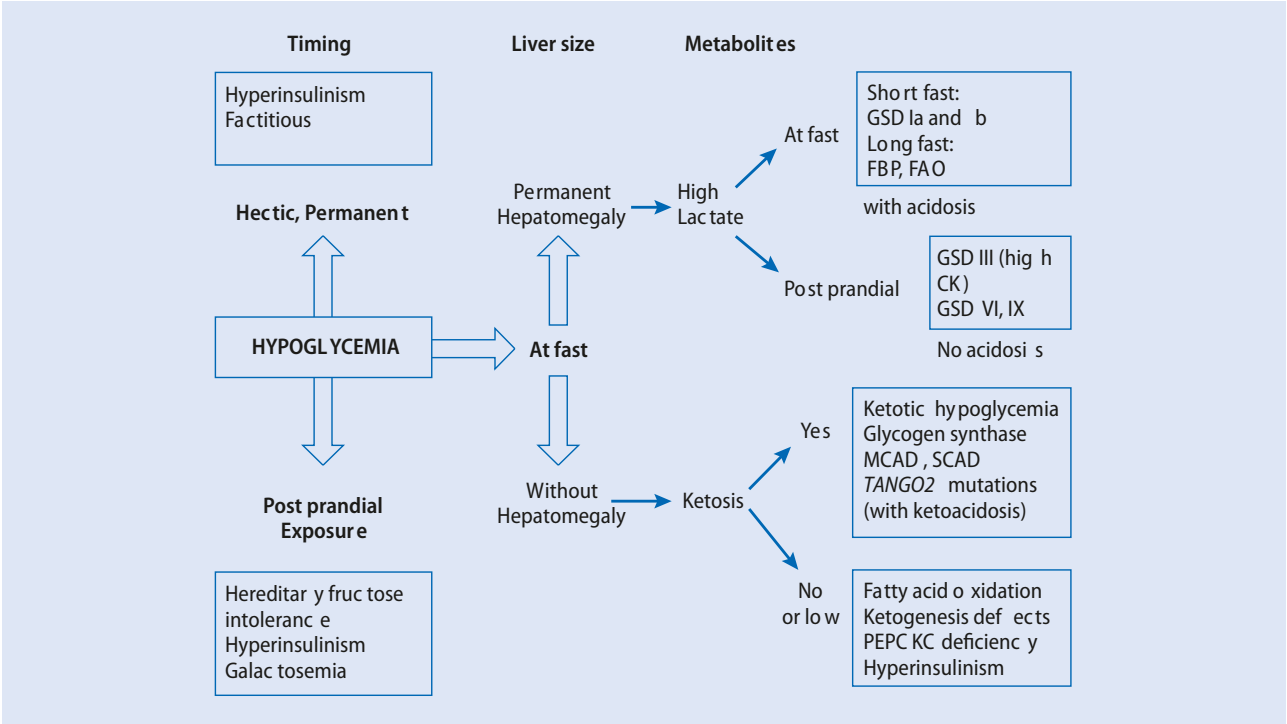

Fig. 1.4 Diagnostic approach to hypoglycaemia in paediatrics based on the timing of hypoglycaemia, the size of the liver and the metabolic profile. GSD glycogen storage disease, FBP fructose biphosphatase, FAO fatty acid oxidation, MCAD medium chain acyl CoA dehydrogenase, SCAD short chain acyl CoA dehydrogenase

1.4.16 Hyperammonaemia

Many IEM can give rise to hyperammonaemia. In the context of acute neonatal encephalopathy, (see Table 1.4) severe hyperammonaemia (>500 µmol/l) is generally caused either by a **UCD** (with respiratory alkalosis, no ketosis and no bone marrow suppression) or by an **OA (PA, MMA, IVA** with metabolic acidosis, ketosis and leuco-thrombocytopenia) (▶ Sect. 18.1.1). Plasma glutamine is generally elevated in **UCD** (>1000 µmol/l) **and LPI** while it is close to normal or low (<500 µmol/l) in **OA**s. Plasma citrulline levels further allows the distinction between mitochondrial and cytoplasmic **urea cycle defects** (▶ Chap. 19). Severe neonatal forms of ornithine aminotransferase defect may mimic ornithine carbamyl transferase deficiency, before ornithine elevation occurs (▶ Sect. 21.1). Hyperammonaemia with hyperornithinaemia and homocitrullinuria is diagnostic for the mitochondrial ornithine transporter defect (**HHH syndrome**) (▶ Sect. 21.2).

Neonatal hyperammonaemia associated with lactic acidosis (>6 mmol/l) and hyperketosis suggests PC (with low glutamine and high citrulline), GOT2 (with high citrulline and low serine) (▶ Sect. 11.11), **MCD** (▶ Chap. 27), or carbonic anhydrase VA deficiencies (▶ Sect. 19.4.2), both with suggestive organic acid profiles.

In a context of severe hypoketotic hypoglycaemia, hyperammonaemia (in general NH_3 <250 µmol/l) suggests a **hyperinsulism/hyperammonaemia syndrome** linked to activating mutations in the glutamate dehydrogenase gene (▶ Chap. 6), or a **FAO defect** with cardiac involvement (▶ Chap. 12). Transient hyperammonaemia with hypoglycaemia may also be observed in premature babies with respiratory distress syndrome. Plasma lysine is elevated in most patients with hyperammonaemia. This is due to impaired lysine metabolism secondary to depletion of 2-ketoglutarate [54]. A low plasma lysine level with low ornithine and arginine, contrasting with a high urinary excretion of these dibasic aminoacids is diagnostic for lysinuric protein intolerance (▶ Sect. 25.3). Mild elevations of NH_3 (<150 µmol/l) may be also a concomitant and accessory finding in MSUD, PDH deficiency and in patients treated by sodium valproate. A paradoxical and moderate elevation of NH_3 only with fasting may be observed in some forms of pyrroline-5-carboxylate synthetase deficiency (▶ Sect. 21.3).

In adults even mild to moderate elevation of NH_3 (>80–150 µmol/l) with mild hepatic disturbances in a context of lethargy, coma or abnormal behaviour should always lead to a suspicion of late onset form of urea cycle disorders mostly OTC in both sexes.

1.4.17 Hyperuricaemias and Hypouricaemias

Normal uricaemia is about 50 mg/l (300 µmol/l) in men, 40 mg/l (240 µmol/l) in women and 30–40 mg/l (180–240 µmol/l) in children. In children a plasma uric acid level >60 mg/l (360 µmol/l) must be always considered abnormal. Hyperuricaemia can result from excessive input, decreased output or both, with regard to the uric acid (UA) pool. Input derives from cellular catabolism of the nucleic acids, purine synthesis and degradation of purines in food. Output results from bacterial intestinal destruction and renal elimination. UA filtered by glomeruli is reabsorbed in the proximal tubule; urinary UA comes from distal secretion which is competitive with organic acids (lactic, MMA, PA…). Several tubular UA transporters have been already described, *SLC22A12* (type I), *SLC2A9* (type II) and GLUT9 acting probably in a multimolecular complex 'transportosome' allowing cooperation between multiple transporters [55, 56].

Secondary hyperuricaemias with low to very low UA excretion are observed in transient neonatal hyperuricaemia and in renal failure from all causes and can be caused by a variety of other disorders: hyperlactataemias, **GSD1**, **OAs such as MMA** (in which gout crisis and hyperuricaemic nephropathy can be a presenting sign), muscular GSDs and **FAO defects** in acute crisis and during treatment with dichloroacetate, and after a fructose load. Hyperuricaemia is a prominent finding in the recently described HUPRA syndrome (Hyperuricemia, pulmonary hypertension, renal failure, and alkalosis) linked to *SARS* 2 mutations (▶Chap. 39).

Primary hyperuricaemias with high UA excretion are seen in primary **classic gout** and in the rare disorders PRPP synthetase superactivity and Lesch-Nyhan syndrome (HGPRT deficiency).

Primary hypouricaemias can result from decreased UA production, as observed in xanthine oxidase and molybdenum co-factor deficiency (with almost no UA in urine) and purine nucleoside phosphorylase, PRPP-synthetase and guanine desaminase deficiency (with low UA excretion), but is more commonly due to decreased renal tubular UA reabsorption. Cytosolic 5′ nucleotidase superactivity results in marked hypouricosuria (▶Chap. 32). Renal hypouricaemia is constant in cystinosis (Fanconi syndrome) (▶Chap. 26). It is also due to renal UA transporter defects characterised by blood uric acid <20 mg/l with high UA excretion. It is usually asymptomatic but may present with acute renal injury and nephrolithiasis and predispose to Parkinson disease.

1.4.18 Isolated Elevated Transaminases

See Later Hepatology ▶Sect. 1.6.6

1.4.19 Glucosuria

Glucosuria should be differentiated from other melituras (galactose, fructose).

Persistent glucosuria is found in the asymptomatic renal glucosuria (▶Sect. 8.2), untreated (decompensated) diabetes mellitus with hyperglycaemia, Fanconi Bickel syndrome (GLUTII) where it is associated with hypoglycaemia (but can be isolated sign in mild form), (▶Sect. 8.5) and more generally in all IEM causing a Fanconi tubulopathy where glucosuria is more marked in postprandial period. Glucosuria associated with proteinuria may be diagnostic sign of cystinosis in a 3–6 month old infant with failure to thrive.

Intermittent glucosuria is found in glycogen synthetase deficiency (with ketotic hypoglycaemia), in glucose galactose malabsorption (with severe diarrhoea), in GLUTII deficiency (mostly in postprandial state) and in some acute attacks of OA (with ketoacidosis) where it can be mistaken for insulin dependent diabetes.

1.5 Chronic and Progressive Neurological Symptoms (Mental Retardation, Developmental Delay, Epilepsy, Neurological Deterioration and Psychiatric Symptoms)

IEMs have a strong predilection for the nervous system.

▪ Diagnostic Approach to Neurological Symptoms Related to Age at Onset

Tables 1.14, 1.15, 1.16, 1.17, 1.18, 1.19, 1.20, 1.21, 1.22, 1.23, 1.24, 1.25, 1.26, 1.27, 1.28, 1.29, 1.30, 1.31, and 1.32 present a general approach to IEM involving neurological and/or mental deterioration. Diseases are classified according to their age at onset, the presence or absence of associated extraneurological signs, and the neurological presentation itself. IEM with neurological signs presenting in the neonate (birth to 1 month; Tables 1.2 and 1.4 and those presenting intermittently as acute attacks of coma, lethargy, ataxia, or acute psychiatric symptoms (Tables 1.6, 1.7, and 1.8), were discussed earlier. Brain development is a dynamic, complex and changing process that determines which category of neurological signs are more likely to appear at a particular age range. According to this idea, in early infancy, IEM tend to produce a global neurological involvement, affecting mostly all functions of the brain (motor, cognitive, behavioural), therefore, the suspicion of an IEM at this period of time should be very high regardless the precise description of the neurological symptoms. By contrast, as children grow up, the brain is more capable to express single or predominant symptoms,

meaning that it is easier to organize the differential diagnosis based on well-defined neurological manifestations. Finally, psychiatric manifestations are more common at late stages of neurodevelopment, being especially relevant in adolescents and adults. This approach is important not only to make a correct diagnosis but also to avoid missing **treatable disorders** (◘ Table 1.30).

1.5.1 Infants (Before 1 year)

Three general categories can be identified:

1.5.1.1 Category 1: Neurological Diseases Associated with Extraneurological Symptoms (◘ Table 1.14)

Visceral signs appear in lysosomal disorders. A cardiomyopathy (associated with early neurological dysfunction, failure to thrive, and hypotonia), sometimes responsible for cardiac failure, is suggestive of respiratory-chain disorders, D-2-hydroxyglutaric aciduria (with atrioventricular block), or CDG (see ▶ Sect. 1.3.7, ◘ Tables 1.11 and 1.12). Abnormal hair and cutaneous signs appear in Menkes disease, Sjögren-Larsson syndrome, elongase 4 and 1 deficiency, **biotinidase deficiency**, and respiratory-chain disorders. Hypopigmentation is found in cell trafficking disorders (▶ Chap. 44). Congenital ichthyosis is frequently observed in many complex lipid synthesis/remodeling disorders (◘ Table 1.32). Peculiar fat pads of the buttocks and thick and sticky skin (tallow, peau d'orange), and inverted nipples are highly suggestive of CDG. A generalized cyanosis, unresponsive to oxygen, suggests methaemoglobinemia, which is associated with hyperkinetic movements, spasticity and microcephaly in cytochrome-b5-reductase deficiency [57]. Kernicterus and athetosis are complications of Criggler-Najjar syndrome. Orthostatic acrocyanosis is suggestive of *ETHE*1 mutations (▶ Sect. 20.9). The presence of megaloblastic anaemia suggests an IEM of **folate and cobalamin** (Cbl) metabolism (▶ Sect. 1.6.5). Abnormal erythrocyte morphology is found in **CAD deficiency** and Rabenosyn-5 defect. Ocular abnormalities can be extremely helpful diagnostic signs, for example cherry-red spot, optic atrophy, nystagmus, abnormal eye movements, and retinitis pigmentosa (▶ Sect. 1.5.7). Erratic head-eye movements (eyes and head move at the same time and in the same direction) are pathognomonic of **GLUT1DS** and appear transiently only during the first months of life.

1.5.1.2 Category 2: Disorders with Specific or Suggestive Neurological Signs (◘ Table 1.15)

Predominant extrapyramidal symptoms are associated with IEM of **biopterin** and **neurotransmitters, antiquitin and pyridox(am)ine phosphate oxidase deficiency**, Lesch-Nyhan syndrome, cytochrome-b5-reductase deficiency, Criggler-Najjar syndrome, the early-onset form of GA type I, **cerebral creatine deficiency (GAMT)** and different causes of Leigh syndrome including Mn transporter deficiency causing SLC39A8-CDG. Dystonia can also be observed as a subtle but presenting sign in X-linked Pelizaeus-Merzbacher syndrome. It can be also associated with psychomotor retardation, spastic paraplegia and ataxia in the **cerebral folate deficiency syndrome** which is more likely to produce a cerebral palsy-like clinical picture. Several new IEM and new categories of diseases cause spastic tetraparesis (+/− microcephaly and other clinical signs) mimicking cerebral palsy during the first year of life: cell trafficking defects, in particular golgipathies, adaptinopathies and tubulinopathies, nucleotide and tRNA synthetase defects, as well as complex lipid defects. Very low folate concentration in the CSF can be due to *FOLR1* mutations, severe MTHFR deficiency, DHFR (▶ Chap. 28), Kearns-Sayre syndrome and POLG mutations (▶ Chap. 10). Macrocephaly with a startle response to sound, incessant crying, myoclonic jerks and irritability are frequent early signs in GM-2 gangliosidosis, Canavan disease (▶ Sect. 22.10), Alexander leukodystrophy, infantile Krabbe disease, all disorders with a severe early neurological regression. Macrocephaly can be also an initial sign in glutaric aciduria type 1, L-2-hydroxyglutaric aciduria and in respiratory-chain disorders due to complex-I deficiency (association with hypertrophic cardiomyopathy) (▶ Sect. 1.5.6).

Recurrent attacks of seizures unresponsive to anticonvulsant drugs occurring in the first year of life is the usual presenting manifestation of the **GLUT1DS** (▶ Sect. 8.3). Recurrent attacks of neurological crisis associated with progressive neurological and mental deterioration suggest Leigh syndrome, which can present at any age from early in infancy to late childhood. Leigh syndrome is not a specific disorder but, rather, the clinical phenotype of any of several IEM involving energy metabolism (▶ Sect. 1.5.6) (▶ Chap. 10). Recurrent stroke-like episodes often associated with anorexia, failure to thrive, and hypotonia can be presenting symptoms in **urea-cycle defects** (mostly **OTC deficiency**), late-onset **MSUD**, **OA**, **GA type I**, CDG, and respiratory-chain disorders. Thromboembolic events can be the presenting sign of **classical homocystinuria** and CDG (see above ▶ Sect. 1.4.1).

1.5.1.3 Category 3: Disorders with Non-Specific Developmental Delay (◘ Table 1.15)

The introduction of whole-exome sequencing (WES) as a diagnostic test for individuals with unexplained neurodevelopmental disorders (NDDs) has led to the identification of dozens of disease-associated genes without metabolic markers. Some involve very general

Table 1.14 Neurological disease with extraneurological symptoms (1–12 months)

Leading symptoms	Other signs	Diagnosis (disorder/enzyme deficiency)
Visceral signs	Hepatosplenomegaly Storage signs, coarse facies	Lysosomal disorders in general GM1, I-cell disease Sialidosis type II, Niemann-Pick A Lactosyl ceramidosis Sandhoff, Salla disease Mucolipidosis II Multiple sulfatase deficiency (*SUMF1*) PPP1R21 (protein phosphatase 1)
	Hepatosplenomegaly Opisthotonos, spasticity	Gaucher type II
	Hepatomegaly Retinitis pigmentosa	Peroxisomal defects CDG Diverse complex lipid synthesis defects
	Hepatomegaly elevated transaminases Recurrent hepatic crises	*IARS* mutations
	Other liver dysfunctions	MEDNIK, ARC (cholestasis) syndromes
Hair and cutaneous symptoms (see also ▶ Sect. 1.6.2)	Steely brittle hair. Neurotrichosis (hair-shaft abnormalities: trichorrexis nodosa, monilethrix, pili torti)	Menkes (X-linked): trichothiodystrophy (TTD) Other causes of TTD: with photosensitivity: mutations in *XPD* (*ERCC2*): microcephaly and hypomyelination **Argininosuccinic aciduria**: trichorrexis nodosa CDG, mitochondrial disorders
	Alopecia, cutaneous rashes Absent eyebrows, sparse eyelashes	**Biotinidase deficiency** Respiratory chain defects Ornithine decarboxylase (*ODC1*)
	Hypopigmentation	Several cell trafficking disorders (▶ Chap. 44) such as: Vici syndrome (*EPG5*) Waardenburg syndrome (*MITF, PAX3*)
	Cutis laxa	Menkes disease (*ATP7A*) CDG, diverse complex lipid synthesis defects, *PI4K2A* mutations (▶ Table 35.1)
	Ichthyosis and spastic paraplegia	Sjögren-Larsson syndrome Fatty acid elongase 4 and 1 (*ELOVL4, ELOVL1*)
	Ichthyosis and optic atrophy	**Serine deficiency syndrome** Multiple sulfatase deficiency Gaucher type II Diverse complex lipid synthesis defects MPDU1-CDG, DK1-CDG, SRD5A3-CDG
	Peculiar fat pads on buttocks	CDG
	Central cyanosis, hypertonicity	Cytochrome b-5 reductase
	Kernicterus, athetosis	Crigler-Najjar disease
	Acrocyanosis, petechiae	EPEMA syndrome (*ETHE1*)
	Subcutaneous nodules	Glutamine synthetase overactivity with cataract and severe regression[a] Farber disease: Painful subcutaneous nodules and progressively deformed joints

Table 1.14 (continued)

Leading symptoms	Other signs	Diagnosis (disorder/enzyme deficiency)
Hematological disorders (see also ▶ Sect. 1.6.5)	Megaloblastic anemia with failure to thrive, RP	**Folate and cobalamin defects** **UMP synthetase**
	Pancytopenia	Gaucher disease type III Niemann-Pick disease type A
	Neutropenia	Barth syndrome, Aspartylglucosaminuria Several cellular traffic defects (▶ Chap. 44) *JAGN1, VPS45, WAS*
	Thrombocytopenia	Niemann-Pick disease type B Some forms of Aicardi-Goutières syndrome **Cobalamin defects** **Holocarboxylase synthetase deficiency**
	Methemoglobinemia and polycythemia	NADH CYB5R (*CYB5R3* mutations): with early-onset encephalopathy
	Abnormal erythrocyte morphology	**CAD** deficiency: acanthocytosis, anisopoikilocytosis and anemia Rabenosyn-5 deficiency: macrocytosis, megaloblastoid erythropoiesis
Immunological Disorders (▶ Sect. 1.6.7)	Recurrent lung infections with DD, failure to thrive, immunodeficiency, leukoencephalopathy, hypohomocysteinaemia. With dysmorphy and developmental delay	*NFE2L2* mutations causing NRF2 accumulation that leads to overtranscription of genes involved in cytosolic redox balance. Several cell trafficking disorders (▶ Chap. 44)
Cardiac symptoms (see also ▶ Sect. 1.6.1)	Cardiomyopathy Heart failure, heart rhythm disorders Cardiomyopathy, cataract Hypertrophic cardiomyopathy with microcephaly, kabuki like facies and syndactyly	D2-hydroxyglutaric acidemia Respiratory chain defects, CDG Sengers syndrome (*AGK* mutations) Serine hydroxymethyltransferase 2 (SHMT2) deficiency
Lung disease	Pulmonary hypertension	Lipoylation defects (with white matter vacuolating encephalopathy) *SARS2* mutations
Ocular symptoms (see ▶ Sect. 1.5.7)	Cherry red spot, hydrops fetalis	GM1-gangliosidosis, Galactosialidosis, Sialidosis type I
	Myoclonic jerks, macrocephaly	Tay-Sachs, Sandhoff disease
	Optic Atrophy, macrocephaly Optic atrophy, Coloboma	Canavan disease MPDU1-CDG (If), Iq (Table 1.28)
	Nystagmus, dystonia, stridor	Pelizaeus-Merzbacher (X-linked)
	Retinitis pigmentosa	See Table 1.28.
	Abnormal ocular movements (oculogyric crises, dystonic ocular movements) See also algorithm specific features of abnormal movements (Table 1.29)	**Neurotransmitter defects** (biogenic amines and *GRIN1* mutations), channelopathies. See-algorithm specific features of abnormal movements Erratic ocular movements with head and eye moving at the same time and direction: pathognomonic of **GLUT1DS**
	Strabismus	PMM2-CDG (1a), many other early neurological disorders
	Supranuclear paralysis	Gaucher, Niemann-Pick disease type C
	Early bilateral cataract with cutaneous nodules and severe regression	Glutamine synthetase overactivity
	Other causes of cataract	Table 1.41

Bold face: treatable disorders
CDG congenital disorder of glycosylation, *RP* retinitis pigmentosa, *UMP* uridine mono phosphate
[a]Glutamine synthetase Gain-of-function variant [58]

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Table 1.15 Prominent neurological involvement (1–12 months)

Leading symptoms/signs	Other signs	Diagnosis (disorder/enzyme deficiency)
With suggestive neurological signs		
Extrapyramidal signs	Hypokinetic-rigid syndrome Dystonia-Parkinsonism Abnormal neurotransmitters	**Inborn errors of biopterin metabolism and neurotransmitters:** AADC, TH, DTD, BH_4 synthesis defects *SLC30A10, SLC30A10* (hypermanganesemia) Mitochondrial disorders, *tWARS* Gaucher disease type 2, INAD *PI4K2A, FIG4, TBC1D24* mutations
	Dystonia, generalized (dystonic tetraparesis) or starting abruptly (sometimes unilateral), plus:	With associated acute/subacute encephalitis: **Glutaric aciduria type 1** BCAA defects: **ECHS1, HIBCH** **Acute decompensations of classic OA and ketolysis defects** With respiratory abnormalities +/– encephalitis Leigh syndrome
	Dystonia	In the context of INAD: PLA2G6 With cerebellar and brainstem involvement: tRNA synthase deficiencies With stridor: Pelizaeus Merzbacher disease (X-linked) PMM2-CDG, synaptic vesicle disorders (several hyperkinetic movements) **GLUT1DS, intracellular Cbl defects,**
	Kernicterus syndrome	Criggler-Najjar syndrome
	Low brain creatine	Creatine deficiency (GAMT)
	Leigh syndrome (see ▶ Sect. 1.5.6)	**PDH,** different mitochondrial genes, **thiamine transporter defect, Biotinidase deficiency** Megdel syndrome (*SERAC1*) Manganese transporter (SLC39A8-CDG) Diverse causes (see neuroimage ◘ Table 1.23)
	Prominent chorea	**GA1, PA, MMA, homocystinurias, GLUT1DS** GAMT, infantile NCL, FOLR mutations, NKH, sulfite oxidase/MOCO deficiency *PI4K2A* mutations (plus cutis laxa), GRIN related disorders, Aconitase deficiency, tWARS, *ATP8A2* and *SNX14* (autophagy defect) L-2-hydroxyglutaric aciduria *PDE10A:* phosphodiesterase 10A (AR form): generalized chorea With self-mutilation: Lesch-Nyhan syndrome
	Complex hyperkinetic movements	Hyperkinetic paroxysmal crises: T3 transport defect (Allan-Herndon-Dudley syndrome) (▶ Sect. 8.9) Complex hyperkinetic movements: ADCY5 (paroxysmal dyskinesia and chorea), GNAO1 (may have also epilepsy), PDE10.

Table 1.15 (continued)

Leading symptoms/signs	Other signs	Diagnosis (disorder/enzyme deficiency)
Neurological regression	Progressive painful pyramidal hypertonia opisthotonos at advanced stage	Krabbe, Gaucher III, Niemann-Pick disease type C, Menkes diseases, untreated **MSUD** and **OAs**, GM3 synthetase deficiency, **arginase deficiency**
	Macrocephaly, startle response to sounds, cherry red spot, myoclonic jerk	Tay Sachs, Sandhoff, other lysosomal disorders: with cherry red spot, Canavan, Alexander disease, vacuolizing leukoencephalopathy Vacuolizing leucoencephalopathy
	Failure to thrive feeding difficulties regression, epilepsy and WM abnormalities	Multiple mitochondrial dysfunction syndromes linked to mutations in the iron–sulfur cluster assembly (ISCA) machinery (▶Chap. 10, ◘ Table 10.4) Diverse causes of infancy onset leukodystrophy (see ▶Sect. 1.5.6)
	Deafness, severe leukodystrophy with brain calcifications	LysyltRNA synthetase gene (*KARS*), which encodes bifunctional LysRS
Severe motor arrest with hypomyelinating leukodystrophy	Early nystagmus, dystonia, spasticity, and profound failure to thrive.	*DEGS1* (sphingolipid desaturase deficiency) Many other disorders with hypomyelination (see ▶Sect. 1.5.6)
Epileptic encephalopathy (ID, epilepsy, spastic quadriplegia, microcephaly, dysmorphic features, and brain abnormalities)		Most IEM that produce neonatal seizures can also start during the first year of life. Consider also **GLUT-1 deficiency**, (▶Sect. 8.3) **Biotinidase deficiency,** Menkes disease, GPI-anchor biosynthesis pathway defects and other defects of complex lipid synthesis such as *FAH2* and *ELOVL4* mutations Malate dehydrogenase (*MDH1* cytosolic) (▶Sect. 11.11)
	With prominent spasticity	NKH, SO, untreated **MSUD** and **OA** **MCD,** Menkes disease, GM3 synthetase deficiency WD-repeat proteins family deficiencies (*WD45B*, *WDR62*-related microcephaly, *WDR4*-related microcephalic primordial dwarfism, *WDR93*-related autism) Purine defects (AMP2, NT5C2, ATIC) *SLC30A9* (zinc transporter) RCDP, complex lipid defects, *MARS2* (aminoacyl-tRNA synthetase defect) *UCHL1* (nucleotide metabolism defect) Cell trafficking defects: adaptinopathies, golgipathies and tubulinopathies
Ocular symptoms	Optic atrophy, incessant crying	Krabbe disease (infantile)
	Retinopathy	Respiratory chain defects, peroxisomal defects, CDG

(continued)

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Table 1.15 (continued)

Leading symptoms/signs	Other signs	Diagnosis (disorder/enzyme deficiency)
Recurrent attacks of neurological crisis	Failure to thrive, hyperventilation attacks	Leigh syndrome (PC, PDH, many nuclear genes involved in respiratory chain and cofactors, mitochondrial machinery, transport, RNA metabolism (*PNPT1, RNASEH1*) *ETHE1, SERAC1, SLC39A8, RANBP2, NUP62* (▶Sect. 1.5.6) MAMEL syndrome (▶Chap. 18) MEGDEL syndrome (▶Sect. 35.3.7) **Thiamine transporter defect: (TBBGD)**
	Stroke-like episodes	**Urea cycle defects, MSUD, OA,** GA I CDG, respiratory chain defects
	Thromboembolic accidents	**Homocystinurias,** CDG
Without suggestive neurological signs		
Evidence of developmental arrest	May associate infantile spasms, hypsarrhythmia and autistic features	Untreated **PKU, biopterin defects,** Peroxisomal defects, Rett syndrome
Nonspecific symptoms, global DD+/− seizures, muscle weakness and hypotonia, +/− distinctive facial features apparently non-progressive disorder	Frequent autistic feature Poor feeding, failure to thrive	**Hyperammonaemia** (late-onset subacute) 4-OH-butyric, L2-OH-, D2-OH-glutaric acidurias, *ODC1, MBOAT7*, Rett-like syndrome (Grinpathies), *GET4* encoding transmembrane domain recognition complex, **BCKDK**, purine defects, SV cycle defects
	Recurrent chest infections with respiratory distress	*PPP1R21* (protein phosphatase 1 regulatory subunit 21)
With diverse neurological findings simulating cerebral palsy		
		Purine and pyrimidine defects. Purine defects (*AMP2, NT5C2, ATIC*) 3-methylglutaconic type 1, Fumarase deficiency, other OA, Creatine deficiency **3-PGD**, 3-phosphoserine phosphatase **Homocystinurias**, Salla disease *SLC30A9* (zinc transporter) RCDP, complex lipid defects, *MARS2* (aminoacyl-tRNA synthetase defect) *UCHL1* (nucleotide metabolism defect) Cell trafficking defects: adaptinopathies, golgipathies and tubulinopathies **Neurotransmitters defects,** **Cerebral folate deficiency due to diverse conditions (DHFR, FOLR1), GLUT1DS**

Bold face: treatable disorders

3-PGD 3-phosphoglycerate dehydrogenase, *ADC* aromatic aminoacid decarboxylase, *CDG* congenital disorders of glycosylation, *DD* developmental delay, *DHFR* dihydrofolate reductase, *DTD* dopamine transporter defect, *FOLR* folate receptor, *GA* glutaric aciduria, *GAMT* guanidino acetate methyltransferase, *INAD* infantile neuroaxonal dystrophy, *MAMEL* methylmalonic aciduria, mitochondrial encephalopathy Leigh-like, *MMA* methylmalonic, *MOCO* molybdenum cofactor, *NCL* neuronal ceroid lipofuscinosis, *OA* organic acidurias, *PA* propionic aciduria, *MSUD* maple syrup urine disease, *OA* organic acidurias, *PC* pyruvate carboxylase, *PDH* pyruvate dehydrogenase, *PGD* phosphoglycerate deshydrogenase, *PNPO* pyridox(am)ine phosphate oxidase, *RCDP rizhomelic, chondrodisplasia punctata, SO* sulfite oxidase, *TH* tyrosine hydroxylase, *WM* white matter

cellular functions such as *TLK2* mutations (encoding a serine/threonine kinase) that cause a distinct neurodevelopmental disorder, hallmarked by mild developmental delay, a variety of behavioural disorders, severe gastro-intestinal problems, and facial dysmorphism [59]. Others involve trafficking machinery the phenotype of which may overlap with some well identified IEM groups such as *PPP1R21* mutations (encoding Protein Phosphatase 1, regulatory subunit 21 involved in endosome function) that more or less mimic a lysosomal storage disorder [60] (▶Chap. 44). An important number of these new genes modulate DNA and RNA metabolism and are histone methyltransferases or similar enzymes that in general cause developmental delay, often associated with microcephaly and dysmorphia.

A large number of IEM present with non-specific early progressive developmental delay, poor growth, hypotonia, some degree of ataxia, frequent autistic features, and seizures. Many IEM can masquerade as a cerebral palsy by presenting as a permanent impairment of movement or posture. Consequently, it is mandatory to systematically screen such children for those IEM which are **at least partly treatable**. In this context, late-onset **subacute forms of hyperammonaemia** (usually **OTC** deficiency in girls) can present with an apparently non-specific early encephalopathy (▶Chap. 19) and **IEM of neurotransmitter synthesis**, especially **dopa-responsive dystonia** due to **cyclohydrolase deficiency, tyrosine-hydroxylase deficiency and sepiapterin reductase deficiency**, as well as other less L-dopa responsive diseases (aromatic-l-amino-acid-decarboxylase deficiency and dopamine transporter defect), can masquerade as cerebral palsy. CSF studies should then be considered in patients with a cerebral palsy-like disorders and any other kind of undetermined neurological disturbances (▶Chap. 30).

1.5.2 Early Childhood to Adolescence (>1 year to 18 years)

In this period, diagnosis becomes easier. Symptoms tend to express in a different manner depending on the age ranges: early childhood (up to 2 years of age), mid to late childhood (2–12 years), adolescence (12–18 years).

Six general categories can be defined according to the accompanying signs and leading symptom: 1-with extraneurological/somatic abnormalities (◘Table 1.16) 2- with predominant epilepsy (◘Tables 1.17 and 1.18) 3- with abnormal movements (◘Table 1.19) 4- with complex motor disorders (◘Table 1.20) 5- with predominant intellectual disability and/or behavioural and neuropsychiatric manifestations, 6- with predominant neuroregression.

1.5.2.1 Category 1: With Visceral, Craniovertebral, Ocular, or Other Somatic Abnormalities (◘Table 1.16)

These symptoms associated with a slowing or regression of development, suggest MPS types I and II, mucolipidosis type III, oligosaccharidosis, multiple sulfatase deficiency, Niemann-Pick disease type C, Gaucher disease type III, and lactosyl ceramidosis, all disorders which are usually easy to recognize. Mucolipidosis type

◘ Table 1.16 Prominent neurological involvement with extraneurological signs >1 year to 18 years

Symptoms	Diagnosis (disorder/enzyme deficiency)
With visceral, craniovertebral, or other somatic abnormalities	
Coarse facies, skeletal change	MPS I, MPS II, MPS III, MLP III, (hirsutism, corneal opacities) AIFM1 mutation (Spondylo-epimetaphyseal dysplasia with severe neurodegeneration)
Coarse facies, subtle bone changes, lens/corneal opacities, hepatosplenomegaly, vacuolated lymphocytes	Mannosidosis (gingival hyperplasia) Fucosidosis (angiokeratoma) Aspartylglucosaminuria (joint laxity) Multiple sulfatase deficiency (ichthyosis)
Hepatosplenomegaly, progressive dementia, myoclonic jerks	Niemann-Pick type C and related disorders (vertical supranuclear ophthalmoplegia)
Splenomegaly + hepatomegaly, osseous lesions, (ataxia, myoclonus)	Gaucher disease type III (supranuclear ophthalmoplegia)
Major visual impairment, blindness	Mucolipidosis type IV (corneal clouding)
Retinitis pigmentosa, deafness	Peroxisomal defects, Usher syndrome type II
Cataract, joint laxity, hypotonia	Pyrroline-5-carboxylase synthetase
Cutis laxa, hypotonia	*PI4K2A* mutation
Congenital cataracts multiple respiratory illnesses, progressive short stature and mild global developmental delay	Phosphatidyl serine decarboxylase deficiency
Recurrent lung infections with developmental delay, failure to thrive, immunodeficiency, leukoencephalopathy	*NFE2L2* mutations causing NRF2 accumulation that leads to overtranscription of genes involved in cytosolic redox balance

IV, which causes major visual impairment by the end of the first year of life, sometimes associated with dystonia, presents with characteristic cytoplasmic membranous bodies in cells. In MPS III, coarse facies and bone changes may be very subtle or absent with only abundant and hirsute hair. Peroxisomal disorders may present at this age, with progressive mental deterioration, retinitis pigmentosa, and deafness, and in a very similar manner to Usher syndrome type II. Pyrroline-5-carboxylate-synthase deficiency presents with slowly progressive neurological and mental deterioration, severe hypotonia, joint laxity, and congenital cataracts (▶ Sect. 21.3). Sjogren Larsson syndrome and ELOVL4 deficiency present with ichthyosis and spastic paraplegia (▶ Sect. 42.4).

1.5.2.2 Category 2: With Predominant Epilepsy (◘ Tables 1.17 and 1.18)

Epilepsy is an important sign of many neurometabolic disorders and reflects the excitatory/inhibitory imbalance of neuronal activity caused by these conditions. In most cases, the semiology of seizures and EEG patterns are determined by the age of presentation: (i) Early myoclonic epilepsy in the neonatal period (▶ Sect. 1.3.2); (ii) Infantile spasms, West syndrome, in severe epilepsies of early and late infancy; (iii) Progressive myoclonic epilepsy phenotype in late childhood, adolescence or young patients.

A large number of diseases may cause epilepsy as a major clinical feature beyond the neonatal period and include all pathophysiological categories of IEM (◘ Table 1.17). Epileptic encephalopathies (refractory seizures leading to neurological deterioration) may be a relevant form of presentation during the first 2 years of life. Some of them are late-onset forms of diseases that are typically seen in the neonatal period. In these severe forms, the EEG may show slow background, multi-focal spikes and burst-suppression pattern. Attenuated forms of **pyridoxine dependent seizures**, **PNPO deficiency** and other **vitamin dependent epilepsies** should be considered also at these onset ages.

In childhood and adolescence, mitochondrial disorders and late infantile and juvenile forms of neuronal ceroid lipofuscinosis (NCL), are amongst the most common neurometabolic conditions with prominent seizures. Progressive myoclonic epilepsies (PMEs) are more likely to appear during these periods of life. They are characterized by progressive myoclonus, epileptic seizures and in most cases, dementia and ataxia. PMEs include Lafora disease, NCLs, sialidosis type I, myoclonus epilepsy and ragged red fibres (MERRF), Gaucher disease type 3, *ASAH1* (N-Acylsphingosine amidohydrolase) associated to spinal muscle atrophy (a specific form of Farber disease), some complex lipid defects such as ceramide synthetase deficiency, and *SCARB* mutations that encodes a cell trafficking protein (LMP2) and causes action myoclonus renal failure syndrome. Other non-metabolic diseases such as Unverricht-Lundborg disease, dentatorubral-pallidoluysian atrophy (DRPLA), neuroserpinosis, and KCNC1 related diseases [61] are also causes of PMEs.

Other types of seizures occasionally raise suspicion for a specific disorder. This is the case with epilepsia partialis continua in *POLG*-related disorder and acute intermittent porphyria, migrating partial seizures of infancy in some CDG syndromes and mitochondrial glutamate transporter defect, drop attacks and Lennox-Gastaut syndrome in **GAMT deficiency**, and myoclonic-astatic seizures in **cerebral folate deficiency**. By contrast, in most cases, the aetiology of seizures cannot be predicted from its semiology. This is the case of many mitochondrial disorders and **GLUT1DS** that present with a wide repertoire of seizure types [62].

Other than treatable disorders (◘ Table 1.17), emerging non-treatable new categories of IEM with prominent epilepsy are complex lipid defects, aminoacyl-tRNA synthetases and cell trafficking disorders, in particular GPI-anchor biosynthesis defects and synaptic vesicle disorders that in general, associate intellectual disability, abnormal movements and neuropsychiatric manifestations.

In infants and children up to 3 years of age, also consider the possibility of genetic epilepsies, such as some forms of KCNQ2, with an excellent response to specific antiepileptic drugs [63].

Other than ◘ Table 1.17 key symptoms that may help in the differential diagnosis are in the ◘ Table 1.18.

1.5.2.3 Category 3: With Predominant Abnormal Movements: Ataxia, Hyper and Hypokinetic Movements (◘ Table 1.19)

- **Movement disorders (MD)**

Are among the most usual neurological symptoms in children with IEMs, present in about 70% of these diseases. MD are classified in hyperkinetic and hypokinetic. Hyperkinetic MD refers to an unwanted excess of movements and include dystonia, choreoathetosis, tremor, myoclonus, tics, and stereotypies. Hypokinetic movements are called hypokinetic-rigid syndrome or Parkinsonism. Ataxia, not initially considered in this classification, is now progressively being included as hyperkinetic MD.

Most IEMs that present with MD exhibit more than one abnormal movement and all types can be present in IEM. However, tics, that are the most prevalent MD in the general population, are almost absent in IEM although can appear in two neurodegenerative disorders: neuroacanthocytosis and Hungtinton's disease.

Table 1.17 IEM with epilepsy as a prominent symptom

Disease group	Onset age	EEG	Other neurological and clinical features	Brain image	Biomarkers
I. Small molecule defects. Some disturb first line metabolic investigations (acidosis, hyperammonaemia, glucose, lactate). Most have plasma, urines or CSF metabolic markers either elevated or lowered with a typical or suggestive metabolic signature. Metabolomics for diagnostic purpose is available in some specialized centres.					First line markers (Table 1.3) Second line: AA, OA, acylcarnitine, metals, porphyrins, purines, pyrimidines, biopterins
Urea cycle disorders **Organic acidurias**	1 1,2	Slow background due to encephalopathy	Abnormal movements, hypotonia, developmental delay	Cortical and subcortical oedema, BBGG hyperintensities	Ammonia, AA
Serine synthesis and transport def. (▶ Sect. 24.2)	1	Hypsarrhythmia (infantile spasms), variable EEG patterns	DD, pyramidal signs, microcephaly, peripheral neuropathy, may have ichthyosis. Progressive microcephaly and spasticity: transport defect	Delayed myelination, atrophy, may have cortical malformations.	Low serine in plasma and CSF
BCAA transport def. ***SLCA5*** (▶ Sect. 25.6)	1,2	Multifocal, partial spikes, variable	Microcephaly, autistic-like and aggressive behaviour, growth retardation	Delayed myelination	Low BCAA in plasma and CSF Only in CSF in transport def (?)
Pyridoxine-dependent **PNPO** (▶ Sect. 29.2) **Hypophosphatasia** (▶ Sect. 29.2.4)	1, 2 (attenuated)	Slow background, multi-focal spikes, burst-suppression pattern Multi-focal spikes	Abnormal movements (myoclonus, segmentary), developmental delay. Mild hyperlactataemia and hypoglycaemia.	From normal to posterior fossa abnormalities and cortical malformations, dysgenetic CC	PDE: High AASA and pipecolic acid; PNPO: Low PLP, high glycine, low NTs, alkaline phosphatase, phosphate, calcium
Biotinidase, HCS deficiency (▶ Sect. 27.1.1)	1 1	Multi-focal spikes, variable	Abnormal movements, developmental delay, myelopathy, dermatitis, alopecia	Normal to WMa, BBGGa and myelopathy changes	Plasma biotinidase activity, organic acids
SLC5A6 (▶ Sect. 27.1.3)	1	Slow background, multifocal spikes	DD, regression, 1 child with peripheral neuropathy	Ca, WM hyperintensity	Ca, WM hyperintensity
Folate defects (▶ Sect. 28.3) **FOLR deficiency** ***SLC46A1*** (absorption)	1,2 1	Multi-focal spikes, variable. Myoclonic-astatic seizures	FOLR: DD, hypotonia, abnormal movements SLC46A1: anaemia, diarrhoea, stomatitis	Normal to BBGG calcification, delayed myelination. ***SLC46A1***: brain calcifications	FOLR: CSF folate SLC46A1: Plasma folate
Cbl C disease (▶ Sect. 28.2.1) **MTHFR deficiency** (▶ Sect. 28.3.7)	1,2,3 1,2,3	Multi-focal and partial spikes, variable	DD, ID, psychiatric symptoms, abnormal movements, parkinsonism.	Vascular stroke, BBGGa may appear. MTHFR: Normal to hydrocephalus	Organic acids, total homocysteine, folate

(continued)

Table 1.17 (continued)

Disease group	Onset age	EEG	Other neurological and clinical features	Brain image	Biomarkers
Monoamine-BH_4 def. (►Sect. 30.5)	1,2,3 TH, AADC, DHPR, PTPS	Multi-focal and partial spikes, variable	Dopa-responsive MD, hypotonia, neonatal hypoglycaemia	In general, normal. Myelination delay and cortical atrophy has been reported. BBGG calcifications in some BH_4 defects.	CSF NT, biopterins plasma phenylalanine
CAD deficiency (►Sect. 32.1.10)	1,2	Irregular slowing, multifocal sharp waves and a periodic sharp wave pattern	Global encephalopathy, swallowing difficulties	Global brain atrophy	Anaemia, acanthocytosis
Menkes disease (►Sect. 34.1.2)	1	Slow background, multifocal waves	DD, pyramidal signs, hypotonia, kinky hair	Vessel tortuosity, subdural collections	Copper, ceruloplasmin
Wilson (►Sect. 34.1.1)	3	Partial spikes (the most common)	Extrapyramidal syndrome, depression, 6% patients present with seizures. Sometimes, status epilepticus.	BBGGa	Copper, ceruloplasmin
Porphyria (AIP) (►Sect. 33.2.2)	3	Focal and generalized spikes. status epilepticus, epilepsia partialis continua	Abdominal pain, sympathetic overactivity, psychiatric manifestations, peripheral neuropathy	Posterior reversible encephalopathy signs may appear	Porphyrins
MOCS1, isolated SUOX (►Sects. 20.10 and 20.11)	1	Slow background, multifocal waves	Hypertonia, hyperekplexia, microcephaly, lethargy	Hypoxic encephalopathy like, ventriculomegaly, BBGa, Ca	Uric acid, sulfite, sulfocysteine, total homocysteine
GS deficiency (►Sect. 24.1) AS deficiency (►Sect. 24.3)	1 1	Slow background, multi-focal spikes, burst-suppression pattern	Severe encephalopathy, microcephaly, erythema, necrotizing (GS)	Cerebellar hypoplasia, cortical malformations	High ammonia, low glutamine, high asparagine
NKH chapter (►Sect. 23.2)	1,2 (attenuated)	Slow background, burst-suppression, multifocal abnormalities	Lethargy, hypotonia, hiccup, myoclonus	Dysgenesis of the CC. T2 hyperintensities and DWI restriction of myelinated tracts	High CSF glycine and CSF/plasma glycine ratio
GABA disorders (►Sect. 30.1)	1,2,3 SSADH 1 GABAT	Slow background, multifocal spikes, burst-suppression pattern	Hypersomnolence, choreoathetosis, DD, behavioural abnormalities. GABAT: macrocephaly	T2 hyperintensities of globi pallidi, dentate and subthalamic nucleus (SSADH)	Hydroxybutyric acid (SSADH); GABA, beta-alanine and homocarnosine (GABAT)

Other small molecule disorders with epilepsy as a prominent feature: AA and related disorders: Canavan disease (►Sect. 22.10), **adenosine kinase deficiency** (high CPK, hypoglycaemia, liver dysfunction, S-adenosylhomocysteine) (►Sect. 32.5.1), HSD10 disease, (►Sect. 18.5), pyrroline-5-carboxylate dehydrogenase deficiency (hyperprolinaemia type 2) (►Sect. 21.3), combined D-2- and L-2-HGA (►Sect. 22.10), folate metabolism: MTHFS (►Sect. 28.3.8), purine defects: ADSL deficiency (►Sect. 32.1.2), ATIC deficiency (►Sect. 32.1.3), ITPAse deficiency (►Sect. 32.1.9), neurotransmitter disorders: GABA and glutamate receptor mutations (►Sect. 30.1), synaptic vesicle disorders (►Sect. 30.6) (see also cell trafficking diseases)

II. Energy metabolism defects. In mitochondrial disorders lactate and other markers may be disturbed mostly in children but often remain normal in adult cases. Diagnosis is complex more and more based on molecular investigations (▶ Sect. 14.5)					First line markers (◘ Table 1.3) (lactate…) AA OA function tests
GLUT1D (▶ Sect. 8.3)	1,2	Atypical absence EEG pattern, multifocal and partial spikes	DD, ID (mild in general), movement disorders	Normal	Low CSF glucose and lactate; low CSF/serum glucose ratio
Creatine deficiency (▶ Chap. 9)	1,2	Variable, as well as the seizure type GAMT: Drop attacks, Lennox-Gastaut	ID, autism, speech disorders, abnormal movements	BBGGa. (GAMT). Low Creatine peak: MRS	Creatine, guanidinoacetate, MRS
HH syndrome (▶ Chap. 6)	1,2,3	Multifocal spikes, myoclonic-absence	ID, leucine sensitive hyperinsulinism	No specific pattern	High ammonia, low glucose
PDH (▶ Sect. 11.3)	1,2	Diffuse or focal slowing, focal and generalized spikes, hypsarrhythmia	Leigh syndrome, DD, ID, abnormal movements PDH deficiency is one of the most epileptogenic mitochondrial disorders	Normal to CC agenesis, periventricular cysts BBGGa	High lactate, pyruvate, normal L/P
BRBG *SLC19A3* (▶ Sect. 29.1.2) *SLC25A19* (▶ Sect. 29.1.3)	1, 2 *SLC19A3* 1 *SLC25A19*	Multifocal spikes; hypsarrhythmia in early onset presentations	Wernicke like encephalopathy and BRBG (SLC19A3); Amish microcephaly (*SLC25A19*)	BBGG abnormalities	Lactate, amino acids, alpha-ketoglutarate, CSF thiamine
Fumarase deficiency (FD)[a] (▶ Sect. 11.8) Aconitase def (▶ Sect. 11.9) **GOT2 def** (▶ Sect. 11.11)	1 1,2	FD, ACO2: Infantile spasms, hypsarrhythmia, variable GOT2: focal, temporal spikes (generalized, tonic, myoclonic seizures)	FD: Hypotonia, irritability, cerebral-palsy mimic. High fat, low carbohydrate diet may modify the disease course. ACO2: from neonatal encephalopathy to attenuated forms, optic atrophy, retinopathy, deafness, ataxia. GOT2: hypotonia, progressive microcephaly	FD: May have cortical malformations: polymicrogyria, thin/absent CC ACO2: progressive cortical and cerebellar atrophy; GOT2; cystic encephalomalacia, cerebellar hypoplasia, thin CC	FD: Fumaric acid, lactate, amino acids GOT: Hyperlactataemia, mild hyperammonaemia and citrullinemia
Mitochondrial defects (multiple genes) **primary CoQ10 deficiency** (▶ Table 10.4)	1,2,3	Various EEG patterns mainly slowing, multifocal or diffuse epileptic abnormalities, or hypsarrhythmia. POLG pattern: RHADS, epilepsia partialis continua	Early-onset cases may have up to 50% of epilepsy Seizure type are diverse and myoclonus is frequent. MERFF (2,3): characteristic myoclonic epilepsy POLG (1,2,3): liver involvement, parkinsonism MELAS (2,3): often, focal epilepsy starts before strokes	BBGGa, cerebellar atrophy, WMa, POLG: progressive atrophy MELAS: stroke-like images	Lactate, pyruvate, AA, organic acids, coenzyme Q10 (muscle, plasma)

(continued)

Table 1.17 (continued)

Disease group	Onset age	EEG	Other neurological and clinical features	Brain image	Biomarkers
Other energy metabolism defects with epilepsy as a prominent feature: mitochondrial glutamate transporter def (early migrating epilepsy), different genes involved in **persistent hyperinsulinaemic hypoglycaemia of infancy (PHHI)** (►Chap. 9); lipoylation disorders: in particular lipoyltransferase 2 def, lipoic acid synthase def, ISCA1 def (high lactate, glycine, WM cavitation) (►Sect. 23.2.3); aspartate-glutamate carrier 1 (*SLC25A19*) def (sporadic tonic seizures) (►Sect. 11.11); FBXL4 deficiency (mitochondrial DNA deletion) (Table 14.2); disorders of glycolysis: Triosephosphate isomerase def (►Sect. 7.3); phosphoglycerate kinase def (►Sect. 7.4); pentose pathway defects: ribose 5-phosphate isomerase def (►Sect. 7.9); ketone body utilization defects: homozygous *MCT1* mutations (►Sect. 13.3)					
III. Complex molecules defects. Many complex molecules accumulation defects like sphingolipidosis, sterols and some peroxisomal disorders may be suspected on clinical grounds and have robust metabolic markers. Most complex molecules synthesis and remodelling defects like complex lipids have no easy to reach metabolic markers and diagnosis is based on molecular investigations. Lipidomics become more and more accessible					VLCFA, plasmalogens, phytanic acid, oxysterols, MPS, oligosaccharides, sulfatides, sialic acid, lipidomics
Lysosomal disorders: many can cause epilepsy, in particular at advanced stages of the disease. In most cases progressive myoclonus epilepsy with a slow EEG. The most frequent lysosomal disorders with prominent epilepsy according to age of presentation are: GM1 gangliosidosis (1) (►Sect. 40.2.3), Gaucher disease type 2 (1) (►Sect. 40.2.1), Sialidosis type 2 (1), Prosaposin deficiency (1) (►Sect. 40.2.9), GM2 gangliosidosis (2) (►Sect. 40.2.4), juvenile GM1 gangliosidosis (2), sialidosis type 1 (3) (►Sect. 41.3.1), Gaucher disease type 3 (3), Niemann-Pick type C (3) (►Sect. 40.2.2); and NCLs (1,2,3) (►Sect. 40.5); acid ceramidase deficiency: spinal muscular atrophy and progressive myoclonic epilepsy (SMA-PME) (1,2,3) (►Sect. 40.3.3); in infantile NCL: photic stimulation leads to occipital spikes and may trigger seizures. In CLN2 epilepsy may mimic Lennox-Gastaut syndrome (akinetic myoclonic petit mal)					
Peroxisomal defects PBD (►Sect. 42.2.9), DBP (►Sect. 42.2.2) RCDP, FAR1, (►Sect. 42.1) ACOX1 def; (►Sect. 42.2.3), ELOVL4 (AR);(►Sect. 42.4.6) X-ALD (►Sect. 42.2.1)	All starting in infancy-early childhood (1) except X-ALD (2,3) and FAR1 (AD) (2)	Peroxisomal biogenesis disorders: Multifocal spikes; hypsarrhythmia Other diseases: variable EEG patterns	RCDP: ID, skeletal dysplasia, cataracts, seizures ELOVL4 (AR): ichthyosis, retinopathy, spasticity, ID mimicking SLS FAR1: cataract, spasticity	X-ALD: pathognomonic confluent WMa with gadolinium enhancement Perisylvian polymicrogyria and pachygyria; hypomyelination; subependymal cysts	VLCFA, plasmalogens, pristanic, phytanic acid Acylcarnitines

Lipid metabolism defects Sphingolipid defects: (▶ Sect. 40.1) *DEGS1, CERS1, CERS2* GM3 synthase def Phospholipids: (▶ Sect. 35.4) *PLA2G6, PNPLA8* FA2H (▶ Sect. 40.1.5) Cholesterol metabolism Squalene synthase def (▶ Sect. 37.3) Sterol and bile acid metabolism: CTX (▶ Sect. 38.4) CK syndrome, (▶ Sect. 37.7.2.2) Others*: BSCL2* (▶ Sect. 35.2.2), ***Mfsd2a*** (▶ Sect. 42.4.13), CK syndrome	DEGS1 (1,2) CERS1,2 (2, 3) GM3 synthase def (1) *PLA2G6* (1,2,3) FA2H (1,2), *PNPLA8* (1) Squalene synthase def (1) CTX (3, adult) CK (1) *BSCL2* (1,2) DHA transport (1)	Variable patterns PLA2G6: In the infantile neuroaxonal dystrophy type, diffuse fast beta-activity in stage I and II of sleep CERS1, 2: progressive myoclonic epilepsy	*DEGS1* (1,2):DD, ID, nystagmus, spasticity *CERS1, 2:* progressive myoclonic epilepsy, ataxia, ID GM3 synthase (1): Early severe epileptic encephalopathy, pharmacoresistant, with choreoathetosis, DD, optic atrophy and hyperpigmented lesions *PLA2G6:* infantile neuroaxonal dystrophy *PNPLA8 (1,2)*: microcephaly, dystonia, spasticity Squalene synthase deficiency (1): brain malformations, dysmorphy, DD CTX: ID, cataracts, ataxia, abnormal movements, peripheral neuropathy CK syndrome: microcephaly, brain malformation, X-linked ID. ***Mfsd2*** DHA transport (1): microcephaly, brain malformation *BSCL2 (1,2):* cardiomyopathy, spasticity, DD, ID	*DEGS1:* hypomyelinating leukodystrophy *FA2H* (2): NBIA features *PLA2G6:* NBIA, cerebellar atrophy CERS1,2: cerebellar and/or brainstem atrophy *PNPLA8*: simplification of the gyral pattern, diffuse atrophy and Ca	*DEGS1:* increased dihydro-sphingolipids in plasma CTX: cholesterol, sterols
Glycogen storage disease	Lafora disease (2,3) (▶ Sect. 5.3.1)	Slow background Progressive myoclonic epilepsy	Neurodegeneration, dementia, ataxia, photosensitive seizures, cortical blindness, apraxia	Brain atrophy	Skin biopsy: PAS + inclusions
Aminoacyl-tRNA synthetases and tRNA metabolism defects (▶ Sect. 39.2.3 ▶ Table 39.1)	All (1)	Variable pattern *DALRD3:* includes myoclonic epilepsy	*DALRD3:* ID, severe seizures (including myoclonic), micro-cephaly, hypotonia, dystonia *YRDC, GON7, LAGE3, OSGEP, TP53RK, TPRKB, WDR4:* Galloway-Mowat syndrome *SARS1*: ID, microcephaly, seizures, ataxia *CARS2, TARS2, NARS2, PARS2*: complex encephalopathy	Hypomyelination, cerebellar and brainstem atrophy are frequent findings (▶ Chap. 37)	High lactate (not constant)

(continued)

Table 1.17 (continued)

Disease group	Onset age	EEG	Other neurological and clinical features	Brain image	Biomarkers
Ribosomal biogenesis (▶ Sect. 39.3)	*UBTF1* (2) *SNORD118* (1)	Variable patterns	*UBTF1:* Cognitive regression after a period of normal development; eventual severe ID *SNORD118:* neurodegeneration, spasticity, dystonia	*UBTF1*: cerebellar and brain atrophy *SNORD118:* angiomatous-like blood vessels, leukoencephalopathy with calcifications	
IV. Cellular trafficking disorders- In general without specific metabolic markers; diagnosis is based on DNA analysis.					
CDG (1,2) (▶Chap. 43)	Epilepsy may appear in different CDG subtypes. PIGOPATHIES, defects in the GPI-anchor-biosynthesis pathway, are amongst the most representative causes of epilepsy (multiple types, dysmorphy, onset-age:1,2); **HPMRS: Hyperphosphatasia and Mental Retardation** Syndrome (global developmental delay with marked involvement of expressive language, coarse facial features, malformations, anorectal abnormalities and other malformations may be present). In PIGW (1 case described) the patient developed West syndrome. **SLC35-A2-CDG:** may respond to D-galactose supplementation				Sialotransferrin in some CDG forms, hyperphosphatasia
Cellular trafficking disorders (▶Chap. 44)	Neonatal: *CNPNY3* (ER/cochaperone), *HCFC1* (ER, nucleus, *ATAD1*: AMPA receptor trafficking defect. *KIF5A* (kinesin, anterograde transport): intractable neonatal myoclonus. *PACS2*: ER-mitochondria MCS defect. Seizures difficult to treat during the first year. Dysmorphism+cerebellar dysgenesis. *SCARB* encodes LMP2 causes action myoclonus renal failure syndrome (AMRF) Most golgipathies and tubulinopathies cause early-onset epilepsy (1,2) and often associate microcephaly, cortical malformation and hypomyelination Epilepsy (onset-age: 1,2) with high CPK levels: *TANGO2* and *TRAPPC* related disorders				

Onset age: *1* newborn to infancy (up to 1 year), *2* early to mid-childhood (from 2 to 12 years), *3* adolescence

AA amino acids, *AADC* L-amino acid decarboxylase deficiency, *ABHD12* monoglycerol lipase, *ACAT1* acetoacetyl-CoA thiolase deficiency, *AD* autosomal dominant, *ADCY5* adenylate cyclase deficiency, *ADL-X* X-linked adrenoleukodystrophy, *ADSL* adenylosuccinate lyase deficiency, *AIP* acute intermittent porphyria, *ALS* amyotrophic lateral sclerosis, *AMACR* α-methylacyl-CoA racemase, *AMPD* AMP deaminase-2 deficiency, *AR* autosomal recessive, *ARALAR* mitochondrial protein involved in glutamate aspartate exchange encoded by SLC25A12, *ARD* (adult Refsum disease) phytanoyl-CoA 2-hydroxylase, *ARG* arginine, *ARSA* arylsulfatase A deficiency, *AS* asparagine synthetase, *ATIC* 5-Amino-4-imidazolecarboxamide-ribosiduria (AICA)-ribosiduria, *Atten* attenuated forms, *ATIC* 5-Amino-4-imidazolecarboxamide-ribosiduria (AICA)-ribosiduria is an exceedingly rare autosomal recessive condition resulting from the disruption of the bifunctional purine biosynthesis protein PURH, *ATP8A2* encodes for a P4-ATPase that actively transports phospholipids across cell membranes ('flipping'), *BBGGa* basal ganglia abnormalities, *B4GALNT1* GM2/GD2 synthase deficiency, *BCAA* branched chain amino acids, *C* cerebellum, *Ca* cerebellar atrophy, *CAD* CAD trifunctional protein, carbamoylphosphate synthetase/aspartate transcarbamylase/dydroorotase, *CC* corpus callosum, *CDG* congenital disorders of glycosylation, *CERS* ceramide synthase, *CIT* citrulline, *CK syndrome* X-linked recessive sterol-4-alpha-carboxylate 3-dehydrogenase deficiency, *CLBP* mitochondrial quality protein control, *CMH* congenital methemoglobinemia, *CMNO* cardiomyopathy, *CP* cerebral palsy, *CPTII* carnitine palmitoyltransferase II deficiency, *CTX* cerebrotendinous xanthomatosis, *CSF* cerebrospinal fluid, *CP* cerebral palsy, *DBP* D-bifunctional protein, *DD* developmental delay, *DEF* deficit, *DHPR* dihydropyrimidine reductase deficiency, *DEGS* dihydroceramide delta4-desaturase, *DHFR* dihydrofolate reductase deficiency, *DLP1* dynamin related protein 1. EAAT1 glutamate aspartate transporter deficiency (SLC25A22), *DWI* diffusion, *ECHS1* mitochondrial short-chain enoyl-CoA hydratase deficiency, *ELOVL* elongation of very long chain fatty acids, *encephalop* encephalopathy, *ENTPD* ectonucleoside trisphosphate diphosphohydrolase I, *EPT1* ethanolamine phosphotransferase, *ETHE1* sulfur dioxygenase deficiency, *FA2H* fatty acid 2-hydroxylase deficiency, *FAR1* fatty-acyl CoA reductase 1 deficiency, *FHD* fumarate hydratase deficiency, *FOLR1* folate receptor alpha deficiency, *GAMT* guanidinoacetate methyltransferase, *GA1* glutaric aciduria type 1, *GABAR* GABA receptor mutations, *GABAT* GABA transaminase deficiency, *GALC* galactocerebrosidase, *GLUT1DS* glut-1 deficiency syndrome, *GORS* Golgi SNAP receptor complex member 2, *GOT2* glutamate oxaloacetate transaminase deficiency, *GRIN* mutations in NMDA glutamatergic receptors, *GS* glutamine synthetase, *GSS* glutathione synthetase deficiency, *GTPCH* guanosine triphosphate cyclohydroxylase, *HCS deficiency* holocarboxylase synthetase deficiency, *HH* hyperinsulinism-hyperammonaemia syndrome, *HHH* hyperornithinaemia-hyperammonaemia-homocitrullinaemia syndrome,

HIBCH 3-hydroxyisobutyryl-CoA hydrolase deficiency, *Homocyst* homocysteine, *HRS* hypokinetic-rigid syndrome, *HSD10* 2-methyl-3-hydroxybutyryl-CoA dehydrogenase deficiency, *ID* intellectual disability, *INAD* infantile neuroaxonal dystrophy, *ITPase* Inosine triphosphatase, *ITPR1* inositol 1,4,5-triphosphate receptor, type1, *IVA* isovaleric aciduria, *L2-HGA* L2-hydroxyglutaric aciduria, *L2HGDH* L-2-hydroxyglutarate dehydrogenase deficiency, *L,D2-HGA* L and 2-D-hydroxyglutaric aciduria, *LBSL* leukodystrophy with brainstem and spinal cord involvement and lactate elevation, *LN* Lesch-Nyhan, *MCGA1* 3-methylglutaconic aciduria type 1, *MECR* Trans-Enoyl-CoA reductase, *MELAS* lactic acidosis, and stroke-like episodes, *MEGDEL* 3-methylglutaconic aciduria with deafness, encephalopathy, and Leigh-like syndrome, *MEPAN* mitochondrial enoyl-CoA reductase protein-associated neurodegeneration, *MERRF* mitochondrial encephalopathy and ragged red syndrome, *MD* movement disorders, *MDH* malate dehydrogenase deficiency, *MLD* metachromatic leukodystrophy, *MMA* methylmalonic aciduria, *MOCS* molybdenum cofactor deficiency, *MPS* mucopolysaccharides, *MRS* brain MRI with spectroscopy, *MSD* multiple sulfatase deficiency, *MSUD* maple syrup urine disease, *MTHFR* methylenetetrahydrofolate reductase deficiency, *MTHFS* 5,10-methenyltetrahydrofolate synthetase, *NADK2* Mitochondrial NAD kinase 2 deficiency, *NARP* neuropathy, ataxia and retinitis pigmentosa, *NAXE* NAD(P)HX epimerase deficiency, *NBIA* neuronal brain iron accumulation, *NCL* neuronal ceroid lipofuscinosis, *NKH* non-ketotic hyperglycinaemia, *NPS* neuropsychiatric symptoms, *NRL* neurological, *NT* neurotransmitter, *NT5C2* 5-prime-nucleotidase, cytosolic II, *ORN* ornithine, *OA* organic acids, *P5CS* δ-1-pyrroline-5-carboxylate synthase deficiency, *PA* propionic aciduria, *PHARC* polyneuropathy, hearing loss, ataxia, retinitis pigmentosa, and cataract, *PC* pyruvate carboxylase, *PCH* pontocerebellar hypoplasia, *PDH* pyruvate dehydrogenase, *PERIV* periventricular, *PLA2G6* phospholipase A2, *PKAN* pantothenate kinase 2 deficiency, *PGK1* phosphoglycerate kinase 1, *PHARC* peripheral neuropathy hearing loss retinitis pigmentosa, cataract, *PME* progressive myoclonic epilepsy, *PMPCA* peptidase, mitochondrial processing, alpha, *PN* peripheral neuropathy, *PNPO* pyridoxamine 5′-phosphate oxidase, *PNP* purine nucleoside phosphorylase deficiency, *PMM2* phosphomannomutase 2 deficiency, *PRO* proline, *PSAP* prosaposin, *RALF* recurrent acute liver failure, *RCDP* rhizomelic chondrodysplasia punctata, *RHADS* rhythmic high amplitude delta with superimposed polyspikes, *RP* retinitis pigmentosa, *RPIA* ribose-5-phosphate isomerase deficiency, *SACS* sacsin, *SCA* spinocerebellar atrophy, *SCP2* sterol carrier protein 2 deficiency, *SDE* syndrome, *SLC25A19* thiamine pyrophosphate transporter, *SHMT2* serine hydroxymethyltransferase type 2, *SLS* Sjögren Larsson syndrome, *SORD* sorbitol dehydrogenase, *SP* spastic paraparesis, *SR* sepiapterin reductase deficiency, *SSADH* succinic semialdehyde dehydrogenase (aldehyde dehydrogenase 5a1, *SUCLA2* succinyl-CoA lyase subunit, *SUCLG1* succinyl-CoA ligase alpha subunit, *SUMF1* gene responsible for multiple sulfatase deficiency, *SUOX* sulphite oxidase deficiency, *SV* synaptic vesicle, *TH* tyrosine hydroxylase deficiency, *TPK1* thiamine pyrophosphokinase deficiency, *UCD* urea cycle disorders, *VLCFA* very long chain fatty acids, *WM* white matter, *WMa* white matter abnormalities, *X-ALD* X-linked adrenoleukodystrophy

[a]Partially treatable; diet modifies the clinical course

Table 1.18 Clinical approach to metabolic epilepsies as the most prominent symptom

Seizures and progressive deterioration	
Rapid regression, myoclonic seizures, spasticity	Schindler disease (optic atrophy, severe osteoporosis), INCL, MERFF, Niemann-Pick disease type C, Gaucher disease type III (ophthalmoplegia, hepatosplenomegaly), Alpers syndrome (hepatic symptoms, hyperlactataemia)
Macular cherry-red spot and myoclonus	Sialidosis, other lysosomal diseases
With spinal muscle atrophy	*ASAH1* mutations (new phenotype of Farber disease)
With +/– microcephaly, spasticity, movement disorders	Golgipathies, tubulinopathies, *TRAPCC* related disorders and *TANGO2* (with high CPK)
Without clear degeneration, often static, other neurological signs associated	
Autistic signs, movement disorders	**Creatine defects** (GAMT, CTD), synaptic vesicle disorders, GABA and glutamate signalling defects, **BCAA defects (BCKDK)**
Ataxia, abnormal movements, worse before meals	**GLUT1DS** (low glycorrhachia)
Late onset pyridoxine dependent seizures	**Antiquitin defect** (elevated pipecolic acid (CSF, plasma, urine) and alpha-aminoadipic semialdehyde (urine).
Intellectual disability, dysmorphia (not constant)	GPI-anchor biosynthesis pathway defects, other cell trafficking disorders

NCL neuronal ceroid lipofuscinosis

MD may have different characteristics according to the age of presentation. In infancy and early childhood, a spectrum or continuum of symptoms is often more common than an isolated sign. In fact, the younger the patients the higher likelihood of detecting combinations of different MD. Hyperkinetic MD are very frequent in infancy and childhood whereas hypokinetic are rare and tend to be complex (associated with other neurological sings). In children with IEM, dystonia is often a prelude to parkinsonism in later childhood and adolescence.

Children with a MD that appear acutely may have an acute injury to the basal ganglia or the cerebral cortex, in most cases with accompanying encephalopathy/coma, as is often seen in Leigh syndrome, glutaric aciduria type 1, and other organic acidurias, and energy defect disorders.

Most IEM with extrapyramidal symptoms exhibit an abnormal brain MRI, although this is usually normal in neurotransmitter defects, GLUT1DS and genetic primary dystonias.

Specific features of MD such as triggers, anatomic localization and the particular characteristics of MD semiology may help in the detection of some particular IEMs (algorithm specific features of MD, Fig. 1.7).

Ataxia

Ataxia, the most frequent MD found in IEM [64], is defined as an inability to maintain normal posture and smoothness of movement while force and sensation are intact. Most IEM cause cerebellar dysfunction and cerebellar hypoplasia or atrophy (Table 1.19). Autosomal recessive cerebellar ataxia (ARCA) with onset in childhood to young adulthood age is the most common type of ataxia [65]. It is important to first consider treatable disorders (most of them partially treatable) such as **vitamin E deficiency, biotinidase deficiency, folate metabolism defects, Hartnup disease, CAD deficiency, some forms of porphyrias, PDH deficiency, cerebrotendinous xanthomatosis, Refsum disease, GLUT-1 deficiency, Niemann-Pick type C, and coenzyme Q10 deficiency** (Table 1.19).

- *Intermittent/episodic ataxias* are mostly caused by channelopathies [66] and have been already addressed at ▶1.3.2 (Table 1.7)
- *Progressive ataxias* is the most common form of ataxia in IEM: the treatable (or partially treatable) conditions **vitamin E-responsive ataxias, Refsum disease, cerebrotendinous xanthomatosis, Niemann-Pick C and Coenzyme Q10 deficiency** (in which the phenotype ataxia with oculomotor apraxia deserves special attention) belong to this category. Other disorders frequently associated with progressive ataxia are mitochondrial syndromes such as MERRF, MELAS, NARP, MILS and KSS. Mitochondrial homeostasis defects, including mtDNA replication and repair are also an important cause of progressive ataxia such as: Frataxin (Friedreich ataxia), POLG (sensory ataxic neuropathy with dysarthria and ophthalmoparesis), TWNK/ C10ORF2 (infantile-onset spinocerebellar ataxia) (▶Chap. 10).

The emerging categories of IEM that cause progressive ataxias are: (i) complex lipid synthesis and remodelling disorders (▶Chap. 35) such as *ABHD5* (Chanarin-Dorfmann syndrome), *PNPLA6* (Laurence-Moon and

Table 1.19 IEM with abnormal movements as prominent clinical manifestation

Disease group	Ataxia	Dystonia	Chorea/athetosis	Myoclonus	Tremor (T)/ Parkinsonism (P)	Other neurological features	Biomarkers /L-Dopa responsive (DR)
I. Small molecule defects. Some result in abnormalities in first line metabolic investigations (acidosis, hyperammonaemia, glucose, lactate). Most disorders have abnormal plasma, urine or CSF metabolic markers with a typical or suggestive metabolic signature. Metabolomics for diagnostic purpose is available in some specialized centres.							First line markers (Table 1.3) second line: AA, OA, acylcarnitines, metals, porphyrins, purines, pyrimidines, biopterins
Monoamine-BH_4 deficiencies (1, 2, 3) (▶ Sect. 30.5)		All of them (3) isolated dystonia may	All	TH, SR, AADC	T: All of them. May be isolated in GTPCH (AR) P: All of them (3). Isolated HRS may appear	Oculogyric crises, dysautonomy, fluctuating SP mimic BBGG calcifications in DHPR def	CSF specific patterns of monoamines and pterins **DR**
Organic acidurias (in particular **MMA, PA** ((▶ Sect. 18.1) **and GA1**) (▶ Sect. 22.5)	(1,2) **MMA, GA1** **IVA** (2,3) (▶ Chap. 18) L/D2-HGA (▶ Sects. 22.8 and 22.9)	All of them	All	(2,3) L2-HGA (▶ Sect. 22.8)	T: (2,3) **IVA** D-2-HGA (▶ Sect. 22.9) P: may appear at late stages	Acute decompensations, In (3): Perioral dyskinesia, tremor dyskinesia may be unilateral.	Organic acids, acylcarnitines Plasma AA
BCAA defects (▶ Chap. 18) **and AA transport defects** (1,2) (▶ Chap. 25) Partially treatable, not all of them	(1,2) **Hartnup** (▶ Sect. 25.4) **MSUD** (▶ Sect. 18.1) (2) **ECHS1,** (▶ Sect. 18.10) **HIBCH** (▶ Sect. 18.7) HSD10 (▶ Sect. 18.5) *SlC25A22* (▶ Sect. 30.2)	(1,2) **MSUD, ECHS1, HIBCH,** HSD10 (2) Malonyl-CoA carboxylase def	(2) HSD10 (▶ Sect. 18.5)	(2) HSD10	T: (1,2) MSUD P: (2,3) HSD10	Hartnup and MSUD: intermittent ataxia Abrupt dyskinesia, DD, ataxia Leigh-like phenotype (excluding MSUD); *SlC25A22*: episodic edema	Lactate, AA acylcarnitines, OA Urine AA: Hartnup
Urea cycle defects (2,3) (▶ Chap. 19)	Arginase def. All **UCD**: in decompensations			HHH (▶ Sect. 21.2)		Spasticity in arginase def and HHH: CP-mimic. BBGGa with thalamic sparing	Ammonia, AA

(continued)

Table 1.19 (continued)

Disease group	Ataxia	Dystonia	Chorea/athetosis	Myoclonus	Tremor (T)/ Parkinsonism (P)	Other neurological features	Biomarkers /L-Dopa responsive (DR)
Homocystinurias (▶ Sect. 20.6) and **Cobalamin (Cbl)** related **disorders** (1,2,3) (▶ Sect. 28.2)	(1,2,3) CblD, (2) Cubulin def (▶ Sect. 28.1.2) (3) CBS (▶ Sect. 20.6), CblG	(2,3) CBS (▶ Sect. 20.6) (1,2,3) intracellular Cbl defects			T: (2,3) CblC P: (2,3) CblD	(2) CblD: spasticity	Homocysteine, AA, B12, OA, acylcarnitines
Sulfur AA defects (▶ Chap. 20) (1,2, 3)	(1,2) *ETHE1* (▶ Sect. 20.9) (2,3) *SUOX* (▶ Sect. 20.11)	Methionine adenosyltransferase I/III deficiency. *ETHE1, SUOX*	(1,2,3) *SUOX* (▶ Sect. 20.11)		T: (1,2) *ETHE1* (▶ Sect. 20.9)	*ETHE1*: BBGGa *SUOX:* extrapyramidal and cerebellar signs, (2,3) cystic leukomalacia	Lactate, OA, acylcarnitines, thiosulfate, homocysteine
Galactosemia (2,3) (▶ Chap. 14)		(3)	(3)	(2)	T: (3)	Learning difficulties Inconstant WM changes	Galactose-1-P (RBC), GALT enzyme activity
Vitamin related diseases (▶ Chaps. 27, 28, and 29) All (2) except: **MTHRF** (3) (2,3) Biotinidase def (3) Vitamin E def (*TTPA*) (2,3) Disorders of thiamine	Biotinidase def (▶ Sect. 27.1.2) *SLC46A1* (▶ Sect. 28.1.1) *FOLR1* (▶ Sect. 28.3.2) DHFR (▶ Sect. 28.3.5) *TPK1*, (▶ Sect. 29.1.4) *SLC25A19* (▶ Sect. 29.1.3) *NADK2, NAXE* (▶ Sect. 11.14) *MTHFR* (▶ Sect. 28.3.7)	*SLC19A3:* (▶ Sect. 29.1.2) Biotin metabolism def *FOLR1* Vitamin E deficiency (*TTPA*)	*FOLR1*		T: Head tremor in Vitamin E def	*SLC19A3:* Abrupt dystonia in catabolic states. *TTPA:* ataxia, retinopathy *SLC25A19:* bilateral striatal necrosis, polyneuropathy Basal calcifications in *SLC46A1 FOLR1 and DHFR*	OA, lactate, free thiamine, folate, blood count, 5-MTHF in the CSF, vitamin E, triglycerides
Metal related disorders (1) ***SLC39A14; SLC30A10*** (▶ Sect. 34.4) Hypermanganesemia (2) **Menkes, Wilson, *SLC39A14; SLC30A10***, *SLC30A9* (3) NBIA, **Wilson**	***ATP7A* (Menkes)** (▶ Sect. 34.1.2) ***ATP7B* (Wilson)** (▶ Sect. 34.1.1) SLC30A9 (▶ Sect. 34.6.3) (▶ Chaps. 2 and 3) NBIA	*SLC39A8* (CDG type) *SLC39A14* *SLC30A10* (▶ Sect. 34.4) NBIA (▶ Sect. 34.2.3) ***ATP7B* (Wilson)** *SLC30A9* (▶ Sect. 34.6.3)		Wilson	T: *SLC39A14, SLC30A10* Wilson P: *SLC39A14, SLC30A10* NBIA, Wilson	Generalized drug-resistant dystonia, suggestive brain MRI finding *SLC39A1:* progressive spasticity and dystonia	Sialotransferrin, manganese Copper, Ceruloplasmin, ASAT/ALAT, blood count
Hem disorders (1, 2) (▶ Chap. 33)	(2) *HMBS* (▶ Sect. 33.4)	(1) CMH (caused by *CYB5R3* mutations)	(1) CMH			*HMBS:* Vertical gaze palsy, nystagmus. SP *CYB5R3:* torticollis, opisthotonos, spasticity, headache, hypomyelination, microcephaly	Congenital: methemoglobin (B) porphobilinogen, aminolevulinic acid, porphyrins

Pyrimidine metabolism (1,2) (▶Chap. 32)	CAD deficiency (▶Sect. 32.1.10)					Developmental encephalopathy, epilepsy	Anisopoikilocyto-sis / anaemia
Amino acid neurotrans-mitter disorders (1, 2, 3) (▶Chap. 30)	(2) SSADH (▶Sect. 30.1) *SLC25A22* (▶Sect. 30.5) (2, 3) NKH (▶Sect. 23.2) GRIN, GABAR (▶Sect. 30.1)	All of them (1) SSADH, NKH GRIN related disorders (▶Sect. 30.2)	(1,2) NKH (1) GRIN, GABAR GABAT	(1) SSADH (▶Sect. 30.1)		SSADH: ID, behav-ioural abnormalities NKH: DD, seizures GRIN1A: oculogyric crises GABAT: hypersomno-lence	OA, AA
Purine disorders (1,2,3) (▶Chap. 32)	All (2): PNP (▶Sect. 32.3.3) ADSL (▶Sect. 32.1.2)	LN disease (1) All (2): β-ureidopropionase deficiency (▶Sect. 32.1.14) *ADCY5* (▶Sect. 32.1.7)	(1,2) LN disease (▶Sect. 32.1.6) (2,3) *ADCY5*			LN: self-aggressive behaviour *ADCY5:* myokymia, paroxysmal chorea, alternating hemiplegia PNP: immunodeficiency. diplegia ADSL: autism, Rett-like	Uric acid, purines PNP: T-lymphocyte deficiency Purines in urine
Proline/Ornithine defect (1)		P5CS (▶Sect. 21.3)	P5CS			P5CS: Prominent spasticity, vessel tortuosity	Ammonia, amino acids
Polyol metabolism def (2)	RPIA (▶Sect. 7.9)					Epilepsy, extensive WM disease, spasticity	Polyols
Metabolite repair (3) (▶Sect. 22.8)		L2HGDH	L2HGDH		T and P: L2HGDH	Epilepsy, DD, leukoen-cephalopathy	Organic acids, aminoacids / lysine
II. Energy metabolism defects. In mitochondrial disorders lactate and other markers may be disturbed mostly in children but often remain normal in adult cases. Diagnosis is complex more and more based on molecular investigations (▶Sect. 14.5)							First line markers (Table 1.3) (lactate…) AA OA function tests
GLUT1DS (1,2,3) (▶Sect. 8.3)	At any age	At any age	At any age	At any age	T: Head tremor may appear in particular in (3) and adults P: Rare although described in (3) and adults	Paroxysmal dystonia, learning disabilities Ataxic-dyspraxic gait, ataxic-spastic gait distal spasticity. Abnormal, pathognomonic eye movements in early infancy	Low glycorrhachia

(continued)

Table 1.19 (continued)

Disease group	Ataxia	Dystonia	Chorea/athetosis	Myoclonus	Tremor (T)/ Parkinsonism (P)	Other neurological features	Biomarkers /L-Dopa responsive (DR)
Creatine defects (2,3) (►Chap. 9)	*SLC6A8* (Creatine transporter) (2,3) (►Sect. 9.1.3) GAMT (3) (►Sect. 9.1.2)	(2,3) *SLC6A8* (2,3) Creatine synth. (3) GAMT	(2,3) *SLC6A8* (3) GAMT			Epilepsy, autism, DD, ID, microcephaly *SLC6A8:* Mild cerebellar atrophy +/ WMa GAMT: BBGG T2 hyperintensity (pallidum)	Creatine, guanidine compounds, brain MRS
Ketone body metabolism (2) (►Chap. 13)	*ACAT1* (►Sect. 13.3)	*ACAT1*		*ACAT1*		Brain MRI: Normal to BBGG T2 hyperintensity	OA, acylcarnitines, ketone bodies, glucose
(1) Mitochondrial/ pyruvate metabolism/ Krebs cycle (►Chap. 11) **(PDH may be treatable)** **CoQ10 synthesis def** (2,3) Mitochondrial carriers, protein import, quality control, fusion, DNA depletion (►Chap. 10)	(1) PC, (►Sect. 11.1) PDH (►Sect. 11.3) NARP (►Table 10.2) Plasma membrane citrate transport, other genes Leigh related, (►Table 10.4, ►Sect. 10.2.1) *twinkle, CLPB, SACS* 2) NARP, *twinkle* **COQ6, COQ8A** C12orf65 release factor def *MSTO1, DNAJC19* *FBXL4, OPA3* (3) Diverse genes PMPCA	(1) PC; diverse Leigh genes. (►Table 10.2) **PDH;** FHD; citrate transporter def; *CLBP* def. *tWARS.SUCLA2, SUCLG1*; (►Sect.11.6) other genes (2) Diverse Leigh genes. FHD, **PDH**, MDH, citrate transporter def, ARALAR1 **COQ8A,** *DNAJC19, POLG, FBXL4, OPA3* (3) Protein import: *TIMM8A, POLG, FARS2*, *MT-ND6*, *MT-TI,* MERRF	(1) Aconitase def, (2) *FBXL4, OPA3 MECR:* (3) Diverse genes	(1) *CLBP* def, (2) CoQ10 def, MERRF (3) Diverse genes MERRF ►Table 10.2)	T: (1) *ATAD3A* (high amplitude tremor (2) DNAJC19, MSTO1 (3) POLG P: (1) *tWARS,* DLP1, PC (2) POLG; (3) *POLG, MTCYB* (complex III), *FARS2*, *MT-ND6* (complex I), *MT-TI*, Mitophagy genes	Aconitase def: cerebellar signs, progressive. *SUCLA2*: deafness (►Sect. 11.6) SACS: Spastic ataxia, Charlevoix-Saguenay type MERRF: myoclonic epilepsy. Progressive spasticity ARALAR1: epilepsy (►Sect. 11.11) *DNAJC19:* Dilated cardiomyopathy with ataxia 3-methylglutaconic aciduria type 5 *POLG:* epilepsy, ID *OPA3:* spasticity *TIMM8A*: Mohr-Tranebjaerg syndrome: dystonia+deafness-optic neuronopathy *PMPCA:* Autosomal recessive spinocerebellar ataxia type 2	Lactate, organic acids, plasma amino acids, citrate Lactate (brain), N-acetylaspartic acid (brain), **POLG may be DR**

Glycolysis metabolism (3)		*PGK1* (▶Sect. 7.4)			T y P: *PGK1*	Haemolytic anaemia, myopathy	Blood count
III. Complex molecules defects. Many complex molecules accumulation defects like sphingolipidosis, sterols and some peroxisomal disorders may be suspected on clinical grounds and have robust metabolic markers. Most complex molecules synthesis and remodelling defects like complex lipids have no easy to reach metabolic markers and diagnosis is based on molecular investigations. Lipidomics become more and more accessible							VLCFA, plasmalogen, phytanic acid, oxysterols, MPS, oligosaccharides, sulfatides, sialic acid, lipidomics
Lysosomal storage disorders (2,3) (▶Chaps. 40 and 41)	(2) CLN subtypes: 2, 3, 5,6,7,8,14 (▶Sect. 40.5) Sphingolipidosis (▶Sect. 40.2) Gaucher type 3, Sandhoff (*HEXA*), Krabbe *SUMF1*; Sialidosis (Salla disease); B-mannosidosis, *NEU* (▶Sect. 41.3) (3) CLN10, *CTSD* Gaucher. Sandhoff, NPC, *SCARB2*, *MAN2B1* (oligosaccharidosis)	(2) CLN2,3,6, (NLC) Niemann-Pick type C (NPC) 1 and 2 (▶Sect. 40.4) Gaucher type 3, GM1 Krabbe, α-fucosidase, *FUCA* (▶Sect. 41.3) (Oligosaccharide accumulation) (3) Sphingolipidosis: Gaucher, GM1 Niemann-Pick C NLC, others (▶Sect. 41.3)	(2) NPC Gaucher 3 NLC	(2) Sialidosis CLN2, 8 Sandhoff Krabbe *NEU* (3) Gaucher *HEXB*	T: (2) *ATP13A2* Sandhoff, Sialidosis (3) *HEXB* P: (2) CLN2,3,6, NPC, Gaucher 3, GM1, Sandhoff (3) Gaucher, GM1, NLC, *HEXB*	NLC: retinal degeneration, PME NPC: vertical ophthalmoplegia, gelastic cataplexy Gaucher: horizontal ophthalmoplegia. β-mannosidosis: mimics SCA Sialidosis: hypotonia at 6 months followed by ataxia, tremor CLN2, Sandhoff: spasticity *FUCA:* generalised and painful dystonia Oromandibular dystonia *CTSD:* prominent spasticity; GM1. Cherry red spot. *SCARB2*: PME, action myoclonus-renal failure syndrome	Sialidosis, CLN3, GM1, Sandhoff: Enzymatic activity, genetic test GM1, *FUCA, NEU*: vacuolated lymphocytes *SUMF1*: Sulfatides, glycosaminoglycans, urine oligosaccharides, enzymatic activity
Glycogen metabolism (2,3) (▶Sect. 5.3.1)	Lafora disease: *EPM2A, EPM2B*	Lafora disease	Lafora disease		T: Lafora disease	Myoclonic epilepsy, Neurodegeneration	Skin biopsy: PAS + inclusions

(continued)

Table 1.19 (continued)

Disease group	Ataxia	Dystonia	Chorea/athetosis	Myoclonus	Tremor (T)/ Parkinsonism (P)	Other neurological features	Biomarkers /L-Dopa responsive (DR)
Lipid metabolism: Phospholipid defects (▶ Chap. 35) Phosphatidylinositol def Phospholipid (PL) remodeling (▶ Sect. 35.4) Fatty acid synthesis elongation (▶ Sect. 42.4) Triglyceride metabolism (▶ Sect. 35.2.3) NBIA syndromes (▶ Sect. 34.2.3)	(1) *ITPR1* (2) Celia's encephalopathy (See Table 35.1) Mevalonate kinase deficiency (MK) (▶ Sect. 37.1) NBIA (▶ Sect. 34.2.3) PL remodelling (▶ Sect. 35.4) A*BHD5*, *PNPLA6* *ABHD12, CYP7B1* *CERS1*,2 (▶ Sect. 40.1.3)	(1) *PLA2G6* (PLAN) (▶ Sect. 35.4.2) P*I4K2A* (dyskinesia) *SERAC1* (▶ Sect. 35.3.7) (2) *MECR* *BSCL2* (seipin) (▶ Sect. 35.2.2) *PLA2G6* (▶ Sect. 35.4.2) *FA2H* (▶ Sect. 40.1.5) (NBIA) (▶ Sect. 34.2.3)	(1) P*I4K2A* *ATP8A2*	(1) *PI4K2A* (2) *MECR* Celia's	T: (2) *ABHD12* (▶ Sect. 42.4) P: (1) PLA2G6 (1) *PI4K2A* (▶ Table 35.1)	ITPR1; congenital non progressive SCA *PLA2G6:* INAD, NBIA2 *PI4K2A* akathisia, epilepsy, cutis laxa *SERAC1*: MEGDEL syndrome MEPAN, *MECR, FA2H* and Celia's encephalopathy: spasticity CERS1,2: PME *ABHD5:* Chanarin-Dorfman *PNPLA6:* retinopathy, hypogonadotropic hypogonadism *ABHD12:* PHARC syndrome *CYP7B1:* sensory ataxia, optic atrophy	OA, lactate, liver enzymes Lactate (brain) ACOX2: Bile acid intermediates *CYP7B1:* hydroxycholesterol *PLA2G6* mutations may present as **DR** parkinsonism
Peroxisomal disorders (▶ Chap. 42)	FA β-oxidation (▶ Sect. 42.2) (2) *ACOX1, ACOX2* (▶ Sect. 42.2.7), *SCP2, ABCD1* (3) *HSD17B4, AMACR* Diverse PEX genes	(2) *ACOX1*, *SCP2* (2) *SCP2*; X-ALD			T: (2) *SCP2*, *AMACR*	*SCP2*: spasmodic torticollis, head tremor *ABCD1:* regression, progressive spasticity *AMACR:* Leukoencephalopathy with dystonia and neuropathy *HSD17B4*: Pseudo-Zellweger (severe) Perrault syndrome type 1 (milder) (▶ Sect. 42.2.2)	VLCFA, pristanic acid, phytanic acid, plasmalogens, pipecolic acid, bile acids
Nucleotide and nucleic acid metabolism disorders (1,2) (▶ Chap. 39)		*TREX1*, *RNASEH2A*, *RNASEH2B*, C *SAMHD1*, *ADAR1* and *IFIH1*, *RNASET2*			P: Rigidity, HRS-like is common in different genes	**Aicardi-Goutières syndrome** (▶ Sect. 39.1.2) (emergency treatment: Baricitinib) Leukodystrophy, basal ganglia calcification	Increased CSF lymphocytes, thrombocytopenia, interpheron signature

Aminoacyl-tRNA synthetases and tRNA processing metabolism disorders (►Sect. 39.2) ►Table 39.1	tRNA aminoacylation (►Sect. 39.2.3) (2) *DARS1,2, MARS2 SARS1, RARS1,2 EPRS1*	(1) *EPRS1, EPRS1, SEPSECS* Other genes (2) *MARS2, WARS2, EPRS1*	(1) tWARS (2) *WARS2*		T: (2) *DARS1* P: (1) tWARS	Some of them with combined oxidative phosphorylation (see also mitochondria) *SARS1:* seizures *DARS1, 2, RARS1,2, EPRS1:* spastic-ataxia, spastic paraparesis (■ Table 39.1) PCH and hypomyelination are common	Lactic acidosis may be present **DR has been described in tWARS**
Ribosomal biogenesis (►Sect. 39.3)	(1) *POLR1C* (type 11) (1) *POL3B* (type 8) (3) *POLR3A* (type 7) (2) *UBTF1*					Hypomyelinating leukodystrophy (type 11) with hypodontia, hypogonadotropic hypogonadism POLR3A: spastic-ataxia *UBTF1*: cerebellar atrophy, regression	
IV. Cellular trafficking disorders: in general, there is no metabolic marker so far but in some CDG. Diagnosis is based on DNA test.							Only in some CDG syndromes
CDG syndromes (1,2,3) (►Chap. 43)	(1) PMM2 (►Sect. 43.2.1) (2,3) Diverse CDG syndromes	(1,2) PMM2 (2) Dolichol metabolism def (►Sect. 43.5)	PMM2	(2) *NGLY1* (►Sect. 43.5.1)	T: (1) GPI anchor defects (►Sect. 43.4) (2) *NGLY1*	Multisystem involvement (►Chap. 43) Often cerebellar hypoplasia/atrophy	Sialotransferins, ASAT/ALAT, coagulation factors

(continued)

Table 1.19 (continued)

Disease group	Ataxia	Dystonia	Chorea/athetosis	Myoclonus	Tremor (T)/ Parkinsonism (P)	Other neurological features	Biomarkers /L-Dopa responsive (DR)
Cell trafficking disorders (1,2,3) (▶Chap. 44)	(1) *SNX14* (disorders of autophagy, ▶Sect. 44.2.1) *SCYL1* (SCA21) *KIF1C* (2) *SNX14* (autophagy defect) *TRAPPC11 TANGO2, VPS13D RUBCN, SIL1, SCYL1, SPTBN2* (3) *WDR45* (autophagy) *VPS13D SIL1*	(1) Synaptic vesicle disorders (▶Sect. 30.6) (2) *TRAPPC* (diverse subunits) *VPS16, VPS4*: early-onset dystonia *ATP1A3 TUBB4A* (3) Autophagy disorder: *WDR45 ATP13A2:* Rufor-Rakeb syndrome *VPS16 ACTB*	(1) *SNX14* ATP8A2 (flippase)	(1) *SNX14* (2) *GORS2* (action myoclonus)	T: (1) *SNX14, KIF1C* (dystonic tremor) (2) *GORS2* (rest tremor) P: (1) *FIG4* PARKIN deficiency (2) Autophagy disorder: *WDR45,* SV endocytic disorders, *ATP6AP2 VPS13D*: SCA4 (3) *WDR45, ATP13A2 KIAA1840; ZFYVE26 STXBP1, TUBB4A VAC14, VPS13C, VPS13D*	*BCAP31*: deafness, dystonia, hypomyelination *ATP8A2*: hypotonia, optic atrophy *SCYL1:* RALF, peripheral Neuropathy *KIF1C:* may also cause spastic ataxia, adult-onset ataxia *WDR45:* β-propeller protein-associated neurodegeneration (BPAN) (▶Sect. 39.2.3) *TUBB4A*: torsion dystonia *TANGO2*: arrythmias, rhabdomyolysis, SP *VPS13D*: Cohen disease, spastic-ataxia *RUBCN:* SCA4; *SIL1:* cataracts, myopathy *SPTBN2:* may cause adult onset SCA *KIAA1840; ZFYVE26, VPS13D:* spastic paraparesis *SYNJ1, DNAJC6, STXBP1* may appear as refractory epilepsy in early childhood and progressively evolve towards parkinsonism	***SYNJ1, DNAJC6, CLCT, VPS35* may be DR**

In black bold: treatable disorders

Onset age: *1* infancy to early childhood, *2* childhood (from 2 years until adolescence), *3* adolescence

AA amino acids, *AADC* L-amino acid decarboxylase deficiency, *ABHD12* monoglycerol lipase, *ACAT1* acetoacetyl-CoA thiolase deficiency, *AD* autosomal dominant, *ADCY5* adenylate cyclase deficiency, *ADL-X* X-linked adrenoleukodystrophy, *ADSL* adenylosuccinate lyase deficiency, *AIP* acute intermittent porphyria, *ALS* amyotrophic lateral sclerosis, *AMACR* α-methylacyl-CoA racemase, *AMPD* AMP deaminase-2 deficiency, *AR* autosomal recessive, *ARALAR* mitochondrial protein involved in glutamate aspartate exchange encoded by SLC25A12, *ARD* (adult Refsum disease) phytanoyl-CoA 2-hydroxylase, *ARG* arginine, *ARSA* arylsulfatase A deficiency, *AS* asparagine synthetase, *ATIC* 5-Amino-4-imidazolecarboxamide-ribosiduria (AICA)-ribosiduria, *Atten* attenuated forms, *ATIC* 5-Amino-4-imidazolecarboxamide-ribosiduria (AICA)-ribosiduria is an exceedingly rare autosomal recessive

condition resulting from the disruption of the bifunctional purine biosynthesis protein PURH, *ATP8A2* encodes for a P4-ATPase that actively transports phospholipids across cell membranes ('flipping'), *BBGGa* basal ganglia abnormalities, *B4GALNT1* GM2/GD2 synthase deficiency, *BCAA* branched chain amino acids, *C* cerebellum, *Ca* cerebellar atrophy, *CAD* CAD trifunctional protein, carbamoylphosphate synthetase/aspartate transcarbamylase/dydroorotase, *CC* corpus callosum, *CDG* congenital disorders of glycosylation, *CERS* ceramide synthase, *CIT* citrulline, *CK syndrome* X-linked recessive sterol-4-alpha-carboxylate 3-dehydrogenase deficiency, *CLBP* mitochondrial quality protein control, *CMH* congenital methemoglobinemia, *CMNO* cardiomyopathy, *CP* cerebral palsy, *CPTII* carnitine palmitoyltransferase II deficiency, *CTX* cerebrotendinous xanthomatosis, *CSF* cerebrospinal fluid, *CP* cerebral palsy, *DBP* D-bifunctional protein, *DD* developmental delay, *DEF* deficit, *DHPR* dihydropyrimidine reductase deficiency, *DEGS* dihydroceramide delta4-desaturase, *DHFR* dihydrofolate reductase deficiency, *DLP1* dynamin related protein 1. EAAT1, glutamate aspartate transporter deficiency (SLC25A22), *DWI* diffusion, *ECHS1* mitochondrial short-chain enoyl-CoA hydratase deficiency, *ELOVL* elongation of very long chain fatty acids, *encephalop* encephalopathy, *ENTPD* ectonucleoside trisphosphate diphosphohydrolase I, *EPT1* ethanolamine phosphotransferase, *ETHE1* sulfur dioxygenase deficiency, *FA2H* fatty acid 2-hydroxylase deficiency, *FAR1* fatty-acyl CoA reductase 1 deficiency, *FHD* fumarate hydratase deficiency, *FOLR1* folate receptor alpha deficiency, *GAMT* guanidinoacetate methyltransferase, *GA1* glutaric aciduria type 1, *GABAR* GABA receptor mutations, *GABAT* GABA transaminase deficiency, *GALC* galactocerebrosidase, *GLUT1DS* glut-1 deficiency syndrome, *GORS* Golgi SNAP receptor complex member 2, *GOT2* glutamate oxaloacetate transaminase deficiency, *GRIN* mutations in NMDA glutamatergic receptors, *GS* glutamine synthetase, *GSS* glutathione synthetase deficiency, *GTPCH* guanosine triphosphate cyclohydroxylase, *HCS deficiency* holocarboxylase synthetase deficiency, *HH* hyperinsulinism-hyperammonaemia syndrome, *HHH* hyperornithinaemia-hyperammonaemia-homocitrullinaemia syndrome, *HIBCH* 3-hydroxyisobutyryl-CoA hydrolase deficiency, *Homocyst* homocysteine, *HRS* hypokinetic-rigid syndrome, *HSD10* 2-methyl-3-hydroxybutyryl-CoA dehydrogenase deficiency, *ID* intellectual disability, *INAD* infantile neuroaxonal dystrophy, *ITPase* Inosine triphosphatase, *ITPR1* inositol 1,4,5-triphosphate receptor, type1, *IVA* isovaleric aciduria, *L2-HGA* L2-hydroxyglutaric aciduria, *L2HGDH* L-2-hydroxyglutarate dehydrogenase deficiency, *L,D2-HGA* L and 2-D-hydroxyglutaric aciduria, *LBSL* leukodystrophy with brainstem and spinal cord involvement and lactate elevation, *LN* Lesch-Nyhan, *MCGA1* 3-methylglutaconic aciduria type 1, *MECR* Trans-Enoyl-CoA reductase, *MELAS* lactic acidosis, and stroke-like episodes, *MEGDEL* 3-methylglutaconic aciduria with deafness, encephalopathy, and Leigh-like syndrome, *MEPAN* mitochondrial enoyl-CoA reductase protein-associated neurodegeneration, *MERRF* mitochondrial encephalopathy and ragged red syndrome, *MD* movement disorders, *MDH* malate dehydrogenase deficiency, *MLD* metachromatic leukodystrophy, *MMA* methylmalonic aciduria, *MOCS* molybdenum cofactor deficiency, *MPS* mucopolysaccharides, *MRS* brain MRI with spectroscopy, *MSD* multiple sulfatase deficiency, *MSUD* maple syrup urine disease, *MTHFR* methylenetetrahydrofolate reductase deficiency, *MTHFS* 5,10-methenyltetrahydrofolate synthetase, *NADK2* mitochondrial NAD kinase 2 deficiency, *NARP* neuropathy, ataxia and retinitis pigmentosa, *NAXE* NAD(P)HX epimerase deficiency, *NBIA* neuronal brain iron accumulation, *NCL* neuronal ceroid lipofuscinosis, *NKH* nonketotic hyperglycinaemia, *NPS* neuropsychiatric symptoms, *NRL* neurological, *NT* neurotransmitter, *NT5C2* 5-prime-nucleotidase, cytosolic II, *ORN* ornithine, *OA* organic acids, *P5CS* δ-1-pyrroline-5-carboxylate synthase deficiency, *PA* propionic aciduria, *PHARC* polyneuropathy, hearing loss, ataxia, retinitis pigmentosa, and cataract, *PC* pyruvate carboxylase, *PCH* pontocerebellar hypoplasia, *PDH* pyruvate dehydrogenase, *PERIV* periventricular, *PLA2G6* phospholipase A2, *PKAN* pantothenate kinase 2 deficiency, *PGK1* phosphoglycerate kinase 1, *PHARC* peripheral neuropathy hearing loss retinitis pigmentosa, cataract, *PME* progressive myoclonic epilepsy, *PMPCA* peptidase, mitochondrial processing, alpha, *PN* peripheral neuropathy, *PNPO* pyridoxamine 5′-phosphate oxidase, *PNP* purine nucleoside phosphorylase deficiency, *PMM2* phosphomannomutase 2 deficiency, *PRO* proline, *PSAP* prosaposin, *RALF* recurrent acute liver failure, *RCDP* rhizomelic chondrodysplasia punctata, *RHADS* rhythmic high amplitude delta with superimposed polyspikes, *RP* retinitis pigmentosa, *RPIA* ribose-5-phosphate isomerase deficiency, *SACS* sacsin, *SCA* spinocerebellar atrophy, *SCP2* sterol carrier protein 2 deficiency, *SDE* syndrome, *SLC25A19* thiamine pyrophosphate transporter, *SHMT2* serine hydroxymethyltransferase type 2, *SLS* Sjögren Larsson syndrome, *SORD* sorbitol dehydrogenase, *SP* spastic paraparesis, *SR* sepiapterin reductase deficiency, *SSADH* succinic semialdehyde dehydrogenase (aldehyde dehydrogenase 5a1, *SUCLA2* succinyl-CoA lyase subunit, *SUCLG1* succinyl-CoA ligase alpha subunit, *SUMF1* gene responsible for multiple sulfatase deficiency, *SUOX* sulphite oxidase deficiency, *SV* synaptic vesicle, *TH* tyrosine hydroxylase deficiency, *TPK1* thiamine pyrophosphokinase deficiency, *UCD* urea cycle disorders, *VLCFA* very long chain fatty acids, *WM* white matter, *WMa* white matter abnormalities, *X-ALD* X-linked adrenoleukodystrophy

Boucher-Neuhauser syndrome), *ABHD12* (PHARC), or ceramide synthase deficiency (with myoclonic epilepsy) amongst others; (ii) Cell trafficking disorders (▶ Chap. 44) such as several disorders of autophagy (*SNX14, WDR45*); (iii) Disorders of aminoacyl-tRNA synthetases and ribosome metabolism disorders (▶ Chap. 39) which are associated with early-onset encephalopathies with cerebellar and brainstem atrophy and hypomyelination.

Hypogonadotropic hypogonadism is a key sign of the neuropathy target esterase (NTE) spectrum that includes Boucher-Neuhäuser syndrome (with chorioretinal dystrophy) and Gordon Holmes syndrome (with peripheral neuropathy) (▶ Sect. 35.4.4).

- *Non-progressive ataxias* are typically found in CDG syndromes, SSADH deficiency and ITPR signalling defects. In these diseases, ataxia can even improve over time [67].

Other than ◘ Table 1.19 key symptoms that may help in the differential diagnosis are depicted in the ataxia algorithm (◘ Fig. 1.5).

Dystonia

Dystonia is defined by the occurrence of sustained/intermittent muscle contractions, causing aberrant, repetitive twisting movements, and abnormal postures. In many different metabolic diseases, dystonia is a major feature. In fact, almost all neurometabolic disorders can cause dystonia at some stage. However, glutaric aciduria type I, Leigh syndrome, metal disorders, neurotransmitter defects and some lysosomal disorders such as Niemann-Pick C are among the most relevant (dystonia algorithm, ◘ Fig. 1.6). Neurodegeneration with Brain Iron Accumulation (NBIA) is a growing group of disorders that is characterized by progressive dystonia and parkinsonism [68] (▶ Sect. 34.2.3).

MOST COMMON GENETIC ATAXIAS

- Friedrich ataxia (accounts for ~25% of all SCARs)
- Autosomal-recessive spastic ataxia of Charlevoix-Saguenay
- SPG7: paraplegin mutations; SYNE1 mutations
- Ataxia telangiectasia (PI3K-family kinase)
- Ataxia with oculomotor apraxia type 1 and 2
- *POLG* and other mitochondrial diseases such as PDH, OPA1
- ITPR1 (inositol 1,4,5-triphosphate (IP3) receptor)

ATAXIA WITH SPASTICITY

Ataxia and spasticity usually coexist as a spectrum of clinical manifestations that correspond to similar mechanisms of disease. See Table 1.20 "Complex Motor Signs"

ATAXIA WITH PROMINENT EPILEPSY

CAD deficiency, GLUT1D, Creatine defects, PDH, Biotinidase deficiency, Cerebral folate deficiency, COQ10 defects, Niemann-Pick C

- NKH, SSADH. GABA receptor mutations and GRINpathies cause ataxic-dyspraxic-uncoordinated gait more than pure ataxia
- Several mitochondrial disorders
- Lysosomal disorders, and in particular NLCs (neuronal lipofuscinosis)
- Complex lipid disorders such as *PI4K2A, PLA2G6* mutations
- Aminoacyl-tRNA synthetases such as *SARS* mutations.
- *UBTF1* mutations (ribosomopathy) with regression
- Cell trafficking disorders and in particular synaptic vesicle defects such as *SYNJ1, DNAJC6, STXBP1* mutations
- Progressive Myoclonic Epilepsies: Lafora disease, NCLs, sialidosis type I, MERRF), Gaucher disease type 3, ASAH1, ceramide synthetase deficiency, *SCARB* mutations, Unverricht-Lundborg disease, dentatorubral-pallidoluysian atrophy (DRPLA), neuroserpinosis, and *KCNC1* related diseases.

ATAXIA WITH OTHER ABNORMAL MOVEMENTS

- **With dystonia: organic acidurias, BCAA disorders, intracellular Cbl defects, creatine defects, biotinidase deficiency, cerebral folate deficiency, vitamin E deficiency, Wilson, GLUT1DS, PDH, CoQ10 defects, Niemann-Pick C,** sulfur amino acid disorders such as *ETHE1, SUOX* mutations, *SLC30A9*, NBIA, SSADH, NKH, GRIN related disorders, several mitochondrial disorders, CLN2,3,6, Niemann-Pick type C 1 and 2, Gaucher type 3, Lafora disease, NBIA syndromes, peroxisomal disorders: ACOX1, SCP2, X-ALD, Aminoacyl-tRNA synthetases such as *EPRS1* mutations, diverse CDG subtypes, cell trafficking disorders such as TRAPPCpathies
- **With choreoathetosis: organic acidurias, creatine defects, cerebral folate deficiency, Wilson, GLUT1DS, Niemann-Pick C.** Diverse mitochondrial disorders such as: Aconitase deficiency, FBXL4, OPA3, MECR. Lysosomal disorders: Gaucher 3, NLC, Lafora disease. CDG syndrome: PMM2. Cell trafficking disorders such as *SNX14* mutations
- **With myoclonus: HHH, ACAT1 (ketone body metabolism defect), Wilson, CoQ10 defects, GLUT1D,** L2-HGA, SSADH, sialidosis, CLN2, 8, Sandhoff, Krabbe, NEU, Gaucher, *HEXB* mutations. Cell trafficking disorders such as *SNX14* mutations
- **With tremor: Isovaleric aciduria, MSUD, CblC, Wilson, vitamin E deficiency and GLUT1D** (head tremor), D-2-HGA, L2HGDH, sulfur metabolism defect such as ETHE1, metal disorders such as *SLC39A14, SLC30A10*, several mitochondrial disorders such as *ATAD3A, DNAJC19, MSTO1., POLG*. Sialidosis, Lafora disease. Complex lipid disorders such as ABHD12 mutations. Peroxisomal disorders such as: SCP2, AMACR.
  Aminoacyl-tRNA synthetases such as DARS1, GPI anchor defects, cell trafficking disorders such as: *SNX14, KIF1C, GORS2* mutations.
- **With parkinsonism: Wilson, CblD, GLUT1DS** (rare, and more common in adults), HSD10 (BCAA defect), metal disorders: *SLC39A14, SLC30A10* mutations, NBIA syndromes, L2HGDH, in several mitochondrial disorders: *tWARS, DLP1, POLG, MTCYB* (complex III), *FARS2, MT-ND6* (complex I), MT-TI, mitophagy genes. Lysosomal disorders such as: CLN2,3,6, NPC, Gaucher 3, GM1, Sandhoff, Gaucher, GM1, NLC, *HEXB* mutations.
  Aminoacyl-tRNA synthetases such as *tWARS*. Cell trafficking disorders such as *WDR45, FIG4, VPS13D*

ATAXIA WITH POLYNEUROPATHY: see table 1.20 "Complex Motor Signs"

◘ **Fig. 1.5 Ataxia algorithm**

ISOLATED DYSTONIA

- *DYT-THAP1*: young-onset generalized dystonia with predominant craniocervical symptoms
- The GAG deletion in *TOR1A*: generalized dystonia with onset in childhood in the lower limbs
- De novo mutations in *GNAL* and *ANO3*: isolated focal and/or segmental dystonia with preference for the upper half of the body and older ages at onset.

IEM with isolated dystonia are rare and in most cases dystonia remains isolated for some time but later on, other symptomos appear.
This is the case for:

Neurotransmitter defects, homocystinurias, early stages of NBIA syndromes and lysosomal disorders.

OTHER GENETIC CAUSES OF EARLY-ONSET DYSTONIA (related to developmental encephalopathies)

Relatively high frequency of the following genetic variants: *KMT2B* (complex childhood onset dystonia), *SGCE* (myoclonus-dystonia), *ADCY5* (dystonia associated with other movement disorders), *ATP1A3* (rapid onset dystonia parkinsonism), *PANK2*, and *ATM* (ataxia-telangiectasia).

Early infancy until 2 years: – Complex hyperkinetic movements: *ADCY5, GNAO1* (may have also epilepsy), *PDE10*. All these disorders dysregulate cAMP signaling. PDE10A: dominant forms: early onset generalized chorea and t2 striatal hyperintensity; recessive: generalized chorea first months of life, normal brain MRI.

Other genes involved in early complex hyperkinetic movements: *SCNA2, SCN8A*; PNKP deficiency (chorea): DNA damage response gene; microcephaly, DD, seizures, oculomotor apraxia and polyneuropathy, *AUTS2, CHD8, ZEB2, DHCR24, GRID2, MORC2, MSL3, PAK1, PPP2R5D, TECPR2, ZMYND1*

MOST COMMON IEM WITH DYSTONIA

- **Neurotransmitter defects**
- **Classic homocystinuria, Cbl and remethylation defects**
- **Maple syrup urine disease**
- **Organic acidurias (propionic academia, methylmalonic aciduria, malonic aciduria, glutaric aciduria type 1)**
- **Vitamin related disorders: Pyridoxine-dependent epilepsy, PNPO deficiency, biotin-thiamine-responsive basal ganglia disease, thiamine pyrophosphokinase deficiency, mitochondrial thiamine pyrophosphate transporter deficiency**
- **Other treatable disorders: GLUT1DS, classic galactosaemia, pyruvate dehydrogenase complex deficiency, COQ8A deficiency, beta-ketothiolase deficiency, Wilson disease, cerebrotendinous xantomatosis**
- Metal related disorders: Aceruloplasminemia, *SLC30A10, SLC39A14, SLC39A8* mutations
- Complex molecule defects: CLN2 disease, Gaucher disease, metachromatic leukodystrophy, X-ADL, Celia's encephalopathy (seipinopathy)

MOST COMMON IEM WITH CHOREOATHETOSIS

Aromatic L-amino acid decarboxylase deficiency, 6-pyruvoyl-tetrahydropterin synthase deficiency, sepiapterin reductase deficiency, dihydropteridine reductase deficiency propionic academia, methylmalonic aciduria due to methylmalonyl-CoA mutase deficiency, glutaric aciduria type 1,
hereditary folate malabsorption, folate receptor alpha deficiency, classic galactosemia, Wilson disease, glucose transporter 1 deficiency, pyruvate dehydrogenase complex deficiency, beta-ketothiolase deficiency, cerebrotendinous xanthomatosis
Dopamine transporter deficiency,
glycine encephalopathy due to glycine decarboxylase deficiency,
glycine encephalopathy due to aminomethyltransferase deficiency,
molybdenum cofactor deficiency, aceruloplasminemia
CLN2 disease
Benign hereditary chorea: *NRX2/TITF-1* (genetic, non metabolic disease). May improve with L-Dopa

Fig. 1.6 Algorithm for IEM with dystonia and choreoathetosis

Specific characteristics of dystonia and dyskinetic movements may suggest some particular IEM. Some of the most common IEM in abrupt with an acute onset of dystonia are OA (such as GA1) and mitochondrial disorders. **GLUT1DS** can cause paroxysmal exercise-induced dyskinesia and also complex MD even without epilepsy. Oromandibular dystonia is common in creatine deficiency, PKAN and other IEMs, whereas head tremor is a typical feature of **Vitamin E, GLUT1DS** and a few other diseases [64] (specific features of MD algorithm, Fig. 1.7).

New and emerging categories of IEM that cause dystonia as a relevant feature are: (i) complex lipid defects such as *PI4K2A* (dyskinesia and parkinsonism), SERAC1 (MEGDEL syndrome with progressive painful dystonia and specific brain MRI images), *ATP8A2* (with optic atrophy), *MECR* (with associated spasticity) and, *BSCL2* (seipin defect, that may also cause spasticity and epilepsy) (ii) Nucleotide metabolism disorders, such as diverse genes that cause Aicardi-Goutières syndrome (dystonia-rigidity) [69]; (iii) Cell trafficking disorders such as synaptic vesicle diseases, *VPS16, VPS4* as forms of early-onset dystonia, TRAPPCpathies and many other genes (Table 1.19).

In all cases it is important to consider first those IEM for which some effective therapeutic intervention is possible, such as **dopa-responsive dystonia syndromes (Segawa disease and other neurotransmitter related deficiencies)**, **creatine deficiency syndromes**, **cerebral folate deficiency**, **Wilson disease**, **homocystinuria**, **biotinidase**, **thiamine defects**, **vitamin E** and **GLUT1** deficiencies.

Other than Table 1.19, key symptoms that may help in the differential diagnosis are in the dystonia and choreoathetosis algorithm, Fig. 1.6.

1

PAROXYSMAL MOVEMENT DISORDERS	ANATOMIC LOCATION	SPECIFIC TYPE OF MOVEMENTS
Triggers: exercise, fever, other stimuli, unknown - Energy defects (dyskinesias, dystonia, ataxia, chorea) GLUT1D, PDH, biotinidase deficiency - Amino acid catabolism + energy metabolism defects (dystonia, choreoathetosis) ECHS1-mitochondrial short-chain enoyl-CoA hydratase 1 deficiency, HIBCH-3-hydroxyisobutyryl-CoA hydrolase deficiency - Neurotransmitter amino acids (ataxia, chorea, dyskinesias) - Non-ketotic Hyperglycinaemia, GABA transaminase deficiency, succinic semialdehyde dehydrogenase - Other amino acids: HTD-tyrosinemia type III (dystonia): Hartnup disease (ataxia) - T3 transport defect (Allan-Herndon-Dudley syndrome) - *PARK2*-Parkin deficiency (dyskinesia) - *B4GALNT1* (GM2/GD2 synthase): fever-induced ataxia with myokymia - FOLR1 – sensory stimulus sensitive drop attacks - Niemann-Pick C-cataplexy while laughing - *ADCY5* – nocturnal - *ATP1A3* – painful tonic spasms - *SLC16A2* – shivering tremor like episodes - *KCNMA1* – complex PNKD (*) with epilepsy	**Oromandibular dystonia** Cerebral creatine deficiency, Segawa disease, NBIA syndromes, aceruloplasminemia, fucosidosis, oligosaccharidosis, hypermanganesemia due to *SLC39A14*, CLN3, GM1, *TIMM8A*-Deafness-Dystonia-Optic Neuronopathy Syndrome, biotin-thiamine responsive basal ganglia disease. Jaw opening dystonia: *PKAN2* Dystonic posturing of the jaw and tongue: *ETHE1* **Head tremor** GLUT1D, diverse spino cerebellar ataxias, PEX6, PEX16, VitE deficiency, Niemann Pick type C disease, NAXE-NAD(P)HX epimerase deficiency, SCP2-sterol carrier protein-2 deficiency **Palatal myoclonus, myoclonus of the tongue:** Krabbe **Facial chorea:** *MECR* **Facial dystonia:** GM1 **Facial dyskinesia:** *ATP8A2*	**Status dystonicus:** *PKAN2, GNAO,* Wilson, AADC and dopamine transporter deficiency **Tics:** neuroacanthocytosis, Huntington disease; **Starting as action dystonia in one limb:** Niemann-Pick C; **Micrographia:** Wilson's disease; **Spasmodic torticollis:** SPC2; **Startle myoclonus:** *ST3GAL5;* **Ballismus:** Lesch-Nyhan disease; **Opisthotonos:** Lesch-Nyhan disease, *CYB5R3;* **Facial-faucial-finger mini-myoclonus:** *ATP13A2;* **Stereotypies:** CDG syndromes, syndromes with prominent autism such as BPAN, purine disorders, creatine defects, SSADH, synaptic vesicle disorders, amino acid neurotransmitter signalling disorders, MAO deficiency

(*) PNKD paroxysmal non-kinesigenic dyskinesia

Fig. 1.7 Movement disorders depending on specific features that may help in the differential diagnosis

Chorea/Choreoathetosis

The term chorea is a derived from the Greek word "choreia" for dancing and refers to involuntary, brief, random, rapid, spasmodic movements of the face, neck and proximal limb muscles. It can migrate from one side of the body to the other. Choreic movements are a prominent feature of GA1, **GLUT-1 deficiency**, Lesch-Nyhan disease, **cerebral folate deficiency**, PKAN and other NBIA syndromes, **homocystinuria and Niemann-Pick C** among others [64, 67] (Fig. 1.6).

Parkinsonism (Rigid Akinetic Syndrome)

IEM constitute an important group amongst the genetic causes of parkinsonism at any age. However, early forms of parkinsonism have distinctive features as compared to parkinsonism in adolescents and adults. Most early forms of parkinsonism lack crucial classical signs like tremor and tend to be associated to other kind of abnormal movements and neurological manifestations such as pyramidal signs. Therefore, the concept "hypokinetic-rigid syndrome" (HRS), "dystonia-parkinsonism", "parkinsonism-plus", or "parkinsonism-like" are more accurate in children [70].

The main IEM causing parkinsonism are (Table 1.19, Fig. 1.8 algorithm parkinsonism): (i) metal-storage diseases such as Wilson's disease, manganese transporter deficiency and NBIA syndromes; (ii) **Neurotransmitter defects** (as already discussed as causes of early onset dystonia and dystonia-parkinsonism); (iii) Lysosomal and complex molecule disorders. In this group, special consideration should be given to ceroid lipofuscinosis (CNL), GM_1, **Niemann-Pick C and cerebrotendinous xanthomatosis (CTX)** 4) Energy metabolism defects. POLG and different mitochondrial diseases may exhibit parkinsonism features. Characteristically they can respond to low L-dopa+carbidopa doses, especially in adolescents and adults [71].

New diseases and emerging categories of IEM that cause prominent parkinsonism are cell trafficking disorders, in particular endocytotic defects of the synaptic vesicle cycle and autophagy disorders such as *WDR45* mutations (▶ Chap. 44), and some aminoacyl tRNA synthetase deficiencies such as tWARS [72]. Some of these IEM may be also dopa-responsive (Table 1.19).

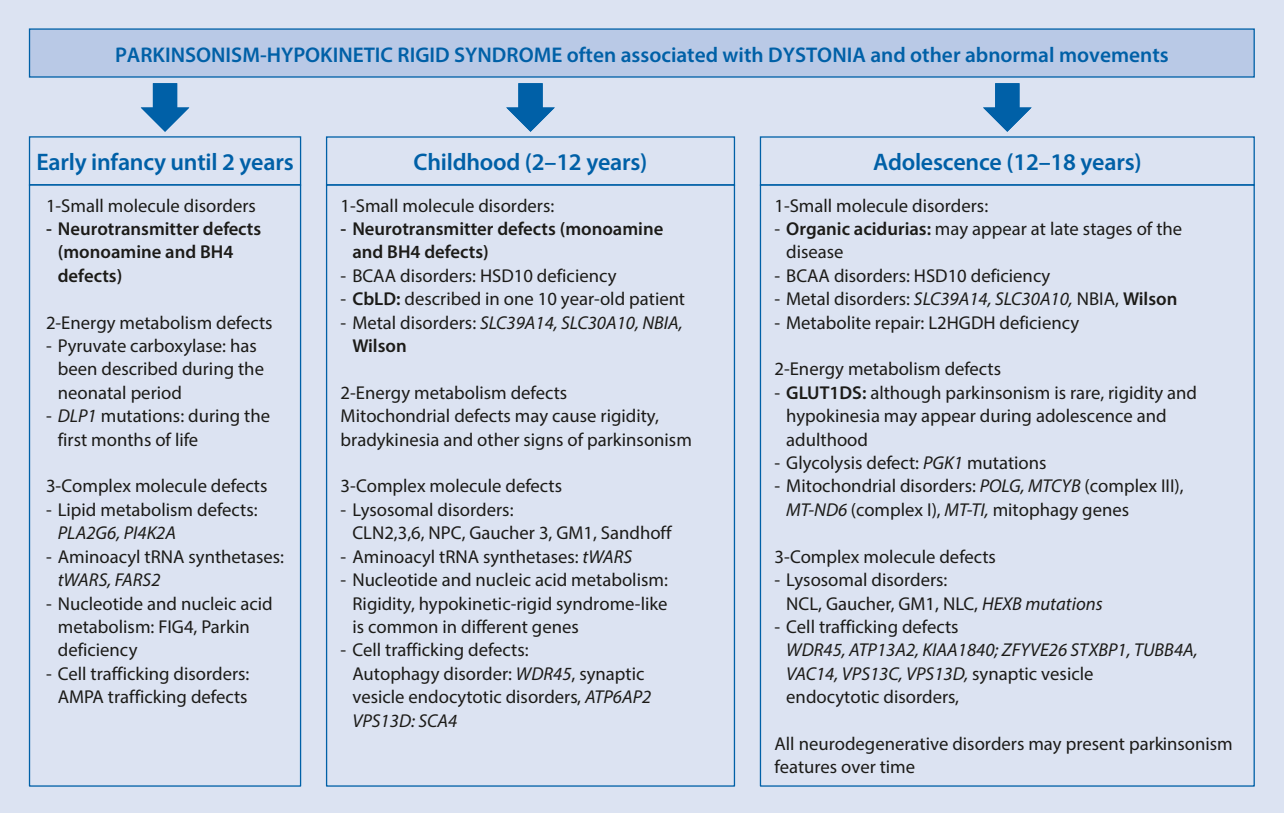

Fig. 1.8 Algorithm for parkinsonism

1.5.2.4 Category 4: With Complex Motor Disorders: Ataxic-Spastic Gait, Predominant Spasticity, and/or Peripheral Nerve/Motor Neuron Involvement [Table 1.20, Fig. 1.9 (Spasticity) and Fig. 1.10 (Peripheral Neuropathy/Motor Neuron Diseases)]

In this category of complex motor disorders, we will include diseases with major involvement of the pyramidal system, which is often connected with the cerebellum, as well as the motor neuron and peripheral nerves.

Ataxic-Spastic Spectrum

Purkinke cells and spinocerebellar tracts (ataxias) and corticospinal tracts (spastic paraparesis, spasticity in general), are often damaged by the same mutated genes causing a continuum spectrum. Ataxia may appear before spasticity or both signs could be detected simultaneously. The classical system of neurogenetic classification of spastic paraplegia (SPG) and autosomal recessive ataxias (ARCAs) were traditionally separated. However, the coincidence of both phenotypes in many genetic conditions has raised awareness about the common mechanisms of disease in these neurological manifestations. Therefore, the concept "spastic-ataxic" disease is being increasingly used [73] The same occurs in IEMs that cause this motor phenomenology and that are included in Table 1.20.

Spasticity

Spasticity is a very common clinical situation in paediatric and adult neurology and is the result of damage to upper motor neurons or to the corticospinal tract. Symptoms may vary from mild stiffness to severe muscle spasms and include hypertonia, brisk deep tendon reflexes, pathological reflexes, clonus and weakness. Depending on the affected anatomical region, spasticity may be more evident or restricted to the lower extremities (spastic diplegia or paraplegia: spastic paraparesis) to one side of the body (spastic hemiplegia) or affecting all four limbs (spastic quadriplegia or tetraparesis).

Recent advances in the molecular characterization of spastic paraplegias (SPGs) have described more than 60 genes that show considerable overlap with other clinical manifestations and share pathophysiological mechanisms as intracellular trafficking, mitochondrial function and lipid metabolism [74].

Regarding IEM, disorders that interfere with myelin metabolism (leukodystrophies), synthesis and remodelling of complex lipids, defects in energy production

Table 1.20 IEM with complex motor clinical manifestations

Disease group	Ataxia-SP spectrum	Spasticity (pure/complex)	Peripheral neuropathy/ motor neuron disease	Other clinical features	Brain image	Biomarkers
I. Small molecule defects. Some cause abnormalities in first line metabolic investigations (acidosis, hyperammonaemia, glucose, lactate …). In most, plasma, urine or CSF metabolic markers are abnormal with a typical or suggestive metabolic signature. Metabolomics for diagnostic purpose is available in some specialized centres.						First line markers (Table 1.3) Second line: AA, OA, acylcarnitine, metals, porphyrins, purines, pyrimidines, biopterins
Urea cycle disorders (▶ Chap. 19)	(2,3) **HHH** (▶ Sect. 21.2)	(1,2) **Arginase def** (complex) (2,3) **HHH**: pure or complex		Acute decompensations, DD, ID. Arginase def: CP-like, microcephaly	Cortical and subcortical edema. BBGGa	Ammonia, AA
Organic acidurias (▶ Chaps. 18 and 22)	**MCGA1** (1,2,3) (▶ Sect. 18.3) L,D-2HGA (1,2) (▶ Sects. 22.8 and 22.9)	**MCGA1** (1), L,D-2HGA (1,2): complex		ID, seizures	MCGA: WMa, BBGGa, Ca; L,D2-HGA: Table X	Organic acids
Vitamin related diseases including folate metabolism	(1,2,3) **CblD** (▶ Sect. 28.2) (1,2,3) **Biotinidase deficiency** (▶ Sect. 27.1.2) (3) ***TTPA*: Vit E def** (1) SHMT2 (▶ Sect. 28.3.10)	Pure spasticity has been described in biotinidase deficiency. All other are complex	**Riboflavin transport** (2,3): motor and sensory neuropathy, distal motor neuropathy, multineuritis with cranial nerve involvement, Guillen-Barré syndrome, ALS mimic SHMT2 (1): axonal neuropathy	**CblD*:*** seizures, psychiatric symptoms, parkinsonism. ***TTPA*:** head tremor, nystagmus, retinopathy; **Biotinidase def:** hypoacusia, dermatitis, myelopathy, seizures. SHMT2: myocardiopathy, ID, congenital microcephaly. **Riboflavin transport:** deafness, pontobulbar palsy	**CblD:** Vascular stroke BBGGa may appear. SHMT2: perisylvian polymicrogyria, thin CC	Organic acids, homocysteine, biotinidase, VitE acylcarnitines
Monoamine-BH_4 def. (▶ Sect. 30.5)	(1,2,3) **SR**	(2,3) **GTPCH** (AD), (1,2,3) **TH:** complex; also pure in TH		Dopa-responsive MD	Usually normal	CSF NT
Proline, ornithine defect: *ALDH18A1* (P5CS) (▶ Sect. 21.3)	Mild cerebellar signs may be present	Pure or with cataracts, skeletal abnormalities, other NRL features AR: 1,2; AD: 3	Axonal neuropathy	Microcephaly, tremor, seizures, ID	Normal, thin/absent CC. C may be affected Atrophy and/or vessel tortuosity	AA: low ORN, CIT, ARG, PRO

Glutamatergic signaling (▶Sect. 30.1)	(1) *GRID2*			ID, tonic upgaze (occasional or persistent)		
Lipoylation defect (▶Chap. 23)	(1) *GLRX5*				Leukodystrophy, upper spinal cord lesions	High plasma glycine levels
Purine defects (▶Chap. 32)	(2,3) *ENTPD1*	(1), *AMPD2,* (2,3) *ENTPD.* (1) *NT5C2,* (1) ATIC: all complex	(1) *AMPD2,* (2,3) *ENTPD1*: axonal neuropathy	*AMPD2*: short stature, amyotrophy; *ENTPD1:*ID, microcephaly; *NT5C2*: ID; ATIC: epilepsy, CP-like	*ENTPD1:* WMa *NT5C2*: WMa, thin CC	Purines
Hem disorders (porphyrias) (▶Chap. 33)		(2) *HMBS*: complex (▶Sect. 38.3) (2) *CYB5R3* (2): complex		*HMBS:* Acute intermittent porphyria. Vertical gaze palsy, nystagmus. *CYB5R3:* Decreased WM volume, hypomyelination	*HMBS:* Leukodystrophy, later cerebellar atrophy *CYB5R3:* Decreased WM volume, hypomyelination	Porphobilinogen, aminolevulinic acid, porphyrins, blood count
Metal defects	(1,2,3) NBIA; *SLC30A9* (▶Sect. 34.6.3) (Zinc metabolism) (2,3)	(2,3) *SLC30A9* progressive complex spasticity		NBIA: other MD, neurodegeneration	NBIA: Eye of the tiger	
Polyol defects: SORD (2,3) (▶Sect. 15.4)	1 patient with spastic-ataxia		Axonal (CMT2) and motor neuropathy		1 patient with cerebellar atrophy	High levels plasma sorbitol
II. Energy metabolism defects. In mitochondrial disorders lactate and other markers may be disturbed mostly in children but often remain normal in adult cases. Diagnosis is complex and increasingly reliant on molecular investigations (▶Sect. 14.5).						First line markers (◘Table 1.3) (lactate…) AA, OA, function tests
Glut-1 deficiency (1,2,3) (▶Sect. 8.3)	Ataxic-spastic gait	Complex: ataxia, other MD		DD, ID, tremor, parkinsonism	Normal	Low glycorrhachia
Mitochondrial defects (▶Chap. 10)	(1,2,3) *OPA1, 3* (1,2,3) **PDH** (▶Chap. 11) (1) *POLR3A, POLR3B* (1) *SACS*, (2,3) *TTC19*, (2,3) Paraplegin, (1) Aconitase def	(2,3) *C12orf65*, (2) *IBA57*: complex (2,3) Paraplegin: pure or complex (2,3) MERFF: complex	(1) *SACS:* axonal demyelinating sensorimotor PN (2,3) *C12orf65,* (2) *IBA57* (2,3) Paraplegin: axonal PN	*POLR3A*, *POLR3B*: hypodontia, hypogonadism *C12orf65:* optic atrophy, PN *IBA57*: optic atrophy, PN Paraplegin: optic atrophy, PN MERFF: myoclonic epilepsy	*POLR3A*, *POLR3*: hypomyelination *TTC19*: BBGGa Aconitase def: Cerebellar atrophy	Lactate, pyruvate, AA, organic acids

(continued)

Table 1.20 (continued)

Disease group	Ataxia-SP spectrum	Spasticity (pure/complex)	Peripheral neuropathy/ motor neuron disease	Other clinical features	Brain image	Biomarkers
III. Complex molecules defects. Many complex molecules accumulation defects such as sphingolipidosis, sterols and some peroxisomal disorders may be suspected on clinical grounds and have robust metabolic markers. Most complex molecules synthesis and remodelling defects such as complex lipids have no readily measured metabolic markers and diagnosis is based on molecular investigations. Lipidomics is becoming increasingly accessible.						VLCFA, plasmalogens, phytanic acid, oxysterols, MPS, oligosaccharides, sulfatides, sialic acid, lipidomics
Lysosomal storage disorders (►Chaps. 40 and 41) *ARSA* (MLD) *GALC* (Krabbe) *GM2* (Gangliosidoses) NPC (Niemann-Pick C) *SUMF1* (MSD) *SLC17A5* (sialin transport)	(1,2) *ARSA* (1,2) *GALC* (1,2) *GM2* (1,2,3) *NPC1,2* (2) *PSAP* (1,2) *SLC17A5*	(1,2) *ARSA:* complex (1,2) *GALC:* complex (1,2) *GM2*: complex (1,2) *SUMF: complex* (2) *PSAP*: complex (1,2) *Sialidosis*: complex	(1,2) *ARSA, GALC*, (2) *PSAP,* (1,2) *SLC17A5*: demyelinating PN	*ARSA, GALC, GM2:* neuroregression, irritability *NPC1,2:* visceral involvement, early Cholestasis, vertical supranuclear gaze palsy *SUMF1: visceral involvement* *GM2, SLC17A5, PSAP, Sialidosis:* Cherry red spot, myoclonus	*ARSA, PSAP:* metachromatic leukodystrophy *GALC:* calcifications, leukodystrophy, optic nerve thickness *NPC1,2:* cerebellar atrophy *SLC17A5*: hypomyelination	*ARSA, GALC*: high CSF proteins Enzymatic tests, peripheral cell blood inclusions, vacuolations *SLC17A*: CSF, urine sialic acid
Peroxisomal defects (►Chap. 42)	(2) *ABCD1*: X-ADL (2,3) *AMACR*	(2) *ABCD1*, (1) RCDP, (1,2) SLS, (1) ELOVL4 biallelic mutations: complex Several PEX genes (►Chaps. 10, 14, and 19): infantile; (2,3) *AMACR* (2) *FAR1* AD inheritance All are complex	(2) *ABCD1*: demyelinating (2,3) PN. *DBP*: sensorimotor neuropathy (3) ARD (Refsum): demyelinating motor and sensory PN (2,3) *AMACR*: demyelinating	RCDP: ID, skeletal dysplasia, cataracts, seizures ARD: RP, cerebellar ataxia, and chronic polyneuropathy; SLS: ID, retinopathy, ichthyosis; ELOVL4: ichthyosis, seizures; *FAR1:* cataracts, seizures; *AMACR:* PR	*ABCD1*: pathognomonic confluent WMa with gadolinium enhancement SLS: periventricular WMa Several PEX genes (►Chaps. 10, 14, and 19): demyelination	VLCFA, plasmalogens, pristanic, phytanic acid acylcarnitines

Lipid metabolism defects Sphingolipid defects (►Chap. 40): *DEGS1, FA2H, B4GALNT1* Phospholipids (►Chap. 35): *BHD12, PLA2G6*, EPT1, *ABHD12, PNPLA6, DDHD1,2* Sterols: *CYAP27A1* CTX *CYP7B1, CYP51A1* Others*: GBA2, ERLIN1, BSCL2, ABHD12, ABHD5*	(2) *FA2H* (1,2) *B4GALNT1* (2,3) *ABHD12* (2,3) CTX (1,2,3) *CYP7B1* (1,2,3) *GBA2* (1,2,3) *PLA2G6* (2,3) *PNPLA6* (2,3) *ABHD5*	(1,2) *DEGS1*: complex (2) *FA2H*: complex (1,2) *B4GALNT1*: complex (2,3) CTX: complex (1,2,3) *CYP7B1*: pure or with cerebellar signs, ID and nystagmus (1,2,3) *GBA2*: complex (1,2,3) *PLA2G6*: complex (3) *DDHD2*: pure or complex (1) *DDHD2*: complex (1,2) *CYP2UI*: complex (1,2,3) *ERLIN:* pure (1,2,3) *BSCL2:* complex (2,3) *ABHD5*: complex (1) EPT1: complex (1) *CYP51A1*: complex	(1,2) *DEGS1*: demyelinating PN. (1,2) *B4GALNT1*: axonal PN (2,3) *ABHD12*: demyelinating sensory motor polyneuropathy (2,3) CTX: axonal PN *GBA2:* polyneuropathy (1,2,3) *PLA2G6*: neuroaxonal dystrophy (1,2,3) *PNPLA6:* axonal motor PN, (1,2) *BSCL2:* axonal neuropathy, ALS *DDHD1:* axonal neuropathy with distal sensory loss *CYP2UI*: axonal PN	*DEGS1*:DD, ID, nystagmus, seizures; *ABHD12:* PHARC *B4GALNT1*: cataracts, PN CTX: cataracts, ID, diarrhoea *GBA2:*ID, nystagmus, cataracts, male infertility *PNPLA6:* retinopathy, cerebellar atrophy *DDHD2*: DD, ID, nystagmus, dysarthria; *DDHD1:* RP *CYP2UI*: DD, ID, nystagmus, dysarthria *BSCL2:* cardiomyopathy, epilepsy, DD, ID *ABHD5:* Chanarin-Dorfman (deafness, cataract, cardiomyopathy) EPT1: ID, RP *CYP51A1*: cataract, liver disease	*DEGS1:* hypomyelinating leukodystrophy *FA2H* (2): NBIA features *GBA2:* thin CC, cerebellar atrophy *PLA2G6:* NBIA, cerebellar atrophy *DDHD1:* thin CC, NBIA *DDHD2*: thin CC, WMa *CYP2UI* (1,2): thin CC, WMa	*DEGS1:* increased dihydrosphingolipids in plasma CTX, *CYP51A1*: Cholesterol, sterols *ABHD5:* CPKs, Jordan's anomaly
Aminoacyl-tRNA synthetases and tRNA metabolism defects (►Sect. 39.2)	(2) *DARS1, 2, RARS1,2, EPRS1*	(2) *DARS1, 2, RARS1,2, EPRS1* (1) *FARS* (2), *MARS2* (1): all are complex	(2) *KARS1*, (1) *AARS1*, *GARS1, HARS1* *YARS1:* Axonal motor PN	DD, ID, cerebellar atrophy, seizures *KARS1:* deafness *HARS1* (AD); Usher syndrome	*DARS*: LBSL	High lactate
Nucleotide metabolism (►Chap. 39)	(1) *EXOSC3*, *UCHL1*	(1) *UCHL1*: complex (3) *ENTPD1*: complex; (1) *ADAR*: pure or complex	(1) *UCHL1:* Axonal sensorimotor PN	*UCHL1*: optic atrophy, nystagmus; *ENTPD1*: ID	PCH type 1B	*ADAR*: interferon signature
IV. Cellular trafficking disorders: in general, there are, as yet no metabolic markers. Diagnosis is based on DNA testing.						Only in some CDG syndromes
CDG syndromes (►Chap. 42)	Ataxia and Spastic-Ataxia spectrum are common. Peripheral axonal and mixed neuropathies are also frequent				Disialotransferrins. Some forms do not have markers	
Cellular trafficking disorders (►Chap. 44)	**Onset-age** **Pure/complex SP**		**Ataxia-SP spectrum disease** **Peripheral neuropathy/motor neuron disease**		**Other symptoms** **Genetic syndromes**	

(continued)

Table 1.20 (continued)

Disease group	Ataxia-SP spectrum	Spasticity (pure/complex)	Peripheral neuropathy/ motor neuron disease	Other clinical features	Brain image	Biomarkers
Onset age: (1): *SPAST, SPART, AP4B1, TECPR2, AP4M1, AP4E1, AP4S1, VPS37A, TFG, USP8, WDR48, ARL6IP1, RAG3GAP2, KLC2, ATL1, KIF1A, REEP2, TUBB4A.* (2): *SPAST, NIPA1, KIF5A, RTN2, REEP1, SLC33A1, SPG11, ZFYVE26, SPART, GJC2, KIF1C, ATL1, KIF1A, REEP2, TANGO2*; (3): *SPAST, NIPA1, WASHC5, KIF5A, RTN2, REEP1, SLC33A1, SPG11, ZFYVE26, GJC2, KIF1C, ATP13A2, KIF1A, TANGO2* -adult onset: *SPAST, NIPA, WASHC51, KIF5A, RTN2, REEP1, SLC33A1, SPG11, ZFYVE26, KIF1C, ATP13A2, KIF1A* Pure: *SPAST, NIPA1, WASHC5, KIF5A, RTN2, REEP1, SLC33A1, SPG1, ZFYVE26, ATL1, KIF1A, REEP2* Complex: *KIF5A, REEP1, SPG11, ZFYVE26, SPART, GJC2, AP4B1, TECPR2, AP4M1, AP4E1, AP4S1, VPS37A, TFG, KIF1C, USP8, ARL6IP1, ATP13A2, KLC2, ATL1, KIF1A, TANGO2*		*Ataxia-SP spectrum disease: ZFYVE26, SPART, GJC2, TECPR2, AP4M1, KIF1C, ATP13A2, KIF1A, TUBB4A* Axonal neuropathy: *KIF5A, SPG11, ZFYVE26, GJC2, TFG, WDR48, ATP13A2, KLC2, ATL1, TMR13, LITAF, MTMR2, 5, SH3TC2, ARL6IP1, ATP13A2, ATP7, TGF,* DGAT2 -Demyelinating neuropathy: *FIG, EGR2, NEFL, FBLN5 ARHGEF* Sensory and autonomic neuropathy: *FAM134B, RAB7, ATL1, DNMT1, ATL3, SCN11A, PRNP, WNK1, KIF1A, IKBKAP, SCN9A, NTRK1, NGF-B, DST, CCT5* ALS: *CHMP2B,* Spactasin*, FIG4, C9orf72, OPTN1, VAP9, VAPB, SigR1, KIF5A, BICAUDAL; DYNC1H1, ATP7*		Cognitive impairment: most of them except pure forms Early-onset complex encephalopathy: *AP4B1, TECPR2, AP4M1, AP4E1, AP4S1, VPS37A, TFG, KLC2* Parkinsonism: *KIF5A, ATP13A2* Ocular symptoms: Cataract: *RAG3GAP2* Nystagmus: *KLC2, ZFYVE26, SPART, GJC2, TECPR2, AP4M1, KIF1C, ATP13A2, KIF1A, TUBB4A* Deafness: *RAG3GAP2* Genetic syndromes: Kjellin syndrome: *ZFYVE26,* Troyer syndrome: *SPART*	

In black bold: treatable disorders.

Onset age: *1* infancy to early childhood, *2* childhood (from 2 years until adolescence), *3* adolescence

AA amino acids, *AADC* L-amino acid decarboxylase deficiency, *ABHD12* monoglycerol lipase, *ACAT1* acetoacetyl-CoA thiolase deficiency, *AD* autosomal dominant, *ADCY5* adenylate cyclase deficiency, *ADL-X* X-linked adrenoleukodystrophy, *ADSL* adenylosuccinate lyase deficiency, *AIP* acute intermittent porphyria, *ALS* amyotrophic lateral sclerosis, *AMACR* α-methylacyl-CoA racemase, *AMPD* AMP deaminase-2 deficiency, *AR* autosomal recessive, *ARALAR* mitochondrial protein involved in glutamate aspartate exchange encoded by *SLC25A12*, *ARD* (adult Refsum disease) phytanoyl-CoA 2-hydroxylase, *ARG* arginine, *ARSA* arylsulfatase A deficiency, *AS* asparagine synthetase, *ATIC* 5-Amino-4-imidazolecarboxamide-ribosiduria (AICA)-ribosiduria, *Atten* attenuated forms, *ATIC* 5-Amino-4-imidazolecarboxamide-ribosiduria (AICA)-ribosiduria is an exceedingly rare autosomal recessive condition resulting from the disruption of the bifunctional purine biosynthesis protein PURH, *ATP8A2* encodes for a P4-ATPase that actively transports phospholipids across cell membranes ('flipping'), *BBGGa* basal ganglia abnormalities, *B4GALNT1* GM2/GD2 synthase deficiency, *BCAA* branched chain amino acids, *C* cerebellum, *Ca* cerebellar atrophy, *CAD* CAD trifunctional protein, carbamoylphosphate synthetase/aspartate transcarbamylase/dydroorotase, *CC* corpus callosum, *CDG* congenital disorders of glycosylation, *CERS* ceramide synthase, *CIT* citrulline, *CK syndrome* X-linked recessive sterol-4-alpha-carboxylate 3-dehydrogenase deficiency, *CLBP* mitochondrial quality protein control, *CMH* congenital methemoglobinemia, *CMNO* cardiomyopathy, *CP* cerebral palsy, *CPTII* carnitine palmitoyltransferase II deficiency, *CTX* cerebrotendinous xanthomatosis, *CSF* cerebrospinal fluid, *CP* cerebral palsy, *DBP* D-bifunctional protein, *DD* developmental delay, *DEF* deficit, *DHPR* dihydropyrimidine reductase deficiency, *DEGS* dihydroceramide delta4-desaturase, *DHFR* dihydrofolate reductase deficiency, *DLP1* dynamin related protein 1. EAAT1, glutamate aspartate transporter deficiency (*SLC25A22*), *DWI* diffusion, *ECHS1* mitochondrial short-chain enoyl-CoA hydratase deficiency, *ELOVL* elongation of very long chain fatty acids, *encephalop* encephalopathy, *ENTPD* ectonucleoside trisphosphate diphosphohydrolase I, *EPT1* ethanolamine phosphotransferase ETHE1, sulfur dioxygenase deficiency, *FA2H* fatty acid 2-hydroxylase deficiency, *FAR1* fatty-acyl CoA reductase 1 deficiency, *FHD* fumarate hydratase deficiency, *FOLR1* folate receptor alpha deficiency, *GAMT* guanidinoacetate methyltransferase, *GA1* glutaric aciduria type 1, *GABAR* GABA receptor mutations, *GABAT* GABA transaminase deficiency, *GALC* galactocerebrosidase, *GLUT1DS* glut-1 deficiency syndrome, *GORS* Golgi SNAP receptor complex member 2, *GOT2* glutamate oxaloacetate transaminase deficiency, *GRIN* mutations in NMDA glutamatergic receptors, *GS* glutamine synthetase, *GSS* glutathione synthetase deficiency, *GTPCH* guanosine triphosphate cyclohydroxylase, *HCS deficiency* holocarboxylase synthetase deficiency, *HH* hyperinsulinism-hyperammonaemia syndrome, *HHH* hyperornithinaemia-hyperammonaemia-homocitrullinaemia syndrome, *HIBCH* 3-hydroxyisobutyryl-CoA hydrolase deficiency, *Homocyst* homocysteine, *HRS* hypokinetic-rigid syndrome, *HSD10* 2-methyl-3-hydroxybutyryl-CoA dehydrogenase deficiency, *ID* intellectual disability, *INAD* infantile neuroaxonal dystrophy, *ITPase* inosine triphosphatase, *ITPR1* inositol 1,4,5-triphosphate receptor, type1, *IVA* isovaleric aciduria, *L2-HGA* L2-hydroxyglutaric aciduria, *L2HGDH* L-2-hydroxyglutarate dehydrogenase deficiency, *L,D2-HGA* L and 2-D-hydroxyglutaric aciduria, *LBSL* leukodystrophy with brainstem and spinal cord involvement and lactate elevation, *LN* Lesch-Nyhan, *MCGA1* 3-methylglutaconic aciduria type 1, *MECR* Trans-Enoyl-CoA reductase, *MELAS* lactic acidosis, and stroke-

like episodes, *MEGDEL* 3-methylglutaconic aciduria with deafness, encephalopathy, and Leigh-like syndrome, *MEPAN* mitochondrial enoyl-CoA reductase protein-associated neurodegeneration, *MERRF* mitochondrial encephalopathy and ragged red syndrome, *MD* movement disorders, *MDH* malate dehydrogenase deficiency, *MLD* metachromatic leukodystrophy, *MMA* methylmalonic aciduria, *MOCS* molybdenum cofactor deficiency, *MPS* mucopolysaccharides, *MRS* brain MRI with spectroscopy, *MSD* multiple sulfatase deficiency, *MSUD* maple syrup urine disease, *MTHFR* methylenetetrahydrofolate reductase deficiency, *MTHFS* 5,10-methenyltetrahydrofolate synthetase, *NADK2* mitochondrial NAD kinase 2 deficiency, *NARP* neuropathy, ataxia and retinitis pigmentosa, *NAXE* NAD(P)HX epimerase deficiency, *NBIA* neuronal brain iron accumulation, *NCL* neuronal ceroid lipofuscinosis, *NKH* nonketotic hyperglycinaemia, *NPS* neuropsychiatric symptoms, *NRL* neurological, *NT* neurotransmitter, *NT5C2* 5-prime-nucleotidase, cytosolic II, *ORN* ornithine, *OA* organic acids, *P5CS* δ-1-pyrroline-5-carboxylate synthase deficiency, *PA* propionic aciduria, *PHARC* polyneuropathy, hearing loss, ataxia, retinitis pigmentosa, and cataract, *PC* pyruvate carboxylase, *PCH* pontocerebellar hypoplasia, *PDH* pyruvate dehydrogenase, *PERIV* periventricular, *PLA2G6* phospholipase A2, *PKAN* pantothenate kinase 2 deficiency, *PGK1* phosphoglycerate kinase 1, *PHARC* peripheral neuropathy hearing loss retinitis pigmentosa, cataract, *PME* progressive myoclonic epilepsy, *PMPCA* peptidase, mitochondrial processing, alpha, *PN* peripheral neuropathy, *PNPO* pyridoxamine 5′-phosphate oxidase, *PNP* purine nucleoside phosphorylase deficiency, *PMM2* phosphomannomutase 2 deficiency, *PRO* proline, *PSAP* prosaposin, *RALF* recurrent acute liver failure, *RCDP* rhizomelic chondrodysplasia punctata, *RHADS* rhythmic high amplitude delta with superimposed polyspikes, *RP* retinitis pigmentosa, *RPIA* ribose-5-phosphate isomerase deficiency, *SACS* sacsin, *SCA* spinocerebellar atrophy, *SCP2* sterol carrier protein 2 deficiency, *SDE* syndrome, *SLC25A19* thiamine pyrophosphate transporter, *SHMT2* serine hydroxymethyltransferase type 2, *SLS* Sjögren Larsson syndrome, *SORD* sorbitol dehydrogenase, *SP* spastic paraparesis, *SR* sepiapterin reductase deficiency, *SSADH* succinic semialdehyde dehydrogenase (aldehyde dehydrogenase 5a1, *SUCLA2* succinyl-CoA lyase subunit, *SUCLG1* succinyl-CoA ligase alpha subunit, *SUMF1* gene responsible for multiple sulfatase deficiency, *SUOX* sulphite oxidase deficiency, *SV* synaptic vesicle, *TH* tyrosine hydroxylase deficiency, *TPK1* thiamine pyrophosphokinase deficiency, *UCD* urea cycle disorders, *VLCFA* very long chain fatty acids, *WM* white matter, *WMa* white matter abnormalities, *X-ALD* X-linked adrenoleukodystrophy

PURE

Although these IMD can present as pure forms, they often have associated signs

SMALL MOLECULE DEFECTS
- Urea cycle defect: HHH
- Biotinidase deficiency
- Neurotransmitter defect: TH
- Ornithine/proline: P5CS

ENERGY DEFECTS
- Mitochondrial disorders: paraplegin

COMPLEX MOLECULE DEFECTS
- Lipid defects: *CYP7B1, DDHD2*
- Nucleotide defects: *ADAR*

CELL TRAFFICKING DISORDERS
SPAST, NIPA1, WASHC5, KIF5A, RTN2, REEP1, SLC33A1, SPG1, ZFYVE26, ATL1, KIF1A, REEP2

WITH ASSOCIATED NRL SYMPTOMS

EPILEPSY (see Table 1.17)

- Organic acidurias: MCGA1, L,D-2HGA; CblD; biotinidase deficiency; -P5CS
- Purine defects: ATIC, others
- Mitochondrial defects: MERRF: myoclonic epilepsy
- Lysosomal disorders: diverse lysosomal diseases cause myoclonic epilepsy
- Peroxisomal defects: FAR1, ELOVL4, diverse PEX genes
- Aminoacyl-tRNA synthetases : diverse genes
- Cell trafficking disorders: in particular synaptic vesicle cycle defects

HYPERKINETIC MOVEMENTS (see Table 1.19)

- Tremor: GTPCH, D-2-HGA, VitE deficiency, *SLC39A14, SLC30A10*, Glut1D, Tay-Sachs, Sandhoff, sialidosis, *DARS1, PHARC, KIF1C*
- Dystonia: SLC39A8 (CDG type), SLC39A14, SLC30A10, homocystinurias, Lesch-Nyhan disease, Glut1D, SERAC1, lysosomal disorders, CLN14, several sphingolipidosis, several nucleotide metabolism defects.
- Chorea: *ADCY5*, NKH, Lesch-Nyhan disease Glut1D, aconitase deficiency, sialidosis, MLD
- Myoclonus: Neurotransmitter defects (TH, SR), L2-HGA, CLN14, Tay-Sachs, Krabbe, Sandof, sialidosis, MERRF, PHARC, *DARS1*

HYPOKINETIC MOVEMENTS: parkinsonism often dystonia-parkinsonism (see Figure 1.8)

- Neurotransmitter disorders: TH, GTPCH, SR; - metal disorders: *SLC39A14, SLC30A10;*
- Lysosomal disorders: Tay-Sachs, Gaucher types 2, 3; CLN2,3,6; NPC, GM1, Sandhoff;
- Nucleotide metabolism: several causes of Aicardi-Goutières syndrome;
- Cell trafficking disorders: *VPS13D,* several synaptic vesicle disorders

PERIPHERAL NEUROPATHY/MOTOR NEURON DISEASE: see table

Many diseases that present prominent spasticity associate
ATAXIA: spastic-ataxia spectrum (see Table 1.20)

WITH ASSOCIATED NRL SYMPTOMS

SKIN

- Ichthyosis: complex lipid disorders such as ELOVL4, Sjögren-Larsson; peroxisomal defects: FAR1, ARD, RCD; lysosomal disorders: Gaucher II, MSD; -Others: serine deficiency
- Melanoderma: X-ADL; - Angiokeratoma: sialidosis II

EYE

- Nystagmus: CblD, *HMBS* (porphyria), many diseases with cerebellar involvement and hypomyelination
- Retinopathy: CblD, several lysosomal disorders (*GM2, SLC17A5, PSAP,* sialidosis)
- Cataract: several disorders of complex lipid defects, peroxisomal defects and cell trafficking
- Optic atrophy: botinidase, several mitochondrial defects (*C12orf65, IBA57*: optic atrophy, Paraplegin)
- Cherry red spot: lysosomal disorders
- Pigmentary retinitis: peroxisomal disorders, some NBIA syndromes, VitE deficiency

BONE

- Plasmalogen defects; - P-Inositides defects; - Cholesterol defects;
- P-Cho, P-Eth, P-Ser defects

VISCERAL

- Several complex molecule disorders: Lysosomal, complex lipid defects
- Arginase and HHH deficiency

Fig. 1.9 Algorithm for spasticity

HEREDITARY MOTOR AND SENSORY NEUROPATHY (HMSN)

AXONAL

- Mitochondrial: NARP, POLG, MELAS, MERRF. From 35–45% of mitochondrial patients have axonal neuropathy, mostly nuclear, PDH; Sacsinopathies
- Fatty acid oxydation: LCHAD, trifunctional protein.
- Lysosomal: Nieman Pick type-C, Gaucher disease, ceroid lipofuscinosis, Pompe disease, Schindler disease, GM2 (*)
- Peroxisomal: Bifunctional protein (neuropathy may be isolated as presentation), peroxisome biogénesis defects, racemase (*)
- Porphyrias: DOSS porphyria; acute intermittent porphyria Hereditary coproporphyria, Variegate porphyria
- Lipid disorders: *B4GALNT1*, CTX, *BSCL2*, *DDHD1*, Neuronal Target Enterase, INAD
- Cell traffic: *KIF5A*, *SPG11*, *ZFYVE26*, *GJC2*, *TFG*, *WDR48*, *ATP13A2*, *KLC2*, *ATL1*, *TMR13*, *LITAF*, *MTMR2, 5*, *SH3TC2*, *ARL6IP1*, *ATP13A2*, *ATP7*, *TGF*, *DGAT2*
- CDGs: in general, axonal neuropathies
- Purine disorders: *PRPS*, *AMPD2*, *ENTPD1*
- Aminoacyl-tRNA and nucleotide metabolism: *KARS1*, *AARS1*, *GARS1*, *HARS1*, *YARS1*, *UCHL1*, *MARS1*
- Triose phosphate isomerase; - Choline presynaptic defect
- Polyol defect: sorbitol dehydrogenase deficiency
- Ornithine/proline metabolism: P5CS; - OAT deficiency (late complications) - Serine synthesis deficiency
- Polyglucosan body disease (*), pyroglutamic aciduria (*)

DEMYELINATING

- Mitochondrial: MNGIE, *SACS*, *SURF1*, *MPV17*; Sacsinopathies
- Homocysteine remethylation defects
- Lysosomal: Krabbe, MLD, Beta-mannosidase, Sandhoff disease, *PSAP*, *SLC17A5*, *SUMF1*, Farber, Krabbe
- Peroxisomal: Zellweger, Adrenoleucodystrophy Refsum, AMACR, several PEX mutations, X-ADL, AMN
- Lipid disorders: *DEGS1*, *ABHD12* (PHARC), CTX, Tangier disease
- Cell trafficking: *FIG*, *EGR2*, *NEFL*, *FBLN5*, *ARHGEF*
- Fatty acid oxidation: LCHAD, TP
- Other genes: *GDAP1*, *MTMR2*, *SBF3*, *MTMR5*, *SH3TC2*, *NDGR1*, *HK1*, Neurofascin

MIXED

- Lysosomal: Galactosialidosis, mucolipidosis, others
- Fatty acid oxydation: LCHAD, trifunctional protein.
- Many other complex molecule defects, in particular those with WM involvement may have mixed neuropathies, also CDG syndromes

HEREDITARY SENSORY NEUROPATHY (HSN) and autonomic (HSAN)

- Mitochondrial: MELAS, MERRF, other genes
- Lipid disorders: SPTLC1 or SPTLC2, *DDHD*
- Cell traffic disorders: *REEP1*, *KIF1A*, *FAM134B*
- Other genes involved in HSN and/or HSAN (most cell traffic disorders): *ELP1*, *FAM134B*, *RAB7*, *ATL1*, *DNMT1*, *ATL3*, *SCN11A*, *PRNP*, *WNK1*, *KIF1A*, *IKBKAP*, *SCN9A*, *NTRK1*, *NGF-B*, *DST*, *CCT5* ; - Painful small fiber neuropathy: Fabry disease

VITAMIN RELATED

$VitB_{12}$: myelopathy, funicular myelosis, proprioceptive dysfunction **Folate related:** progressive distal sensory neuropathy. **Thiamine:** distal axonal sensory motor. **$VitB_6$:** distal axonal sensory motor. **Biotin:** myelopathy: **VitE/abetalipoproteinemia:** distal symmetric with severe sensory ataxia. **Riboflavin transport:** motor and sensory neuropathy, distal motor neuropathy, cranial nerve involvement, Guillan-Barré, ALS

HEREDITARY MOTOR NEUROPATHY (HMN)
Lower motor neuron disease: SMA-like, ALS

Neonatal, early infancy SMA-like - Mitochondrial defects: TK2, other genes leading to mtDNA depletion, ACO2; - Peroxisomal disorders; - Traffic defects: *BICD2*, *VAPB*, *ATP7*; - Others: PCH1, *AGTPBP1*.
Late SMA with progressive myoclonic epilepsy: acid ceramidase deficiency
ALS: - Traffic defects: *CHMP2B*, spactasin, *FIG4*, *C9orf72*, *OPTN1*, *VAP9*, *VAPB*, *SigR1*, *KIF5A*, *BICAUDAL*, *DYNC1H1*, *ATP7*; - DNA/RNA metabolism: *FUS*, *TARDBP*; Others: *SOD1*, riboflavin transport defects (ALS mimic)
HMN, distal SMA: - Polyol defect: SORD; - Glycolitic defect: TPI; - Cell traffic disorders: *DCTN1*, *FIG*, *SPG11*, *RAB7*, *DNM2*, *RAB18, 39B*, *RAB7*, *RAB18, 39B*, *RAB7*, *RAB3GAP1*, *connexin32 (C32)*, *BSCL2*, *REEP1*, *SIGMAR1*, *DCTN1*, *SPTAN1*, *PLEKHG5*, *SYT2*; - Lipid disorders: *PNPLA6*, presynaptic choline transporter *(CHT)*, GM2 synthase deficiency; - RNA and DNA metabolism: *FBX038*, *SETX*, *IGHMBP2*, *AIFM1* - tRNA aminoacylation: *AARS*, *HARS*, *GARS*, *WARS*, *HINT1*; - Ion channels and transporters: *SLC12A6*, *SLC25A21*, *TRPV4*, *SLC5A7*, *ATP7A*
Others: *ABCD1* (adrenomyeloneuropathy), combined spinal cord degeneration in homocysteine remethylation defects, *PNPLA6* (spinal cord atrophy)

Fig. 1.10 Algorithm for peripheral neuropathy and motor neuron disease

or small toxic molecules often cause pyramidal tract lesions. In fact, almost all progressive neurometabolic diseases end up manifesting different degrees of spasticity. Only those IEM in which spasticity is the dominant or one of the most prominent signs in the clinical picture are depicted in ◘ Table 1.20 (complex motor disorders).

Metachromatic leukodystrophy and infantile neuroaxonal dystrophy (INAD) (*PLA2G6* mutations) present between 12 and 24 months of age with flaccid paraparesis, hypotonia, and weakness (▶ Sect. 35.4.2). CSF protein content and nerve conduction velocity are disturbed in the former but normal in the latter (however axonal neuropathy can be present). Schindler disease is roughly similar to neuroaxonal dystrophy, though it is often associated with myoclonic jerks (▶ Sect. 41.3).

Spasticity in IEMs is in general associated with involvement of additional neurological functions or organs (◘ Fig. 1.9 spasticity algorithm). However, some disorders can start with isolated spastic paraparesis such as **X-linked adrenoleukodystrophy, remethylation defects of homocysteine metabolism**, **biotinidase deficiency, HHH syndrome** (hyperammonaemia, hyperornithinemia, homocitrullinuria), **arginase deficiency, Segawa disease** and some lipid metabolism disorders, in particular phospholipid synthesis and remodeling defects (◘ Table 1.20 complex motor disorders, ◘ Fig. 1.9 spasticity algorithm).

New IEMs with prominent spasticity belong to cell trafficking disorders, most of them included in classical genetic spastic paraparesis and complex lipid defects.

▪ Peripheral Neuropathy

The diagnosis of peripheral neuropathies rely on clinical and electrophysiological criteria. The general classification depends on whether there is an involvement of large fibers (motor weakness, loss of deep reflexes, muscle atrophy, sensory ataxia), or small fibers (autonomic dysfunction, abnormal temperature, sensibility pinprick loss) and whether the neuropathy is predominantly demyelinating or axonal (◘ Fig. 1.10 algorithm neuropathies).

Although peripheral neuropathies can be found in all IEM categories, complex molecule defects, and in particular, lipid storage disorders, and energy metabolism defects are the most common. In lipid storage disorders, both the peripheral and central myelin can be involved, leading to a low nerve conduction velocity (NCV) and leukoencephalopathy on brain MRI. In contrast, defects of energy metabolism are mostly responsible for axonal peripheral neuropathies with normal NCV and are usually associated with other signs of energy metabolism defects (optic atrophy and cerebellar ataxia in the case of respiratory chain disorders). Many exceptions to this schematic view however exist. MNGIE syndrome (myoneurogastrointestinal neuropathy) caused by thymidine phosphorylase deficiency is typically responsible for a demyelinating polyneuropathy (▶ Sect. 32.6.4). Some lipid storage disorders such as cerebrotendinous xanthomatosis (▶ Sect. 38.3), adrenomyeloneuropathy and other peroxisomal diseases (▶ Chap. 42) may cause polyneuropathies that can be axonal, demyelinating or both (algorithm peripheral neuropathy).

Peripheral neuropathies in IEM are in most cases syndromic opposite to non-syndromic classic CMT (Charcot-Marie-tooth disease). In fact, they are often found in IEM that show other motor manifestations involving first motorneuron and the spinal cord. However, there are some particular IEMs in which peripheral neuropathy is the only clinical manifestation. This is the case of the recently described **SORD (sorbitol dehydrogenase) deficiency** (▶ Sect. 14.4), some **late-onset riboflavin metabolism defects,** some forms of **trifunctional protein defects** and those related to cell trafficking disorders. **Porphyrias** and **tyrosinemia type I** can present with acute attacks of polyneuropathy mimicking Guillain-Barre syndrome. Patients with sphingosine-1-P lyase deficiency (▶ Sect. 40.3.4) are distinct from other AR-CMT2 subtypes and are characterized by acute/subacute onset, unilateral motor deficit (one patient), and episodes of mononeuropathy with a tendency for improvement. The deterioration pattern is atypical for axonal CMT. Rather, it is observed in acquired neuropathies, hereditary neuropathies with liability to pressure palsies, or hereditary neuralgic amyotrophy [75] (▶ Chap. 40).

EM (Electromyography) can reveal myotonia-like discharges as typical finding in juvenile type II glycogenosis.

▪ Motor Neuron Disease

Motor neuron diseases refer to lower motor neuron dysfunction (second motor neuron). These are hereditary motor neuropathies (HMN) and may present as distal SMA (spinal muscle atrophy) with peroneal muscular atrophy and weakness without involvement of sensory dysfunction, or more complex and severe SMA-like clinical pictures.

A few metabolic diseases cause HMN and tend to be complex or atypical, that means that they may have predominance in the upper limbs or involvement of upper motor neurons, and vocal cord and/or diaphragm paralysis, among other features. Lipid disorders and cell trafficking defects are the most representative IEM with this clinical manifestation. SMA-like are found in energy, peroxisomal and complex lipid defects (◘ Table 1.20 complex motor disorders, and ◘ Fig. 1.10 neuropathy/motor neuron algorithm). On the other hand, Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease that causes degeneration of the lower and upper motor neurons and is the most prevalent motor neuron disease in the general population. This disease is characterized by muscle weakness, stiffness, and hyperreflexia. Most cases are sporadic, with only 10% of the

cases being genetic. Only a few IEM may present with ALS and they include **SORD deficiency, Riboflavin transport defects**, cell trafficking and DNA/RNA metabolism defects and dominantly acting *SPTLC1* (▶ Chap. 40 note added in proof) (◘ Fig. 1.20).

1.5.2.5 Category 5: With Predominant Intellectual Disability and/or Behavioural, Neuropsyhiatric Manifestations (Algorithm)

Intellectual disability (ID) is characterized by significant limitations in both intellectual functioning and adaptative behaviour, that originates before the age of 18 [76] and affects about 2.5% of the population. It is defined by an IQ below 70 at 5 years of age or older. Developmental delay (DD) is the term used below 5 years, defined as deficits in two or more developmental domains (e.g. motor skills and speech) [77]. It is estimated that IEMs account for 1–5% of non-specific ID, although no recent systematic studies have been performed to validate these numbers.

Almost all IEMs that affect the nervous system at paediatric ages will cause DD or ID. However, in the great majority of cases, this is a syndromic ID that associates other neurological and extra-neurological symptoms. In this section we discuss IEM with isolated or predominant ID, although most of them are accompanied by behavioural problems (autistic features, hyperactivity, aggressive and self-injurious behaviours, executive dysfunction problems) (◘ Fig. 1.11 ID algorithm).

All pathophysiological categories of IEM may cause isolated or predominant ID and some are treatable (◘ Fig. 1.11). In most of them DD/ID remains the only sign for a certain period of time before the appearance of more evocative signs such as in small molecule disorders (**AA related disorders, OAs**) and mitochondrial defects. Autism is a frequent sign in disorders with predominant ID. This is the case with **AA and creatine defects**, mitochondrial disorders, attenuated forms of **Smith-Lemli-Opitz syndrome**, and other diseases that mimic genetic conditions. Disorders that mimic Rett syndrome are BPAN (beta-propeller associated neurodegeneration, an autophagy disorder) [78] (▶ Sect. 44.3.1), choline kinase deficiency (▶ Sect. 35.3.1), disorders of the Synaptic Vesicle and GABA/Glutamate signalling diseases (▶ Chap. 44). Choline kinase deficiency may also mimic Angelman syndrome.

Behavioural disturbances, psychosis, and schizophrenia-like syndrome can be also the first clinical signs of a metabolic disorder even in the absence of ID [79]. This is more frequent in late childhood, adolescence and adulthood. **OTC deficiency** can present with episodes of abnormal behaviour and personality change until hyperammonaemia and coma reveal the true situation (see ▶ Sect. 1.4.1). **Homocystinuria** due to **MTHFR deficiency** has presented as isolated schizophrenia. Searching for these treatable disorders is mandatory including also **CTX** and **Wilson disease** (see ◘ Fig. 1.11).

1.5.2.6 Category 6: With Neuroregression

Most diseases that cause progressive intellectual and neurological deterioration (PIND) present in childhood: 81% before the age of 5 years [80].

Most of these IEMs represent storage disorders, but other categories are represented as well. In a recent review over half were categorized in the group "storage disorders" mainly due to different types of MPS (type I, II, IIIA, IIIB, IIIC, IIID, VII) and NCL (neuronal ceroid lipofuscinosis) which represent about half of the storage diseases [81]. Sanfilippo disease is the classic example with regression of high-level achievements, loss of speech, and agitation usually beginning later than 5 years. Other complex molecule disorders such as peroxisomal diseases (in particular X-ALD, where regression appears in childhood but can also starts at early adolescence, in particular in frontal forms), complex lipid defects, cell trafficking disorders and diseases of nucleotide metabolism will invariably lead to a regression at different moments of late infancy, childhood and adolescence. However, they can previously have some kind of neurological abnormalities (cerebral palsy like symptoms) that could make deterioration difficult to appreciate. This is also the case for some metal, purine and pyrimidine disorders. Canavan disease and some mitochondrial disorders such as MELAS (mitochondrial encephalopathy and lactic acid acidosis) have also a notable regression.

Finally, neurodevelopment and neurodegeneration can behave as opposites throughout disease evolution, but they may also run in parallel [10]. Additionally, the lack of natural history studies for long periods of life in most metabolic diseases, do not allow concise knowledge about possible regression in adulthood or in the elderly.

Other neurological signs such as chronic/recurrent headache appear in just a few IEM: MELAS, Hyperammonaemias, α-Methylacyl-CoA racemase (AMACR) deficiency (▶ Sect. 42.2.4), *NFE2L2* mutations leading to NRF2 accumulation with hypohomocysteinaemia. Hemiplegic migraine has been described in **GLUT1DS**, and in the following mutated genes: *CACNA1A, ATP1A2, SCN1A.*

1.5.3 Onset in Adulthood (>15 years to >70 years)

(See ▶ Chap. 2)

- **Specific Neurosensorial, Neurophysiological and Neuroradiological Signs and Symptoms (at any Age)**

Neuroimaging, neurophysiology and ophthalmological investigations are helpful for elucidating neurologic and psychiatric syndromes. The most significant findings are

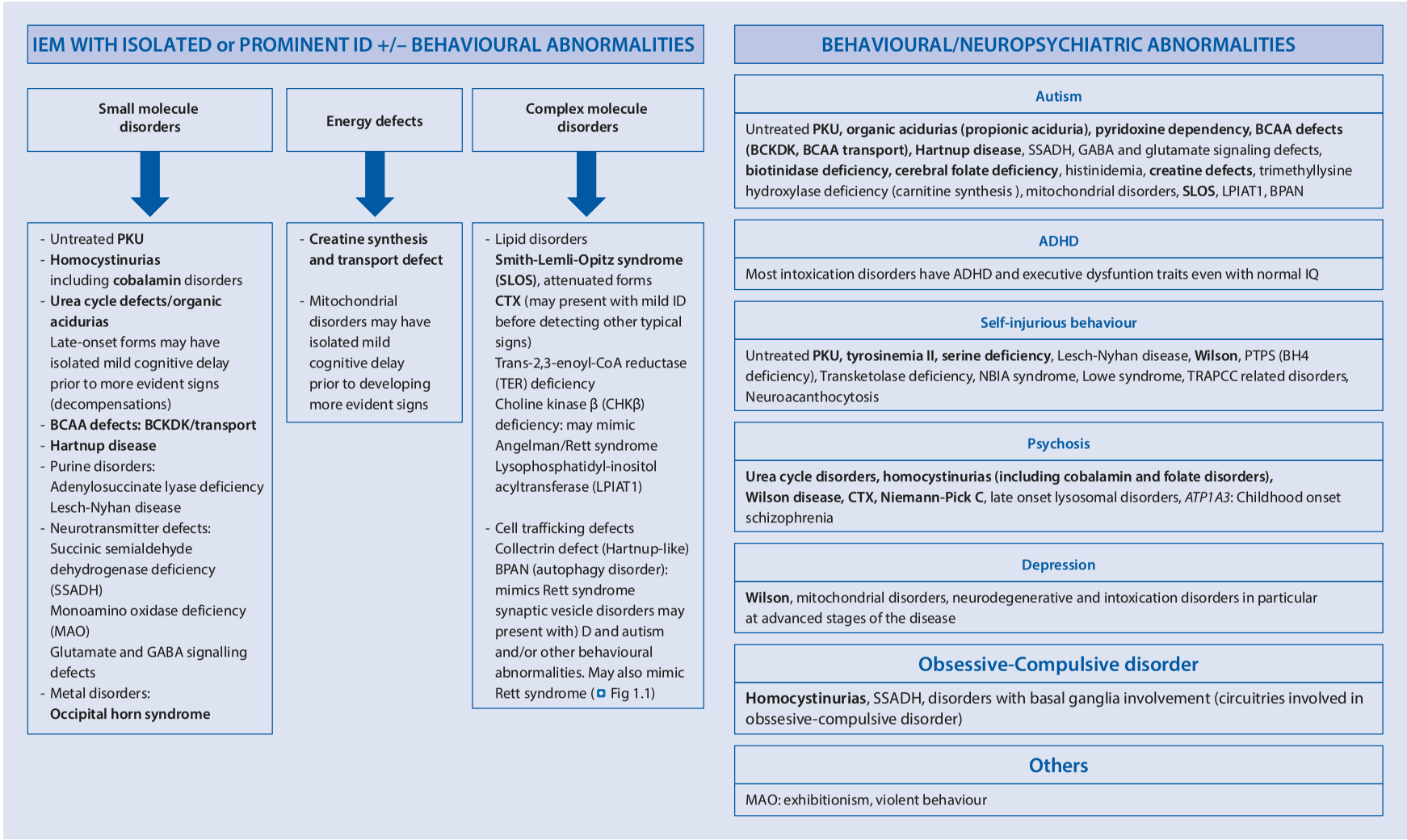

Fig. 1.11 IEM with predominant intellectual disability +/– behavioural and psychiatric manifestations

presented in ◘Tables 1.21, 1.22, 1.23, 1.24, 1.25, 1.26, 1.27, 1.28, 1.29, 1.30, 1.31, and 1.32. Some are sufficiently distinctive to make a clinical diagnosis.

1.5.4 Deafness

The hearing loss in metabolic disorders is mostly sensorineural, symmetrical and (at least initially) high frequency. Mutations in connexins *GJB2* (CNX26) and *GJB6* (CNX3) cause up to 50% of recessive non-syndromic deafness. Mitochondrial syndromic and non syndromic defects are also amongst the most common causes. Many genetic syndromes can mimic a metabolic disease; some are listed in ◘Table 1.21.

1.5.5 Head Circumference, Cephalhematomas, Subdural Hematomas (◘Table 1.22)

Macrocephaly: Congenital macrocephaly may be an isolated early marker of **GA1** and a few other cerebral OA (►Chap. 22).

Microcephaly: There are many untreated IEM in which microcephaly results from a progressive non-specific cerebral atrophy. A few disorders present with an antenatal (congenital) microcephaly, among them mild forms of **serine synthesis defects** may be treatable (►Sect. 24.2). Mutations in *MFSD2A*, required for omega-3 fatty acid transport in brain, have been recently shown to cause a lethal microcephaly syndrome (►Sect. 42.4.13). Mutations in *PYCR2*, encoding pyrroline-5-carboxylate reductase 2, cause a unique syndrome of postnatal microcephaly with severe hypomyelination (►Sect. 21.4). Many Golgipathies present with severe microcephaly (congenital and post natal onset) (►Chap. 44).

1.5.6 Neuroimaging Signs

Morphological evaluation is best undertaken by MRI. Cranial computer tomography (CT scan) is still important when looking for calcifications or in an emergency. Proton MR spectroscopy is a tool for assessing brain metabolites but is diagnostic only in a few disorders, including cerebral creatine disorders (absence of creatine peak), Canavan disease (high peak of N-acetyl aspartate) and some complex lipids/fatty acid defects such as Sjogren-Larsson syndromes and ELOV4 in which there is an additional lipid peak (►Sect. 42.4) Mutations in at least 15 genes causing neurodegeneration with brain iron accumulation (NBIA) have been identified so far (►Sect. 34.2.3).

Specific signs as listed in the following tables:

- Basal ganglia, brain stem hyperintensities: ◘Table 1.23
- Brain deposits: ◘Table 1.24
- White matter abnormalities ◘Table 1.25
- Brain dysplasia/malformation: ◘Table 1.26
- Posterior fossa abnormalities: ◘Table 1.27

1.5.7 Neuro-ophthalmological Signs (◘Tables 1.28 and 1.29)

▪ Abnormal fundoscopy findings (◘Table 1.28)

Retinal dystrophies encompass retinitis pigmentosa, Leber congenital amaurosis (LCA), early onset retinal dystrophy and Stargardt disease. There are over 400 known inherited diseases in which the retina, macula or choroids are substantially involved [82]. Most of metabolic causes involve complex molecules (mostly lipids) and energetic processes. LCA, which is associated with several gene mutations, is a severe retinal dystrophy with infantile onset and is one of the most frequent cause of congenital blindness. Mutations in *NMNAT* (coding for NAD synthetase) have been recently described identifying a new disease pathway for retinal degeneration [83] (►Sect. 24.1.2).

Hereditary optic atrophy is common in neurodegenerative diseases due to IEM, especially white matter diseases and energy deficiencies, and deserves an extensive metabolic work-up. Optic atrophy is a frequent early presenting sign of primary (Leber hereditary optic neuropathy, RCD, PDH deficiency, biotinidase deficiency, Costeff optic atrophy syndrome) or secondary (organic acidurias) mitochondrial dysfunction.

▪ Ophthalmoplegia, abnormal eye movements (◘Table 1.29)

Abnormalities of eye movements can be an important diagnostic clue in many IEM. In few defects they can be the presenting sign, as with progressive external ophthalmoplegia (PEO) in several mitochondrial DNA disorders or vertical gaze palsy as in Niemann-Pick disease type C. PEO associated with peripheral neuropathy is suggestive of mitochondrial nuclear genes mutations. Furthermore, oculogyric crisis is an important feature in disorders affecting dopamine synthesis or transport (►Sect. 30.5.7). Nystagmus, oculomotor apraxia (failure of saccade initiation) and oculogyric crisis are the most common abnormal eye movements in IEM [84].

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Table 1.21 Sensorineural deafness

Detectable in neonatal to infancy	Acyl-CoA oxidase deficiency Acetyl-CoA transporter (*SLC33A1* mutations) Adenylate kinase 2 (reticular dysgenesis) Alport syndrome (*COL4A* mutations) Aminoacyl-tRNA synthetase deficiencies (mito and cytoplasmic), *KARS* Bjornstad syndrome (with pili torti) (*BCSIL* mutations) Cockayne syndrome Connexin Cx26 (*GJB2*) mutations (with ichthyosis) Encephalopathy with hyperkinurininuria *ELOVL1* mutation (with ichthyosis and spasticity) Forkhead transcription factor *FOXI1* (with distal tubular acidosis) H+ -ATPase (V-ATPase) (with distal tubular acidosis) Galactose-4-epimerase deficiency Heimler syndrome (*PEX 1* and *7* mutations) MEDNIK syndrome PRPPS (phosphoribosylpyrophosphate synthetase deficiency) **Riboflavin transporters (2 and 3) defects** Rhizomelic chondrodysplasia punctata Sphingosine-1-P lyase deficiency Zellweger and variants Several cell trafficking disorders such as Waarderburg syndrome (with hypopigmentation): *WS4A* Ribosome defect: Treacher-Collins syndrome (conductive hearing loss) *WFS1* mutations: Neonatal/infancy-onset diabetes, congenital sensorineural deafness, and congenital cataracts.
Detectable in early to mid childhood	Aminoacylase deficiency **Biotinidase deficiency (biotin responsive) (untreated or treated late)** Chanarin-Dorfman syndrome Infantile Refsum disease (pseudo-Usher syndrome) Kearns-Sayre syndrome MEDNIK syndrome MPS type I, II, III (may be presenting sign) and IV α-Mannosidosis Mucolipidosis type II (I cell disease) **Megaloblastic anemia, diabetes and deafness (B1-responsive)** PHARC syndrome Perrault syndrome PRPP synthetase overactivity **Riboflavin transporter defects** Sphingolipidoses Mitochondrial non syndromic deafness: mutations in *MTTS1* and *MTRNR1*: Mitochondrial DNA mutations (syndromic deafness): NARP, Pearson, Wolfram, MELAS, MERFF, Kearns-Sayre, MIDD, OPA1, DDP Dystonia-Deafness: Mohr-Tanebjaerg (TIMM8A) *KARS1* mutations Several cell trafficking disorder (▶Chap. 44) *PIK3C2A* mutations (with congenital cataracts, nephrocalcinosis)
Detectable in late childhood to adolescence	β-Mannosidosis Fabry disease Refsum disease (adult form) Usher syndrome type II MERFF and other mitochondrial DNA mutations, Kearns-Sayre syndrome *FDXR* mutations (mitochondrial Fe-sulfur synthesis defect) **Riboflavin transporter defects** *PRPS1* mutations (X-linked nonsyndromic sensorineural hearing deafness)

Table 1.22 Head circumference

Macrocephaly	Microcephaly Congenital and not congenital but progressive after birth
1. Small molecule disorders	
Glutaric aciduria type 1 Canavan disease (acetylaspartaturia) L-2-hydroxyglutaric aciduria	Infant born to mother with untreated PKU **BCKDK (branched chain dehydrogenase kinase defect): not constant** **Serine synthesis defects improved by serine and brain serine transporter** including Neu Laxova syndrome Glutamine synthesis defects Asparagine synthesis defects PYCR2 (pyrroline-5-carboxylate reductase 2 deficiency) Sulfite oxidase deficiency **Cerebral folate deficiency due to *FOLR1* mutations and DHFR deficiency** Amisch lethal microcephaly (mitochondrial TPP transporter) *SepSec* (selenium metabolism defect) *MFSD2A* mutationn (omega3 fatty acid transport defect) *CYB5R3* mutations (congenital methemoglobinemia)
2. Energy metabolism defects	
Some mitochondrial disorders	ACO2 (aconitase deficiency) GOT2 deficiency **GLUT1DS and PDH** Mitochondrial encephalopathies (in particular, severe early onset mitochondrial DNA depletions), POLGpathies.
3. Complex molecule disorders	
GM2 gangliosidosis (Sandhoff, Tay-Sachs) Krabbe disease (infantile form) Some phosphatidylinositol defects: *PIK3CA* (hemimegalencephaly), *PIK3R2* (with polymicrogyria), *INPPL1* mutations Desmosterolosis (may also produce microcephaly)	PI4K2A (with cutis laxa) Desmosterolosis Congenital ceroid lipofuscinosis Aminoacyl-tRNA synthetases and tRNA processing metabolism disorders *RPL10, UTBF1* (ribosomopathies) Many neurodegenerative disorders with progressive brain atrophy
4. Cell trafficking disorders	
Golgipathies (like *RAB39B, HERC STRADA* mutations) *TBCK* mutations *RAC1* mutations (may also cause microcephaly)	Dolichol kinase deficiency, DPM 13 (dolichol recycling defects) Different CDG syndromes such as *COG1,2,4- 8,SLC35,TMEM 165* mutations (with post-natal microcephaly) Golgipathies related to RAB GTPases and their molecular partners (▶ Chap. 44) Adaptinopathies (*AP4* …), TRAPPCopathies Cytoskeleton disorders (tubulinopathies and others) *IER3IP1*: microcephaly, epilepsy and diabetes Vici syndrome WD-repeat proteins superfamily (*WDR45B* …)
Other genetic conditions:	
Alexander leukodystrophy Megalencephalic leukodystrophy with subcortical cysts Phacomatoses, *PTEN* (with autism, hamartomas). Overgrowth syndromes (mTOR1-AKT hyperactivation) NuRD complex mutations: *CHD3*, *CHD4*, and *GATAD2B* (neurodevelopmental disorders with macrocephaly and ID)	A large number of neurogenetic conditions that disrupt neurodevelopment. Rett syndrome due to *MECP2* and Rett like mutations including *CDKL5* and *FOXG1* are amongst the most frequently described
Cephalhematomas: **Glutaric aciduria type 1** Menkes disease	

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Table 1.23 Basal ganglia & brainstem hyperintensities

Leigh syndrome	Other types of hyperintensities	Hyperintensity of the Inferior olivary nucleus (brainstem)
1. Small molecule disorders		
ECHS1 mutations (enoyl CoA hydratase) Hydroxy-isobutyryl-CoA hydrolase 3-methylglutaconic aciduria 1 and 4 (► Chap. 18, Table 18.1). EPEMA syndrome (*ETHE1* mutations) Sulfite oxidase deficiency Lipoylation defects including *LIPT1* **Vitamin related diseases** (overlap with energy defects): **Biotinidase deficiency** **Mutations in *SLC19A3* (thiamine-biotin responsive basal ganglia disease)**	SSDH (pallidum) L-2-hydroxyglutaric aciduria **MMA** (pallidum) *NAXE and NAXD* mutations *MCT1* mutations (with thalami involvement) ***SLC19A3* mutations** **Wilson disease**[a] Cyt b5 reductase type II	**Wilson disease** Dihydropyrimidine dehydrogenase deficiency
2. Energy defects		
CoQ10 deficiency (including ***SQOR*** encoding sulfide: quinone oxidoreductase) Fumarase deficiency *SUCLA2* (succinyl CoA synthetase) mutations Pyruvate carboxylase deficiency **Pyruvate dehydrogenase deficiency** *KGD4* (coding for α-ketoglutarate dehydrogenase) >70 genes involved in oxidative phosphorylation, cofactors, and mitochondrial machinery including *PTCD3* coding for one of the mitochondrial ribosomal proteins (►Chaps. 14 and 37)	Mitochondrial cytopathies[a] **PDH deficiency**[a]	**PDH** *POLG* mutations Leber hereditary optic neuropathy
3. Complex molecule defects		
SERAC1 (MEGDEL syndrome: involvement of the putamen: 'eye' on the dorsal putamen) (also mitochondrial/energy dysfunction) (►Sect. 35.3.7)	**CTX**[a] GM1 Gangliosidosis[a] (may produce T2 hypointensity) PDE10 deficiency *AIMP1, AIMP2* (BG signal and hypomyelination)	Niemann Pick type C Salla disease Infantile neuroaxonal dystrophy Pontocerebellar hypoplasia (*TSEN, EXOSC3* mutations)
4. Cell trafficking disorders		
	MEDNIK	PMM2-CDG
Other genetic diseases:		
	KMT2B (histone methyltransferase): pallidum Acute thiamine depletion related to different causes (nutritional, alcoholism): **Wernicke encephalopathy**[a] (thalami, brain stem)	Ataxia telangiectasia

Legend: [a]Observed in adulthood
BG basal ganglia, *PDE* phosphodiesterase

Table 1.24 Basal ganglia & brain deposits

Calcifications on CT scan	Metals
1. Small molecule defects: Carbonic anhydrase deficiency (*CA2*) (▶ Sect. 19.4.2) **Biopterin metabolism defects (DHPR)** **Folic acid metabolism defects:** *FOLR1, SLC46A1*, *MTHFR, DHFR* NBIA syndromes (▶ Sect. 34.2.3) 3-hydroxyisobutyric aciduria Congenital hypomagnesemia 2. Energy defects: Kearns-Sayre syndrome MELAS and other respiratory chain disorders Congenital lactic acidemias 3. Complex molecule defects: Krabbe disease GM2 Gangliosidosis *KARS, FARSA, FARSB* and *SNORD118* (calcifications and cysts) (▶ Sect. 39.2.3) Aicardi-Goutières related genes (▶ Sect. 39.1.2) Cyt P450 hydroxylase (CYP2U1: SPG 56) (▶ Sect. 35.4.6) Other genes with diverse functions: Primary familial brain calcifications (previously known as Fahr's disease) (*SLC20A2, PDGFB, PDGFRB, XPR1, MYORG, JAM2 genes*)[a] *CSFR1* (AR inheritance: paediatric forms) *JAM3*: haemorrhagic destruction of the brain, subependymal calcification, and cataracts Collagen IV related disorders: *COL4A1, COL4A2* Cockayne and other DNA repair defects **Hypoparathyroidism**	Copper: **Wilson disease**[a] MEDNIK Manganese: **Hypermanganesemia with cirrhosis** Iron: Neurodegeneration with brain iron accumulation (NBIA) (▶ Sect. 34.2.3): *FTL* Neuroferritinopathy[a] (pallidum, putamen, caudate) low ferritin PANK2[a] (PKAN defect, HARP syndrome) tiger eye, RP COASY (CoA synthetase) similar to PKAN C2orf37/DCAF17 *PLA2G6* mutations (pallidum>substantia nigra) cerebellar atrophy *FA2H* mutations (pallidum>substantia nigra) *CPa* **aceruleoplasminaemia**[a] (diffuse hypointensity) low Cu and CER *C19orf12* mutations (pallidum > substantia nigra) optic atrophy *WDR45* (substantia nigra > pallidum) X linked dominant with MR *ATP13A2* (caudate, putamen) *GTPBP2*

Legend: [a]Observed in adulthood
RP retinitis pigmentosa, *MR* mental retardation, *Cu* copper, *CER* ceruloplasmin

Table 1.25 White matter abnormalities

With increased head circumference

Glutaric aciduria type I (bitemporal atrophy)
L-2-hydroxyglutaric aciduria
Canavan disease (▶ Sect. 22.10)
Mucopolysaccharidosis (with vacuoles)
Megalencephalic leukodystrophy with subcortical cysts (MLC1)
Vacuolizing leukoencephalopathy
Alexander disease (anterior)

Hypomyelination	Periventricular	U fibres/other locations	Pyramidal tracts/ cavitating leukoencephalopathies	MRS lipid peak

(continued)

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Table 1.25 (continued)

1. Small molecule defects: **Cerebral folate transport** (*FOLR1* **mutations) and folate metabolism defects** Untreated **galactosemia** **Serine synthesis defects** **PYCR2 (pyrroline-5-carboxylate reductase 2 deficiency)** S-Adenosylhomocysteine hydrolase (SAHH) Methionine S-adenosyltransferase deficiency Ribose-5-phosphate isomerase[a] (arabitol, ribitol peaks) *SLC 25 A12* (aspartate glutamate carrier) (▶ Sect. 11.11) Glutathione peroxidase 4 defect *CYB5R3* mutations (congenital methemoglobinemia) 2. Energy defects: Mitochondrial HSP 60 chaperonopathy 3. Complex molecule defects: Fucosidosis Sialidosis 4H syndrome (hypomyelination, hypogonadotropic hypogonadism, hypodontia; *POLR3A, B* genes) tRNA synthetases: *KARS* *RARS1* (with thin corpus callosum), *DARS1* (with supratentorial, brainstem, cerebellum, and spinal cord lesions), *EPRS1, AARS1* *AIMP1, AIMP2* (+brain atrophy) 4. Cell trafficking defects: GJA12 /GJA13 connexins (Pelizaeus-Merzbacher like) Oculodentodigital dysplasia (GJA1) HABC (hypomyelination with cerebellum atrophy and atrophy of the basal ganglia, *TUBB4A* gene) Other cell trafficking defects: *VPS11; SLC33A; FIG4* Other genetic defects Pelizaeus-Merzbacher disease (myelination arrest, PLP1) *TMEM106B* (Pelizaeus-Merzbacher-like disease) *TMEM63A* (mechanosensitive ion channel) *SPTAN1* (beta spectrin) Trichothiodystrophy with photosensitivity (*ERCC2, 3, GTF2H5, MPLK1P* genes) Cockayne syndrome (*ERCC6,8*) Clinically, hypomyelination presents as nystagmus, spasticity/mixed movement disorder, ataxia and ID. Low choline is a marker in MRS	1. Small molecule defects **Homocysteine remethylation defects**[a] **Glutaric aciduria type I**[a] **PKU (untreated, reversible)**[a] Menkes disease 3-Methylglutaryl-CoA lyase defect[a] Thymidylate kinase deficiency (*TYMK*) a novel vanishing white matter disease *NFE2L2* mutations (supratentorial multiple hyperintense lesions mimicking MS (▶ Chap. 35) 2. Energy defects Mitochondrial cytopathy Kearns-Sayre syndrome MNGIE (with supratentorial cortical atrophy) 3. Complex molecules: **CTX**[a] Cockayne (with calcifications) Metachromatic leucodystrophy[a] PBD[a], PEX-7 Polyglucosan body disease[a] Aicardi Goutières syndrome (with calcifications) Other genes: CACH (vanishing white matter disease) Cockayne (with calcifications) Hypomyelination with congenital cataracts (FAM126A)	U fibres: 1. Small molecules MCT1 homozygous mutations L-2-hydroxyglutaric **Glutaric aciduria I**[a] **Homocysteine remethylation defects**[a] 3-methylglutaryl-CoA lyase deficiency[a] 2. Energy defects: Mitochondrial cytopathies 3. Complex molecule defects: Polyglucosan body disease[a] Other locations: Occipital WM: GM3 synthase heficiency **X-ALD (posterior)** Peroxisomal disorders Frontal WM: Alexander disease Metachromatic leukodystrophy Neuroaxonal leukodystrophy with axonal spheroids	With pyramidal tract involvement: 1. Small molecule defects Lipoilation defects (especially *NDUFS1, NDUFA2:* tigroid-like cavitation) 2. Energy defects Mitochondrial cytopathies[a] COX deficiency due to mutations in *APOPT1* Pyruvate metabolism defects (**PDH**, pyruvate carboxylase deficiency) Mitochondrial A83446 mutation 3. Complex molecule defects: **Cerebrotendinous xanthomatosis**[a] Adrenomyeloneuropathy[a] Krabbe disease[a] Cavitating leukoencephalopathies: Cystic leukoencephalopathy without megalencephaly (RNASET2-deficient leukoencephalopathy) Vanishing white matter disease (*EIF2B2*) With cystic leukomalacia (WM cysts): SOX (not only periventricular) Glutaric aciduria (in adults) *GOT2* mutations PC, PDH With focal and asymmetric WM lesions (vasculopathies): RNASET2-related leukodystrophy NGLY1 related congenital disorder of deglycosylation Cathepsin A-related arteriopathy with strokes and leukodystrophy	DDHD2, (SPG54 with thin corpus callosum) FALDH DDHD2, (SPG54 with thin corpus callosum) FALDH (Sjogren Larsson syndrome) CPT 2 and several other FAO defects X-ALD and several other peroxisomal defects Chanarin Dorfmann and several other complex lipid synthesis defects Gaucher and NP C disease CTX and Smith Lemli Opitz syndrome

Legend: [a]Observed in adulthood

CPT carnitine palmitoyl transfease, *FAO* fatty acid oxidation defects, *CTX* cerebrotendinous xanthomatosis, *ALD* adrenoleucodystrophy, *MS* multiple sclerosis, *PBD* peroxisome biogenesis defect

Table 1.26 Brain dysplasia and malformations

Gyration abnormalities	Corpus callosum hypoplasia/agenesis
1. Small molecule defects: Glutamine Synthetase deficiency **Serine synthesis defects** Asparagine synthesis defects Serine hydroxymethyltransferase type 2 MFSD2A defect (omega3 fatty acid transport defect) *SLC35A2* (encoding an UDP-galactose transporter) 2. Energy metabolism defects: CPTII, MAD (multiple acyl-CoA dehydrogenase) Fumarase deficiency 3. Complex molecule defects: Peroxisomal disorders (Zellweger and others) CK syndrome (cholesterol metabolism defect): polymicrogyria or pachygyria Squalene synthase deficiency Perisylvian polymicrogyria: *PIK3R2* (phosphoinositide 3 kinase) mutations 4. Cell trafficking disorders: O-glycosylation disorders: Muscle eye brain disease (POMGnT) Walker Warburg syndrome (*POMT1*) Fukuyama syndrome (fukutin) Congenital muscular dystrophy: DMC1C (fukutin related protein) DMC1D (protein large) CEDNIK syndrome (snare protein mutation) *FIG4* (Temporo-occipital polymicrogyria) Most golgipathies with microcephaly *NDE1* (dynamin): microhydranencephaly, lissencephaly Tubulin defects (*TUBA1A, TUBB2B, TUBB3*) Reelin gene (*RELN*) encoding an extracellular matrix associated glycoprotein Other genes: Different lissencephaly genes (*L1S1, DCX, ARX, VLDLR*) Periventricular nodular heterotopia (bilateral due to *FLN1* mutations) *FOXG1* defects	With gyration abnormalities 1. Small molecule defects: Non ketotic hyperglycinemia MCT1 (homozygous mutations) MOCS2 (molybdenum cofactor; 1 case corpus callosum agenesis) 3-hydroxyisobutyric aciduria Brain serine transporter defect Asparagine synthetase deficiency Serine hydroxymethyltransferase type 2 Glutathionuria (cc agenesis) 2. Energy metabolism defects: Complex II mitochondrial cytopathies (with leukodystrophy) PDH defect (with basal ganglia abnormalities) Fumarase deficiency (cc agenesis) 3. Complex molecule disorders Aicardi Goutières syndrome (with calcifications) *EARS2* mutations Phospholipids synthesis /remodelling defects associated with spastic paraplegia: SPG 28 (*DDHD1*), SPG 49 (*CYP2U1*),SPG 54 (*DDHD2*), SPG 35 (*FA2H*), SPG 46 (*GPA2*), Desmosterolosis, lathosterolosis Hemizygous EBP deficiency in males 4. Cell trafficking disorders: *EPG5* mutations (Vici syndrome) (cc agenesis) Most golgipathies with microcephaly Tubulinopathies with hypomyelination Others: *ATP6AP2* *CDK5RAP2*, (cc agenesis) Genes that disrupt mid-brain development ACTH deficiency In general, all amino acid synthesis defects may present corpus callosum hypoplasia Any disease with hypomyelination/white matter disturbances may have cc hypoplasia

1.5.8 Neurophysiological Signs

Neurophysiologic studies are important for diagnosis and follow-up in IEM, especially if epilepsy or neuropathy are part of the clinical picture.

The diagnosis of peripheral neuropathies has been already addressed in ▶ Sect. 1.5.2 and in the ◘ Fig. 1.10 algorithm of peripheral neuropathies.

Electroencephalographic abnormalities (see also ◘ Tables 1.17 and 1.18 and section epilepsy).

Burst suppression pattern is frequently observed in several IEM in the neonatal period (see above ▶ Sect. 1.3.2). In the neonatal manifestation of maple syrup urine disease, EEG displays comb-like rhythms. In infantile neuronal ceroid lipofuscinosis (NCL), the first abnormality in the electroencephalogram (EEG) is the disappearance of eye opening/closing reaction, followed by a loss of sleep spindles. Subsequently the EEG becomes rapidly flat. In CLN2 disease, an occipital photosensitive response to photic stimulation at 1–2 Hz with eyes open is present. In patients with homocystinuria, centrotemporal spikes are often present resembling the epileptiform potentials in benign epilepsy with centrotemporal spikes. In Alpers disease, the EEG is very valuable in the early stage of the disease and displays, albeit not in all patients, **r**hythmic **h**igh-**a**mplitude **d**elta with

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Table 1.27 Posterior fossa (and olivo-ponto-cerebellar)

Hypoplasia	Progressive atrophy	Dentate nuclei/ cerebellar cortex hyperintensities
Global cerebellar hypoplasia: NKH, mitochondrial disorders, PDH, Zellweger, mucopolysaccharidoses (type I and II) Ribosomopathies: *POLR1C, POLR3A, POLR3B* CDG syndrome Joubert syndrome Dystroglycanopathies PCH (pontocerebellar hypoplasias): Asparagine mynthetase deficiency *SEPSECS* *COASY* (soenzyme A synthetase) Aminoacyl-tRNA synthetases and tRNA processing metabolism disorders *AMPD2* *EXOSC3, EXOSC8, EXOSC9, VPS53, 51, TBC1D23, PCLO* (with optic atrophy) Dandy-Walker malformation: Hemizygous EBP deficiency in males (sterol metabolism) *AP1S2, KIAA1109*: trafficking defects Other cerebellar dysplasia: *PACS2*	1. Small molecule defects: L-2-hydroxyglutaric aciduria Mevalonic aciduria Adenylosuccinase deficiency *SepSec* (selenium metabolism) Glutamine synthetase (GS) deficiency SSDH deficiency 3-Methylglutaconic aciduria type 1 and CLPB mutations 2. Energy metabolism defects Mitochondrial cytopathies[a] Leigh syndrome 3. Complex molecule defects X-linked adrenoleukodystrophy[a] GM2 gangliosidosis[a] Niemann-Pick disease type C[a] Cerebrotendinous xanthomatosis[a] Sialidosis type 1[a] Ceroid lipofuscinosis[a] *DEGS1* mutations (with hypomyelination) *FA2H* mutations (with leukodystrophy) Neuroaxonal dystrophy (infantile) and other phospholipid synthesis defects Schindler disease Smith-Lemli-Opitz Nucleotide, tRNA and ribosome metabolism defects: *QARS1, AIMP1, AIMP2, UBTF1,* 4. Cell trafficking disorders: *ZNHIT3* (PEHO-Like syndrome), *AP4E1, SLC9A6* Others: Galloway-Mowat, cerebellar atrophy: *YRDC, GON7, LAGE3, OSGEP, TP53RK, TPRKB, WDR4:* SCA in general[a] Dentatorubralpallidoluysian atrophy[a] Progressive myoclonus epilepsies[a] Spinal and bulbar muscular atrophy[a]	Dentate nuclei hyperintensities: L-2-hydroxyglutaric aciduria Semialdehyde succinate dehydrogenase deficiency[a] **Wilson disease**[a] NBIA (some late onset cases together with hypointensities)[a] Mitochondrial encephalopathy[a] Cerebrotendinous xanthomatosis[a] Polyglucosan body disease[a] Cerebellar atrophy and cerebellar cortex T2-hyperintensity: Some mitochondrial disorders **Coenzyme Q10 deficiency** Infantile neuroaxonal dystrophy PMM CDG Late infantile NCL pontocerebellar hypoplasia type 7 Marinesco-Sjörgen syndrome Christianson syndrome

Stroke-like episodes: MELAS, POLG1, complex1, mt DNA mutations, CoQ10 defects, KSS, *Twinkle*, *TK2, CABC1; SCA* spinocerebellar atrophy

Cerebrovascular strokes: homocystinurias, CDGs, hyperammonaemias, ITPA mutations (venous thrombosis)

[a]Observed in adulthood

superimposed (poly)spikes (RHADS), usually over the posterior regions.

Somatosensory evoked potentials (SSEP) are helpful in delineating posterior column involvement, e.g. in Friedreich ataxia or cobalamin deficiency. Giant SSEP are found in some of the progressive myoclonic epilepsies including late infantile neuronal ceroid lipofuscinosis.

Visual evoked potentials help to detect early involvement of the optic nerve, e.g. in mitochondrial disorders, infantile neuroaxonal dystrophy, Alpers disease and in hypomyelinating disorders.

The **electroretinogram (ERG)** is important in detecting retinal involvement which is of use in the differential diagnosis of neurodegenerative disorders. Retinal involvement is part of the neuronal ceroid lipofuscinoses, but also of panthotenate-associated neurodegeneration (PKAN), mitochondrial disorders and many others (see also Table 1.28 Abnormal Fundoscopy).

Table 1.28 Retinal manifestations

Cherry red spot	Retinitis pigmentosa and others	Optic atrophy
Cytochrome C oxidase deficiency Niemann-Pick diseases type A, B Galactosialidosis (neuraminidase deficiency) Gangliosidosis GM1 (landing) Gangliosidosis GM2 (Sandhoff, Tay Sachs) Nephrosialidosis Sialidosis type I Farber disease	**Retinitis pigmentosa:** **Methylene tetrahydrofolate dehydrogenase defect (MTHFD1)** **CblC**[a] Respiratory chain disorders (Kearns Sayre, NARP, mtDNA deletions)[a] ACO2 (aconitase deficiency) **Abetalipoproteinemia** **Vitamin E malabsorption (tocopherol carrier)** **LCHAD deficiency** CDG Peroxisomal defects[a] **Refsum disease**[a] Mucopolysaccharidoses Ceroid lipofuscinosis Panthothenate kinase deficiency (Harp syndrome) *HARS* mutations Dehydrodolichol diphosphate synthase deficiency Fatty acid 2-hydroxylase (FA2H) deficiency **Ceramide kinase-like (*CERKL*) mutation** PHARC syndrome (*ABHD12*) *VAC14* mutations *VPS13B:* Cohen syndrome, ID, obesity, neutropenia and retinopathy *ACBD5*: retinal dystrophy with leukodystrophy Inosine-5′-monophosphate dehydrogenase1 **Taurine tranporter deficiency (SLC6A6)**	**MMA and PA** Sulfite oxidase (infantile) ***ATAD3A* mutations** **Biotinidase deficiency** **CblC**[a] Ribose-5-phosphate isomerase[a] Canavan disease (early sign) LHON (Leber due to mitochondrial DNA deletions[a]) DOA autosomal-dominant optic atrophy (*OPA1*) Leigh syndrome (all causes) Mitochondrial cytopathies[a] ACO2 (aconitase deficiency) Iron sulfur cluster disorders Ceroid lipofuscinosis (CLN3[a], CLN4[a]) Krabbe disease (infantile) Metachromatic leukodystrophy Infantile Neuroaxonal dystrophy (*PLA2G6*) Schindler disease Fatty acid 2-hydroxylase (*FA2H*) deficiency Pelizaeus-Merzbacher disease (presenting sign early in infancy) Peroxisomal biogenesis defects[a] **Pyruvate dehydrogenase deficiency**[a] SOPH syndrome (*NBAS* mutation) X-ALD[a] 3-Methylglutaconic aciduria type 3 (Costeff due to OPA mutations) Dolichol synthesis/recycling defects: SRD5A3-CDG (Ig) (with nystagmus colobomas, cataracts, glaucoma, micro-ophthalmia DPM1-CDG, MPDU1-CDG *SLC25A46* mutations *CDC42* (cell trafficking disorder): Takenouchi-Kosaki syndrome
	Gyrate atrophy with OAT deficiency **Aceruloplasminemia**[a] Mucolipidosis type IV Heterozygous *ELOVL4* mutations (AD juvenile form of macular degeneration Stargardt type 3) Sjögren-Larsson syndrome (FALDH) macular dystrophy with very suggestive retinal crystalline inclusions	
	Extinguished ERG with normal fundoscopy: NAD synthase deficiency (NMNAT1) Leber congenital amaurosis	
	Other findings: thin retina and reduced vascularization in **GLUT1DS** with normal ERG	

[a]Observed in adulthood. LHON, Leber congenital optic atrophy; SOPH syndrome: Short stature, optic atrophy, Pelger-Huet anomaly

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Table 1.29 Ophthalmoplegia, ptosis, eye movements, strabismus

Supranuclear gaze palsy	**Niemann-Pick C** (vertical), Gaucher (horizontal) *ATP13A2, GBA* PANK2 (horizontal and vertical supranuclear gaze palsy)
Oculogyric crisis	**Neurotransmitter defects, Pyridox(am)ine-5-phosphate oxidase deficiency GLUT1DS, Wilson**, PKAN, diverse causes of dystonia-parkinsonism syndromes, *GRIN1* mutations. Other genetic conditions: channelopathies, Rett syndrome, *H-ABC*, ataxia-telangiectasia
Nystagmus	**Vit E deficiency**, **SLC19A3** (biotin-thiamine responsive BBGG disease), *DNAJC12*, L-2-hydroxyglutaric aciduria **Abetalipoproteinemia** (with progressive gaze disturbances and a characteristic pattern of dissociated nystagmus) Leigh syndrome, *SACS*, *MECR*, *FA2H*, ECHS1, *HIBCH* PHARC, *CYB5R3* (porphyria), NLC, *GBA* (Gaucher) *SPTBN2* (cell trafficking). Chanarin-Dorfman syndrome (*CGI58*), *ELOVL1* mutations Paelizaeus-Merzbacher disease (*PLP1*) Disorders with ataxia, cerebellar dysfunction and hypomyelination may have nystagmus.
Oculomotor apraxia	*PIK3R5*, Gaucher types 2 and 3, Vitamin E deficiency, PDH, CDG, Lesch-Nyhan, Dopamine transporter deficiency. Ataxia telangiectasia, ataxia with oculomotor apraxia (*AOA,2, 3,4*) Diseases with cerebellar involvement may have oculomotor apraxia
External ohphtalmoplegia	Mitochondrial disorders: mitochondrial deletions and point mutations including spastin *SPG7*, Christianson syndrome
Others	Ocular flutter: PC, **GLUT1DS,** *DGUOK*, some tRNA synthetase defects, CDG, Dopamine transporter deficiency, **MSUD** Eyelid myoclonus: **DHPR** Palpebral ptosis: **neurotransmitter defects**, mitochondrial disorders, diseases with muscle involvement Abnormal saccade pursuits: common in diseases with cerebellar involvement (i.e.**Niemann-Pick C, cerebrotendinous xanthomatosis**)

1.5.9 Recommended Laboratory Tests in Neurological Syndromes

Table 1.30

1.6 Specific Organ Signs and Symptoms

In the following sections medical specialities are listed by alphabetical order. Where possible, disorders are classified in tables according to pathophysiological groups. The following diagnostic checklist is primarily based upon the authors' personal experience and information from the chapters of this book. It is, of course not exhaustive and should be progressively updated according to the personal experiences of all readers.

1.6.1 Cardiology

All pertinent information on cardiac failure, cardiomyopathies and heart rythym disorders is presented in ▶ Sects. 1.3.7 and 1.4.8 and Tables 1.11 and 1.12.

Orthostatic hypotension is a presenting symptom in dopamine β-Hydroxylase deficiency and other extremely uncommon monoamine defects such as cytochrome b561 deficiency and norepinephrine transporter deficiency (▶Sect. 30.5). It is also frequent in occipital horn syndrome (▶Sect. 34.1.2).

Cardiac valve disease with thickening of valves leading to dysfunction (insufficiency and/or stenosis) is most common in MPS with accumulation of dermatan sulfate (MPS I, II, VI and VII), this being the most abundant GAG in heart valves (▶Chap. 41).

1.6.2 Dermatology

- **Hair Abnormalities (Table 1.31)**
- **Hyperkeratosis/Ichthyosis (Table 1.32)**

Most are linked to complex molecules or trafficking disorders and not or poorly treatable.

The classification of erythroderma and inherited ichthyosis is clinically based and distinguishes between syndromic and non-syndromic ichthyosis forms [85] although this can be difficult to determine at birth and even in infancy. Bullous ichthyosis/epidermolytic

Table 1.30 Main neurological syndromes from childhood to adolescence and recommended laboratory tests (focused on treatable disorders)

Predominant neurological syndrome	Laboratory tests (rational approach based on associated clinical signs and treatable disorders)	Treatable disorders
Isolated developmental delay/ intellectual disability (ID)	Basic laboratory tests[a]: blood glucose, acid-base status, blood counts, liver function, creatine kinase, uric acid, thyroid function, alkaline phosphatase Plasma: lactate, ammonium, amino acids, total homocysteine, folate, biotinidase activity, copper, ceruloplasmin Urine: creatine metabolites, organic acids (including 4-hydroxybutyric acid), amino acids, glycosaminoglycanes (GAGs) and oligosaccharides purines, pyrimidines, Consider maternal phenylalanine	Phenylketonuria (PKU), homocystinurias, urea cycle defects, amino acid synthesis defects, thyroid defects, biotinidase deficiency, Hartnup disease, occipital horn syndrome
With dysmorphic features	Consider also: plasma sterols, peroxisomal studies (very long-chain fatty acids, phytanic acid, plasmalogens), transferrin isoelectric focusing for glycosylation studies (CDG), oligosaccharides and GAGs in urine For the study of ID +/– dysmorphic features, genetic tests (cytogenetic studies, microarrays, NGS, and targeted studies) have the highest diagnostic yield.	Peroxisomal diseases (only partially by some supplements), SLO
Behavioural and psychiatric manifestations including autistic signs	Basic laboratory tests[a] Plasma: ammonium, amino acids, homocysteine, total homocysteine, folate, sterols (including oxysterols), copper, ceruloplasmin Urine: GAGs and oligosaccharides, organic acids (4-hydroxybutyric acid), amino acids, purines, creatine, creatinine and guanidinoacetate Depending on additional clinical signs and brain MRI pattern: peroxisomal studies, lysosomal studies, consider CSF for BCAA studyin spite of normal plasma BCAA (transport defect)	PKU, urea cycle disorders, homocystinurias, folate metabolism defects, Wilson disease, BCKDH kinase deficiency, BCAA transport defect, CTD, mild forms of SLO, Niemann-Pick disease type C, X-ALD (at some stages), Hartnup disease
Epilepsy	Basic laboratory tests[a] adding calcium, magnesium, phosphate, manganese, peripheral erythrocyte morphology Plasma: lactate, ammonium, amino acids, total homocysteine, folate, biotinidase activity, copper and ceruloplasmin, VLCFA Urine: organic acids, creatine, creatinine and guanidinoacetate, sulphite test, purines and pyrimidines, pipecolic acid and 5-AASA CSF: glucose, lactate, amino acids, 5-methyltetrahydrofolate (5-MTHF), pterins, biogenic amines, citrate, GABA Consider lysosomal studies and targeted tests if PME Consider genetic tests for GPI-anchor biosynthesis pathway defects and other defects of complex lipid synthesis (FA2H, ELOVL4, GM3 synthetase)	GLUT1DS, homocystinurias, IEM of folate metabolism, organic acidurias, biotinidase deficiency, sodium dependent multivitamin transporter, creatine synthesis defects, serine biosynthesis defects, BCKDK defects, MoCo deficiency, CAD deficiency, Menkes disease (only partially treatable), late onset forms of pyridoxine dependent epilepsy, pterin defects (DHPR), AADC deficiency, consider SLC35-A2-CDG *SLC13A5* mutations may respond to acetazolamide *KCNQ2* mutations may respond to carbamazepine

(continued)

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Table 1.30 (continued)

Predominant neurological syndrome	Laboratory tests (rational approach based on associated clinical signs and treatable disorders)	Treatable disorders
Ataxia	Basic laboratory tests[a] adding albumin (for AOA1 and AOA4), cholesterol, triglycerides, and alpha-foetoprotein (for AT, AOA2, AOA4), blood smear for acanthocytes Plasma: lactate, pyruvate, ammonium, amino acids, biotinidase activity, vitamin E, sterols (including oxysterols), ceruloplasmin, peroxisomal studies (including phytanic acid), coenzyme Q10, transferrin electrophoresis Urine: organic acids (including 4-hydroxybutyric and mevalonic acids), amino acids, purines CSF: glucose, lactate, pyruvate Consider lysosomal/mitochondrial/NBIA studies depending on the clinical and brain MRI signs Consider lipidome studies (plasma, CSF) Consider genetic panels of inherited ataxias and other NGS techniques	PDH deficiency (thiamine-responsive; ketogenic diet), biotinidase deficiency, GLUT-1, abetalipoproteinemia, CTX, Refsum disease, coenzyme Q10 deficiencies, Hartnup disease, CAD deficiency, Niemann-Pick disease type C Consider channelopathies, *PRRT2* and *ATP1A3* mutations (may respond to acetazolamide, carbamazepine)
Dystonia-Parkinsonism	Basic laboratory tests[a] Plasma: lactate, pyruvate, ammonium, amino acids, total homocysteine, biotinidase activity, sterols (including oxysterols), copper, ceruloplasmin, uric acid, manganese Urine: organic acids, uric acid, creatine, creatinine and guanidinoacetate, purines, GAGs, oligosaccharides CSF: glucose, lactate, pyruvate, amino acids, 5-methyltetrahydrofolate, pterines, biogenic amines, GABA Consider lysosomal/mitochondrial/NBIA studies depending on the clinical and brain MRI signs Consider genetic panels of inherited dystonias, parkinsonism, and other NGS techniques	Neurotransmitter defects, GLUT-1 deficiency, thiamine transport defects (TBBGD), PDH defects, organic acidurias, homocystinurias, IEM of folate metabolism, defects of creatine biosynthesis, Wilson disease, biotinidase deficiency, Niemann-Pick disease type C, CTX, manganese defects Consider Dopa responsive conditions (sometimes only transitory response): *POLG, SYNJ1, DNAJC6, CLCT, VPS35, PLA2G6, tWARS*
Chorea	Basic laboratory tests[a] Plasma: lactate, pyruvate, ammonium, amino acids, total homocysteine, folate, biotinidase activity, sterols (including oxysterols), copper, ceruloplasmin, uric acid, galactose -1-P, transferrin electrophoresis Urine: organic acids, uric acid, creatine, creatinine and guanidinoacetate, purines, galactitol, sulphite test CSF: glucose, lactate, pyruvate, amino acids, 5-methyltetrahydrofolate, pterins, biogenic amines, GABA Consider NCL studies and GPI-anchor synthesis defect genetic tests Consider genetic panels of inherited choreas, and other NGS techniques	GA1 and other classic organic acidurias (MMA, PA), GAMT, GLUT1DS, homocystinurias, pterin and neurotransmitter defects, Niemann-Pick disease type C, Wilson disease, galactosaemia, cerebral folate deficiency due to *FOLR* mutations, MoCo deficiency, NKH (attenuated, late-onset forms can be partially treatable)

Table 1.30 (continued)

Predominant neurological syndrome	Laboratory tests (rational approach based on associated clinical signs and treatable disorders)	Treatable disorders
Spasticity	Basic laboratory tests[a] Plasma: lactate, pyruvate, ammonium, amino acids, total homocystinuria, folate, biotinidase activity, vitamin E, triglycerides, cholesterol, sterols, peroxisomal studies, Urine: organic acids, amino acids, GAGs, oligosaccharides, sialic acid CSF: glucose, biogenic amines, pterins and 5-MTHF Consider lysosomal/mitochondrial/ NBIA studies depending on clinical and MRI findings Consider genes related to HSP and plasma, CSF lipidome	HHH, arginase deficiency, ornithine amino transferase deficiency, homocysteine remethylation defects, biotinidase deficiency, cerebral folate deficiencies, GLUT1D, dopamine synthesis defects (atypical TH), CTX, vitamin E deficiency
Peripheral neuropathy	Basic laboratory tests[a] Plasma: lactate, pyruvate, ammonium, amino acids, folate, vitamin E, triglycerides, cholesterol, acylcarnitines, sterols, peroxisomal studies, transferrin electrophoresis, sorbitol levels Urine: amino acids, GAGs, oligosaccharides, thymidine, porphyrins Consider lysosomal/mitochondrial/ NBIA studies depending on clinical and MRI findings	Refsum disease, X-ALD (treatable at some stages), homocysteine remethylation defects, CTX, abetalipoproteinemia, LCHAD, trifunctional protein, PDH, vitamin E malabsorption, ornithine amino transferase, serine deficiency, porphyrias, sorbitol dehydrogenase deficiency

AADC amino acid decarboxylase, *AOA* ataxia with oculomotor apraxia, *AT* ataxia telangiectasia, 5-*AASA* 5-aminoadipic semialdehyde, *BCKDH* branched chain ketoacid dehydrogenase, *CTX* cerebrotendinous xanthomatosis, *DHPR* dihydropteridine reductase, *HHH* hyperammonaemia, hyperornithinaemia, homocitrullinuria, *FOLR* folate receptor, *GA1* glutaric aciduria type 1, *GAG* glycosamineglycan, *GAMT* guanidinoacetate methyltransferase, *GTPCH* GTP cyclohydrolase I, *LCHAD* long-chain 3-hydroxyacyl-CoA dehydrogenase, *HSP* hereditary spastic paraparesis, *MMA* methylmalonic aciduria, *MoCo* molybdenum cofactor deficiency, *NGS* next generation sequencing, *NKH* nonketotic hyperglycinaemia, *PA* propionic acidaemia, *PDH* pyruvate dehydrogenase deficiency, *PME* progressive myoclonus epilepsy, *X-ALD* X-linked adrenoleukodystrophy, *TH* tyrosine hydroxylase

[a]These basic laboratory tests should be considered as a routine screening in every neurological syndrome

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Table 1.31 Hair abnormalities: mostly onset in neonatal period and infancy

Alopecia, absent eyebrows and sparse eyelashes	Brittle Hair	Pili Torti, Hirsutism	Trichorrhesis Nodosa
Small molecule metabolism defects			
Acrodermatitis enteropathica (zinc) **Biotin-responsive MCD (fatty acids)** **Calciferol metabolism defects (vit D)** **Essential fatty acid deficiency** Menkes disease (Copper) **Methylmalonic and propionic acidurias** **OAT deficiency** (peculiar fine, sparse, straight hair) Ornithine decarboxylase defect (►Sect. 21.8) **Porphyrias (congenital erythropoietic, Hepatoerythropoietic Cutanea tarda (in adults)** **Zinc deficiency**	**Argininosuccinic aciduria** **Citrullinemia** Menkes syndrome	**Pili torti:** Menkes disease	**Argininemia** **Argininosuccinic aciduria** **Lysinuric protein intolerance** Menkes disease
Complex molecule defects and miscellaneous			
RFT1-CDG Conradi-Hunermann syndrome Ehlers-Danlos type IV IFAP syndrome (►Sect. 37.11) Netherton syndrome (*SPINK5*) Steinert disease (adult) Woodhouse-Sakati syndrome (adult) Many trafficking disorders involving vesicular and organelles trafficking, cytoskeleton and autophagy (►Chap. 44)	Pollitt's syndrome Trichothiodystrophy Mucopolysaccharidosis Bjornstad syndrome	**Pili Torti:** Netherton syndrome Bjornstad syndrome **Hirsutism:** MPS III (and to a lesser extent several other MPSs) Oliver-McFarlane syndrome	Netherton syndrome

Many defects are linked to small molecules disorders and are treatable
IFAP ichthyosis follicularis, atrichia, and photophobia, *OAT* ornithine amino transferase deficiency

hyperkeratosis has been redefined as keratinopathic ichthyosis. Collodion babies present with a tight shiny cast that cracks after some time, resulting in irregularly branched fissures [86].

AR congenital ichthyosis (ARCI) refers to harlequin ichthyosis, lamellar ichthyosis, and congenital ichthyosiform erythroderma. About 40 inherited disorders of complex lipids (►Chaps. 35 and 40), cholesterol (►Chap. 37) and complex fatty acids synthesis, remodelling, catabolism, and transport (►Chap. 42) presenting with ichthyosis have been described. The vast majority present as neuro-cutaneous syndromes, chondrodysplasia punctata or multiple congenital anomalies, as do CDG or serine synthesis defects (including Neu Laxova syndrome) (►Sect. 24.2). Among the neuro-cutaneous syndromes comprising spastic paraparesis, **Sjögren-Larsson syndrome** presents at birth with a very severe pruriginous ichthyosis that responds dramatically

Table 1.32 Hyperkeratosis, ichthyosis

Hyperkeratosis	
CEDNIK (neuro-cutaneous syndrome: keratosis on palms and soles) (▶ Sect. 44.3.2) MEDNIK syndrome: keratodermia (see below) (▶ Sect. 34.1.4) Ichthyosis (see below) **Tyrosinemia type II** (keratosis on palms and soles) (▶ Sect. 17.3) SAM syndrome (*DSG1*) (keratosis on palms and soles and ichthyosiform erythrodermia) Erythropoietic protoporphyria (seasonal palmar keratoderma)	
Angiokeratosis	
Aspartylglucosaminuria Beta-mannosidosis, fucosidosis **Fabry disease** (presenting sign) Galactosialidosis Schindler disease (adult form) (▶ Sect. 41.3.1) Kawasaki disease	
Ichthyosis (with Congenital Erythrodermia)	
Lysosomal diseases	X-linked steroid sulfatase (non pruritic)
	Austin disease: multiple sulfatase deficiency
	Gaucher disease type II (collodion baby)
Complex lipids synthesis and remodelling Phospholipids (▶ Chap. 35) Sphingolipids (▶ Chap. 40) Non mitochondrial fatty acid metabolism including peroxisomal defects (▶ Chap. 42)	Early ARCI: phospholipase A1 deficiency (*PNPLA1*) *ABHD12* (harlequin ichthyosis) Ceramide synthesis defects *KDSR, CERS3, CYP4F22*
	Late ARCI: epidermal lipase N deficiency (*LIPN*)
	Chanarin Dorfmann syndrome (*ABDH5*)
	Sjogren Larsson syndrome (FADH) (pruritic)
	Elongase 4 and 1 deficiency (*ELOVL4*, *ELOVL1*)
	Serine synthesis defects
	Sphingosine-1-phosphate Lyase deficiency (▶ Sect. 40.3.4)
	SLC27A4 (Ichthyosis prematurity syndrome) (▶ Sect. 42.4.2)
	Adult Refsum disease
	Chondrodysplasia punctata (CDP I, II, III)
Cholesterol synthesis defect (▶ Chap. 37)	Conradi Hunermann syndrome (X-linked) (transient)
	CHILD syndrome (unilateral)
	Sterol-C4 methyl oxidase deficiency (spares palms and soles)
	IFAP syndrome
Dolichol synthesis and recycling defects (▶ Chap. 43)	MPDU1-CDG: Mannose-P-dolichol utilization defect 1
	SRD5A3-CDG: with eye findings
	DK1- CDG: with loss of hair eyebrows and eyelashes
Trafficking disorders	CEDNIK syndrome (▶ Sect. 44.3.2) MEDNIK syndrome (*AP1S1* mutations) (▶ Sect. 34.1.4) Other vesicular trafficking (*SNAP29, SUMF1, VIPAS39*)
Organic acidurias	**Holocarboxylase synthetase deficiency**, **biotinidase deficiency, MMA**
Primary immuno deficiencies	Omenn, Wiskott Aldrich, graft versus host disease
Others	Netherton syndrome: debilitating pruritis with bamboo hair Connexin Cx26 (*GJB2*) with deafness Disorders of pre-rRNA processing (dyskeratosis congenita) (▶ Chap. 39)

IFAP ichthyosis follicularis, atrichia, and photophobia, *SAM syndrome*, severe dermatitis, multiple allergies and metabolic wasting linked to mutations in Desmoglein 1

to Zileuton (▶Sect. 42.4.5). **Sterol methyl-C4 oxidase**, a sterol metabolism disorder, is another treatable ichthyosis with a spectacular improvement on statin and cholesterol supplement (▶Sect. 37.7.1) Ichthyosis and keratodermia are also cardinal signs of CEDNIK (▶Sect. 44.3.2) and MEDNIK syndromes (▶Sect. 34.1.4).

- **Vesiculobullous Lesions/Skin rashes/Photosensitivity (Table 1.33)**

The majority are linked to small molecule disorders leading either to an accumulation of a toxic compounds (such as in **porphyrias and organic acidemias**) or deficiency of an essential small molecules (as in **Hartnup, LPI** or **Zinc deficiency**).

- **Cutis laxa/Nodules/Xanthomas/Miscellaneous (Table 1.34)**

Most are linked to primary complex molecules synthesis or trafficking disorders.

Ehlers-Danlos syndromes (EDS) are collagenopathies that comprise a clinically and genetically heterogeneous group of heritable connective tissue disorders. Its principal clinical features reflect varying degrees of connective tissue fragility, affecting mainly the skin, ligaments, blood vessels, and internal organs. There are 16 EDS variants described so far, which include defects in noncollagenous proteins including genes involved in glycosaminoglycans (GAG) synthesis. Deficiency of galactosyltransferase I and II affects the initial steps in the formation of the GAG chains [87] (▶Sect. 41.1).

Cutis laxa syndrome forms a group of diseases, mostly elastinopathies characterized by wrinkled, redundant, inelastic and sagging skin due to defective synthesis of elastic fibres and other proteins of the extracellular matrix. Syndromic forms of cutis laxa are caused by diverse genetic defects, mostly coding for structural extracellular matrix proteins [88]. A number of metabolic disorders are associated with inherited cutis laxa among them copper metabolism defects such as Menkes disease (▶Sect. 39.1.2), GLUT10 (▶Sect. 8.6), combined disorder of N- and O- linked glycosylation (mutations in *ATP6V0A2,* COG7-CDG and other CDG defects ▶Chap. 43), proline synthesis defects (▶Sect. 21.3), and *PI4K2A* mutations (▶Chap. 35, ▶Table 35.1). All are also neurologic disorders.

Table 1.33 Vesiculous bullous lesions, photosensitivity

Photosensitivity & skin rashes	Vesiculo bullous lesions	Acrocyanosis
Infantile Porphyrias: (▶Chap. 33) **Congenital erythropoietic porphyria** **Erythrohepatic porphyria** **Erythropoietic protoporphyria** **Adult Porphyrias:** **Hereditary coproporphyria** **Porphyria variegata** **Porphyria cutanea tarda** **5ALA dehydratase** **Biotinidase deficiency** (▶Chap. 27) *ETHE1* mutations (▶Sect. 20.9) **Hartnup disease** (▶Sect. 25.4) ***CLTRN2*** (▶Sect. 25.5) Mevalonic aciduria (with fever) (▶Sect. 37.1) *SECISPB2* mutations (▶Sect. 34.5) **Zinc deficiency** (▶Sect. 34.6) Respiratory chain disorders NAXE and NADP (▶Sect. 11.14) Glutamine synthetase deficiency (▶Sect. 24.1.1) Prominent Mongolian blue spots and a characteristic papular rash are prominent in severe MPS II (▶Sect. 41.2)	**Acrodermatitis enteropathica** (▶Sect. 34.6.1) **Biotin responsive disorders** (▶Chap. 27) **Congenital erythropoietic porphyria (sun exposed skin)** **LPI (lupus like skin lesions)** (▶Sect. 25.3) **MMA, PA** (isoleucine deficiency) (▶Sect. 18.1) Lipin 2 deficiency (Majeed syndrome) (▶Sect. 35.1.4) NAXE and NADP deficiency **Zinc deficiency**	(*ETHE1* mutations) (orthostatic) ▶Sect. 20.9 Aicardi Goutières syndrome (Chilblains) (▶Sect. 39.1.2)

LPI lysinuric protein intolerance, *ALA* aminolevulinic acid

1.6.3 Endocrinology (Table 1.35)

IEM may be associated with endocrine dysfunction, the most frequent being disorders of carbohydrate metabolism (diabetes and hyperinsulinism) (▶Chap. 6). Diabetes may occur with iron overload, mitochondriopathies, and thiamine sensitive disorders. The clinical spectrum of some forms of IEM changes from hypoglycaemia in childhood to diabetes in adulthood. Mitochondriopathies can be associated with all types of endocrine disorders, the most frequent being diabetes and dysthyroidism. Hypothyroidism is encountered in mitochondriopathies, cystinosis, primary hyperoxaluria and the Allan-Herndon-Dudley syndrome (▶Sect. 8.9).

Long term consequences of IEM on fertility, reproduction and bone metabolism are still poorly understood and should be prospectively investigated.

Table 1.34 Cutis laxa and laxity, nodules, xanthoma, and miscellaneous

Cutis laxa, skin laxity	Xanthoma	Nodules, lipodystrophy and lipomatosis	Miscellaneous
Copper defects (▶Sect. 34.1) Menkes disease, occipital horn syndrome Proline synthesis defects (de Barsy syndrome): P5C-synthetase (cutis laxa type III), (▶Sect. 21.3) P5-phosphate reductase CDG syndromes (▶Chap. 43) *ATP6V0A2,* GALNT1-CDG Phospholipids synthesis defects *PTDSS1* (▶Sect. 35.3.3) *PI4K2A* (▶Table 35.1) GLUT 10 (▶Sect. 8.6) TALDO (transient) (▶Sect. 7.10)	Hyper lipoproteinemias (▶Chap. 36): Apo CII defect (eruptive) Apolipoprotein A1 defect (planar) **Autosomal dominant hypercholesterolaemia** Autosomal recessive hypercholesterolaemia Dysbetalipoproteinemia (hyperlipoproteinaemia type III) Hepatic lipase Lipoprotein lipase (eruptive) Sitosterolaemia (childhood) **Cerebrotendinous xanthomatosis** (▶Sect. 38.3) Niemann Pick A (▶Sect. 40.2.2)	**Nodules** PMM2-CDG syndrome (▶Chap. 43) Farber disease (▶Sect. 40.2.8) *PSMB8* (mutations in Proteasome) Gain-of-function of glutamine synthetase (erythematic subcutaneous nodules)	**Telangiectasias, purpuras, petechiae** **Ethylmalonic aciduria** (*ETHE1* mutations) (▶Sect. 20.9) Prolidase deficiency (▶Sect. 31.4.1)
			Pachydermia with seborrhoea and hyperhydrosis Hypertrophic osteoarthropathy: *SLC2A1* and *15PGDH* deficiencies (▶Sect. 42.5.2)
Laxity, dysmorphic scarring, easy bruising Ehlers-Danlos syndrome (16 types) of which *B4GALT7B-3GALT6* and *CHST14*		**Lipodystrophy and lipomatosis** Triglycerides synthesis defects (▶Sect. 35.2): Perilipin deficiency *AGPAT II* and *SEIPIN* mutations Phospholipids synthesis defects (▶Sect. 35.3): *PCYT1A* mutations (with SMD) *PIK3CA*-related overgrowth spectrum Mitochondrial defect: MERFF syndrome: multiple lipomas (▶Chap. 10) *POLR3A* variants (Wiedemann-Rautenstrauch syndrome)	**Hyper and hypo-pigmented skin maculae** (salt and pepper syndrome) GM3 synthetase deficiency (▶Sect. 40.1.6)
			Ulceration (skin ulcers) Prolidase deficiency (▶Sect. 31.4.1) HSAN type 1 (▶Sect. 40.1.1)
			Inflammatory dermatosis Sweet syndrome, Majeed syndrome (▶Sect. 35.1.4) Aplasia cutis congenita: *EOGT-CDG* Progressive reticular dys pigmentation: *POGLUT1-CDG* Salt and pepper syndrome: *ST3GAL5-CDG* H syndrome (*SLC29A3*[a] mutations) **Mastocytosis:** in encephalopathy due to *GNB1* mutations (G protein signalling pathway defect)

H syndrome: hyperpigmentation, hypertrichosis, and induration: SLC29A3 encodes the human equilibrative nucleoside transporter 3
SMD spondylometaphyseal dysplasia, *HSAN* Hereditary sensory and autonomic neuropathy

1

Table 1.35 Endocrine abnormalities

Pancreas	Thyroid/parathyroid and growth hormone	Adrenal/sex glands
Diabetes (and pseudodiabetes) Abnormal proinsulin cleavage Aceruleoplasminemia (▶Sect. 34.2.3.1) **Diabetes, deafness and TRMA syndrome** (▶Sect. 29.1.1) Diabetes type II: FAO? *Kir 6.2*, glucokinase (*GCK*) mutations (▶Chap. 6) **Hemochromatosis** (adult) *MCT1* mutations **MMA, PA, IVA, ketolytic defects)** (▶Chap. 18) Respiratory chain defects (▶Table 10.1) Untreated cystinosis Wolfram syndrome (▶Table 10.2) Woodhouse-Sakati syndrome (C2orf37) (▶Sect. 34.2.3.9)	**Hyperthyroidism** GlutarylCoA oxidase deficiency? Allan-Herndon-Dudley syndrome (peripheral thyrotoxicosis with high T3) (▶Sect. 8.9) **Hypothyroidism** Allan-Herndon-Dudley syndrome (low brain T3) (▶Sect. 8.9) Respiratory chain defect (▶Table 10.1) **Cystinosis** (▶Chap. 26) **Fabry disease** (▶Sect. 40.2.7) Selenoprotein defect (▶Sect. 34.5) Sphingosine-1-phosphate lyase insufficiency syndrome (▶Sect. 40.3.4) **Hypoparathyroidism** **LCHAD deficiency** (▶Sect. 12.1) Respiratory chain defect **Trifunctional enzyme deficiency** Kenny-Caffey syndrome (with primary bone dysplasia syndrome linked to autosomal dominant or recessive mutations in *FAM111A* or *TBCE* respectively) *STX16* Pseudohypoparathyroidism **Growth hormone deficiency** Respiratory chain defects	**Hypogonadism, sterility** PMM2-CDG **Galactosemia** *PLA2G6* mutation spectrum (▶Sect. 35.4.2) Kalman syndrome Perrault syndrome (several mitochondrial genes: *C10orf2*, *CLPP*, *HARS2*, *LARS2*, *HSD17B4*) (▶Table 10.2) D-bifunctional protein deficiency (▶Sect. 42.2.2) X-linked ALD (▶Sect. 42.2.1) **Fabry disease** **Cystinosis (males)** Alstrom disease **Hemochromatosis** (▶Sect. 34.2.1) Endosomal ferrireductase defect Selenoprotein defect 4H syndrome (▶Sect. 39.3.2) Several syndromes caused by vesicular trafficking defects (▶Table 44.2) Sphingosine-1-phosphate lyase insufficiency syndrome (SPLIS) Non-lysosomal β-glucosidase (GBA2) deficiency (testicular hypotrophy) (▶Sect. 40.3.1)
Hyperinsulinism (▶Chap. 6) ***SUR1 and KIR6.2* mutations** Glucokinase overactivity **GDH overactivity** **SCHAD deficiency** MCT1 overactivity		**Sexual ambiguity** Congenital adrenal hyper- and hypoplasia Disorders of adrenal steroid metabolism
		Salt-losing syndrome **Disorders of adrenal steroid metabolism** **FAO** (CPT II) (▶Sect. 12.1.1) Respiratory chain (mt DNA deletions)

GDH glutamodehydrogenase

Hypogonadism is almost constant in women with classic galactosaemia, frequent in CDG syndromes, cystinosis, and iron overload and in some complex lipids disorders such as in PNPLA 6 mutations spectrum (▶Sect. 35.4.4). Sphingosine Phosphate Lyase Insufficiency Syndrome (SPLIS) is a multisystem disorder responsible for several endocrinopathies (▶Sect. 40.3.4).

Many IEM may interfere with growth. Several involve various overlapping mechanisms, many of them involving the mechanistic target of rapamycin (mTOR) signalling pathway [89]. They can lead either to congenital short stature or severe failure to thrive that can be presenting sign. In almost all IEM short stature is associated with other signs such as visceral failure, metabolic disturbances (hypoglycaemia, acidosis, hyperlactataemia, hyperammonaemia, liver disturbances …), dysmorphic features, abnormal head circumference, immunohaematological findings or neurological involvement. There are many monogenic causes of isolated short stature that are beyond the scope of this chapter [90].

1.6.4 Gastroenterology and Nutritional Findings

Persistent anorexia, feeding difficulties, chronic vomiting, failure to thrive, frequent infections, osteopenia, and generalized hypotonia isolated or in association with chronic diarrhoea may be the presenting features in a number of IEM in infancy. They are easily misdiagnosed as cow's milk protein intolerance, celiac disease, chronic ear, nose & throat infections, late-onset chronic pyloric stenosis etc. Congenital immunodeficiencies are also frequently considered, although only a few presents early in infancy with this clinical picture [91].

There are two groups of IEM presenting with chronic diarrhoea and failure to thrive:

- Disorders of the intestinal mucosa or the exocrine function of the pancreas with almost exclusive intestinal effects, for example **congenital chloride diarrhoea, glucose-galactose malabsorption, lactase** and **sucrase-isomaltase deficiencies, abetalipoproteinemia type II (Anderson disease), enterokinase deficiency, acrodermatitis enteropathica** and **selective intestinal malabsorption of folate** and **vitamin B_{12}**, the latter also causing systemic disease. A new congenital diarrhoea disorder linked to mutations in *DGAT1* involved in triglycerides synthesis has been recently described (▶Sect. 35.2.1).
- Systemic disorders which also give rise to GI and nutritional abnormalities.

In clinical practice, these groups are sometimes very difficult to distinguish, because a number of specific intestinal disorders can give rise to various systemic clinical abnormalities and vice versa. There are also several metabolic phenocopies linked to chronic deficient intake in a specific essential nutrient (mostly vitamins), as summarized in ◘Table 1.36.

Abdominal Pain (Recurrent)

See acute symptoms section ▶Sect. 1.4.7 and ◘Table 1.10.

Acute Pancreatitis

- Hyperlipoproteinemia type I and IV, familial lipoprotein lipase deficiency (▶Table 36.2)
- **Lysinuric protein intolerance** (▶Sect. 25.3)
- **Organic acidurias** (MMA, PA, IVA, MSUD) (▶Sect. 18.1)
- Respiratory chain disorders (Pearson, MELAS) (▶Table 10.2)
- Citrin deficiency (▶Sect. 19.3.2)

Chronic Diarrhoea, Failure to Thrive, Osteoporosis

Hypocholesterolaemia

- **Abetalipoproteinemia type I and II** (▶Table 36.2)
- Chylomicron retention disorder (▶Table 36.2)
- PMM2-CDG (▶Sect. 43.2.1)
- Infantile Refsum disease (▶Sect. 42.2.9)
- Mevalonic aciduria (▶Sect. 37.1)
- Smith-Lemli-Opitz syndrome (▶Sect. 37.10)
- **Tangier disease** (alpha-lipoprotein deficiency) (▶Sect. 36.3)

HELLP Syndrome (Mothers Whose Babies have…)

- Carnitine palmityl transferase I deficiency (▶Sect. 12.1)
- LCHAD deficiency and other fatty acid β-oxidation disorders, (▶Sect. 12.2.3)
- Respiratory chain defects (▶Chap. 10).

Intestinal Obstruction

- MNGIE syndrome is a mitochondrial cytopathy due to mutations in thymidine phosphorylase and other mitochondrial genes (▶Table 10.2)
- Intestinal ulcerations
- Cytosolic *PLA2G4A* mutations (▶Sect. 42.5.1)

Inflammatory Bowel Disease

- Glycogenosis type Ib (▶Sect. 5.1.2)
- Mevalonate kinase (▶Sect. 37.1)
- Chronic granulomatosis (X-linked)
- SCID: ADA, RIPK1 (receptor-interacting serine/threonine kinase 1) … (▶Sect. 32.3)

Recurrent inguinal and umbilical hernia are a frequent finding in MPS I, II and VI (▶Sect. 41.2)

1.6.5 Haematology

Red Blood Cell Disturbances

Many IEM can cause anaemia (◘Table 1.37). Over 95% of macrocytic anaemias are secondary to acquired deficiencies of folate or vitamin B_{12}, but many IEM of vitamin B_{12} and folate metabolism also present with macrocytic anaemia (with the notable exception of MTHFR deficiency) (▶Chap. 28) and one thiamine transporter deficiency (▶Chap. 29). Haemolytic anaemias are due to deficiencies of glycolytic, glutathione oxidoreduction and pentose phosphate shuttle enzymes (some of which are

1

Table 1.36 Chronic diarrhoea, poor feeding, vomiting, failure to thrive

Leading symptoms	Other signs	Age of onset	Diagnosis (disorder/enzyme deficiency)
Severe watery diarrhoea, attacks of dehydration	Nonacidic diarrhoea, hypochloraemic alkalosis	Congenital to infancy	**Congenital chloride diarrhoea**
	Acidic diarrhoea, reducing substances in stools	Neonatal	**Glucose galactose malabsorption** (▶Sect. 8.1) **Lactase deficiency**
	Acidic diarrhoea, reducing substances in stools after weaning	Neonatal to infancy	**Sucrase isomaltase deficiency**
	Skin lesions, alopecia	Neonatal or post weaning	**Acrodermatitis enteropathica** (▶Sect. 34.6.1)
Protein losing enteropathy	Non bloody, watery diarrhoea	Neonatal	*DGAT* mutations (▶Sect. 35.2.1) Plasmalemma vesicle associated protein (*PVAP*)
	Cholangitis crisis	Infancy	**MPI-CDG (Ib),** ALG8- CDG (Ih), ALG6-CDG (Ic) (▶Sect. 43.2)
	Hypoglycaemia		PMM2-CDG (1a)
Fat-soluble vitamins malabsorption, severe hypocholesterolaemia Osteopenia, steatorrhea	Cholestatic jaundice	Neonatal to infancy	**Bile acid synthesis defects** (▶Chap. 38)
			Infantile Refsum (▶Sect. 42.2.9)
	Ichthyosis, keratodermia, deafness, MR		MEDNIK (▶Sect. 34.1.4)
	Hepatomegaly, hypotonia, retinitis pigmentosa, deafness	Infancy	Infantile Refsum
			PMM2-CDG (1a)
	Abdominal distension, ataxia, acanthocytosis, peripheral neuropathy, retinitis pigmentosa	Infancy	**Abetalipoproteinemia I and II** (no acanthocytes, no neurological sign in type II) (▶Table 36.2)
	Pancreatic insufficiency, neutropenia, pancytopenia	Early in infancy	Pearson syndrome (▶Table 10.2)
			Schwachman Diamond syndrome (*SBDS, DNAJC21, EFL1, SRP54*) (Ribosomopathies: ▶Chap. 39, ▶Table 39.1)

Table 1.36 (continued)

Leading symptoms	Other signs	Age of onset	Diagnosis (disorder/enzyme deficiency)
Severe failure to thrive, anorexia, poor feeding, with predominant hepatosplenomegaly	Severe hypoglycaemia, inflammatory bowel disease, neutropenia,	Neonatal to early infancy	**Glycogenosis type Ib** (no splenomegaly) (▶Sect. 5.1.2)
	Hypotonia, vacuolated lymphocytes, adrenal gland calcifications	Neonatal	**Wolman disease** (▶Sect. 36.3)
	Cardiomyopathy, retinopathy, micronystagmus	Infancy	Chylomicron retention disorder (no splenomegaly)
	Recurrent infections, inflammatory bowel disease,	Infancy	**Chronic granulomatosis** (X-linked)
	Megaloblastic anaemia, neuropathy, homocystinuria, MMA	1–5 years	**Intrinsic factor deficiency** (▶Sect. 28.1.1)
	Leuconeutropenia, osteopenia, hyperammonaemia, interstitial pneumonia,	Infancy	**Lysinuric protein intolerance** (▶Sect. 25.3)
	Recurrent fever, inflammatory bowel syndrome, hyper-IgD	Infancy	Mevalonate kinase (▶Sect. 37.1)
Recurrent infections Lymphopenia	Inflammatory bowel syndrome, intractable diarrhoea	Early infancy	**SCID** (adenosine deaminase, *RIPK1* mutations) (▶Sect. 32.3)
Severe failure to thrive, anorexia, poor feeding, with megaloblastic anaemia	Oral lesion, neuropathy, infections, pancytopenia, homocystinuria, MMA	1–2 years	**TC II deficiency** (▶Sect. 28.1.4)
			Intrinsic factor deficiency
	Stomatitis, peripheral neuropathy, infections, intracranial calcifications	Infancy	**Congenital folate malabsorption** (▶Sect. 28.3.1)
	Severe pancytopenia, abnormal marrow precursors, lactic acidosis	Neonatal	Pearson syndrome (▶Table 10.2)
Severe failure to thrive, anorexia, poor feeding, no significant hepato-splenomegaly, no megaloblastic anaemia	Severe hypoproteinaemia, putrefaction diarrhoea	Infancy	**Enterokinase deficiency**
	Diarrhoea after weaning, cutaneous lesion (periorificial), low plasma zinc	Infancy	**Acrodermatitis enteropathica**
	Ketoacidotic attacks, vomiting	Infancy	Organic acidurias (**MMA**, **PA**) (▶Sect. 18.1)
			Mitochondrial DNA deletions (▶Sect. 10.4.1)
	Vomiting, lethargy, hypotonia, hyperammonaemia	Infancy	**Urea cycle defects** (▶Chap. 19) (mainly OTC)
	Frequent infections, lymphopenia	Infancy	**Adenosine deaminase defect** (▶Sect. 32.3)
	Developmental delay, relapsing petechiae, orthostatic acrocyanosis	Infancy	*Ethe1* mutations (▶Sect. 20.9)
	Skin laxity, pili torti, hypothermia, hypotonia, seizures, facial dysmorphism		Menkes disease (▶Sect. 34.1.2) Occipital horn syndrome

Bold face: treatable disorders
MMA methylmalonic acidaemia, *PA* propionic acidaemia, *CDG* congenital disorder of glycosylation, *OTC* ornithine transcarbamylase, *MR* mental retardation

1

Table 1.37 Red blood cells disturbances

Acanthocytosis polycythemia	Anaemias: macrocytic	Anaemias: non macrocytic, haemolytic, congenital dyserythropoietic or due to combined mechanisms
Acanthocytosis Abetalipoproteinemia (▶Table 36.2) Panthothenate kinase deficiency (▶Sect. 34.2.3) Inborn errors of cobalamin **(Cbl C)** (▶Sect. 28.2.1.3) Wolman disease (▶Sect. 36.1) **Polycythemia** Inherited manganism (▶Sect. 34.4.1)	**Cobalamin metabolism defects** (▶Sects. 28.1 and 28.2) **Imerslund-Gräsbeck disease** **Intrinsic factor deficiency** **TC II deficiency** **Cbl C, Cbl D, Cbl E, Cbl F, Cbl G deficiencies** **Methionine synthase (CblG)deficiency** **Folate metabolism defects** (▶Sect. 28.3) Dihydrofolate reductase deficiency Glutamate formimino transferase deficiency **Congenital folate malabsorption** **MTHDF1-deficiency** **Others** **Hereditary orotic aciduria** (▶Sect. 32.3.6) Mevalonic aciduria (▶Sect. 37.1) Pearson syndrome (▶Table 10.2) (dyserythropoiesis) Respiratory chain disorders **Thiamine responsive megaloblastic anaemia** (▶Sect. 29.1.1) **Blackfan diamond anaemia** (▶Sect. 39.3.4) **Congenital methaemoglobinemia** (▶Sect. 1.5.1, ◘Table 1.14)	Abetalipoproteinemia (▶Table 36.2) Adenylate kinase deficiency (▶Sect. 32.3.4) Adenosine triphosphatase deficiency **Carnitine transport defect** **Congenital erythropoietic porphyria** (▶Sect. 33.2.3) **Erythropoietic protoporphyria** (▶Sect. 33.9) *SLC11A2* mutations (▶Sect. 34.2.2) Endosomal ferri reductase (STEAP 3) (▶Sect. 34.2.2) **Galactosaemia** (▶Chap. 14) **GLUT1DS (haemolysis triggered by exercise)** (▶Sect. 8.3) **Glycolytic and PPP deficiencies** (▶Chap. 7) Hemochromatosis (▶Sect. 34.2.1) IRIDA (▶Sect. 34.2.2) Lecithin cholesterol acyltransferase deficiency (▶Table 36.1) Majeed syndrome (dyserythropoietic) (▶Sect. 35.1.4) Mevalonic aciduria (▶Sect. 37.1) MLASA syndrome (▶Table 10.2) PNPO and PLP deficiency (▶Sect. 29.2) **Porphyrias (diverse types)** (▶Chap. 33) Glutathione synthetase deficiency (▶Sect. 31.3.2) Pyrimidine 5-nucleotidase deficiency (▶Sect. 32.3.7) *SEC23B-CDG* (congenital dyserythropoietic anaemia II) (▶Chap. 43) Severe liver failure (all causes) Sitosterolaemia (with stomatocytes) (▶Table 36.2) Transaldolase deficiency (▶Sect. 7.10) **Wilson disease** (▶Sect. 34.1.1) Wolman disease (▶Table 36.2)
		Sideroblastic anaemia **Isolated** (see also ▶Sect. 33.2) **X-linked sideroblastic anaemia (B_6 responsive)** (▶Sect. 33.1) *GLRX5* (iron sulfur cluster) (adults) **Syndromic:** Mitochondrial disorders Pseudouridine synthase 1 (*PUS1*) and mitochondrial tyrosyl-tRNA synthase (YARS2): MLSA syndrome (▶Table 10.2) Pearson syndrome (▶Table 10.2) MLSA plus syndrome: *MT-ATP6*, NDUFB*11* mutations *SLC25A38* mutations (▶Chap. 10) *TRNT1* mutations (▶Chap. 39)

associated with neurological signs) (▶ Chaps. 7 and 31), abnormal erythrocyte nucleotide metabolism (▶ Chap. 32) porphyrias (▶ Chap. 33), and disorders of lipid metabolism or hypersplenism. Sideroblastic anaemias are observed in mitochondrial disorders such as Pearson syndrome or mitochondrial tyrosyl-tRNA synthetase deficiency presenting with MLASA: myopathy, lactic acidosis, and sideroblastic anaemia syndrome (▶ Chaps. 10 and 39). The **pyridoxine-responsive anaemia** (or X-linked sideroblastic anaemia) presents in the second decade of life; 90% of patients are B_6 responsive (▶ Sect. 33.1). Microcytic anaemia is a prominent sign in five disorders with iron deficiency (▶ Sect. 34.2.2). Blackfan Diamond anaemias (many of them with congenital anomalies) belong to the newly classified group of ribosomopathies (▶ Sect. 39.3.5).

White Blood Cells Disturbances (Table 1.38)

Isolated neutropenia involve specific mechanisms as in Glycogenosis type B in a context of hypoglycaemia, frequent infections and colitis (▶ Sect. 5.1.2), in Dursun syndrome (G6PC3 deficiency) in a context of pulmonary arterial hypertension and cardiac abnormalities or in Barth syndrome in a context of cardiomyopathy (▶ Sect. 35.3.8).

Pancytopenia in IEM may result from many mechanisms some of them complex and not fully understood. Discounting 'peripheral' pancytopenia (or bicytopenia) linked to an exaggerated destruction of blood cells in

Table 1.38 White blood cells

Pancytopenia - thrombocytopenia - leucopenia	Vacuolated lymphocytes	Miscellaneous
Peripheral causes: All conditions with major hepato/splenomegaly causing hypersplenism (▶ Sects. 1.6.6 and 1.3.5): **Gaucher** disease type I and III (mostly anemia and thrombopenia), Niemann Pick disease type A and B, – MPS, MLP Oligosaccharidoses, Wolman disease	LSD Multiple sulfatase deficiency (▶ Sect. 41.2) Ceroid lipofuscinosis (▶ Sect. 40.5) I-cell disease (▶ Sect. 41.3) GM1-gangliosidosis (▶ Sect. 40.2.3) Niemann-Pick Ia (▶ Sect. 40.2.2) MPS (▶ Sect. 41.2) Pompe disease (▶ Sect. 5.2.3) Sialidosis (▶ Sect. 41.3) Wolman disease (▶ Sect. 36.1) Neutral lipid storage (Jordan anomaly) (▶ Sect. 35.2.3)	**Hyperleucocytosis (>100.000):** Leucocyte adhesion deficiency syndrome (*SLC35C1-CDG* (IIc): GDP fucose transporter 1) (▶ Table 43.2)
Isolated neutropenia Barth syndrome (myocardiopathy) (▶ Sect. 35.3.8) **Glycogenosis type Ib** (▶ Sect. 5.1.2) Dursun syndrome (G6PC3) (▶ Sect. 5.1.2)		**Pelger-Huet anomaly** (▶ Sect. 37.6) Lamilin mutations Mother of foetus with Greenberg lethal dwarfism (▶ Sect. 37.6) SOPH syndrome (▶ Sect. 44.3.2) *NBAS* mutations (▶ Sect. 44.3.2)
Pancytopenia, Bicytopenia **Cobalamin metabolism defects** (▶ Sects. 28.1 and 28.2) **Folate metabolism defects** (▶ Sect. 28.3) **Lysinuric protein intolerance** (▶ Sect. 25.3) **Organic acidurias (MMA, PA, IVA)** in acute attacks) (▶ Sect. 18.1) Pearson syndrome (▶ Table 10.2) Respiratory chain disorders Transaldolase deficiency (▶ Sect. 7.10) Adenylate kinase 2 deficiency (with deafness) (▶ Sect. 32.3.4) SCID: ADA, *RIPK*1 (lymphopenia) (▶ Sect. 32.3.1) Hemophagocytic lymphohistiocytosis (see right column) Ribosomopathies: Schwachman Diamond syndrome (*SBDS, DNAJC21, EFL1, SRP54*) (▶ Chap. 39) Trafficking disorders: Congenital neutropenia (*JAGN1, VPS45, WAS*), Cohen syndrome *DNM2* mutations (neutropenia with CMT) (▶ Chap. 44) *CDG: SLC35A-CDG (IIf)* (▶ Table 43.2)		**Hemophagocytic lymphohistiocytosis:** **Cobalamin C** **Gaucher disease** **Lysinuric protein intolerance** Niemann-Pick disease **Propionic acidaemia** **Methylmalonic aciduria** Trafficking disorders (▶ Table 44.2)

the spleen as in Gaucher disease, four groups of mechanisms leading to metabolic 'central pancytopenia' may be identified

1. Failure to make stem cells that turn into blood cells because of lack of appropriate availability of indispensable compounds to make nucleic acids such as B vitamins (B_1, B_{12}, Folate, B_6), purine/pyrimidines (several defects of which responsible for SCID), essential amino acids (BCAA, or Lysine in LPI), or defective energy supply (as in Pearson syndrome)
2. Fibrosis or scarring of bone marrow cells due to myelofibrosis, osteopetrosis, or aplastic anaemias like in ribosomopathies (such as Blackfan Diamond and Schwachman Diamond syndrome) (▶Sect. 39.3) or CDG and trafficking disorders leading to severe congenital neutropenia or Cohen syndrome (▶Sect. 44.3.2).
3. Immune system destroying healthy bone marrow cells as in hemophagocytic lymphohistiocytosis (HLH) where there is marked inappropriate and ineffective T cell activation that leads to an increased hemophagocytic activity. The T cell activated macrophages engulf erythrocytes, leukocytes, platelets, as well as their progenitor cells (▶Sect. 44.3.2). Several mechanisms may lead to HLH.
4. Suppression of bone marrow function due to illness or toxic coumponds like in acute episodes of organic acidurias where bone marrow suppression is an important concern and, diagnostic sign and is rapidly reversible on acute treatment (▶Sect. 18.1.1).

1.6.6 Hepatology

- **Cholestatic Jaundice and Cirrhosis (Table 1.39)**

- **Liver Failure (Ascites, Edema) See ▶Sects. 1.3.5) and 1.4.9), and Tables 1.6 and 1.13)**

Acute liver failure is defined as the rapid development of severe impairment of hepatic synthetic function including hypoalbuminaemia (responsible for ascites and oedema) and the development of coagulopathy (prolonged blood prothrombin time and/or a prolonged blood activated partial thromboplastin time). When acute neurologic symptoms are present it may mimic a Reye like episodes (▶Sect. 1.4.5). Less severe liver dysfunction includes abnormal biochemical markers of liver function (mostly alanine ALT and aspartate AST aminotranferases) but without clinical symptoms (no ascites, no haemorrhagic syndrome). In *TMEM 199* mutations the adolescent individuals presented with a mild phenotype of hepatic steatosis, elevated aminotransferases and alkaline phosphatase, hypercholesterolemia, low serum ceruloplasmin and abnormal N- and mucin-type O-glycosylation (▶Chap. 43, ▶Table 43.2).

- **Hepatomegaly and Hepatosplenomegaly Without Prominent Hepatic Dysfunction (▶Sect. 1.3.5)**

There are four mechanisms by which IEM can lead to hepatomegaly in paediatrics:

- Storage (glycogen, neutral lipids, complex lipids)
- Cholestasis, (bile retention)
- Fibrosis/cirrhosis, and
- Inflammatory and immune processes

A few clinical criteria allow an initial diagnostic approach:

- Consistency of the liver (rock hard: cirrhosis; firm to hard: fibrosis and cholestasis; soft to normal: storage with or without splenomegaly),
- Ultrasound findings (nodules, hyperechoic liver suggesting steatosis, others),
- Clinical context: coarse facies and dysostosis, neurological deterioration, failure to thrive and gastrointestinal signs, inflammatory, immunologic or hematologic signs, and hypoglycaemia.

A firm or rock-hard consistency may indicate **tyrosinemia type I, galactosaemia**, GSD type IV, severe neonatal hemochromatosis (▶Sect. 34.2.1.5), α_1-antitrypsin deficiency, **Wilson disease** (▶Sect. 34.1.1), **cystic fibrosis, Niemann-Pick and Gaucher disease** (▶Sect. 40.2).

When the liver consistency is normal or soft and there is associated splenomegaly (HSM), a LSD should be considered; coarse facies, bone changes, joint stiffness, ocular symptoms, vacuolated lymphocytes, and neurologic deterioration are strongly suggestive of the mucolipidoses (mannosidosis, ISSD, sialuria, sialidosis) and **MPSs I, II, VI and VII** (▶Chap. 41). Failure to thrive, anorexia, poor feeding, severe diarrhoea, hypotonia, hypothermia, and frequent infections are presenting signs in Niemann-Pick type A, Farber, Gaucher type II (▶Sect. 40.2), and Wolman diseases (▶Sect. 36.1), and also in chronic granulomatous disease, **intrinsic factor deficiency** (▶Sect. 28.1.1)**, GSD type Ib, lysinuric protein intolerance** (▶Sect. 25.3) and familial histiocytosis [92] (▶Chap. 39). HSM can be the only presenting sign in **Gaucher disease type I and in Niemann-Pick disease type B** (with asymptomatic interstitial pneumonia in the latter) and is observed in several lipoprotein disorders like apolipoprotein C-II, Apo AV, α & β LCAT deficiencies), or cholesteryl ester storage disorder (▶Chap. 36). HSM with liver failure in early infancy is the presenting sign of PMP deficiency due to *ABCD3* mutations (▶Sect. 42.2.6). **Familial lipoprotein lipase** presents with HSM, abdominal pain, xanthomas, acute pancreatitis and massive hypertriglyceridemia (▶Chap. 36, ▶Table 36.2). In late infancy or childhood, HSM associated with myoclonic jerks, ophthalmoplegia, and neurologic deterioration strongly suggest the late-onset forms of Niemann-Pick type C (▶Sect. 40.4) or subacute neu-

Table 1.39 Cholestatic jaundice and cirrhosis

Cholestatic Jaundice	Cirrhosis
Small molecule accumulation disorders causing intoxication (with metabolic marker)	
Arginase deficiency (▶Sect. 19.2) **Galactosemia** (▶Sect. 14.1) **Tyrosinemia type I** (▶Sect. 17.1)	**Arginase deficiency** **Argininosuccinate lyase deficiency** (▶Sect. 19.2) **Galactosaemia** **HFI** (▶Sect. 15.2) **Hypermanganesemia with dystonia** (▶Chap. 34). Hemochromatosis (▶Sect. 34.2.1) SAH hydrolase deficiency (▶Sect. 20.4) **Tyrosinemia type I** **Wilson disease** (▶Sect. 34.1.1)
Complex molecule catabolism, synthesis, or trafficking disorders (most with metabolic marker)	
α-1-antitrypsin deficiency **Bile acid metabolism disorders** (▶Chap. 38) Byler disease Cystic fibrosis CDG including COG 6 and 7-CDG (▶Table 43.2) N-Glycanase deficiency (▶Sect. 43.5.1) **Cerebrotendinous xanthomatosis** (▶Sect. 38.3) Cholesterol synthesis defects (Smith-Lemli-Opitz syndrome) (▶Sect. 37.10) Mevalonic aciduria (▶Sect. 37.1) **MEDNIK syndrome** (▶Sect. 34.1.4) MEGDHEL syndrome (filipin test) (▶Sect. 35.3.7) **Niemann-Pick type C disease (filipin test)** (▶Sect. 40.4) Peroxisomal disorders including ACOX2 and PMP 70 deficiency and methylacyl-CoA racemase deficiency (▶Sect. 42.2.4) *SCYL1* variants (Calfan syndrome with low gamma GT) (▶Sect. 44.3.2 and ▶Table 44.2)	α-1-antitrypsin deficiency CDG syndromes several types including **MPI-CDG (Ib)** (▶Sect. 43.2.2) Cholesterol ester storage disease (▶Sect. 36.3) Cystic fibrosis *FARSB* (▶Sect. 39.2.3 and ▶Table 39.1) **Gaucher disease** (▶Sect. 40.2.1) Glycogenosis type IV (▶Sect. 5.1.4) GPDH1 mutation (▶Sect. 35.1.1) Niemann-Pick disease (▶Sects. 40.2.2 and 40.4) Peroxisomal disorders (▶Chap. 42) *SCYL1* variant (Calfan syndrome) (▶Sect. 44.3.2) Wolman disease (▶Sect. 36.1)
Energy deficiency and other mechanisms	
CPT1 deficiency (late infantile to adult) (▶Sect.12.2.2) **LCHAD deficiency** (early infancy) (▶Sect. 12.2.3) Transaldolase deficiency (neonatal) (▶Sect. 7.10)	**LCHAD deficiency** Transaldolase deficiency Alpers progressive infantile poliodystrophy (▶Table 10.2)

ronopathic Gaucher type III diseases (▶Sect. 40.2.1). *CCDC 115* mutations may present with a storage-disease-like phenotype involving hepatosplenomegaly which regresses with age like several other trafficking disorders that involve vesicular, organelle and interorganelle trafficking including the trichohepatoenteric syndrome (▶Chap. 44, ▶Table 44.2).

When hepatomegaly is not associated with splenomegaly, three clinical circumstances should be considered. Situations with fasting hypoglycaemia suggest **GSD type I or type III** (in which the liver can extend down to the iliac crest) or **Fanconi-Bickel syndrome** (in which glycogenosis is associated with tubulopathy) (▶Sect. 8.5); these patients have a doll-like appearance and short stature. **FBPase deficiency** is considered when hypoglycaemia is associated with recurrent attacks of lactic acidosis triggered by fasting or by intercurrent infections (▶Sect. 15.3). In **argininosuccinic aciduria** there can be hepatomegaly and failure to thrive that can mimic hepatic GSD (▶Sect. 19.2).

Isolated hepatomegaly with a protuberant abdomen is a presenting sign of GSD type VI and IX but may be also the only presenting sign in GSD type III. It is also observed in the rare entities, cholesteryl ester storage disease, Tangier disease, (▶Chap. 36) and neutral lipid storage disorders (▶Sect. 35.2.3. Cytoplasmic glycerol 3 phosphate dehydrogenase 1 deficiency, presenting with isolated soft asymptomatic hepatomegaly and transient hypertriglyceridemia in infancy, has been recently described (▶Sect. 35.1.1).

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Paediatric Fatty Liver Disease [Non-alcoholic Fatty Liver Disease (NAFLD)]

Steatosis is defined by the presence of fat in hepatocytes when examined under light microscopy and can be classed as microvesicular or macrovesicular. It is highly suspected based on a hyperechoic liver ultrasound. The known causes of steatosis in children may be classified according to their typical clinical presentation [93]: (i) acute liver failure (▶Sects. 1.4.9 and 1.4.5); (ii) neonatal or infantile jaundice; (▶Sect. 1.3.5) (iii) hepatomegaly, splenomegaly or hepatosplenomegaly; (above) (iv) developmental delay/psychomotor retardation (▶Sect. 1.5) and perhaps most commonly v) the asymptomatic child with incidental discovery of abnormal liver enzymes (below).

Isolated Elevated Transaminases

A careful clinical and echographic (hyperehoic liver suggesting steatosis) evaluation is required. Always rule out a myopathy, haemolysis and a macrotransaminemia. Look at gamma-glutamyl transpeptidase. Search first for **Wilson disease** (after 3 years), alpha-1-antitrypsine deficiency, cystic fibrosis, **glycogenosis, Wolman disease and hereditary fructose intolerance**. Other causes are listed below in alphabetical order

- **Acute Intermittent Porphyria**
- **Bile acid synthesis defects (cholestasis with normal GGT) Including ACO2**
- **Bile acid conjugation defects**
- CDG syndromes (including congenital disorder of N-linked deglycosylation
- Chanarin-Dorfman syndrome
- Cellular trafficking disorders (*NBAS, RINT1 SCYL1*….)
- **Fatty acid oxidation defects**
- Glycerol-3-phosphate deshydrogenase deficiecy
- **Glycogenosis (mostly types VI and IX)**
- **Hemochromatosis**
- **Hereditary fructose intolerance and fructose biphosphatase deficiency**
- **Lipoprotein metabolism defects**
- **Lysosomal storage: Wolman, Gaucher, Niemann Pick disease type C and B**
- **Methionine demethylation defects**
- Mevalonate kinase deficiencg
- Mitochondrial aminoacyl t-RNA synthetases defects (*LARS, MARS, HARS*)
- Mitochondrial respiratory chain defects (mostly TRMU, ribonucleotide reductase, mt-DNA depletion
- Transaldolase deficiency
- **Urea cycle defects, citrin and HHH syndrome**
- **Wilson disease**

1.6.7 Immunology (See Also Neutropenia ◘Table 1.38)

The main immunologic manifestations of IEM are (i) Combined immunodeficiencies (CID, SCID) involving T and B cells (ii) phagocytes deficiencies involving polymorphonuclear, monocytes or mastocytes (iii) diseases of immune dysregulation and (iv) auto-inflammatory disorders.

Some disorders are restricted to the immune system while some other are associated with extraimmune manifestations like deafness, anaemia, dermatologic, osseous, or neurologic signs that may be preponderant.

Inflammatory Syndrome, Recurrent Fever

- Hyper-IgD syndrome and mevalonate kinase deficiency (▶Sect. 37.1)
- Aicardi Goutières syndrome (altered cytokine expression) (▶Sect. 39.1.2)
- Majeed syndrome (*LPIN2* mutations) (▶Sect. 35.1.4)
- **Fabry disease**: bouts of fever (▶Sect. 40.2.7)
- *PSMB8* mutations in proteasome: nodular erythema, muscular weakness and lipodystrophy
- *HOIL1/LUBAC* mutations: autoinflammation, immunodeficiency, and amylopectinosis
- *RBCK1* (E3 ubiquitin ligase) autoinflammation with recurrent episodes of sepsis,
- *COG7-CDG* (malignant hyperthermia)
- Tumour necrosis factor (TNF) receptor-associated periodic syndrome (TRAPS)
- Cryopyrin-associated periodic syndromes (CAPS)
- Histiocytosis-lymphadenopathy plus syndrome (*SLC29A3* mutations) (▶Chap. 39)

Macrophage Activating Syndrome, Haemophagocytosis

- **Gaucher disease**
- **Lysinuric protein intolerance**
- Niemann-Pick disease type A and B
- **Propionic acidemia**
- **Familial histiocytosis**
- ***TRMU* mutation (with transient infantile liver failure)**

Severe Combined Immune Deficiency (SCID)

As a predominant presenting sign (▶Chap. 32)

- **Adenosine deaminase 1 deficiency** (with costochondral abnormalities)
- **Purine nucleoside phosphorylase** (with hypouricemia and developmental delay)

- **Adenylate kinase 2** (reticular dysgenesis with deafness)
- Cytidine deaminase deficiency (autosomal recessive type II hyper-IgM syndrome)
- Transferrin receptor 1 deficiency
- *RIPK1* mutation (with early-onset inflammatory bowel disease, and progressive polyarthritis), lymphopenia and altered production of various cytokines.

As an associated finding:
- α-Mannosidosis
- **Hereditary orotic aciduria** (with megaloblastic anemia) (▶Chap. 32)
- VICI syndrome (▶Chap. 44)
- NRF2 superactivity
- **Folate and B_{12} disorders**(▶Chap. 28):
  - Hereditary folate malabsorption
  - Transcobalamin II deficiency
  - Methylene tetrahydrofolate dehydrogenase deficiency (MTHFD1)

Deletions of the *PLCG2* encoding phospholipase Cγ(2), an enzyme expressed in B cells, natural killer cells, and mast cells present with cold urticaria, immunodeficiency and autoimmunity (▶Chap. 35)

1.6.8 Myology

Many IEM can present with severe hypotonia, muscular weakness, and poor muscle mass. These include most of the late-onset forms of UCD and many OA. Severe neonatal hypotonia and progressive myopathy with or without nonobstructive idiopathic cardiomyopathy, can be the specific presenting findings in a number of inherited energy deficiencies; the most frequent conditions are mitochondrial RCD and other congenital hyperlactataemias, FAO defects, PBD, muscular GSD, alpha-glucosidase deficiency, and some other LSD (▶Sects. 1.3.3 and 1.3.7). Hypotonia, generalized weakness, reduced muscle mass and developmental delay are also the presenting features of the Allan-Herndon-Dudley syndrome (▶Sect. 8.9). Several defects of cytoplasmic triglycerides and phospholipids synthesis present with congenital progressive myopathy including choline kinase deficiency (▶Sect. 35.3.1). Severe neonatal hypotonia with elevated CK and brain dysfunction are major findings in most of the dolichol synthesis and recycling defects (▶Chap. 43). A congenital myasthenic syndrome pyridostigmine responsive can be a presenting sign in ALG2, ALG14, DPAGT1, GFPT1, and GMPPB-CDGs that bridges myasthenic disorders with dystroglycanopathies (▶Chap. 43). *ISPD* mutations (coding for isoprenoid synthase containing domain) are a common cause of congenital and limb girdle muscular dystrophy [94].

Exercise Intolerance, Myoglobinuria, Cramps, Muscle Pain, Elevated CK

See acute symptoms ▶Sect. 1.4.6.

Myopathy (Progressive)

There are many metabolic myopathies but only a few have an effective treatment. Disorders are listed in alphabetical order:
- Allan-Herndon-Dudley syndrome (monocarboxylate transporter 8 deficiency) (▶Sect. 8.9)
- Adenylate deaminase deficiency (▶Sect. 32.4.1)
- **Carnitine transport defect and fatty acid oxidation disorders**
- **Creatine synthesis defect linked to AGAT deficiency**
- Choline kinase deficiency (▶Sect. 35.3.1)
- CDG syndromes: **DPAGT1- CDG**, ALG14 –CDG and ALG 2-CDG (myasthenic syndrome),
- Dolichol synthesis defects (▶Chap. 43, ▶Table 43.4)
- **ETF, ETF dehydrogenase, FAD synthase and mitochondrial FAD transporter deficiencies** (▶Chap. 12)
- Glycogenosis type II (alpha glucosidase deficiency), Danon disease (LAMP-2) (▶Chap. 5)
- **Glycogenosis type III**, IV, 0b (muscle type), *AMPK* mutations
- Glycogenosis type V (dominant *PYGM* mutation in adult)
- *ISPD* mutations (isoprenoid synthase containing domain): limb girdle muscular dystrophy
- Neutral Lipid Storage Diseases: ATGL and CGI-58 Deficiencies (▶Sect. 35.2.3)
- Phosphoglucomutase deficiency (▶Sect. 43.4.6)
- *RBCK1* mutations (E3 ubiquitin ligase)
- Respiratory chain disorders (Kearns-Sayre, MLASA syndrome and others) (Table 14.2)
- Vici syndrome (Table 44.2)

1.6.9 Nephrology (Table 1.40)

Nephrolithiasis/nephrocalcinosis, polycystic kidneys, tubulopathy, abnormal urine colour/odour) are the main renal manifestations of IEM. Atypical Haemolytic Uremic Syndrome (HUS), nephrotic syndrome and tubulointerstitial nephropathy may also be presenting signs. DGKE mutations responsible for HUS with

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Table 1.40 Nephrology

Nephrolithiasis (stone composition) See also ▶Sect. 36.2, Nephrocalcinosis	Tubulopathy	Urines (colour, odour)	Miscellaneous
APRT deficiency (2–8 dihydroxy adenine) (▶Sect. 32.2.3) **Cystinuria** (cystine) (▶Sect. 25.1) Hereditary hyperparathyroidism (calcium) Hereditary renal hypouricemia (uric acid) **Hyperoxaluria type I and II** (oxalic acid) Lesh-Nyhan (uric acid) (▶Sect. 32.1.6) Molybdenum cofactor deficiency (xanthine) (▶Sect. 20.10) PRPP synthase superactivity (uric acid) RTA type I **XO** (xanthine) (▶Sect. 32.2.2) Familial juvenile hyperuricemic nephropathy (uromodulin) (▶Sect. 32.2.4) 5 Oxoprolinuria (▶Sect. 31.3.2)	**RTA type I / II** PC deficiency *SURF1* (with Leigh) **MMA** **GSDI** **CPT I deficiency** Dent disease CA II (proximal) Forkhead transcription factor *FOXI1* and H+ -ATPase (V-ATPase) (both with dRTA and early deafness)	**Abnormal odour** DMGHDH (fish) 3-MCG (cat) **GAII** (sweaty feet) **IVA** (sweaty feet) **MSUD** (maple syrup) **PKU** (musty odor) **TMAU** (fish) (▶Sect. 31.1) **Tyr I** (boiled cabbage) MAT I/III deficiency (▶Sect. 20.1) MTO defect (extraoral halitosis) (▶Sect. 20.2)	**Nephrotic syndrome**: Respiratory chain disorders (coenzyme Q synthesis defects) DGKE (▶Sect. 35.1.5) SPLIS (steroid resistant) **Sphingosine-1-phosphate lyase deficiency** (▶Sect 40.3.4) Disorders of pre-rRNA processing (dyskerin and NOP10 defects) (▶Sect. 39.3.3)
			Polycystic kidneys: CDG syndromes *PMM2* promotor mutations (HIPKD syndrome) **CPT II deficiency** GAII Zellweger syndrome
	Hypochloraemic alkalosis Bartter and Gitelman syndromes Congenital chloride diarrhea Hupra syndrome (▶Table 10.2)	**Abnormal colour** Alkaptonuria (black) Indicanuria (blue) Myoglobinuria (red) **Porphyria** (red) Beets lovers (red)	**Nephropathy (tubulo-interstitial)** : **GSD1** **MMA** RCD (pseudo Senior-Loken syndrome) X-prolyl aminopeptidase 3 (nephronophtisis like) Ciliopathies (*TMEM67*…)

CPT carnitine palmitoyl transferase, *DMGDH* dimethylglycine dehydrogenase, *GSD* glycogenosis, *GAII* glutaric aciduria type 2, *HUS* hemolytic uremic syndrome, *HFI* hereditary fructose intolerance, *HIPKD* hyperinsulinism polycystic renal disease, *MAT* **methionine S-adenosyltransferase,** *MCG* methylcrotonylglycinuria, *MMA* methylmalonic aciduria, *MTO* methane thiol oxidase, *dRTA* distal tub ular acidosis, *PC* pyruvate carboxylase, *RCD* respiratory chain disorder, *RTA* renal tubular acidosis, *SPLIS* sphingosine-1-phosphate lyase insufficiency syndrome (▶Chap. 40), *TMAU* trimethylaminuria, *XO* xanthine oxidase

nephrotic syndrome provides an interesting new mechanism of atypical HUS (▶Sect. 35.1.5).

Oxalurias and Oxalosis: Glyoxylate Detoxification Disorders

Primary hyperoxaluria type 1 (PH1) is a disorder of glyoxylate metabolism characterized by the accumulation of oxalate due to a deficiency of the peroxisomal hepatic enzyme L-alanine: glyoxylate aminotransferase (AGT) . The defect in AGT, which normally converts glyoxylate to glycine, results in an increase of the glyoxylate pool, which is converted to oxalate (poorly soluble) and glycolate (without associated pathology). Differential diagnosis includes primary hyperoxaluria type 2 (PH2), primary hyperoxaluria type 3 (PH3), Dent disease, and familial hypercalciuria-hypomagnesemia-nephrocalcinosis (▶Sect. 34.3) as well as secondary forms of hyperoxaluria (enteric hyperoxaluria, dietary hyperoxaluria), and idiopathic calcium oxalate urolithiasis. PH2 is due to mutations in *GRHPR* coding for a cytosolic enzyme with hydroxypyruvate reductase, glyoxylate reductase, and D-glycerate dehydrogenase catalytic activities. The enzyme has widespread tissue expression. PH3 is caused by mutations in *HOGA1* which codes for the mitochondrial enzyme 4-hydroxy-2-oxoglutarate aldolase 1.

Clinical presentation of PH1 is variable, ranging from occasional symptomatic nephrolithiasis to nephrocalcinosis and end-stage renal disease with systemic involvement. PH3 has a less severe course than PH1 or PH2 and may be silent. Diagnosis of PH1-3 is suspected on clinical features along with pure calcium oxalate monohydrate stone composition and confirmed by urine

oxalate: creatinine ratio, L-glycerate excretion, molecular genetic testing and infrequently by enzyme catalytic activity from liver biopsy. In a proportion of patients with primary hyperoxaluria type 1, treatment with pyridoxine (vitamin B_6) may decrease oxalate excretion and prevent kidney stone formation [95].

1.6.10 Neurology and Psychiatry

See ▶Sects. 1.3.1, 1.3.2, and 1.4.3.

1.6.11 Ophthalmologic Signs

See also neuro-ophthalmologic signs, ▶Sect. 1.5.7.

Cataracts

Congenital cataracts may occur as isolated defects or may be associated with other anterior chamber developmental anomalies such as microphthalmia or aniridia. Both the structure and stability of the lens crystallins and maintenance of strong cellular homeostatic systems are necessary to maintain normal lens function. Recent evidence suggests that newly synthesized proteins might be present in the lens nucleus, and that central nuclear fibre cells, largely restricted to glycolysis as an intrinsic energy source, receive metabolic support from the anterior epithelium through circulation of fluid. As lens crystallins make up over 90% of soluble lens proteins, their short-range ordered packing in a homogeneous phase is important for the maintenance of lens transparency [96]. Most cataracts caused by IEM are syndromic although some of them may appear as presenting sign or even remained isolated. The pathophysiology, involving intoxication mechanism (as in galactosaemias), lipid membrane disturbances or energy process required for maintenance of lens crystallins transparency, is still poorly understood. The recent description of *SLC7A8* mutations in congenital cataracts highlights the role of transporters required to import all amino acids involved in lens cell metabolism and in particular also those required for the synthesis of the tripeptide glutathione (GSH), which is a vital antioxidant known to be important for the preservation of lens transparency [97] (▶Chap. 25).

Metabolic causes of cataracts according to age of onset (◘Table 1.41).

Corneal Clouding (◘Table 1.42)

Ectopia Lentis (Dislocation of the Lens)

- **Classical homocystinuria** (▶Sect. 20.6)
- Sulfite oxidase deficiency (▶Sect. 20.11)
- Marfan syndrome
- Marchesani syndrome

Keratitis with Corneal Opacities

These are presenting signs of two treatable disorders:

- **Tyrosinemia type II**
- **Fabry disease (X-linked)**
- IFAP syndrome (AD or X-linked both with photophobia) (see ◘Tables 1.29, 1.30 and ▶Sect. 37.11)

Miscellaneous

- Alacrimia: N-glycanase 1 deficiency (▶Chap. 43)
- Conjunctivitis, blepharitis: **Acrodermatitis enteropathica, cystinosis, tyrosinemia type II, PA, MCD**
- Colobome: **Familial hypomagnesemia** (▶Chap. 39), trafficking disorders (*ACTB, TBC1D23, PACS1*) (▶Table 44.2)
- Microcornea: Ehlers Danlos type IV
- Macular telangiectasia type 2: Serine Palmitoyltransferase (subunit 1 or 2) (▶Sect. 40.1.1)

1.6.12 Orthopaedic Signs (◘Table 1.43)

The updated nosology of genetic skeletal disorders comprises 461 disorders classified within 42 different groups [98]. Of these, many are caused by IEM.

- Multiplex dysostosis is a characteristic hallmark of a number of LSD (MPS, oligosaccharidosis and mucolipidosis) (▶Chap. 41). Conversely glycosaminoglycans (GAG) synthesis defects (many of them classified in O-glycosylation disorders) (▶Chap. 43) display major clinical features that include short long bones, joint dislocations or laxity and scoliosis and additional suggestive features such as skin laxity with or without atrophy and blue sclerae [99] (▶Chap. 41).
- Severe osteopenia is a major feature in many treated or untreated IEM.
- Bone dysplasia (primary or syndromic) is a preponderant sign in complex lipid synthesis and remodelling defects. These comprise plasmalogen and peroxisomal defects (mostly presenting with rhizomelic chondrodysplasia punctata) (▶Sect. 42.1), most cholesterol synthesis defects with polymalformative syndromes, (▶Chap. 37), and phospholipid (PL) metabolism disorders (▶Chap. 35). PL disorders may present as (1) hyperostotic dwarfism as in Lenz Majewski syndrome, (2) Pure spondylometaphyseal dysplasia (SMD), as in opsismodysplasia or syndromic SMD associated with cone rod dystrophy, (*PCYT1A, PLCB3*, *PISD* mutations: Liberfarb syndrome), or sphingomyelin synthase 2 deficiency (▶Sect. 40.1.9). Many trafficking disorders including

Table 1.41 Cataracts

Categories of disorders	Detectable at birth (<1 month) (Congenital)	Detectable early in infancy (<1 years)	Detectable in childhood / adolescence (1–15 years)	Detectable in adulthood (>15 years)
Carbohydrate metabolism	**Galactosemias** (▶ Chap. 14) Marginal maternal galactokinase deficiency Peripheral epimerase deficiency	Galactitol or sorbitol accumulation of unknown origin **Galactokinase deficiency** Hypoglycaemias	**Diabetes mellitus**	**Heterozygotes for GALT and galactokinase deficiency** **Lactose malabsorption**
Cholesterol Fatty acids Complex lipids Peroxisome disorders	SLS (may be isolated) (▶ Sect. 42.4.5) PSD deficiency (▶ Sect. 35.3.5) *SLC7A8* (▶ Sect. 25.7) GBA2 II deficiency (▶ Sect. 40.3.1) Acetyl CoA carrier defect (*SLC33A1*) (▶ Sect. 34.1.4) Conradi-Hunermann syndrome and CYP51A1 (▶ Sects. 37.5 and 37.8.1) PBD defects (▶ Sect. 42.2.9) PGDH deficiency (▶ Sect. 24.2) RCDP (▶ Sect. 42.1) SLO syndrome (▶ Sect. 37.10) Sengers syndrome (may be isolated) (▶ Sect. 35.3.6)	Steroid 5-α-reductase 3 deficiency Chanarin-Dorfman syndrome (▶ Sect. 35.2.3) Sterol-C4-methyl oxidase deficiency (▶ Sect. 37.7) ELOVL4 deficiency (Stargartd macular dystrophy) (▶ Sect. 42.4.8) AD fatty acid oxidoreductase (de novo *FAR1* variants) (▶ Sect. 42.1.4)	Chanarin Dorfman syndrome (▶ Sect. 35.2.3) Mevalonic aciduria (▶ Sect. 37.1) PHARC syndrome (▶ Sect. 35.4.1) SLS (retinal crystalline inclusions (white glistening dots) (▶ Sect. 42.4.5)	*PEX 7* mutations (▶ Sect. 42.1.1) Refsum disease (▶ Sect. 42.3) **Cerebrotendinous xanthomatosis** (▶ Sect. 38.3) Mevalonate kinase defect (▶ Sect. 37.1) **Fabry disease** (▶ Sect. 40.2.7) **Tangier disease** (▶ Sect. 36.3)
Complex molecules and trafficking disorders (▶ Chap. 44)	B4GALNT1 (rare) Cockayne syndrome Lowe syndrome Vici syndrome *ATAD3* duplication	Alpha-mannosidosis (▶ Sect. 41.3) Sialidosis (▶ Sect. 41.3)		Carriers for Lowe syndrome Steinert dystrophy (cataract can be presenting sign)
Miscellaneous Including IEM disturbing redox state in lens	GSH reductase deficiency (▶ Sect. 31.3.6) Glutamine synthetase overactivity (▶ Sect. 24.1)	P5C synthetase deficiency (▶ Sect. 21.3) Respiratory chain disorders (▶ Chap. 10)	**Hypoparathyroidism** LPI (▶ Sect. 25.3) **Wilson** disease (▶ Sect. 34.1) Dominant cataract with high serum ferritin	G6PD deficiency **Homocystinurias** (▶ Sect. 20.6, ▶ Sects. 28.2.1, 28.2.3, 28.3.7) Mitochondrial cytopathies **OAT deficiency** (▶ Sect. 21.1)

AD autosomal dominant, *G6PD* glucose-6-phosphate dehydrogenase, *GALT* galactose uridyl transferase, *GBA2* non-lysosomal β-Glucosidase**,** *GSH* glutathion, *LPI* lysinuric protein intolerance, *RCDP* rhizomelic chondrodysplasia punctata, *OAT* ornithine amino transferase, *PGDH* phosphoglycerate dehydrogenase, *PSD* phosphatidyl-serine decarboxylase, *SLO* Smith-Lemli-Opitz, *PBD* peroxysome biogenesis defects, *P5C* Δ1-pyrroline 5-carboxylate, *SLS* Sjogren- Larsson syndrome

Table 1.42 Corneal clouding: mostly lysosomal storage disorders

Visible in early infancy (3–12 months)	Visible in late infancy to early childhood (1–6 years)	Visible in late childhood, adolescence to adulthood
Tyrosinemia type II (presenting sign) (▶ Sect. 17.3) **Cystinosis** (presenting sign) (▶ Chap. 26) **Hurler, Scheie** (MPS I) and Maroteaux-Lamy (MPS VI) (▶ Sect. 41.2) I-cell disease (▶ Sect. 41.3) Steroid sulfatase deficiency	Mucolipidosis type IV (presenting sign) (▶ Sect. 41.3) Alpha-mannosidosis defect (late-onset form) (▶ Sect. 41.2) Lecithin cholesterol acyl-transferase deficiency (▶ Sect. 32.3) Morquio disease (MPS IV) (▶ Sect. 41.2) Pyroglutamic aciduria (presenting sign) (▶ Sect. 31.3.2) **Tangier disease** (▶ Sect. 32.3)	**Fabry disease** (X-linked) (▶ Sect. 40.2.7) Galactosialidosis (juvenile form) (▶ Sect. 41.3) **Wilson disease** (green Kaiser-Fleischer ring) also observed in cholestatic liver disease (▶ Sect. 39.1.1)

MPS mucopolysaccharidosis

Table 1.43 Orthopaedic presentations

Bone dysplasias	Punctate epiphyseal calcifications Chondrodysplasia punctata	Exostosis and hyperostosis
Cholesterol defects (▶ Chap. 37) Phospholipids defects (▶ Chap. 35) GAG synthesis defects (▶ Sect. 41.1) Many trafficking disorders (▶ Chap. 44) Mitochondrial disorders (*AIFM1* mutation, mt chaperonopathies such as even-plus and CODAS syndromes) Miscellaneous (prostaglandins, purines, ribosomopathies (▶ Chap. 39), Glutathione peroxidase 4 **Lysosomal storage disorders** (Dysostosis multiplex) (▶ Chap. 41)	Cholesterol synthesis disorders (▶ Chap. 37) X-linked dominant chondrodysplasia punctata (Conradi-Hunermann syndrome) Child syndrome X-linked steroid sulfatase deficiency Peroxisome disorders (▶ Chap. 42) Peroxisomal RCDP types I, II, III (with RCDP) PBD disorders Familial resistance to thyroid hormone Betaglucuronidase deficiency Spondylo enchondromatosis Warfarin embryopathy	**Exostosis** O-glycosylation defects (EXT1-EXT2) **Hyperostosis** Hyperphosphatemic hyperostosis syndrome (GALNT3-CDG) Osteopetrosis (ARO) T-cell immune regulator1 (*TCIRG1*) Osteopetrosis (ADO2) chloride channel 2 (*CLNC2*) Dysosteosclerosis (DOS) nucleoside transporter 3 *SLC29A3* (Histiocytosis lymphadenopathy plus syndrome) Carbonic anhydrase 2 (with dRTA and cerebral calcifications Lenz Majewski syndrome (▶ Sect. 35.3.3)
Overgrowth: Phospholipid defects: PIK3CA-related overgrowth spectrum (▶ Chap. 35)	**Osteopenia** Small molecules defects: **Galactosemia** (long term) **Homocystinurias** (▶ Fig. 20.2) **LPI** (▶ Sect. 25.3) **All organic acidurias (long term)** Complex molecule defects: **CTX** (▶ Sect. 38.3) CDG (▶ Chap. 43) **GSD type I** (▶ Sect. 5.1.2) **Gaucher type 1** (▶ Sect. 40.2.1) Sphingomyelin synthase 2 (▶ Sect. 40.1.9) I-cell disease (MLP II) (▶ Sect. 41.3) Infantile Refsum disease (▶ Sect. 42.2.9)	**Bone infarction and inflammatory presentations** **Gaucher type 1** (▶ Sect. 40.2.1) Majeed syndrome (Lipin 2/3 mutations (▶ Sect. 35.1.4) Farber disease (pseudo arthritis) (▶ Sect. 40.2.8) Aicardi Goutières syndrome (chronic progressive deforming arthropathy) (▶ Sect. 39.1.2)
Carpal tunnel syndrome MPS VI (▶ Sect. 41.2) Mucolipidosis type III (▶ Sect. 41.3)		

CTX cerebrotendinous xanthomatosis, *LPI* lysinuric protein intolerance, *MLP* mucolipidosis, *GSD* glycogenosis, *PBD* peroxisome biogenesis defects, *RCDP* rhizomelic chondrodysplasia punctata

the new group of Golgipathies present with spondylometaphyseal dysplasia, such as the Dyggve-Melchior-Clausen syndrome linked to Dymeclin mutations (▶Chap. 44).
- The molecular aetiology of somatic overgrowth syndromes with lipomatosis has been recently clarified and allowed the clinical delineation and natural history of the PIK3CA-related overgrowth spectrum (◘ Table 35.1).
- Osteopetrosis characterized by sandwich vertebrae sign [100] is observed in a few metabolic disorders. Generalised increase in bone density and wormian bones of the skull are seen in pycnodysostosis (▶Sect. 41.3).
- Bone infarction may be a presenting sign in **Gaucher** type I and III disease and a major therapeutic concern. It is also observed in Majeed syndrome (recurrent aseptic osteomyelitis) (▶Sect. 35.1.4.2). Bone cysts may be seen in congenital lipodystrophy (▶Sect. 35.2.2). The presentation of some patients with Farber disease mimics juvenile idiopathic arthritis.

Many other IEM may display various bone abnormalities the pathophysiology of which remains poorly explained. Some of them are distinctive syndromes like primary hypertrophic osteoarthropathy linked to prostaglandin defects (▶Chap. 42), or the nail cartilage syndrome linked to ribosomopathy (▶Chap. 39).

In others IEM bone abnormalities are only accompanying signs as in about half of the patients with adenosine deaminase deficiency (▶Sect. 32.3.1).

1.6.13 Pneumology

- **Hyperventilation Attacks**
- Hyperammonaemias
- Joubert syndrome
- Leigh syndrome in acute attacks (due to many inborn errors)
- Metabolic acidosis
- Rett syndrome (girls only)

- **Interstitial Pneumopathy Is a Frequent Complication and May Occasionally Be the Presenting Sign in the Following Disorders**
- **Gaucher disease** (▶Sect. 40.2.1)
- **Lysinuric protein intolerance** (▶Sect. 25.3)
- **Niemann-Pick disease type B** (▶Sect. 40.2.2)
- **CblC deficiency** (▶Sect. 28.2.1)
- *MARS* mutations (methionyl t-RNA synthetase) (▶Sect. 39.2.3)
- *FARSB* (▶Sect. 39.2.3)

- **Stridor**
- **Biotinidase deficiency** (▶Sect. 27.1.2)
- Hypocalcemia
- Hypomagnesemia
- **MADD** (riboflavin responsive) (▶Sect. 12.1.1.4)
- Pelizaeus-Merzbacher
- Farber disease (hoarseness) (▶Sect. 40.2.8)

- **Pulmonary Hypertension**
- Dursun syndrome (*G6PC3* mutations) with neutropenia (▶Sect. 5.1.2)
- **Glycogenosis type I** (▶Sect. 5.1.2)
- Non ketotic hyperglycinemia and lipoate disorders (▶Chap. 23)
- HUPRA syndrome (with hyperuricaemia (▶Sect. 1.4.16 and ▶Chap. 39)
- Gaucher disease (▶Sect. 40.2.1)
- Mitochondrial disorders: *NFU1, LIPT1, TMEM70* mutations (▶Chap. 10)
- *FARSB* mutation

1.6.14 Psychiatry

(See ▶Sect. 1.4.3 and ◘ Table 1.8, and ▶Sect. 1.5.2 and ◘ Fig. 1.11)

1.6.15 Rheumatology

- **Arthritis – Joint Contractures – Bone Necrosis**
- Aicardi Goutières syndrome (chronic progressive deforming arthropathy with chilblains and contractures) (▶Sect. 39.1.2)
- Alkaptonuria (▶Sect. 17.6)
- *ANKH* mutations (progressive ankylosis with deafness, mental retardation and hypophosphataemia)
- Familial gout
- Farber disease (▶Sect. 40.2.8)
- **Gaucher disease type I** (▶Sect. 40.2.1)
- **Homocystinuria** (▶Sect. 20.6)
- I-Cell disease, mucolipidosis type III (▶Sect. 41.3)
- Lesch-Nyhan syndrome (▶Sect. 32.1.6)
- Mevalonic aciduria (recurrent crisis of arthralgia) (▶Sect. 33.1)
- Mucopolysaccharidosis type I S (▶Sect. 41.2)
- *NT5E* mutations and arterial and joint calcifications
- PRPP synthetase, HGPRT defects (▶Sect. 32.1)
- Uromoduline mutation (familial hyperuricaemic nephropathy)
- Majeed syndrome (*LPIN2*) (▶Sect. 35.1.4)
- *RIPK1* mutations (progressive polyarthritis)

- **Pain in Extremities**

Bone Crisis: See ▶Sect. 1.4.10).

1.6.16 Stomatology

- **Glossitis, Stomatitis, Mouth Ulcers**
  - **Folate and Cobalamin disorders** (▶Chap. 28)
  - CblF deficiency
  - **Folate malabsorption**
  - **Intrinsic factor deficiency**
  - **Transcobalamin II**
  - **Riboflavin deficiency** (▶Sect. 12.2)
  - Aicardi Goutières syndrome (▶Sect. 39.1.2)
- **Macroglossia (and Thickening of the Lips)**
  - Beckwith-Wiedemann syndrome
  - Congenital muscular dystrophies (DMC1C)
  - Complex IV deficiency
  - Pompe disease (▶Sect. 5.2.3)
  - **Hurler syndrome** (MPS 1S) and to a lesser degree several other MPSs (▶Sect. 41.2)
  - GM1 gangliosidosis (▶Sect. 40.2.3)
- **Delayed Dentition and Hypodontia**
  - Leucoencephalopathy with ataxia
  - POLR3-related leukodystrophy and 4H syndrome (■Table 1.23) (▶Sect. 39.3.2)
  - Heimler syndrome (*PEX 1,6* mutations): enamel hypoplasia (▶Sect. 42.2.9)
  - **Congenital erythropoietic porphyria (erythrodontie)**
  - Pycnodysostosis (▶Sect. 41.3)

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Inborn Errors of Metabolism in Adults: A Diagnostic Approach to Neurological and Psychiatric Presentations

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Contents

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2

Introduction

Late-onset forms of IEM presenting initially in adulthood are often unrecognised, so that their exact prevalence is unknown [1]. Most often they have psychiatric or neurological manifestations, including atypical psychosis or depression, unexplained coma, peripheral neuropathy, cerebellar ataxia, spastic paraparesis, dementia, movement disorders and epilepsy [2–5].

Physicians caring for adult patients with IEM are also involved in the management of those with early onset forms who reach adulthood. The transfer of such patients from paediatric to adult care raises a number of medical, dietetic and social concerns.

A further important issue is the diagnosis of adult patients who had their first clinical signs in childhood but for whom the diagnosis was missed, either because IEM were not considered or because the disease or its mild clinical form had not been described at that time.

2.1 Differences Between Paediatric and Adult Phenotypes

Adults' physicians who want to specialise in IEM are faced with the fact that, with the exception of several review articles, most existing books and diagnostic algorithms refer to paediatric forms of these diseases. Late-onset forms of IEM tend to display attenuated phenotypes, which in some instances are associated with one or more clinical manifestations that differ from the classic clinical picture described in children. Phenotypic severity and age of onset are expected to be modulated by differences in residual protein activity possibly driven by various genetic factors [6]. Phenotypic variability may also occur in the context of similar protein expression, which suggests the intervention of environmental, ontogenic, and aging factors [6].

Table 2.1 gives some examples of differences between childhood and adult onset presentations.

Table 2.1 Phenotypic differences between childhood-onset and adult-onset forms of IEM

Disease	Classic presentation in childhood	Adult-onset forms
AGAT deficiency	Psychomotor delay, severe language impairment, failure to thrive and autistic-type behaviour	Mild intellectual disability with myopathy
AMACR deficiency	Neonatal cholestasis, intellectual disability, retinitis pigmentosa	Recurrent encephalopathy, cerebellar ataxia, polyneuropathy, rhabdomyolysis
α-Mannosidosis	Intellectual disability, deafness, upper airways infections, dysmorphic features	Episodes of psychosis, confusion, cerebellar ataxia, posterior leukoencephalopathy
Biotinidase deficiency	Hypotonia, lethargy, grand mal and myoclonic seizures, ataxia, stridor, skin lesions, deafness	Bilateral optic atrophy, spastic paraparesis, motor neuropathy
Cerebral glucose transporter (GLUT1) deficiency	Epilepsy, psychomotor delay, ataxia, acquired microcephaly, complex motor disorder (gait dyspraxia, chorea, dyskinesias)	Isolated seizures, exercise-induced dyskinesia, dystonia
Cobalamin C deficiency	Progressive encephalopathy, abnormal movements, epilepsy, coma, multisystem pathology (renal failure, hepatic dysfunction, cardiomyopathy), retinopathy, macrocytosis	Psychiatric problems, confusion, subacute myelopathy, peripheral neuropathy, thromboembolic events, isolated haemolytic uremic syndrome or isolated pulmonary hypertension
Coenzyme Q10 deficiency	Leigh syndrome, myoglobinuria, encephalopathy	Cerebellar ataxia, seizures, myopathy
Cerebrotendinous xanthomatosis	Intellectual disability, chronic diarrhoea, epilepsy, childhood onset cataract, neonatal cholestasis	Tendon xanthomas, cerebellar ataxia, spastic paraparesis, dementia, psychiatric signs
Cystathionine β-synthase deficiency	Intellectual disability, marfanoid habitus, epilepsy, autism, lens dislocation, scoliosis	Strokes (internal carotid dissection), deep vein thrombosis, psychiatric disorders
Fatty acid β-oxidation defects	Non-ketotic hypoglycaemia, cardiomyopathy, liver disease, rhabdomyolysis, peripheral neuropathy, retinitis pigmentosa (LCHAD)	Rhabdomyolysis, proximal myopathy, isolated cardiomyopathy (VLCAD)
Fabry disease	Crises of acroparaesthesia	Strokes, vertigo, cardiomyopathy, hearing loss, proteinuria
GAMT deficiency	Epilepsy, movement disorders, intellectual disability, behavioural problems	Isolated myopathy

Table 2.1 (continued)

Disease	Classic presentation in childhood	Adult-onset forms
Glutaric aciduria type 1	Encephalopathic crises with movement disorders, bilateral lesions of basal ganglia, dystonia and chorea	Leukoencephalopathy with subependymal nodules, spastic paraparesis, cephalalgia, dysexecutive syndrome, peripheral neuropathy
Glycogenosis type IV (glycogen branching enzyme deficiency)	Neuromuscular form, combined hepatic and myopathic form	Polyglucosan body disease: Spastic paraparesis, peripheral neuropathy, leukoencephalopathy with spinal cord atrophy
GM1 gangliosidosis	Dysmorphic features, organomegaly, macular cherry red spot, progressive spasticity, seizures, decerebrate posturing	Generalised dystonia, parkinsonism, dysarthria, kyphoscoliosis, vertebral and hip dysplasia. MRI: High signal of posterior putamen
GM2 gangliosidosis	Motor weakness, visual loss, progressive spasticity, macular cherry red spot, epilepsy	Psychosis, lower motor neuron disease, cerebellar ataxia, dystonia, sensory neuronopathy
Krabbe disease	Progressive encephalopathy, hyperaesthesia, tonic spasms, signs of peripheral neuropathy, blindness, loss of bulbar function, seizures	Spastic paraparesis with or without peripheral neuropathy, specific leukoencephalopathy involving cortico-spinal tracts
Lesch-Nyhan syndrome	Severe generalised dystonia, cognitive disability and self-injurious behaviour	Isolated dystonia, mild cognitive or behavioural problems
L-2-Hydroxyglutaric aciduria	Seizures, progressive ataxia, spasticity, intellectual disability, progressive macrocephaly, leukoencephalopathy with cerebellar atrophy	Epilepsy, progressive dystonia and parkinsonism, leukoencephalopathy involving the subcortical white matter, malignant brain tumours
MERRF	Myoclonic epilepsy, generalised epilepsy, cerebellar ataxia	Cerebellar ataxia, hearing loss, peripheral neuropathy, lipomatosis
Metachromatic leukodystrophy	Progressive gait problems, hypotonia, peripheral neuropathy, spasticity in all four limbs, optic atrophy, cerebellar ataxia	Psychiatric form: Psychosis-like features (mimics schizophrenia), cognitive decline Motor form: Spastic paraparesis, cerebellar ataxia, dystonia, demyelinating polyneuropathy
3-Methylglutaconyl-CoA hydratase deficiency	Intellectual disability and/or motor delay, movement disorders, febrile seizures	Ataxia, dementia, optic atrophy, spasticity, leukoencephalopathy
MTHFR deficiency	Progressive encephalopathy with apnoea, epilepsy, microcephaly	Psychiatric disorders, spastic paraparesis, thromboembolic events, polyneuropathy
Neurotransmitter defects (dopamine synthesis)	Intellectual disability, oculogyric crises, abnormal movements (dystonia-parkinsonism)	Focal or generalised dopa-responsive dystonia or parkinsonism
Niemann-pick disease type C	Liver disease, hypotonia, psychomotor delay, epilepsy, spasticity, ataxia, cataplexy, vertical supranuclear gaze palsy	Psychosis, cognitive decline, cerebellar ataxia, vertical supranuclear gaze palsy, dystonia, isolated splenomegaly
Non ketotic hyperglycinemia	Epilepsy with suppression bursts, encephalopathy	Paroxysmal choreic movement disorders, confusion triggered by fever, intellectual disability with aggressive behaviour
PDH deficiency	Lactic acidosis, corpus callosum agenesis, Leigh syndrome, polyneuropathy	Episodic ataxia triggered by fever, optic neuropathy, MRI can be normal
Peroxisome biogenesis defects	Mental retardation, liver disease, deafness, cerebral malformations, dysmorphic features (high forehead, epicanthic folds), skeletal abnormalities, retinopathy, cataracts, seizures	Various combinations of peripheral neuropathy, spastic paraparesis, cerebellar ataxia, hearing loss, retinitis pigmentosa, leukoencephalopathy
Phenylketonuria (untreated)	Intellectual disability, autistic behaviour, seizures, movement disorders	Spastic paraparesis, optic atrophy, dementia, parkinsonism
POLG mutations	Severe encephalopathy with intractable epilepsy and hepatic failure	Ptosis, ophthalmoplegia, sensory neuronopathy, cerebellar ataxia

(continued)

Table 2.1 (continued)

Disease	Classic presentation in childhood	Adult-onset forms
Thiamine transporter (SLC19A3) mutations	Biotin responsive basal ganglia disease (encephalopathy, coma, epilepsy, generalised dystonia)	Wernicke-like encephalopathy
Serine deficiency	Intellectual disability, epilepsy, microcephaly	Polyneuropathy
Sialidosis type 1	Dysmorphic features, intellectual disability, progressive encephalopathy	Action and stimulus-sensitive myoclonus, cerebellar ataxia
SSADH deficiency	Epileptic encephalopathy (50% patients), intellectual disability with behavioural and psychiatric disorders	Behavioural/psychiatric disorders, isolated seizures
Wilson disease	Hepatic failure	Psychiatric signs, tremor, parkinsonism, dystonia, dysarthria

AGAT arginine:Glycine amidinotransferase deficiency, *AMACR* α-methylacyl coenzyme A racemase, *GAMT* guanidinoacetate methyltransferase, *MERRF* myoclonic epilepsy associated with ragged-red fibres, *MTHFR* N(5,10)-methylenetetrahydrofolate reductase, *PDH* pyruvate dehydrogenase, *POLG* polymerase gamma, *SSADH* succinate semialdehyde dehydrogenase
Disorders for which specific treatments are available are shown in boldface type

Although the limited information available about adult forms of IEM makes the specialty new, the diagnostic approach in adults is facilitated by the fact that the nervous system is already mature. Therefore, clinical presentations are more homogeneous than in children, in whom clinical signs usually differ depending on their stage of maturation (► Chap. 1).

2.2 General Approach to IEM in Adulthood

As stated above, adult-onset presentations of IEM are essentially neurological or psychiatric. The typical situation is that of a patient with an unexplained and unusual neurological or psychiatric problem in whom the usual aetiologies have been excluded by appropriate tests. The diagnostic approach in such a situation is always based on the two questions; when to suspect an IEM and if suspected, what type of metabolic investigations must be performed [7].

Some general clinical features are highly suggestive of an IEM: fluctuating clinical signs or symptoms, especially when triggered by fasting, exercise, fever, catabolic circumstances or post-partum; clinical signs that suggest a diffuse disease including neurological signs plus systemic signs (eye or skin problems, organomegaly etc.) or involvement of different parts of the nervous system (optic nerves and cerebellum; leukoencephalopathy and polyneuropathy).

In addition, some clinical signs are highly suggestive of a particular IEM or of a particular group of IEM. Some of these 'red flags' are listed in Table 2.2.

Unfortunately, in many circumstances, highly specific signs or symptoms are lacking and the presentation is that of a less specific neurological or psychiatric disorder (epilepsy, cognitive decline, psychiatric signs). In such situations, the diagnostic approach is based on the type of clinical signs, their clinical course (acute, acute-relapsing, with diurnal variations, progressive, static), brain MRI findings, eye findings and electroneuromyography. Some matching between clinical, imaging, ophthalmological and electrophysiological findings and IEM is shown in the text and tables below.

There are now almost 1500 IEM described in the literature [8]. As recently outlined in a simplified classification of IEM based on a pathophysiological approach, metabolic diseases involving the nervous system can be divided into five main categories, all of which display some similarities in clinical presentation, diagnostic methods and treatment strategies: accumulation or deficiency of small molecules, accumulation or deficiency of complex molecules, and disorder of energy metabolism [9]. A sixth category has also been recently described and encompasses cellular trafficking of complex molecules metabolism [9] (► Chap. 44), but so far, few disorders have been reported in adults.

2.2.1 Accumulation of Small Molecules

Accumulation of small molecules cause acute or progressive "intoxication". In adults, these disorders include porphyrias, urea cycle defects, organic acidurias, aminoacidopathies and homocysteine remethylation defects. Signs result primarily from accumulation of the compound and can reverse as soon as it is removed. Crisis can be induced by food or catabolism. However, in mild adult forms, symptoms can be progressive giving rise to

Table 2.2 Examples of syndromes or signs with very high diagnostic value (see also ► Chap. 1)

Syndromes	Metabolic pathways involved
Neurological	
Recurrent coma of unknown cause	Urea cycle disorders (mainly)
Dopa-responsive dystonia	Monoamine synthesis defects
Acute or subacute myelopathy	Homocysteine remethylation defects
Exercise-induced paroxysmal dyskinesia	GLUT1 deficiency
Brain MRI	
Abnormally high signal of basal ganglia on T_2-weighted sequences (Leigh syndrome)	Energy metabolism defects (pyruvate dehydrogenase, respiratory chain, coenzyme Q10)
Abnormally low signal of basal ganglia on T_2-weighted sequences	Neurodegeneration with brain iron accumulation
Abnormally high signal of basal ganglia on T_1-weighted sequences	Disorders of manganese metabolism, Porto-systemic shunts
Stroke-like episodes	Energy metabolism defects (mitochondrial DNA mutations, *POLG* mutations)
Ophthalmological	
Supranuclear gaze palsy	Lysosomal diseases (Gaucher, Niemann pick C)
Bilateral optic neuropathy	Energy metabolism defects (pyruvate dehydrogenase, respiratory chain, biotinidase deficiency, organic acidurias)
Macular cherry red spot	Sialidosis
Cataract	Cerebrotendinous xanthomatosis, *GBA2* mutations
Retinitis pigmentosa	Energy metabolism defects (respiratory chain), peroxisomal disorders, complex lipids disorders
Cutaneous	
Progressive dysmorphia	Lysosomal diseases
Angiokeratoma	Lysosomal diseases
Xanthomata (Achilles tendons)	Cerebrotendinous xanthomatosis
Ichthyosis	Sjögren Larsson syndrome, Refsum disease, *ELOVL4* mutations
Visceral	
Splenomegaly	Lysosomal diseases, Tangier disease
Venous and arterial thrombosis	Hyperhomocystinemia
Gout, nephrolithiasis, tophi	Purine salvage (Lesch-Nyhan syndrome)
Past history of neonatal cholestasis	Sterols metabolism (Niemann-pick C, hereditary spastic paresis type SPG5, cerebrotendinous xanthomatosis, alpha-methyl-acyl-CoA racemase deficiency), citrin deficiency, *SERAC1* mutations

leukoencephalopathies, epilepsy, psychiatric disorders or spastic paraparesis.

This category also includes metal storage disorders with Wilson disease (interfering with copper metabolism), the group of NBIA (neurodegeneration with brain iron accumulation) such as neuroferritinopathy, aceruloplasminaemia, *PANK2*-associated neurodegeneration and *PLA2G6* mutations (interfering, even if only partially, with iron metabolism) and a recently identified disorder of manganese metabolism. The hallmark of these diseases is the metal deposits that occur in the basal ganglia and that are visible on brain MRI (► Chap. 1 ► Sect. 1.5.2). The main presentations are movement disorders because of the primary involvement of the basal ganglia. Treatments, when they exist, are mainly based on metal chelators.

2

2.2.2 Deficiency of Small Molecules

Symptoms result primarily from the defective synthesis or transportation of an essential molecule, which includes amino acids synthesis or transport into the brain, fatty acids synthesis and transport, and metals. Clinical signs are, at least in theory, treatable by providing the missing compound. These disorders are mainly paediatric as they often cause neurodevelopment disruption and mimic disorders of complex molecules synthesis.

In adults, this category is mostly represented by disorders of neurotransmitter metabolism and especially the synthesis of serotonin and dopamine. Clinical signs are related to dopamine deficiency (dystonia, parkinsonism, oculogyric crisis), noradrenergic deficiency (ptosis, myosis, hypotension) or serotonin deficiency (sleep disturbance, dysthermia, behavioural disturbance). Dopa-responsive dystonia or parkinsonism is highly suggestive. Diurnal fluctuations of symptoms are also characteristic, with improvement in the morning and worsening during the day. Diagnosis of these disorders relies on analysis of neurotransmitter metabolism in the CSF.

2.2.3 Accumulation of Complex Molecules

Complex molecules are neither water soluble nor diffusible, such as glycogen, triglycerides, sphingolipids (SPL), phospholipids (PL), bile acids, glycosaminoglycans, oligosaccharides, glycoproteins, glycolipids and nucleic acids. To this group, very long chain FA and cholesterol have been added since they can be a source of complex molecules, such as triglycerides, glycolipids, PL, SPL and cholesteryl esters. Catabolism defects lead typically to storage of a visible compound that accumulates in the cytoplasm (eg, glycogenosis, steatosis), or in lysosomes (eg, lysosomal storage disorders). They are the most typical and historical group (such as sphingolipidoses, mucopolysaccharidoses or glycoproteinopathies) in which signs and symptoms primarily result from the abnormal accumulation of compound(s) proximal to the block.

In adults, these disorders are mainly represented by sphingolipidoses (Krabbe disease, metachromatic leukodystrophy, Niemann Pick A and B, Fabry disease and Gaucher disease), peroxisomal disorders (X-linked adrenoleukodystrophy/adrenomyeloneuropathy, Refsum disease, disorders of pristanic acid metabolism, peroxisome biogenesis disorders) and sterols disorders (cerebrotendinous xanthomatosis, Niemann-Pick C, spastic paraplegia type 5 and Tangier disease) Given the great proportion of lipids in the nervous system, these diseases can produce almost all kinds of symptoms but spastic paraparesis is very common. Leukodystrophy and demyelinating polyneuropathy are hallmarks of disorders interfering with myelin formation or maintenance. A past history of prolonged neonatal jaundice is suggestive of disorders of sterols metabolism (▶ Chap. 1 ▶ Sect. 1.3.1). Splenomegaly is highly suggestive of Niemann-Pick B and C, Gaucher disease and Tangier disease.

2.2.4 Deficiency of Complex Molecules

These disorders encompass the newly described group of metabolic diseases affecting the synthesis and remodelling of phospholipids (mutations in *PLA2G6*, *DDHD1*, *DDHD2*, *NTE*, *CYP2U1*, *ABHD12*) and sphingolipids (mutations in *FA2H*, *GBA2*, *B4GALNT1*) [10, 11] (▶ Chaps. 35 and 40). PL and GSL synthesis and remodelling defects lead to a variety of progressive neurodegenerative symptoms, myopathy and cardiomyopathy (like in Barth or Sengers syndrome), orthopaedic signs (bone and chondrodysplasia, malformation), syndromic ichthyosis, and retinal dystrophy. Most are not treatable. Only few have metabolic markers such as peroxisome and cholesterol disorders. For all others, the diagnosis is mostly based on NGS. Untargeted metabolomics and lipidomics are promising methods.

2.2.5 Disorders of Energy Metabolism

These consist of IEM with symptoms due, at least in part, to a deficiency in energy production or utilization within the liver, myocardium, muscle, brain, and other tissues. Diagnosis can be orientated by functional tests measuring glucose, lactate, ketones and other energetic molecules (amino acids, organic acids, acylcarnitines) in blood, CSF and urines and confirmed by enzyme assays and molecular testing. These disorders include: (i) membrane carriers of energetic molecules such as glucose, lactate and ketone bodies involved in GLUT1 deficiency syndrome or MCT1 deficiency (▶ Chap. 8); (ii) cytoplasmic energy defects such as creatine metabolism disorders (▶ Chap. 9) and, (iii) mitochondrial defects such as respiratory chain disorders (▶ Chap. 10), pyruvate dehydrogenase deficiency and Krebs cycle deficiencies, (▶ Chap. 11) β-oxidation defects (▶ Chap. 12) and disorders involving co-factors (biotin, riboflavin (▶ Chaps. 27 and 29), coenzyme Q synthesis defects (▶ Chap. 10). Acute manifestations are often triggered by infections and encompass Leigh syndrome, acute optic neuropathy, acute cerebellar ataxia, pseudo-strokes or status epilepticus. Chronic presentations often involve muscles, cer-

ebellum, basal ganglia (parkinsonism, dystonia), cortex (epilepsy, myoclonus) or the peripheral nervous system (axonal polyneuropathy). In adults, the brain white matter is less involved and spastic paraparesis is uncommon.

2.3 Specific Approaches to Neurometabolic Presentations in Adults

The clinical diagnostic strategies are illustrated in the Sections below, starting from the main neurological and psychiatric syndromes seen in adults with IEM. For each syndrome, the signs (clinical or radiological) indicative of an IEM and the approach leading to the specific metabolic investigations are discussed (see also ► Chap. 1 ► Sect. 1.5.1).

2.3.1 Encephalopathies/Comas

In a patient with an unexplained encephalopathy or coma, certain features are highly suggestive of an IEM, firstly when the encephalopathy is triggered by an external factor (surgery, fasting, exercise, high protein intake, new medication) and secondly when specific brain lesions are present on brain MRI [5] (see also ► Chap. 1 ► Sect. 1.4.1).

Two main groups of IEM are responsible for encephalopathies in adults: intoxication syndromes and energy metabolism defects (■ Table 2.3). In the first group MRI is usually normal or shows nonspecific features (brain oedema, generalised leukoencephalopathy), whereas in the second group MRI is often abnormal, showing bilateral lesions of basal ganglia (Leigh syndrome) or stroke-like lesions.

In addition, some clinical signs suggest specific diagnoses. Encephalopathies in the context of urea cycle disorders, organic aciduria and aminoacidopathies are usually associated with gastrointestinal symptoms such as nausea or vomiting. Porphyria crises are associated with abdominal pain, acute neuropathy or hyponatraemia. Homocysteine remethylation defects cause acute or subacute myelopathy and are often preceded by psychiatric symptoms lasting for months or years.

Fatty acid oxidation disorders usually cause muscular symptoms; however, patients with MCAD deficiency can present with isolated encephalopathies starting in adolescence or adulthood with normal MRI.

Lastly, α-methyl-acyl-CoA racemase (AMACR) deficiency can cause a very severe relapsing encephalopathy. Patients with this disease often have characteristic MRI findings including abnormal signals of the thalami and brain stem, with cortical lesions mimicking infectious encephalitis or pseudo-strokes (► Chap. 42).

■ **Table 2.3** Diagnostic approach to metabolic causes of encephalopathy, strokes or pseudo-strokes (see also ► Chap. 1 ► Sect. 1.4.1)

Diseases	Encephalopathy/coma	Strokes or pseudo-strokes
Energy metabolism disorders		
Respiratory chain disorders (MELAS and others)	+	+
Thiamine transporter (SLC19A3 mutations), PDH deficiency	+	
MCAD deficiency	+	
Small molecules accumulation		
Urea cycle disorders	+	+
Homocysteine remethylation defects	+	+
CBS deficiency		+
Acute intermittent porphyrias	+	
Lysinuric protein intolerance	+	
MSUD	+	
Non ketotic hyperglycinemia	+	
Complex molecules accumulation		
AMACR deficiency	+	+
Fabry disease		+
Pompe disease		+

AMACR α-methyl-acyl-CoA racemase, *CBS* cystathionine-β-synthase, *MCAD* medium-chain acyl-CoA dehydrogenase, *MELAS* mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes, *MSUD* maple syrup urine disease. Disorders for which specific treatments are available are shown in boldface type

2.3.2 Strokes and Pseudo-Strokes

Some IEM cause ischaemic strokes in adulthood (see also ► Chap. 1 ► Sect. 1.4.1). This is the case in Fabry disease and homocystinurias. In the former, strokes typically involve small arteries of the vertebrobasilar system, leading to acute deafness, vertigo, diplopia, hemiplegia. In homocystinurias (cystathionine β-synthase deficiency) or homocysteine remethylation defects, thrombosis or dissection or large vessels (carotid arteries) is observed. Ischaemic brain lesions have also been reported in patients with Pompe disease. In addition, acute focal

neurological signs mimicking strokes (pseudo-strokes) are very suggestive of mitochondrial diseases, especially mitochondrial DNA mutations (MELAS, MERRF, NARP) and can also be seen in patients with urea cycle disorders and AMACR deficiency. These pseudo-strokes differ from true strokes in that they do not correspond to usual arterial territories and are often associated with signs of encephalopathy, including cephalalgia, confusion and epileptic seizures.

2.3.3 Movement Disorders

In patients with movement disorders, an IEM should be suspected in several situations: (1) when a patient displays several types of abnormal movements (example dystonia + parkinsonism); (2) when movement disorders are associated with other neurological signs (epilepsy, dementia, etc.); (3) when dystonia involves the orofacial region; (4) when bilateral lesions of the basal ganglia are observed on brain MRI; and (5) when paroxysmal movement disorders are triggered by fasting and exercise [12] (Tables 2.2 and 2.4).

Generally, a particular movement disorder can be seen in many different IEM, and conversely, a given IEM can present with different abnormal movements [13]. As a consequence, the classic phenomenological diagnostic approach to movement disorders (i.e. dystonia, parkinsonism, chorea, myoclonus) is less applicable to the diagnosis of IEM. As in the case of acute encephalopathies, brain MRI can help the diagnostic approach. A T2 hypersignal of the basal ganglia suggests an energy metabolism disorder (see above) whereas a T2 hyposignal of the pallidum suggests NBIA [14] (Table 2.5). Diffuse T2-hypersignal involving thalami, brain stem and cerebellar peduncles are suggestive of Wilson disease.

When MRI is normal, the diagnostic approach can be based on the course of the disease. Dystonia or parkinsonism with diurnal fluctuations suggests a neurotransmitter metabolism defect. Paroxysmal dystonia triggered by exercise is highly suggestive of GLUT1 deficiency but

Table 2.4 Diagnostic approach to metabolic causes of movement disorders

Disease	Parkinsonism	Dystonia	Chorea	Myoclonus	Paroxysmal dystonia
Energy metabolism disorders					
Respiratory chain disorders	+	+	+	+	
Adenylate cyclase (*ADCY5*)		+	+	+	+
PDH deficiency	+	+	+		+
GLUT1 deficiency		+	+		+
BBGD (*SLC19A3* mutations)		+			
Vitamin E deficiency		+			
Complex molecules accumulation					
Cerebrotendinous xanthomatosis	+	+	+	+	
Niemann-pick type C	+	+	+	+	
GM1 gangliosidosis	+	+			
GM2 gangliosidosis	+	+	+		
Gaucher disease	+	+		+	
Ceroid-lipofuscinosis	+	+		+	
Sialidosis				+	
Complex molecules deficiency					
CYP2U1 mutations (SPG56)		+			
FA2H mutations (SPG35)		+			
B4GALNT1 mutations (SPG26)		+			
PLA2G6 mutations	+	+			

Table 2.4 (continued)

Disease	Parkinsonism	Dystonia	Chorea	Myoclonus	Paroxysmal dystonia
Small molecules accumulation					
Phenylketonuria	+				
Homocystinuria	+	+	+		
L-2-Hydroxyglutaric aciduria	+	+			
Wilson disease	+	+	+		+
Aceruloplasminaemia	+	+	+		
Panthotenate kinase deficiency	+	+	+		
Neuroferritinopathy	+	+	+		
Manganese metabolism disorder *(SLC30A10* mutations)	+	+			
Lesch-Nyhan disease		+	+		
Small molecules deficiency					
GTP cyclohydrolase-1 deficiency	+	+			+
Tyrosine hydroxylase deficiency	+	+			
Dopamine transporter deficiency	+	+			
PTP synthase deficiency		+			+
Sepiapterin reductase deficiency	+	+			
Non-ketotic hyperglycinaemia			+		

BBGD biotin-responsive basal ganglia disease, *PDH* pyruvate dehydrogenase, *PTP* 6-pyruvoyl-tetrahydropterin. Disorders for which specific treatments are available are shown in boldface type

Table 2.5 Diagnostic approach to metabolic causes of basal ganglia lesions on brain MRI

Diseases	Palli-dum	Thala-mus	Puta-men	Brain stem nuclei	Dentate nuclei
Energy metabolism disorders					
Respiratory chain disorders	+	+	+	+	+
BBGD (*SLC19A3* mutations)			+	+	
PDH deficiency	+	+	+	+	+
Co-enzyme Q10 deficiency			+	+	
Mitochondrial thiamine pyrophosphate transporter (*SLC25A19 mutations*)			+		
CoA synthase deficiency (COASY)	+	+	+		
Complex molecules accumulation					
Cerebrotendinous xanthomatosis	+				+
AMACR deficiency		+		+	
GM1 gangliosidosis			+		
Fabry disease		+			

(continued)

Table 2.5 (continued)

Diseases	Palli-dum	Thala-mus	Puta-men	Brain stem nuclei	Dentate nuclei
Complex molecules deficiency					
PANK2 mutations	+				
PLA2G6 mutations	+			+	
FA2H mutations (SPG35)	+				
Small molecules accumulation					
Methylmalonic/propionic aciduria	+				
SSADH deficiency	+				+
Urea cycle disorders	+				+
Glutaric aciduria type 1			+		
Wilson disease	+	+	+	+	+
Aceruloplasminaemia	+	+	+	+	+
Neuroferritinopathy	+		+	+	+
Manganese metabolism intoxication disorder (*SLC30A10*)	+		+		+

AMACR α-methylacyl coenzyme A racemase, *BBGD* biotin-responsive basal ganglia disease, *SSADH* succinic semialdehyde dehydrogenase. Disorders for which specific treatments are available are shown in boldface type

can also be observed in PDH deficiency. In addition, paroxysmal dyskinesias (not triggered by exercise) have been observed in several IEM, including Wilson disease and neurotransmitter metabolism defects.

2.3.4 Peripheral Neuropathies

Peripheral neuropathies in the context of IEM are often labelled 'Charcot-Marie-Tooth disease'. These types of neuropathy are characterised by long-standing chronic, predominantly motor, distal and symmetrical polyneuropathy with claw toes, diffuse, and severe homogeneous electrical abnormalities. IEM should be suspected in such patients if the neuropathy is associated with other incongruous neurological signs (leukoencephalopathy, ataxia, pyramidal signs, psychiatric or visual signs) or with systemic manifestations (skin problems, xanthomas, splenomegaly, cataract). In some cases, peripheral neuropathies may be acute or relapsing, and may involve multiple nerves (mononeuropathy, multiplex), motor neurons or dorsal root ganglia [15] (Table 2.6).

Two main groups of metabolic diseases give rise to peripheral neuropathies: complex molecules disorders and energy metabolism defects. When complex molecules accumulate, both the peripheral and central myelin can be involved, leading to a leukoencephalopathy seen on brain MRI. By contrast, defects of energy metabolism are mostly responsible for axonal peripheral neuropathies and are usually associated with other signs of energy metabolism defects (cerebellar ataxia in the case of respiratory chain disorders). Many exceptions to this schematic view exist, however. MNGIE (mitochondrial neurogastrointestinal encephalomyopathy) syndrome caused by thymidine phosphorylase deficiency (▶ Chap. 32) is typically responsible for a demyelinating polyneuropathy. Some complex lipid storage disorders, such as cerebrotendinous xanthomatosis (▶ Chap. 38), adrenomyeloneuropathy and other peroxisomal diseases, may cause polyneuropathies that can be axonal, demyelinating or both. Metabolic neuropathies may also present as autosomal dominant diseases such as the hereditary sensory and autonomic neuropathy type 1 (HSAN1) related to mutations in the gene encoding serine palmitoyltransferase and leading to toxic accumulation of abnormal sphingolipids [16] (▶ Chap. 40) and mutations in the a-N-acetyl-glucosaminidase (*NAGLU*) gene associated with a late-onset painful sensory neuropathy [17]. Acute polyneuropathies mimicking Guillain-Barré syndrome can be observed in acute attacks of porphyria and in pyruvate dehydrogenase deficiency, acute exacerbations of Refsum disease or untreated tyrosinaemia type 1. Painful peripheral neuropathy involving small fibres is reminiscent of Fabry disease, Tangier disease, GM2 gangliosidosis, HSAN1 and porphyria. Motor neuron involvement mimicking spinal muscular atrophy is characteristic of late-onset Tay-Sachs disease. Lastly, involvement of dorsal root ganglia is highly suggestive of *POLG* mutations (mtDNA polymerase γ). In summary, the type of metabolic investigations is mainly based on the type of the peripheral neuropathy (demyelinating versus axonal), its topography and course and on brain MRI results.

Table 2.6 Diagnostic approach to metabolic causes of peripheral nervous system

Diseases	Demyelinating	Axonal	Motor neuron	Small fibres	Dorsal root ganglia	Acute	Mononeuropathy multiplex
Energy metabolism disorders							
Respiratory chain disorders		+			+		
MNGIE	+						
PDH deficiency		+				+	
β-Oxidation defects (LCHAD, TFP)		+			+		
Biotinidase deficiency			+			+	
Riboflavin transporter deficiency			+			+	
Complex molecules accumulation							
Cerebrotendinous xanthomatosis	+	+					
GM2 gangliosidosis			+	+			
Fabry disease				+			
Metachromatic leukodystrophy	+						
Krabbe disease	+	+					
Adrenoleukodystrophy/adrenomyeloneuropathy	+	+					
Refsum disease	+					+	+
AMACR deficiency	+	+					
Peroxisome biogenesis defects	+	+					
Tangier disease				+		+	+
β-Mannosidosis	+						
Serine palmitoyltransferase mutations (HSAN1)		+	+	+			
APBD		+	+				
Complex molecules deficiency							
NTE mutations (SPG39)		+					
ABHD12 mutations (PHARC)	+						
PLA2G6 mutations		+					
CYP2U1 mutations (SPG56)		+					
Cellular trafficking defects (► Chap. 44)		+	+	+			
GBA2 mutations (SPG46)		+					
B4GALNT1 mutations (SPG26)		+					
Presynaptic choline transporter (*SLC5A7*)		+					
Small molecules accumulation							
Tyrosinaemia type 1						+	
Homocysteine remethylation defect	+	+			+		

(continued)

Table 2.6 (continued)

Diseases	Demyelinating	Axonal	Motor neuron	Small fibres	Dorsal root ganglia	Acute	Mononeuropathy multiplex
Acute porphyrias		+		+		+	+
Sorbitol dehydrogenase deficiency (*SORD*)		+					
Small molecules deficiency							
Serine deficiency		+					
Vitamin E deficiency		+			+		

AMACR α-methylacyl coenzyme A racemase, *APBD* adult polyglucosan body disease, *CDG* congenital disorders of glycosylation, *HSAN1* dominant hereditary sensory and autonomic neuropathy, *LCHAD* long-chain 3-hydroxyl-CoA dehydrogenase, *PDH* pyruvate dehydrogenase, *MNGIE* mitochondrial neurogastro intestinal encephalomyopathy, *TFP* trifunctional protein. Disorders for which specific treatments are available are shown in boldface type

2.3.5 Leukoencephalopathies

The first step in the diagnostic approach of leukoencephalopathies is to search for acquired, potentially treatable causes. These causes are numerous and include inflammatory, infectious, metabolic, neoplastic, paraneoplastic, toxic or vascular diseases. In metabolic leukoencephalopathies, lesions are usually bilateral and symmetrical involving specific white matter tracts (pyramidal tracts, cerebellar peduncles, U-fibres, etc.). Furthermore, the existence of an associated polyneuropathy is highly suggestive of an IEM [18, 19].

The diagnostic approach to genetic leukoencephalopathies should be guided by the clinical examination, the MRI aspect and electroneuromyographic studies (see also ▶ Chap. 1 ▶ Sect. 1.5.2). Some IEM are responsible for a specific pattern of leukoencephalopathy (Table 2.7). In general, two main groups of IEM are responsible for leukoencephalopathies: accumulation of small or complex molecules.

2.3.6 Epilepsy

Although epilepsy is a frequent presentation of IEM in neonates and children, several IEM may also manifest in adults with onset of epileptic seizures, but these are usually observed as part of a larger clinical spectrum.

In a patient with epilepsy, several clinical, radiological or electrophysiological features suggest an IEM: (1) the form of epilepsy does not match with any classic epileptic syndrome, i.e. atypical electroclinical presentation, mixture of generalised and partial epileptic manifestations (e.g. association of myoclonus and partial seizures); (2) progressive myoclonic epilepsy; (3) association with other neurological impairments (cerebellar, pyramidal, etc.), with unexplained mental retardation, or with other organ disorders; (4) seizures that are related to the times of eating (fasting, protein rich meal); (5) inefficacy of or worsening with classic antiepileptic drugs; (6) unexplained status epilepticus; (7) abnormalities on proton magnetic resonance spectroscopy, e.g. creatine deficiency or increased lactate; (8) slowing of the background activity on the EEG, photo-paroxysmal responses during the photic intermittent stimulation at low frequencies (1–6 Hz) [20].

The three main groups of IEM presenting with epilepsy in adults are disorders of energy metabolism, accumulation of small or complex molecules (Table 2.8). Myoclonic epilepsy suggests lysosomal disorders or certain respiratory chain disorders (MERRF syndrome). Partial motor or occipital seizures are frequent in respiratory chain disorders together with slow waves predominating in posterior brain regions.

2.3.7 Psychiatric Disorders

IEM frequently present with psychiatric diseases in adolescents or adults. Retrospective analysis of patients with various IEM shows that psychiatric signs may remain isolated for years before more specific organic involvement becomes obvious. Since psychiatrists' awareness of these rare disorders is low, IEM presenting with a pure psychiatric illness are often missed. In most cases, treatments are more effective at the 'psychiatric stage' of the disease, before the development of irreversible neurological lesions.

The diagnosis is especially difficult when psychiatric signs are initially isolated, without a family history or

Table 2.7 Diagnostic approach to metabolic causes of leukoencephalopathies

Diseases	Periventricular	Pyramidal tracts	Cerebellum	Spinal cord	Juxtacortical	Brain stem	Corpus callosum
Complex molecules accumulation							
Metachromatic leukodystrophy	+						+
Adrenoleukodystrophy		+	+			+	+
Krabbe disease		+					+
Cerebrotendinous xanthomatosis	+	+	+			+	+
Refsum disease	+						
AMACR deficiency						+	
Peroxisome biogenesis disorder	+	+				+	
α-Mannosidosis	+						
APBD	+	+	+	+		+	
Small molecules accumulation							
Homocysteine RD	+			+	+		
Phenylketonuria	+						+
Glutaric aciduria type 1	+				+		
L-2-Hydroxyglutaric aciduria					+		
3-methylglutaconyl-CoA hydratase deficiency					+		
Wilson disease			+			+	
Disorders of energy metabolism							
Respiratory chain	+	+	+		+	+	+
MNGIE	+				+		
AARS2 mutations	+	+					+
DARS2 mutations	+	+	+	+		+	
EARS2 mutations			+			+	+
LARS2 mutations		+	+			+	+

AMACR α-methylacyl coenzyme A racemase, *APBD* adult polyglucosan body disease, *MNGIE* mitochondrial neurogastrointestinal encephalomyopathy. Disorders for which specific treatments are available are shown in boldface type

clinical somatic involvement. In addition, it is sometimes difficult, in a patient with physical signs, to determine whether psychiatric problems are related to the same disease or not. Furthermore, physical signs may not be evident after a simple clinical examination (as examples, leukodystrophies may be missed if a brain MRI is not performed, peripheral neuropathy, cataract or xanthomas may not be symptomatic, organomegaly is often missed clinically in an adult). It is therefore important to determine which psychiatric symptomatology points to an IEM and should lead to further investigations (Table 2.9). Diseases can be schematically classified into three groups [21, 22].

Group 1 includes diseases with acute and recurrent attacks of confusion and behavioural changes, which are usually associated with physical signs (gastrointestinal signs, cephalalgia, dysautonomia, pyramidal signs, alteration of consciousness). This group corresponds mainly to intoxications (urea cycle defects, homocysteine remethylation defects and porphyrias) but also energy defects (mitochondrial diseases). Therefore, plasma ammonia, homocysteine and lactate should be measured in unexplained acute psychiatric presentations.

Group 2 is made up of diseases with isolated psychiatric signs arising in adolescence or adulthood in a pre-

Table 2.8 Diagnostic approach to metabolic causes of epilepsy

Diseases	Generalised or focal epilepsy	Progressive myoclonic epilepsy
Energy metabolism disorders		
Respiratory chain disorders (MERRF, MELAS, NARP, *POLG* and others)	+	+
GAMT deficiency	+	
GLUT1 deficiency	+	+
***SLC19A3* mutations**	+	
Complex molecules accumulation		
Cerebrotendinous xanthomatosis	+	
Niemann-pick C	+	+
Gaucher type 3	+	+
Ceroid lipofuscinosis	+	+
LIMP2 deficiency	+	+
Sialidosis	+	+
Lafora disease	+	+
Small molecules accumulation		
Homocysteine remethylation defect	+	
L-2-Hydroxyglutaric aciduria	+	
SSADH deficiency	+	
Acute intermittent porphyrias	+	
Hyperinsulinism-hyperammonaemia	+	+

GAMT guanidinoacetate N methyl transferase, *LIMP2* lysosomal integral membrane protein type 2, *MELAS* mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke, *MERRF* myoclonic epilepsy with ragged red fibers, *NARP* neuropathy, ataxia, and retinitis pigmentosa, *SSADH* succinate semialdehyde dehydrogenase. Disorders for which specific treatments are available are shown in boldface type

viously non symptomatic patient. This group includes hyperhomocystinaemia (homocysteine remethylation defects and cystathionine β-synthase deficiency) and lipid metabolism disorders (metachromatic leukodystrophy, GM2 gangliosidosis, Niemann-Pick type C disease, adrenoleukodystrophy and cerebrotendinous xanthomatosis). Patients in this group may initially present with recurrent psychotic attacks, chronic delusion or disorganised behaviour, which may mimic schizophrenia. It also includes behavioural and personality changes. The diagnosis is particularly difficult in this group given the relative non-specificity of psychiatric signs, especially when they remain isolated for years or decades. However, catatonias, visual hallucinations, fluctuating symptoms, mental confusion, resistance or even deterioration with treatments and associated cognitive decline constitute atypical features that suggest an IEM.

Group 3 includes patients with mild mental retardation from childhood and disorders of behaviour or personality without a definite psychiatric syndrome. This group includes chronic intoxications (homocystinurias, non ketotic hyperglycinaemia, succinic semialdehyde dehydrogenase deficiency), some neurotransmitter metabolism defects (monoamine oxidase A deficiency), as well as creatine transporter deficiency, α- and β-mannosidosis and MPS III.

Given the important number of IEM presenting with chronic psychiatric symptoms, minimal investigations include brain MRI, ophthalmological examination, abdominal ultrasonography, electromyogram as well as plasma biomarkers (ammonia, lactate, homocysteine, copper and ceruleoplasmin, cholestanol, very long chain fatty acids, lysosphingomyelin, and hexosaminidase A).

2.3.8 Spastic Paraparesis

Spastic paraparesis is a general term describing progressive stiffness and weakness in the lower limbs caused by pyramidal tract degeneration. This clinical situation is frequently encountered in adult neurology. The diagnostic strategy (Table 2.10) is usually limited to searching for acquired causes (spinal cord compression, inflammatory, metabolic, infectious diseases) and the so-called hereditary spastic paraplegias (HSP). To date, about 80 forms of HSP have been identified, with various modes of inheritance [23]. HSP are clinically classified as *uncomplicated* (or *pure*) when symptoms are limited to spastic paraparesis and as *complicated* (or *syndromic*) when accompanied by other neurological or systemic signs.

However, although poorly recognised by neurologists, spastic paraparesis is also one of the many presentations of IEM in children and adults [18, 24]. Pyramidal signs are usually included in a diffuse neurological or systemic clinical picture, but in some cases spastic paraparesis remains the only symptom for years. In a patient with spastic paraparesis some signs are suggestive of an IEM: (1) when a polyneuropathy is pres-

Table 2.9 Diagnostic approach to metabolic causes of psychiatric disorders

Diseases	Adult-onset psychiatric disorders without mental retardation	Behavioural/psychiatric disorders with mental retardation
Energy metabolism disorders		
Respiratory chain disorders	+	+
Creatine transporter deficiency		+
Small molecules accumulation		
Urea cycle disorders	+	+
Homocysteine remethylation defects	+	+
CBS deficiency	+	+
Acute intermittent porphyrias	+	
Non ketotic hyperglycinemia		+
SSADH deficiency		+
Phenylketonuria	+	+
Wilson disease	+	
Aceruloplasminaemia	+	
Neuroferritinopathy	+	
Complex molecules accumulation		
Niemann-pick C	+	
GM2 gangliosidosis	+	
Metachromatic leukodystrophy	+	
Adrenoleukodystrophy	+	
Cerebrotendinous xanthomatosis	+	+
β-Mannosidosis		+
α-Mannosidosis	+	+
Ceroid lipofuscinoses	+	+
MPS type III (san Filippo syndrome)		+
AMACR deficiency	+	

AMACR α-methyl-acyl-CoA racemase, *CBS* cystathionine-β-synthase, *MPS* mucopolysaccharidoses, *SSADH* sucinate semialdehyde dehydrogenase. Disorders for which specific treatments are available are shown in boldface type

ent on EMG; (2) when a leukoencephalopathy is present on MRI; (3) when the course is acute or subacute, with sensory ataxia suggesting subacute degeneration of the spinal cord.

Three groups of IEM mainly give rise to spastic paraparesis: (1) complex molecules accumulation, (2) complex molecules deficiency and (3) small molecules accumulation. It should be noted that dopamine synthesis defects (guanosine-5′-triphosphate [GTP] cyclohydrolase and tyrosine hydroxylase deficiencies) can produce dystonia mimicking spastic paraparesis in the lower limbs. In such cases, treatment with levodopa is highly effective in alleviating the symptoms. The first metabolic autosomal dominant form of HSP has also been recently identified in patients with mutations in *ALDH18A1* encoding delta-1-pyrroline-5-carboxylate synthetase (P5CS) who present with hypocitrullinaemia [25] (► Sect. 21.2).

2

Table 2.10 Diagnostic approach to metabolic causes of acute myelopathy or spastic paraparesis (without leukoencephalopathy – for spastic paraparesis with leukoencephalopathy, see Table 2.7)

Diseases	Chronic	Acute
Complex molecules accumulation		
Cerebrotendinous xanthomatosis	+	
CYP7B1 mutations (SPG5)	+	
Adrenomyeloneuropathy	+	
Peroxisomal biogenesis disorder	+	
Sjögren-Larsson disease	+	
Adult Polyglucosan body disease	+	
Complex molecules deficiency		
DDHD1 mutations (SPG28)	+	
DDHD2 mutations (SPG54)	+	
CYP2U1 mutations (SPG56)	+	
NTE mutations (SPG39)	+	
FA2H mutations (SPG35)	+	
GBA2 mutations (SPG46)	+	
B4GALNT1 mutations (SPG26)	+	
PLA2G6 mutations	+	
ELOVL4 mutations	+	
PCYT2 mutations	+	
Cellular trafficking defects (► Chap. 44)	+	
Small molecules accumulation		
Phenylketonuria	+	
Arginase deficiency	+	
Triple H syndrome	+	
ALDH18A1 mutations	+	
Homocysteine remethylation defect	+	+
L-2-Hydroxyglutaric aciduria	+	
Small molecules deficiency		
GTP cyclohydrolase 1 deficiency	+	
Tyrosine hydroxylase deficiency	+	

Disorders for which specific treatments are available are shown in boldface type

2.3.9 Cerebellar Ataxia

Except for focal cerebellar lesions, the many causes of cerebellar ataxia include inflammatory diseases, paraneoplastic disorders, acquired metabolic disorders, alcohol intoxication, multiple system atrophy, and genetic diseases (Friedreich ataxia, dominant spinocerebellar ataxias etc.). Cerebellar ataxia in the context of IEM may be acute, triggered by fever (PDH deficiency, respiratory chain disorders or *SLC19A3* mutations), or chronic [26] (Table 2.11).

2.3.10 Myopathy

Metabolism within muscles is very different from that of the nervous system (Table 2.12). Except for mitochondrial disorders that can affect both the muscle and the nervous system, diseases affecting the muscle usually spare the nervous system. Hallmarks of metabolic myopathies are exercise intolerance (exertional cramps or fatigue) and recurrent rhabdomyolysis [27, 28] (see also ► Chap. 1 ► Sects. 1.4.6 and 1.6.8). However, presentations may be less specific, with progressive proximal myopathy or cardiomyopathy. Muscle histology may be suggestive of a metabolic disorder when it shows ragged red fibres, lipid droplets or high glycogen content with PAS staining. The three main groups of metabolic diseases affecting muscle are (1) energy metabolism disorders (respiratory chain defects and fatty acids β-oxidation defects, (2) complex molecules accumulation (glycogen storage disorders, adipocyte triglyceride lipase or *PNPLA2* deficiency) and (3) complex molecules deficiency (complex lipid synthesis disorders).

Mitochondrial diseases may show a wide range of manifestations including exercise intolerance with premature fatigue or myalgia out of proportion to weakness. These symptoms are frequently associated with progressive external ophthalmoplegia, which is highly suggestive of *POLG* mutations or other mtDNA deletion syndromes.

Fatty acid oxidation defects may manifest with rhabdomyolysis triggered by prolonged exercise or prolonged fasting, but progressive proximal weakness with lipid storage is also a common presentation in adults. Lipin deficiency 1 has been observed only in few adults so far (► Chap. 35).

Table 2.11 Diagnostic approach to metabolic causes of cerebellar ataxia

Diseases	Chronic cerebellar ataxia	Spinocerebellar ataxia	Episodic or acute ataxia	Myoclonic ataxia
Energy metabolism disorders				
Respiratory chain disorders	+	+	+	+
PDH deficiency	+		+	
GLUT1 deficiency	+	+	+	+
Co-enzyme Q10 deficiency	+			
***SLC19A3* mutations**			+	
Complex molecules accumulation				
Cerebrotendinous xanthomatosis	+	+		
Niemann-pick type C	+			+
GM2 gangliosidosis	+			
Gaucher type 3	+			+
Adrenoleukodystrophy		+		
Refsum disease	+			
DBP deficiency	+			
Peroxisomal biogenesis disorder	+	+		
α-Mannosidosis	+			
Sialidosis				+
Complex molecules deficiency				
NTE mutations (SPG39)	+	+		
PLA2G6 mutations	+	+		
GBA2 mutations	+	+		
B4GALNT1 mutations	+	+		
ELOVL5 mutations		+		
Cellular trafficking defects (► Chap. 44)	+	+		
Small molecules deficiency				
Vitamin E deficiency	+	+		
Abetalipoproteinaemia	+	+		
Small molecules accumulation				
Urea cycle disorders			+	
Mevalonate kinase deficiency	+			

PDH pyruvate dehydrogenase, *DBP* D-bi-functional protein. Disorders for which specific treatments are available are shown in bold-face type

2

Table 2.12 Diagnostic approach to metabolic causes of myopathies

Diseases	Permanent weakness	Exercise intolerance and/or myglobinuria	Cardiomyopathy
Energy metabolism disorders			
MELAS	+	+	+
MERRF	+	+	+
MNGIE	+		
PEO-Kearns Sayre	+	+	+
POLG mutations	+		+
Cytochrome B deficiency		+	
VLCAD deficiency	+		+
ETF and ETFDH deficiencies	+	+	
TFP deficiency		+	
CPT2 deficiency		+	
Primary carnitine deficiency	+		+
AGAT deficiency	+		
GAMT deficiency	+		
Glycolysis defects		+	+
Complex molecules accumulation			
McArdle disease (GSD-V)		+	
Pompe disease (GSD-II)	+		+
Debranching enzyme (GSD-III)	+		
Branching enzyme (GSD-IV)	+		+
AMACR deficiency		+	
ATGL deficiency (PNPLA2)	+	+	+
Complex molecules deficiency			
Lipin 1 deficiency (*LPIN1*)		+	
Barth syndrome (*TAZ*)	+		+
CHKB mutations	+		+
PNPLA2 mutations	+		+

AGAT I-arginine glycine amidinotransferase, *AMACR* α-methyl-acyl-CoA racemase, *ATGL* adipocytes triglyceride lipase deficiency, *CPT2* carnitine palmitoyltransferase 2, *ETF* electron transfer flavoprotein, *ETFDH* electron transfer flavoprotein dehydrogenase, *GAMT* guanidinoacetate N-methyltransferase, *MELAS* mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke, *MERRF* myoclonic epilepsy with ragged red fibers, *MNGIE* mitochondrial neurogastrointestinal encephalomyopathy, *PEO* progressive external ophthalmoplegia, *TFP* trifunctional protein, *VLCAD* very long-chain acyl-CoA dehydrogenase. Disorders for which specific treatments are available are shown in boldface type

Clinical presentations of muscle glycogenoses are various, ranging from exercise intolerance to isolated progressive muscle weakness. Patients with McArdle disease typically exhibit premature fatigue and contractures, frequently accompanied by muscle breakdown. A sign considered pathognomonic of this disease is the *second wind phenomenon*, which is a marked improvement in exercise tolerance about 10 minutes into aerobic exercise involving large muscle masses (jogging or cycling).

Adipocyte triglyceride lipase (ATGL) deficiency (*PNPLA2* mutations) gives neutral lipid storage with myopathy typically presenting in young adults with weakness and fatty infiltration of muscle or with cardiomyopathy. Weakness can be proximal, distal or generalized. The disease is progressive: some patients are athletic in childhood. Jordan anomaly on a blood smear is highly diagnostic (► Chap. 35).

2.3.11 Sensorial Disorders

Table 2.13.

Table 2.13 Diagnostic approach to metabolic causes of deafness/visual problems

Diseases	Deafness	Corneal deposits	Retinitis	Macula cherry red spot	Optic nerve disorders	Cataract
Energy metabolism disorders						
Respiratory chain disorders	+		+		+	+
β-Oxidation defects (LCHAD, TFP)			+			
Biotinidase deficiency	+				+	
Riboflavin transporter deficiency	+				+	
PDH deficiency					+	
Complex molecules accumulation						
Niemann-pick C	+					
Metachromatic leukodystrophy					+	
Krabbe disease					+	
Adrenoleukodystrophy	+				+	
Fabry disease	+	+				+
Cerebrotendinous xanthomatosis						+
AMACR deficiency			+			
Refsum disease	+		+			+
Peroxisome biogenesis disorder	+		+		+	+
Mucopolysaccharidosis	+	+	+			+
α-Mannosidosis	+					
β-Mannosidosis	+					
Sjögren-Larsson disease			+			+
Ceroid lipofuscinoses			+		+	
Sialidosis type 1				+		
CDG syndrome (PMM2-CDG)			+			

(continued)

2

Table 2.13 (continued)

Diseases	Deafness	Corneal deposits	Retinitis	Macula cherry red spot	Optic nerve disorders	Cataract
Complex molecules deficiency						
ELOVL4 mutations			+			
DDHD1 mutations (SPG28)			+			
DDHD2 mutations			+			
NTE mutations (SPG39)			+			
CYP2U1 mutations			+			
ABDH12 mutations	+		+			+
FA2H mutations (SPG35)					+	
GBA2 mutations (SPG46)						+
PLA2G6 mutations					+	
Cellular trafficking defects (► Chap. 44)	+	+	+			+
Small molecules accumulation						
Homocysteine remethylation defect			+		+	
CBS deficiency						+
Phenylketonuria					+	
Galactokinase deficiency						+
Wilson disease		+				+
Aceruloplasminaemia			+			
Small molecules deficiency						
Vitamin E deficiency			+			
Abetalipoproteinemia			+			
Panthotenate kinase deficiency			+			

AMACR α-methylacyl-CoA racemase, *CBS* cystathionine β-synthase, *CDG* congenital disorders of glycosylation, *LCHAD* long-chain L-3 hydroxyacyl-CoA dehydrogenase, *PDH* pyruvate dehydrogenase, *RD* remethylation defects, *TFP* trifunctional protein. Disorders for which specific treatments are available are shown in boldface type

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Diagnostic Procedures

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Contents

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3

Introduction

Unlike most other genetic disorders, IEMs are usually diagnosed from biochemical analyses prior to molecular testing. Basal metabolic investigations remain the gold standard for many clinical presentations (hypoglycaemia, liver disease, epilepsy, neurodevelopmental delay, movement disorders, neuro-sensorial deficit, peripheral neuropathy, etc.). If an IEM is suspected, then blood, urine and cerebrospinal fluid should be collected for the appropriate investigations (▶ Chap. 1, ▶ Fig. 1.1). If no material is available or if the results are inconclusive, a provocative test that challenges a metabolic pathway may provide clues to a diagnosis and indicate which specific enzymatic or genetic analysis could be undertaken. Functional tests are dynamic investigations based on the measurement of intermediary metabolites in body fluids. They are most useful in disorders that give rise to toxicity or energy deficiency. The best functional test is elicited by nature itself during episodes that cause metabolic stress, including birth, acute infection, inadvertent fasting, or consumption of a nutrient that induces a metabolic intolerance.

With its unprecedented throughput, scalability, and speed, next-generation sequencing changes profoundly our diagnostic approaches in rare diseases, including IEMs. Dedicated gene panels, but also whole exome or genome sequencing, are now becoming first line investigations in some diagnostic centres. However, due to very complex dataset resulting from such large testing – including variants in several candidate genes – the strength of metabolic investigations relies on their ability to functionally validate uncertain genetic variants. Metabolic biomarkers are also invaluable for the therapeutic follow-up of many IEM.

3.1 Basal Metabolic Investigation

3.1.1 Amino and Organic Acids

The initial evaluation of amino acid disorders usually requires contemporaneous analysis of amino acids in blood and urine. For acute disorders, samples should be taken as early as possible after the patient's arrival. For chronic disorders, a fasting blood sample (in the morning or at least 4 hours after last meal) and 24-hour urine collection are usually preferred. Samples collected under different conditions (e.g. post-prandial blood) or using other fluids (mainly CSF) may also be useful (e.g. encephalopathy).

Similarly, for the initial investigation of organic acid disorders, a urine sample should be collected as soon as possible after the onset of acute symptoms for analysis or for storage at −20 °C if the analysis cannot be performed immediately. In non acute situations, a 24-h or a 12-h overnight collection is usually the first investigation.

For these analyses, strict conditions for collection, handling and storage of the samples are necessary to prevent artefactual changes. For example, in a plasma sample that is badly preserved, cystine and homocystine will bind to protein, leading to a falsely low level, and hydrolysis of asparagine and glutamine will result in falsely high concentrations of aspartic and glutamic acids and low concentrations of glutamine. Specific total homocysteine measurement for detecting a slight increase in homocystine is better than an amino acid chromatography which detects only free homocystine (▶ Chap. 20).

The concentration of each metabolite should be considered not only in absolute terms but also compared with the laboratory's age-related reference values and relative to other constituents. In many cases, the final interpretation needs knowledge of the clinical and nutritional status, and the conditions of sampling. This is best achieved by close co-operation between clinician and biochemist.

Some clues for the interpretation of the main variations in amino acids are given in ◘ Table 3.1. The main abnormal organic acids that may be found in IEM are given in ◘ Table 3.2. Abnormal acylcarnitine profiles are discussed in ▶ Chaps. 12 and 18.

3.1.2 Metabolic Profile over the Course of the Day

Indications

This assessment may be performed when an initial or recurrent clinical presentation is associated with a disturbance in intermediary metabolism such as hypoglycaemia (▶ Sect. 1.4.14), hyperlactataemia (▶ Sect. 1.4.13), hyperketosis or hypoketosis (▶ Sect. 1.4.12). In these situations, it should always be undertaken before any provocative test that might lead to metabolic decompensation. The metabolic profile is also used for monitoring treatment in many disorders.

Procedure

Blood samples from an indwelling venous catheter (kept patent with a saline infusion) are taken before and after meals, and once during the night, as outlined in ◘ Table 3.3. To allow a reliable interpretation of the results, the correct method of sampling and processing of blood and urine is specified in ◘ Table 3.3. It is important to record the conditions under which

Table 3.1 Interpretation of main plasma and urine amino acids variations

Plasma Amino Acid	Variation	Other Plasma Amino Acids	Investigations in other fluids	Diagnoses
Alanine	↑	See Gln, Pro, Gly	U: Lac ↑, Fumarate, Succinate	Hyperlactatemia, mitochondrial disorders, PEPCKC, FBP Hypoxia
Arginine	↑	Gln ± ↑, Cit ± ↓, Orn ↓	U: ± Arg/Lys/Orn/Cys, Orotic acid ↑	Arginase deficiency
	↓	Gln ± ↑, Pro ↓, Cit ↓, Orn ± ↓		P5CS deficiency
		Orn ↓, Lys ↓	U: ↑, Orn ↑, Lys ↑ UOA: Orotic ↑/N	LPI Other UCDs
Argininosuccinic acid	±↑	Gln ± ↑, Cit ± ↑	U: ASA ↑	ASLD late-onset form
	↑	Gln ↑, Cit ↑	U: ASA ↑++	ASLD neonatal form
Asparagine	↓	All normal	CSF ↓	Asn synthetase deficiency
Branched chain AA	↑	No AIle, other AA ± ↓		Starvation
		No AIle, other AA ± ↑. Val > Leu		Fed state
		AIle +++, Ala ↓	U: ↑	MSUD and mild variant (PP2Cm) [1]
		AIle± ↑, Ala ↑, Gln ↑	UOA: Lac ↑, 2KG ↑	E3 deficiency
	↑	AIle -	UOA: Alphaketoacids ↓/N	BCAT2 deficiency
	↓	All normal	CSF ↓	BCKAD kinase deficiency
		Met ↑, Tyr ↑, Pipecolic ac ↑	UOA: Phenolic acids↑	Hepatic failure
		Cit ↑, Cys2 ↑, 3Mhis ↑		Renal failure
Citrulline	↑	See Gln	U: ASA ↑/N	UCDs: ASSD, ASLD- Citrin deficiency, PC, TMEM70
	↓	See Gln	U: Orotic ↑/N/↓	NAGS, CPS, OTC
	↓	All normal	U: Orotic N	Short bowel syndrome Intestinal failure
	↓	Gln ± ↑, Pro ↓, Arg ↓, Orn ± ↓		P5CS deficiency
	↓	All normal	UOA: Lac ↑, Krebs cycle metabolites	ATP synthase deficiency (NARP), other mitochondrial disorders
	↑	Cys2 ↑, 3MHis ↑, BCAA ↓ Alone ± ↑		Renal failure Heterozygote ASS
Cystine	↓+++	SulfoCys ↑	U: SulfoCys ↑, tau ↑	Sulfite oxidase deficiency
	↑	Cit ↑, BCAA ↓, 3MHis ↑		Renal failure
	N	All normal	U: Cys ↑, Lys ↑, Orn ↑, Arg ↑	Cystinuria

(continued)

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Table 3.1 (continued)

Plasma Amino Acid	Variation	Other Plasma Amino Acids	Investigations in other fluids	Diagnoses
Glutamine	↑	Cit ↓, Orn ↓, Arg ↓	UOA: Orotic ↑ (OTC, OAT)	Mitochondrial UCD, neonatal OAT deficiency
		Cit ↓ or N, Ala ↑, Pro ↑	P: Lac ↑ UOA: Orotic N, 3-OHP ↑, PG ↑, MC ↑, 3-MCG ↑	CA-VA deficiency
		Cit ↑+++	U: Cit ↑+++, Orotic acid ↑	ASSD
		Cit ↑, ASA ↑	U: Cit ↑, ASA ↑, orotic acid ↑	ASLD
		Cit N, Arg ↑	U: Arg N or ↑ Orotic acid ↑	ARGD
		Cit ↓, Orn ↑, Homocit ↑	U: Orn N or ↑, Homocit ↑, Orotic acid ↑	HHH
		Cit ± ↑, Orn ↓, Lys ↓, Arg ↓	U: Cit ↑, Orn ↑, Lys ↑, Arg↑, Orotic acid ↑	LPI
	↑++	All normal		Glutaminase deficiency (tandem repeat expansion)
	N or ↓	Cit ± ↑, Ala ↑, Lys ↑ ± Ser↓		PC deficiency MAS deficiency
		Cit ↑, Thr ↑, Orn ↑, Lys ↑, Arg ↑		Citrin deficiency
		Cit N, Gly↑, Ala↑, Lys↑	U: Abnormal organic acids	Organic acidurias
	↓ +++		CSF: ↓ +++	Glutamine synthesis deficiency
Glycine	↑	Alone	CSF: ↑, U ↑	NKH Neonatal disseminated herpes virus infection. (Gycine is extremely elevated) (► Chap. 23)
		Ala ↑	CSF: N, U ↑	Valproate
		Ala ↑±, 2-aminoadipate	CSF: ↑, U ↑ P: Lac ↑ UOA: Lac ↑, 2KG ↑, 2KA ↑	Lipoic acid synthesis deficiency (LIAS, BOLA3, GLRX5, NFU1)
		Gln N, Ala ↑, Lys ↑	CSF: N, U ↑, UOA	Organic acidurias
		Thr ↑ ±	CSF: Gly ↑ ±, Thr ↑ ±	PNPO and PLPBP deficiency
Glycine	N/↓	Ser ↓ +/++	CSF: Gly ↓	Serine deficiency
Histidine	↑	Alone	U: ± ↑	Histidase deficiency
Homocys-tine	± ↑	All normal		Secondary to B_{12}, folate deficiency. MTHFR polymorphism
	↑	See methionine		MTHFR deficiency, cobalamin disorders
	↓		P: Creatine ↓	NRF2 [2]

Table 3.1 (continued)

Plasma Amino Acid	Variation	Other Plasma Amino Acids	Investigations in other fluids	Diagnoses
Lysine	↑ ++	Orn ↓	U: Lys, Pipecolic/Sacca↑ Orn ↓	Hyperlysinemia type I/II
	↑	Gln ↑, NH3 ↑		Urea cycle disorders
		Gln ↓, NH3 ↑, Ala ↑, Cit ↑±		PC deficiency
	↑		↑ Lys in urine/CSF ↑ Lactate, ↑pipecolic	Mutations NADK2 [3]
	↓	Gln ↑, Cit ± ↑, Orn ↓, Arg ↓	U: Cit ↑, Orn ↑, Lys ↑, Arg ↑ UOA: Orotic ↑	LPI
Methionine	↓	HCy2 ↑	U: HCy2 ↑ alone	MTHFR deficiency
			UOA: MMA ↑	Cobalamin metabolism
	↑	Alone		MATD, SAHHD, ADKD, GNMTD
		HCy2 ↑, Cys2 ↓, Disulfide	U: HCy2 ↑	CBS deficiency
		Tyr ↑, BCAA ↓, Pipecolic ac ↑	U: Phenolic acid derivatives	Hepatic failure
Ornithine	↑	Alone	U: ± ↑	OAT deficiency
		Gln ↑, Cit ↓, HomoCit ± ↑	U: Orn ± ↑, HomoCit ↑ UOA: Orotic acid↑	HHH syndrome
	↓	Gln ± ↑, Pro ↓, Cit ↓, Arg ↓		P5CS deficiency
		Arg ↓, Lys ↓	U: Cit ↑, Orn ↑, Lys ↑, Arg ↑ UOA: Orotic ↑	LPI
		Gln↑, Cit ↓, Arg ↓	UOA: Orotic ↑ (OTC, OAT)	Mitochondrial UCD, neonatal OAT deficiency
Phenylalanine	↑ +++	Tyr ↓	UOA phenolic acids	PKU
	↑ ±	Tyr ± ↓		Biopterin synthesis disorders and DNAJC12 [4]
Pipecolic	↑	All normal	CSF: Pip ± ↑, P: VLCFA CSF: Pip ± ↑, P and U: αAASA	Peroxisomal diseases αAASADH deficiency
		Met↑, Tyr ↑, BCAA ↓	UOA: Phenolic acids	Hepatic failure
Proline	↑	Alone	U: N or ↑. Aminoaciduria	Hyperprolinemia I
		Alone	U: N or ↑, P5C ↑	Hyperprolinemia II
		Arginine, ornithine N/↑		SLC25A22 deficiency
		Ala ↑		Hyperlactatemia. Mitochondrial disorders
	↓	Cit ↓, Orn ↓, Arg ↓		P5CS deficiency
Serine	↓	All others N Citrulline ↑ ±	CSF: ↓	Serine synthesis deficiency MAS deficiency
Sulfocysteine	↑	Cys2 low Cys2 very low	U: Sulfocys 0, all AA normal U: Sulfocys ↑, tau ↑	Anticoagulant Sulfite oxidase and molybdenum cofactor deficiencies
Taurine (see ► Chap. 25.8)	↓+++		U:↑ 8-oxo-7,8-dihydroguanosine	Taurine transporter deficiency
Threonine	↑	See glycine		PNPO deficiency

(continued)

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Table 3.1 (continued)

Plasma Amino Acid	Variation	Other Plasma Amino Acids	Investigations in other fluids	Diagnoses
Tyrosine	↑ +++	Alone	U: ↑ alone, phenolic acids	Tyrosinemia type II, III
	↑	Met ↑, BCAA ↓	UOA: Succinylacetone 0, phenolic acids	Hepatic failure
			UOA: Succinylacetone +, phenolic acids	Tyrosinemia type I
Urine amino acid	**Variation**	**Plasma amino acids**	**Investigations in other fluids**	**Diagnoses**
Iminopeptiduria	↑	N		Prolidase deficiency
Neutral amino acids	↑	N		Hartnup and collectrin deficiency [5]

± slightly modified, *2-aminoAd* 2-aminoadipate, *2KA*, 2 ketoadipate, *2KG* 2-ketoglutarate, *3-MCG* 3-methylcrotonylglycine, *3MHis* 3-methylhistidine, *3-OHP* 3-hydroxy-propionic, *AA* amino acids, *ADKD* adenosine kinase deficiency, *AIle* alloisoleucine, *Ala* alanine, *Arg* arginine, *ARGD* arginase deficiency, *ASA* argininosuccinic acid, *ASLD* argininosuccinic lyase deficiency, *Asn* asparagine, *ASSD* argininosuccinic synthetase deficiency, *BCAA* branched chain amino acids (valine, isoleucine, alloisoleucine, leucine), *BCKAD* branched-chain ketoacid dehydrogenase, *BCKDD* branched-chain ketoacid dehydrogenase kinase deficiency, *CA-VA* carbonic anhydrase, *CA-VA* carbonic anhydrase VA, *CBS* cystathionine-β-synthase, *Cit* citrulline, *Cys2* cystine, *Disulfide* disulfide cysteine-homocysteine, *FBP* fructose biphosphatase, *Gln* glutamine, *Gly* glycine, *GNMTD* glycine N-methyltransferase deficiency, *Hcy2* homocystine, *HHH* triple H syndrome (Hyperammonemia, Hyperornithinemia, Homocitrullinuria), *HomoCit* homocitrulline, *Lac* lactate, *LIAS* lipoic acid synthetase, *LPI* lysinuric protein intolerance, *Lys* lysine, *MATD* methionine s-adenosyltransferase deficiency, *MC* methylcitrate, *Met* methionine, *MMA* methylmalonic acid, *MSUD* maple syrup urine disease, *MTHFR* methylene tetrahydrofolate reductase, *NARP* neuropathy, ataxia, and retinitis pigmentosa, *NH3* ammonia, *NKH* non ketotic hyperglycinemia, *NRF2* NF-E2-related factor 2, *OAT* ornithine aminotransferase, *OAT* ornithine aminotransferase, *Orn* ornithine, *P5C* Δ1-pyrroline-5-carboxylate, *P5CS* Δ1-pyrroline-5-carboxylate synthase, *PEPCKC* Phosphoenol pyruvate carboxykinase cytoplasmic, *PC* pyruvate carboxylase, *PG* propionylglycine, *Pip* pipecolic, *PKU* phenylketonuria, *PNPO* pyridox(am)ine 5′-phosphate oxidase, *PNPO* pyridox(am)ine-5-phosphate oxidase, *Pro* proline, *SAHHD* S-adenosylhomocysteine hydrolase deficiency, *SLC25A22* potential transporter of P5C, glutamate-γ-semialdehyde or glutamate, *SulfoCys* sulfocysteine, *Tau* taurine, *Thr* threonin, *Tyr* tyrosine, *UCD* urea cycle disorder, *Def* deficiency, *UOA* urine organic acids, *VLCFA* very long chain fatty acids, *αAASA* alpha-amino adipic semi aldehyde, *αAASADH* alpha-aminoadipic semialdehyde dehydrogenase

Table 3.2 Interpretation of urine organic acids analysis

Principal OA	Other OAs	Causes of variation	Other investigations
Adipic	Very high isolated	Non metabolic (plastifier?)	
	See 3-OH- butyric and EMA lines		
	If Adipic > Sebacic	Ketosis, beta oxidation disorder	Acylcarnitines
	If Adipic < Sebacic	MCT supplementation	
Dicarboxylic acids (DCA)	See Adipic, 3-OH-n-butyric and EMA lines		
Branched chain dicarboxylic acids		Squalene synthase deficiency	Sterols
Dimethylsulfoxide and Dimethylsulfone,	Methanethiol	Methanethiol oxidase deficiency	
3,6-Epoxyoctanedioic	Other epoxy (C10, C12, C14), 2-OH-sebacic, DCA with ad>sub	Peroxisomal diseases	
	Idem but ad < sub	MCT supplementation	

Table 3.2 (continued)

Principal OA	Other OAs	Causes of variation	Other investigations
Ethylmalonic	>20 µmol/mmol C alone	SCAD deficiency	Acylcarnitines
	>20 µmol/mmolC ± IBG, 2MBG, IVG	Valproate, RC, GAII, ETHE1	
	>100 µmol/mmolC + IBG, nBG, 2MBG, IVG, HG, SG, 2OHG, DCA, glut	Glutaric aciduria type II	
	>100 µmol/mmolC + nBG	SCAD deficiency	
	20–100	Ethylmalonic encephalopathy (ETHE1)	
Fumaric	High ± succinate, malate	Fumarase deficiency, PEPCKC	
	± high with other KC derivatives + lactate	Respiratory chain disorders, PEPCKC	
Glutaric	3-OH-glutaric	Glutaric aciduria type I	Acylcarnitines
	EMA, 2-CH3succinic, IBG, nBG, 2MBG, IVG, HG, SG, DCA, 2-OHG	Glutaric aciduria type II/III	
Glyceric	Glyceric	Glycerate kinase deficiency	
	L isomer	D-glycerate dehydrogenase or glyoxylate reductase def (hyperoxaluria type II)	
Glycerol	No	Glycerol kinase deficiency Chromosome Xp21 deletion syndrome	
Glycolic	Oxalic	Type I oxalosis	
	4HB	SSADH deficiency	
	Lactic, ethyleneglycol	Ethyleneglycol intoxication	
Hexanoylglycine	High ± and SG ±	Mild or asymptomatic MCAD deficiency	Acylcarnitines
	High + SG + DCA	MCAD deficiency	
	High + SG + DCA + EMA + glut + IBG + EMBG + IVG + nBG	Glutaric aciduria type II	
Homogentisic	Alone	Alkaptonuria	
3-Hydroxy-n-butyric	High ++, AcAc, DCA	Ketosis (starvation, diabetes) Ketolysis defects	AACp
	High ±, DCA, 3HDC	Hepatic failure	AACp
	Low, DCA, 3HDC, ± acylglycines	Fatty acid oxidation defects	Redox + acylcarnitines
4-Hydroxy-butyric	Alone	Drug addiction	
	4,5 diOH-hexanoic lactone and acid, 3,4-diOH-butyric, 2,4-diOH-butyric, glycolic	Succinic semialdehyde dehydrogenase deficiency	
3-Hydroxy-dicarboxylic acids (3HDC)	See 3-Hydroxy-n-butyric line		
2-Hydroxy-glutaric	Very high. 2KG (D-glutaric ac,)	D or L-2-OH glutaric aciduria	
	High ± acylglycines	Glutaric aciduria type II	Acylcarnitines
	Moderately high	Respiratory chain disorders	
3-Hydroxy-glutaric	Glutaric normal or high	Glutaric aciduria type I	
	3-OH-butyric elevated	Ketosis	

(continued)

Table 3.2 (continued)

Principal OA	Other OAs	Causes of variation	Other investigations
3-Hydroxy-isobutyric	2-Ethylhydracrylic	3-OH-isobutyric dehydrogenase def	
2-Hydroxy-isovaleric	2-OH-3-CH3Val, 2-OH-isocaproic, 2-oxo-isovaleric, 2-oxo-3-CH3Val, 2-oxo-isocaproic, AcLeu, AcIle	Maple syrup urine disease	AACp
3-Hydroxy-isovaleric	Slightly elevated Elevated	Valproate treatment Biotinidase deficiency	
	See: 3-Hydroxy-propionic Isovalerylglycine 3-methyl-crotonylglycine 3-Methyglutaconic 3-Methyl-3-OH-glutaric		
3-Hydroxy-propionic	Alone	Bacterial infections	AACu
	PG, TG, MC, (2M3KB, 2M3HB, 3HIV)	Propionic acidemia	
	PG, TG, MC, 3MCG, (2M3KB, 2M3HB, 3HIV)	Biotinidase or holocarboxylase synthetase deficiency	
	Lactic, PG, MC, 3MCG	CA-VA deficiency	Ammonia plasma AACp Redox
	MMA, PG, TG, MC, (2M3KB, 2M3HB, 3HIV)	Methylmalonic aciduria (different causes)	AACp+u
Isovalerylglycine	3-OH-isovaleric	Isovaleric acidaemia	Acylcarnitines
	Other acylglycines, glutaric, EMA	Glutaric aciduria type II, *ETHE1* mutations	
2-Ketoglutaric	Alone	2-KGD deficiency TPK1 deficiency SLC25A19 transporter deficiency E3 deficiency DOOR syndrome	Redox
	Lactic, BCKA, BCHA	E3 deficiency	Redox AACp
	Lactic, KC der	Respiratory chain disorders	Redox
	Lactic, glut, TG, 2OHG,3OHG, 2-oxoAD, 2OHAd	Lipoic acid synthesis deficiency	AACp Redox
Lactic	Alone	Bacterial infections Mitochondrial disorders	AACp+u
	2HIB, 2HB, Pyr, KC derivatives	Respiratory chain disorders	Redox AACp
	KC derivates +3MG	Pearson, respiratory chain disorders	CAAp+u
	High KB, low or very low KC derivatives	PC deficiency	AACp Redox
	3-OHProp, PG, MC, 3MCG, 3HIV	CA-VA deficiency	Ammonia plasma AACp Redox
	Glut, TG, 2OHG,3OHG, TG, 2-oxoAD, 2-OH-adipic, 2KG	Lipoic acid synthesis deficiency	AACp Redox
	Other specific organic acids	Organic acidurias	

Table 3.2 (continued)

Principal OA	Other OAs	Causes of variation	Other investigations
Malonic	Alone	Malonyl-CoA decarboxylase deficiency	
	+ Methylmalonic	ACSF3 def	
Methanethiol	Dimethylsulfide, Dimethylsulfoxide and dimethylsulfone	Methanethiol oxidase deficiency	
3-methyl-crotonylglycine	3-OH-isovaleric	3-CH3-crotonyl-CoA carboxylase deficiency	Acylcarnitines
3-methyl-glutaconic	Very high +3-CH3-glutaric	3-CH3-glutaconyl-CoA hydratase deficiency	
	3-CH3-glutaric ±	Costeff syndrome *CLPB* mutations MEGDEL (*SERAC* mutations) *POLG* mutations Barth syndrome ATP synthase (*TMEM70* mutations) ATAD3 mutations	
	3-CH3-glutaric, lactate, KC derivatives	Respiratory chain disorders, Pearson	
	3-CH3-glutaric, 3-OH-3-CH3-glutaric, 3HIV	HMG-CoA lyase deficiency	
2-Methyl-2,3-dihydroxybutyric		Short-chain enoyl-CoA hydratase (ECHS1) and 3-hydroxyisobutyryl-CoA hydrolase (HIBCHD)	Acylcarnitines
2-Methyl-3-hydroxy-butyric	3-OHProp, PG, TG, MC	Propionate metabolism defects	
	3-OH-nBut, AcAc, 2-CH3–3-oxo-but, TG	β-Ketothiolase deficiency	
	Tiglylglycine	MHBD deficiency (HSD10)	
3-Hydroxy-3-methyl-glutaric	3HIV, 3MG, 3-CH3-glutaric	HMG-CoA lyase deficiency	
Methylmalonic	15 to 250 µmol/mmol crea, isolated	SUCLA2/SUCLG1 Methylmalonyl-CoA racemase deficiency	
	15 to 250 µmol/mmol crea + 3HIB, 3-OHProp	Methylmalonic semialdehyde dehydrogenase deficiency	
	High (>250) with same OA as propionic acidemia (not always)	Methylmalonic acidurias: methylmalonyl-CoA mutase deficiency CblA, CblB IF, IGS, TCII, CblC, D, F, J CblX (*HCFC1* deficiency) Nutritional B_{12} deficiency	AACp (met↓, Hcy+)
Mevalonolactone	Mevalonic	Mevalonate kinase deficiency	
N-acetylaspartate	Alone	Canavan disease or aspartoacylase deficiency	
Orotic		Urea cycle disorders	AACp
		UMP synthase deficiency (hereditary orotic aciduria)	
Phenolic compounds: (Phenyllactic)	Phenylacetic, mandelic, phenylpyruvic, 4-OH-phenylacetic, 4-OH-phenyllactic, 4-OH-phenylpyruvic	Phenylketonuria	AACp
	Phenylpyruvic, 4-OH-phenylacetic, 4-OH-phenyllactic, 4-OH-phenylpyruvic, N-AcTyr	Hepatic insufficiency	AACp

(continued)

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Table 3.2 (continued)

Principal OA	Other OAs	Causes of variation	Other investigations
Pyroglutamic (Oxoproline)	Alone, very high	Glutathione synthetase or oxoprolinase deficiency	
	± High	Secondary: Amino acid infusion, UCD, paracetamol intoxication	
Suberylglycine	See Hexanoylglycine		
Succinylacetone	Several peaks, Succinylacetoacetic, 4-OH-phenyllactic, 4-OH-phenylpyruvic, N-AcTyr	Fumarylacetoacetate lyase def (Tyrosinemia type I) Maleylacetoacetate isomerase deficiency (screening, asymptomatic)	AACp: Not specific
Uracil	Pyroglu, Orotate	Urea cycle disorders	AACp
	Thymine	Dihydropyrimidine dehydrogenase def	
Vanillactic	Vanilpyruvic	Transitory in newborns	
		Dopa treatment	
		Aromatic amino acids decarboxylase deficiency Pyridoxine-related disorders	Neurotransmitters in CSF
Xanthurenic acid		Coeliac disease, chronic dialysis, malabsorption	

2HB 2-hydroxy-n-butyric, *2HIB* 2-hydroxy-isobutyric, *2KG* 2-ketoglutaric, *2KGD* 2-ketoglutarate dehydrogenase, *2M3HB* 2-metyl-3-hydroxy-butyric, *2M3KB* 2-methyl-3-ketobutyric, *2MBG* 2-methylbutyrylglycine, *2-OH-3CH3Val* 2-hydroxy-3-methylvaleric, *2-OHAd* 2-hydroxy-adipic, *2OHG* 2-hydroxyglutaric, *2-OH-isocaproic* 2-hydroxy-isocaproic, *2-oxoAd* 2-oxo-adipic, *3HDC* 3-hydroxydicarboxylic acids (3-OH-adipic, 3-OH-suberic, 3-OH-sebacic, 3-OH-dodecanedioic, 3-OH-tetradecanedioic), *3HIB* 3-hydroxy-isobutyric, *3HIV* 3-hydroxy-isovaleric, *3MCG* 3-methyl-crotonylglycine, *3MG* 3-methylglutaconic, *3OHG* 3-hydroxyglutaric, *3-OH-n-But* 3-hydroxy-n-butyric, *3-OHProp* 3-hydroxy-propionic, *4HB* 4-hydroxy-butyric, *AAC* amino acid chromatography (*p* plasma, *AcAc* acetoacetic, *AcIle* acetylisoleucine, *AcLe*u acetylleucine, *Ad* adipic, *BCHA* branched-chain 2-hydroxy acids, *BCKA* branched-chain keto acids, *CA* carbonic anhydrase, *Cb* cobalamin variant, *CSF* cerebrospinal fluid, *DCA* dicarboxylic acids (adipic, suberic, sebacic, dodecanedioic, tetradecanedioic), *def* deficiency, *DOOR syndrome* deafness, onychodystrophy, osteodystrophy, mental redardation, *E3* common protein of 2-ketoacid dehydrogenase complexes, *EMA* ethylmalonic acid, *ETHE1* ethylmalonic encephalopathy, *GA* glutaric aciduria, *Glut* glutaric, *HG* hexanoylglycine, *HMG* 3-hydroxy-3-methyl-glutaric, *IBG* isobutyrylglycine, *IF* intrinsic factor, *IGS* Imerslund-Gräsbeck syndrome (cubilin/amnionless deficiency), *IVG* isovalerylglycine, *KB* ketone bodies (3-hydroxy-n-butyrate + acetoacetate), *KC der* Krebs cycle derivatives, *MAS* malate aspartate shuttle, *MC* methylcitrate, *MCAD* medium-chain acyl-CoA dehydrogenase, *MCT* medium chain triglycerides, *MDH* malate dehydrogenase, *MHBD* 2-Methyl-3-hydroxybutyryl-CoA dehydrogenase, *MMA* methylmalonic acid, *MMSA* methylmalonic semialdehyde, *MSUD* maple syrup urine disease, *N-AcTyr* N-acetyltyrosine, *nBG* n-butyrylglycine, *PC* pyruvate carboxylase, *PG* propionylglycine, *Pyr* pyruvate, *PyroGlu* pyroglutamic (or oxoproline), *RC* respiratory chain, *Redox* simultaneous measurement of plasma lactate, pyruvate, 3-OH-butyrate and aceto-acetate, *SCAD* short-chain acyl-CoA dehydrogenase, *Seb* sebacic, *SG* suberylglycine, *SLC25A19* transporter (thiamine carrier) (Amisch lethal microcephaly), *SSADH* succinic semialdehyde dehydrogenase, *SUCLA* succinyl-CoA synthetase, *SUCLG* succinyl-CoA ligase, *TC II* transcobalamin II, *TG* tiglylglycine, *TPK1* thiamine pyrophosphate kinase 1, *u* urine, *UCD* urea cycle deficiency, *UMP* uridyl-monophosphate, *β-AIB* β-aminoisobutyric, *β-Ala* β-alanine, *β-ox def* fatty acids β-oxidation defects

Table 3.3 Assessment of intermediary metabolism over the course of the day. The protocol of investigation is adapted to the clinical situation for each patient

Parameters in blood	Breakfast		Lunch		Dinner		Night
	Before	1 h after	Before	1 h after	Before	1 h after	04 h
Glucose[1]	X	X	X	X	X	X	X
Acid-base	X	X					
Lactate[2]	X	X	X	X	X	X	X
Pyruvate[2]	X	X	X	X	X	X	X
Free fatty acids	X	X	X	X	X	X	X
Ketone bodies	X	X	X	X	X	X	X
Ammonia	X	X	X	X	X	X	X
Amino acids	X						
Carnitine	X						
Acylcarnitines	X						
Hormones[3]	X	X	X	X	X	X	X
Urine 24 h collection[4]	Amino acids, organic acids, ketone bodies, urea, creatinine						

[1]Glucose should be determined immediately.
[2]Immediate deproteinization (with perchloric acid) at the bedside is the only way of ensuring that the results for calculating redox potential ratios can be accurately interpreted.
[3]Hormones (insulinemia, cortisol, growth hormone) are useful in the investigation of hypoglycaemia
[4]Urine samples are collected both overnight and during the day and should be frozen immediately

sampling is undertaken, for example local or general anoxia may influence the results for lactate, lactate/pyruvate ratio and ammonia measurements.

Continuous glucose monitoring over a period of 2–3 days during normal activities, using a highly portable subcutaneous probe and recording device, is commonly used in the assessment of individuals with known glycogen storage disease, but is also useful in the investigation of patients who have symptoms at home that may or may not be related to hypoglycaemia [6].

Interpretation

This investigation may show abnormalities in the metabolic and endocrine profiles throughout the day or specifically only during either the fasting or fed states. The data must be compared to age related reference values [7, 8]. All physiological (food refusal) or pathological conditions (malnutrition, cardiac, renal or liver failure) that may influence the results need to be taken in account.

1. In glycogenosis (GSD) type I and in disorders of gluconeogenesis, blood glucose and lactate move in opposite directions, with hypoglycaemia and hyperlactataemia more pronounced in the fasted than in the fed state. In GSD type III, VI and IX, glucose and lactate change in parallel, with a moderate increase of glucose and lactate in the post-prandial state. Fasting hypoglycaemia and ketosis with postprandial hyperlactataemia and postprandial hyperglycaemia is usual in glycogen synthase deficiency (► Chap. 5). Repeated assays are required for glucose and insulin in primary hyperinsulinism, as hyperinsulinemia is frequently erratic and difficult to prove. An insulin level >3 μU/ml with a glucose concentration lower than 2.8 mmol/l should be considered abnormal (► Chap. 6).

2. In patients with pyruvate dehydrogenase (PDH) deficiency, plasma lactate, in association with pyruvate, may be persistently raised, but usually decreases during fasting (► Chap. 11). Lactate may be normal, moderately raised or very high in mitochondrial respiratory chain (RC) disorders [7] (► Chap. 10). It may be difficult to distinguish a moderate elevation of lactate from a falsely raised level due to difficult sampling. However, the presence of a lactaturia with an elevation of alanine in blood is very suggestive of a true hyperlactatemia (the upper threshold for lactate reabsorption is at approx 4 mmol/l). Lactate measurement in cerebrospinal fluid (CSF) may also be of help in patients with neurological disorders.
3. Measurements of ketone bodies are useful for the diagnosis of hyperketotic states, i.e. ketolysis defects or some RC disorders. The simultaneous measurement of blood glucose, free fatty acids and ketone bodies is necessary for the diagnostic and therapeutic evaluation of hypoketotic states, i.e. disorders of fatty acid oxidation (FAO) or ketogenesis (► Chaps. 12 and 13); data must be interpreted with regard to age and length of fasting (Fasting Test [below] and also ► Fig. 1.3). See also ► Sect. 1.4.12.
4. The lactate/pyruvate ratio (L/P), normally around 10:1, and the 3-hydroxy-butyrate/acetoacetate ratio (3OHB/AcAc), normally >1 after an overnight fast and <1 in the fed state, reflect the redox states of the cytoplasm and the mitochondrion, respectively, and may provide additional information [8] as follows: (see also ► Sect. 1.4.13).

   - L/P increased, 3OHB/AcAc normal or decreased: pyruvate carboxylase (PC) deficiency or 3-ketoglutarate dehydrogenase deficiency.
   - L/P and 3OHB/AcAc both increased with persistent hyperlactatemia: RC disorders.
   - L/P normal or low and 3OHB/AcAc normal, with varying hyperlactatemia: PDH deficiency, pyruvate carrier defect.

The usual metabolic abnormalities observed in lactic acidosis due to IEM are summarized in ◘ Table 3.4 (also ► Table 1.3).

3.2 Functional Tests

When performing a functional test, it is important to adhere to a strictly defined protocol in order to attain the maximum amount of interpretable diagnostic information and to minimise the risk of metabolic complications. Some provocative tests are now used infrequently, since simpler direct assays of metabolites and DNA have reduced their diagnostic value. Some have fallen into total disuse and are not considered here. These include the galactose and fructose loading tests, the glucagon test for the differentiation of glycogen storage diseases, the fat loading tests for the differentiation of fatty acids oxidation (FAO) defects and the phenylpropionate loading test for diagnosis of medium-chain acyl-coenzyme A dehydrogenase deficiency (the latter has recently been proposed in some cases of MCAD variants with uncertain pathogenicity) .

3.2.1 Fasting Test

Indications

This test [9] has been used for the clarification of hypoglycaemia observed in disorders of gluconeogenesis, fatty acid oxidation and ketogenesis, ketolysis and in some endocrinopathies. However, as it can be a highly dangerous procedure, its indications are now restricted to unexplained hypoglycaemia when basal metabolic investigations (organic acids analysis, acylcarnitines profile, enzymatic or molecular studies) have ruled out a FAO disorder or adrenal failure, or as a means to assess fasting tolerance during the treatment of certain disorders.

Procedures

The fasting test should only be performed in a specialized metabolic unit and under close medical supervision. The results of the basal investigations should be known before the test is planned. If permanent abnormalities exist, the diagnostic work-up should be adjusted accordingly. During the three days before the test the patient should be adequately fed and the energy intake appropriate for his age. No intercurrent infection or metabolic incident should have occurred during the preceding week.

Fasting tolerance differs considerably depending on the age of the patient and on the disorder. The recommended period of fasting is as follows: 12 h for children less than 6 months of age, 20 h for those 6–12 months, and 24 h from age one year onwards. The test should be planned to ensure that the final and most important period (during which complications may arise) takes place during the daytime, when the best facilities for close supervision are available.

An indwelling venous catheter with a saline drip is inserted at zero time. The patient is encouraged to drink plain water during fasting. ◘ Table 3.5 gives the time

Table 3.4 Main metabolic abnormalities in lactic acidosis due to inborn errors of metabolism (From [7])

	Gluconeogenesis defects (G6Pase, FBPase, PEPCKC deficiencies)	GSD type III, VI	PDH deficiency	PC and MAS defects[1]	KGDH deficiency	Fumarase deficiency	E3 deficiency	Respiratory chain defects
Hyperlactatemia	Maximum during fasting and when hypoglycaemic	Only in fed state	Permanent, maximum in fed state; can be moderate	Permanent	Permanent	Moderate	Permanent	Permanent, maximum in fed state very variable
L/P ratio	<15	<15	<10	>30	15–30	<15	15–30	>20
Ketone bodies	↑ at fast or N Low in PEPCKC	Only at fast	Absent	+ (post-prandial)	+	N	N	↑ + or N
3OHB/AcAc	N	N	N	↓↓	N or ↓	N	N	↑
Glucose	↓ at fast	↓ at fast	N	N or ↓	N	N	N	N or ↓
Ammonia	N	N	N	↑	N or ↑	N	N	N
Alanine	↑ at fast	N	↑ post- prandial	N or ↓	N	N	↑	↑
Glutamine	N	N	N	↓	↑	N	↑	↑
Proline	N	N	N or ↑	↑	N	N	↑	↑
BCAA	N	N	N	↓	N	N	↑	N
Citrulline	N	N	N	↑	N	N	N	N or ↓
Organic acids In urine	Lactate fumarate and succinate in PEPCKC	Lactate	Lactate, pyruvate	Lactate KB	αKG, lactate fumarate	Fumarate	Branched-chain keto acids	N or lactate ± Krebs intermediates, methylglutaconic acid

It should be noted that all metabolic abnormalities are highly variable and that many patients affected with respiratory chain defects have no hyperlactatemia

[1]MAS defects (malate aspartate shuttle defects) include aspartate glutamate carrier (AGC 1), malate oxoglutarate carrier (OGC), mitochondrial malate dehydrogenase (MDH 1 and 2), and glutamate oxaloacetate transaminase (GOT 2) (► Chap. 11)

BCAA branched chain amino acids, *FBPase* fructose-1,6-bisphosphatase, *G6Pase* glucose-6-phosphatase, *KB* ketone bodies, *αKG* α-ketoglutarate, *KGDH* α-ketoglutarate dehydrogenase, *3OHB/AcAc* 3-hydroxybutyrate/acetoacetate, *PC* pyruvate carboxylase, *PDH* pyruvate dehydrogenase, *PEPCKC* phosphoenolpyruvate carboxykinase cytosolic, *N* normal, ↑ increased, ↓ decreased

schedule for the laboratory investigations. The main metabolic monitors for continuing the test safely are glucose and HCO_3^- concentrations in blood. Blood for a complete metabolic and endocrine profile is collected at the start of the test and twice before the end. If glucose drops below 3.2 mmol/l, glucose and HCO_3^- should then be determined at 30-min intervals. If glucose drops below 2.6 mmol/l and/or HCO_3^- drops below 15 mmol/l, or if neurological symptoms develop, the test should be stopped immediately. At that time, blood is taken for the complete metabolic and endocrine profile. The urine is collected and kept on ice for each 8 h period of the fast and for a further 4 h period after the end. From each 8 h or 4 h collection, a sample of 10 ml should be frozen at −70 °C for the determination of lactate, ketone bodies, amino acids and organic acids.

Table 3.5 Fasting-test flow sheet. The duration of the test is adapted to the age of the patient or is determined by the length of time for the onset of spontaneous symptoms (text). A complete sample is taken at the end of the fast if the test is stopped before 24 h

	Time (h)	0	8	12	16	20	24
Blood	Glucose	+	+	+	+	+	+
	HCO_3^-	+	+	+	+	+	+
	Lactate	+	+	+	+	+	+
	3-Hydroxybutyrate	+	+	+		+	+
	FFA	+	+	+	+	+	+
	Carnitine	+		+		+	+
	Acylcarnitines	+	+	+	+	+	+
	Amino acids	+		+		+	+
	Insulin	+		+		+	+
	Cortisol	+					+
	ACTH	+					+
	Growth hormone	+					+
Urine	Organic acids	0–8 +			16–24 +		

ACTH adrenocorticotrophic hormone, *FFA* free fatty-acids

Interpretation

The interpretation of this investigation is difficult and the results must be compared with the normal values for the particular age (Table 3.6).

Blood measurements: the tentative diagnoses are as follows:

- Hyperinsulinaemia: glucose <2.8 mmol/l, insulin >3 mU/l, and FFA <0.6 mmol/l, simultaneously. Ketone bodies (KB) remain very low during the fast. Of note in insulin signalling disorders while patients present with hypoketotic hypoglycaemia, insulin remains low to undetectable (▶ Chap. 6).
- Fatty-acid oxidation and ketogenesis defects: glucose <2.8 mmol/l, increased FFA and low KB with FFA/KB ratio >2 (normal <1) and glucose in mmol/l × total KB in mmol/l <4.
- Gluconeogenesis defect: glucose <2.8 mmol/l and lactate >3.0 mmol/l simultaneously.
- Ketolysis defect: ketone bodies are already high in the basal state and increase dramatically during the fast, with possible acidosis. Glucose × total KB >10.
- PDH defect: high lactate (L) and pyruvate (P) with normal L/P ratio, L and P decrease during the fast. The fasting test is usually not useful for this disorder.
- Defects of the citric-acid cycle and the respiratory chain: variable levels of lactate and KB. The fasting test is usually not informative for these disorders.

Table 3.6 Metabolic profiles during fasting tests in children of different ages (from [9]). Normal blood values of hormones at the end of the fast or when the patient is hypoglycaemic, irrespective of age, are: insulin <3 mU/l at a glucose level of <2.8 mmol/l; cortisol >120 ng/ml; adrenocorticotrophic hormone (ACTH) <80 pg/ml; growth hormone >10 ng/ml

	Less than 12 months	1–7 years		7–15 years	
	20 h	20 h	24 h	20 h	24 h
Glucose (mM)	3.5–4.6	2.8–4.3	2.8–3.8	3.8–4.9	3.0–4.3
Lactate (mM)	0.9–1.8	0.5–1.7	0.7–1.6	0.6–0.9	0.4–0.9
FFA (mM)	0.6–1.3	0.9–2.6	1.1–2.8	0.6–1.3	1.0–1.8
KB (mM)	0.6–3.2	1.2–3.7	2.2–5.8	0.1–1.3	0.7–3.7
3OH-B (mM)	0.5–2.3	0.8–2.6	1.7–3.2	<0.1–0.8	0.5–1.3
3OH-B/AcAc	1.9–3.1	2.7–3.3	2.7–3.5	1.3–2.8	1.6–3.1
FFA/KB	0.3–1.4	0.4–1.5	0.4–0.9	0.7–4.6	0.5–2.0
Carnitine (free; µM)	15–26	16–27	11–18	24–46	18–30

AcAc acetoacetate, *FFA* free fatty-acids, *3OH-B* 3-hydroxybutyrate, *KB* ketone bodies

- Adrenal-cortex insufficiency: glucose <2.8 mmol/l and cortisol <250 nmol/l simultaneously. Adrenocorticotrophin hormone (ACTH) deficiency: ACTH <80 pg/l. The fasting test is dangerous and contraindicated if this disorder is suspected.
- Human growth hormone (hGH) deficiency: glucose <2.8 mmol/l and hGH <10 ng/ml simultaneously. The fasting test is usually not useful for this disorder.
- Urine measurements: the best approach is to compare the results of the last period with those of the first.

Complications

Hypoglycaemia, metabolic acidosis, cardiac dysrythmia, cardiomyopathy, organ failure may occur. Fluids and medication must be immediately available in the patient's room.

3.2.2 Oral Glucose Loading Test

Indications

This test is used for elucidation of hypoglycaemia or moderate/intermittent hyperlactatemia of unknown origin.

Procedures

It should follow a period of fasting of 3 to 8 h, depending on the age and the patient's usual interval between meals. In the case of previously recorded hypoglycaemia, the test is started at a plasma glucose concentration between 3.3 mmol/l and 2.8 mmol/l. An indwelling venous catheter is inserted 30 min before the expected start of the test and kept patent with a saline drip. A glucose load (2 g/kg with a maximum of 50 g), as a 10% solution in water, is administered orally or through a nasogastric tube over 5–10 min. The blood is sampled from the indwelling venous catheter twice at zero time (just before glucose administration) and every 30 min thereafter until 3–4 h.

All blood samples are assayed for glucose, lactate, pyruvate, 3OHB and AcAc. A urine sample collected just before the test and a second aliquot from a sample collected during the 8 h after glucose administration are tested for lactate, ketone bodies and organic acids.

Interpretation

- Glucose: a short-lived increase followed by a precipitous decrease may be observed in some cases of hyperinsulinism.
- Lactate: a marked decrease from an elevated fasting level occurs in disorders of gluconeogenesis and glucose-6-phosphatase deficiency (GSD type I) [10]. An exaggerated increase from a normal fasting level occurs in other GSDs including glycogen-synthase deficiency. Lactate remains increased or increases even further after glucose administration in PDH deficiency and RC disorders [8, 11]. Any increase in lactate must be compared to control values. The L/P ratio, normally around 10:1, is usually increased in PC deficiency and in mitochondrial disorders and remains normal or low in PDH deficiency and in mitochondrial pyruvate carrier defect.
- Ketone bodies: Ketone bodies may increase paradoxically in PC deficiency (with a low 3OHB/AcAc ratio) and in RC disorders (with a high 3OHB/AcAc ratio). Fasting ketone bodies are very low in hyperinsulinism and insulin signalling disorders.

Complications

The test should be stopped if plasma glucose drops below 2.6 mmol/l. The complete metabolic profile should be taken at that time.

In patients with PDH deficiency, a glucose load may precipitate lactic acidosis.

3.2.3 Glucagon Test

Indications

The glucagon test has now been abandoned for the investigation of conditions associated with fasting hypoglycemia where it can be dangerous. However, it remains very useful in patients with spontaneous and repeated hypoglycemias in the fed state, where hyperinsulinism is suspected. For these patients, it is a rapid diagnostic method and also a useful therapeutic test.

Procedure

Intra-muscular injection of glucagon (0.5 mg in newborn and infant, 1 mg in children) with measurements of blood glucose before and after 10, 20 and 30 minutes.

Interpretation

An increase in blood glucose >50% of the basal value is considered significant and suggestive of an hyperinsulinism.

Complications

The test is contraindicated in the fasted state where it can be dangerous and may precipitate catabolic decompensation.

3.2.4 Exercise Test

Indications

The exercise test is used to identify patients suspected of having a metabolic myopathy. Several methods exist:

- A non-ischemic forearm-exercise test [12, 13].
- Bicycle ergometer test [14, 15].
- Treadmill test.

The best exercise test for the widest age range is the treadmill test.

Exercise testing may help in the diagnosis of glycogen storage disorders [16] affecting muscle (▶ Chap. 5) and for AMP deaminase deficiency. In mitochondrial myopathies, an exercise test is neither sufficiently specific nor sensitive for diagnosis [17]. This test may also be suitable for assessing the effects of therapeutics or exercise training in glycogen storage disorders [18] (▶ Chap. 5) and mitochondrial myopathies (▶ Chap. 10).

Procedures

The forearm and the bicycle test are only applicable in adults and older children who are able to adhere to the protocols. The treadmill test has the advantage that it can be used from the age at which the child is able to walk. All exercise tests should be carried out at a submaximal workload. This is a safeguard to prevent severe complications, such as rhabdomyolysis, myoglobinuric anuria and metabolic acidosis.

The original forearm test, and its semi-ischemic modification have been abandoned and replaced by a more accurate non ischemic test [12, 13, 15].

In the bicycle ergometer test, the duration of the exercise and a submaximal workload associated with a pulse rate below 150 beats/min for adults or between 150 and 180 beats/min for children are adapted to the patient's condition [14].

In the treadmill test, the speed of the belt and its angle of inclination can be manipulated to a walking velocity of 3–5 km/h and a pulse rate of 150–180 beats/min. Exhaustion arises rapidly in those with myopathies due to defects of glycolysis and in defects of the citric-acid cycle and the respiratory chain. It occurs later in patients with defects of fatty-acid oxidation (after the exhaustion of energy from glycogen via aerobic and anaerobic glycolysis). The interpretation of the results of each exercise test should take this time sequence into consideration. In plasma and urine, the parameters to be compared before, during and after exercise are the following:

- Plasma: lactate, pyruvate, throughout the study. Acylcarnitines, ammonia, creatine kinase (CK) and potassium (K+) at the start and the end of the test.
- Urine: lactate, organic acids.

Interpretation

Lactate normally rises during muscle contraction reflecting a disturbed equilibrium between its production from glycolysis and its expenditure in the citric-acid cycle. No increase in lactate reflects deficient glycolysis which can be caused by phosphorylase deficiency and other more rare muscle glycolysis defects (▶ Chap. 7). Abnormally high elevations of lactate can be found with mitochondriopathies and muscle AMP deaminase deficiency.

Ammonia normally rises owing to deamination of adenosine monophosphate (AMP) during muscle contraction. There is no increase in ammonia in muscle AMP deaminase deficiency (▶ Chap. 32). The exercise test may reveal specific acylcarnitine accumulation in fatty acid oxidation disorders. An elevation of CK and K^+ reflects abnormal myolysis.

3.3 Metabolomic Analyses for Diagnosis of IEM

The concept of metabolomics encompasses the study of small molecules, lipids (lipidomics) and carbohydrates (glycomics), with different technical approaches. In the last decade, remarkable advances led to the development of high throughput analytical techniques based on NMR spectroscopy and mass spectrometry coupled to different separation techniques, mainly chromatographic [19]. There are two categories of metabolomic analyses; the targeted and untargeted approaches. There are some intrinsic characteristics of metabolomic studies that differentiate it from genomic studies, since the former is much more variable due to environmental factors such as diet, treatments or the microbiome effect that can remarkably modify the metabolic profiles. It is calculated that after an untargeted metabolomic analysis, around 10,000 different "metabolic features" can be obtained [19] and some of them will be classified as FUS (features of uncertain significance), a concept analogous to VUS (variants of uncertain significance) driven after next generation sequencing.

Targeted Metabolomics

This analytical approach is based on hypothesis-driven measurements of metabolites in body fluids, and a limited number of metabolites (typically around 100 or less) can be analyzed in one run. The main advantages are that methods are robust and allow absolute metabolite quantitation. Moreover, the deep knowledge accumulated over decades allow to screen healthy versus altered states and to a direct (biomedical) metabolic profile interpretation. Importantly, interlaboratory comparison schemes (such as ERNDIM program), are fully implemented. As disadvantages, since it focuses on

only target metabolites, no new/unexpected findings and biomarkers are possible to be discovered and thus, it will only provide a partial vison of the metabolic scenario [20].

- **Untargeted Metabolomics**

This analytical approach is based on hypothesis-generating measurements, analysing as many metabolites as possible in body fluids (typically around 10,000). The advantages are that these techniques have the potential to screen known and novel metabolites providing massive information about the overall genomic/environmental interaction. Thus, the discovery of new biomarkers for known and new IEM, either for diagnosis and treatment follow-up is possible. As disadvantages, the metabolite quantitation is less robust when compared with targeted techniques. There is a lack of external quality control schemes for interlaboratory comparison. Finally, data processing parameters are not fully validated, and relay on the use of complex software and mathematical approaches [20]. It means, for example, that incorporation of new professional profiles in clinical laboratories dealing with untargeted metabolomics analysis, such as engineers or mathematicians expert in big data, is mandatory.

The untargeted metabolomics techniques are approaching maturity to be applied for the diagnosis and follow-up of patients with IEM [19, 21], although there are still relevant aspects to improve. Moreover, integration of metabolomics data in "multi-omics" platforms (with phenotypic features, proteomic, transcriptomic and genomic data, amongst others) is a challenge for the near-future [22] and will require international efforts to integrate all these data in data bases (it is advisable to visit the human metabolome database). Other aspects that are promising tools to elucidate the pathophysiological basis of IEM are intracellular metabolomic analyses that need to be fully standardized.

Proteomics gives insight into the composition, structure, and function of the proteome that allows the identification of specific proteomic signatures [23]. At present, technological advances allow to detect around 6000 different proteins in a given cell type. The main limitations of proteomics are tissue selection. Sensitivity is limited as well due to the large dynamic range of protein concentrations across sample tissues [22]. Regarding biological fluids, the proteomic approach is one step behind the metabolomic one, at least from diagnostic point of view feasibility. In any case, it has tremendous potential in the discovery of new biomarkers, as has already been demonstrated in different inherited metabolic diseases [23].

3.4 Next Generation Sequencing and Gene Panels

The important cost reduction and the possibility to accelerate diagnoses make next generation sequencing (NGS) increasingly attractive in the complex journey of patients with rare diseases, such as IEM. Some centres have even implemented these techniques as first-line biological investigations as soon as a genetic disease is suspected. Other centres have elected to pursue performing metabolic investigations before targeting one candidate gene or several candidate genes, often grouped in the so-called gene panels.

Gene panels allow analysing simultaneously several genes that belong to a common clinical or radiological or biochemical pattern, while ensuring an optimal sequencing coverage in order to reduce the number of false negative and false positive analyses. Gene panels have been successfully developed for patients with neurodevelopmental delay, neuro-sensorial deficit, peripheral neuropathy and leukodystrophy (▶ Chap. 2). More specifically in the field of IEM, gene panels are also being developed for major biochemical entries such as hyperhomocystinaemia, hyperammonaemia as well as peroxisome biogenesis disorders and lysosomal disorders. Although gene panels allow the analysis of a more restrictive number of genes than whole exome approaches, they do not preclude the use of standard genetic validation approaches, especially the analysis of variants frequency in the general population, the analysis of variants segregation in the family and functional analyses when possible. Furthermore, the possibility for a given gene to be associated with both dominant (heterozygous mutation) and recessive (homozygous or compound heterozygous mutations) inheritance, as reported in a growing number of metabolic disorders [24–26] further complicates the interpretation of NGS datasets. However, such global approaches may help further understanding the additional pathological effects of variants in distinct genes that encode proteins belonging to the same pathway. For whole exome sequencing, it is important to keep in mind that the chances to identify disease genes significantly increase in either of the 3 situations: family consanguinity, affected siblings or trio analyses (the patient and his/her parents) in the case of a sporadic patient.

Altogether, instead of replacing metabolic investigations, the development of NGS is increasingly likely to require the use of functional tools in order to validate genetic variants before they can be implicated in a patient's disease. Moreover, some biochemical investigations should remain first-line tools for certain clinical presentations as they are cheap to perform and very

sensitive to diagnosis, especially in adult medicine where the access to NGS is still somewhat limited. Of importance, the interpretation of datasets obtained by NGS requires the access to advanced bioinformatics pipelines in order to sort out the complexity of the genetic information of a given patient.

3.5 Postmortem Protocol

Since the first description of a post-mortem protocol by Kronick [27], some refinements have become available to enhance the diagnostic value of the original recommendations [28, 29]. In the protocol given below, the time schedule for proper preservation of specimens determines the sequence of the diagnostic procedures.

3.5.1 Cells and Tissues for Enzyme Assays

Liver (minimum 10–20 mg wet weight) and muscle (minimum 20–50 mg wet weight) biopsies are taken by needle puncture or, preferably, by open incision. The tissues are immediately frozen in small plastic cups in liquid nitrogen, followed by storage at −80 °C. Part of the liver biopsy should be fixed for histological and electron-microscopic investigation prior to freezing (»Autopsy« below). A total of 20 ml of blood is collected by peripheral or intracardiac puncture in a heparin-coated syringe; 10 ml is transferred to the laboratory for isolation of erythrocytes or white blood cells, and the biochemist is notified. At least 3–5 ml is conserved for chromosome analysis and DNA extraction.

3.5.2 Cells and Tissues for Chromosome and DNA Investigations

Of the 10 ml of fresh heparinized blood collected, 1–2 ml is reserved for chromosome analysis; the remaining 3–5 ml can be used for DNA extraction. Additionally, blood spots dried on filter paper (as in the Guthrie test) are useful for many investigations and should always be collected. These samples as well as paraffin-embedded tissues can also be used for DNA analysis.

3.5.3 Skin Fibroblasts

At least two biopsies (diameter 2–3 mm) are taken under sterile conditions as early as possible; one from the forearm, one from the upper leg (fascia lata above). Although a delay decreases the chance of successful fibroblast cultivation, fibroblasts may often be cultivated even from biopsies taken many hours after death. A biopsy may also be taken from the pericardium in case of delayed autopsy. These samples are conserved in culture medium or, if not immediately available, on sterile gauze wetted in sterile saline and sealed in a sterile tube for one night at room temperature.

3.5.4 Body Fluids for Chemical Investigations

Plasma from the centrifuged blood sample, urine (~10 ml), and cerebrospinal fluid (~4 ml) are immediately frozen at −20 °C or at −80 °C if available (Table 3.7). If no urine can be obtained by suprapubic puncture or catheterization, the bladder may be filled with 20 ml of saline solution and diluted urine may be harvested. Alternatively, vitreous humor can also be collected (by intraocular puncture) and frozen. This liquid is comparable to blood plasma with respects to its solubility for organic acids. Bile, readily available at autopsy, has been found to be useful material for the post-mortem assay of acylcarnitines [30].

Many biochemical parameters are impossible to interpret post-mortem due to rapid tissue lysis. These include lactate, ammonia, carnitine (total and free), and amino acids, all of which rapidly increase without any specific significance. In contrast, the acylcarnitine-ester profile, determined from dried blood spots or from bile, may be highly diagnostic for many disorders of fatty-acid oxidation and for organic acidurias.

3.5.5 Autopsy

The autopsy is important, particularly in undiagnosed patients and foetus, where it may give important clues to the underlying disorder. It should be as complete as soon as possible and include the cranium, provided that the parents have given permission [31]. The pathologist freezes fresh samples of liver, spleen, muscle, heart, kidney and brain and conserves important tissues for histology and electron microscopy in buffered formaldehyde (4%) and Karnofski fixative, respectively.

If a complete autopsy is refused, it is important to obtain permission to take photographs, X-rays, blood, urine and CSF samples, skin biopsies, and to do needle biopsies of liver and muscle. A kit containing all the material necessary for collecting and conserving specimens is highly recommended as a means of ensuring that the post-mortem protocol is completed as fully and as quickly as possible.

Table 3.7 Collection, processing and storage of blood, urine, and cerebrospinal fluid (CSF) for metabolic and endocrine investigation. The volumes of blood, urine, and CSF are subject to local practice, which must be taken into account

Blood	Urine	Cerebrospinal fluid
Hematology: 0.5 ml in EDTA tube	pH, amino acids, organic acids, ketone bodies, lactate, reducing substances: 5 ml (at least), freeze at −20 °C	Cells, protein, glucose: 0.5 ml in plastic tube
Blood gases: 0.5 ml on heparin-coated syringe (eject air bubble, cap syringe immediately)		Lactate/pyruvate: 1 ml, add to 0.5 ml perchloric acid (18% v/v, keep on ice), centrifuge under refrigeration, store supernatant at −20 °C
Electrolytes, urea, creatinine, urate, total protein, liver function tests: 1–2 ml (centrifuge after clotting)		Amino acids: 0.5 ml in plastic tube
Glucose: 0.3 ml fluoride-heparin tube (dry heparin and fluoride salts, no solution)		Culture: 1 ml in sterile tube
Lactate/pyruvate and 3OHB/AcAc: 1 ml blood (no forcing), mix immediately with 0.5 ml perchloric acid (18% v/v, keep on ice, centrifuge under refrigeration, store supernatant at −20 °C)		
Ammonia: 0.5 ml in heparin-coated syringe on ice (eject air bubble, cap syringe immediately)		
Amino acids: 1–2 ml in EDTA or heparin tube		
Carnitine: 1–2 ml in EDTA tube on ice, centrifuge under refrigeration, store at −20 °C		
Free fatty acids: 0.3 ml in fluoride-heparin tube (dry heparin and fluoride salts, no solution)		
Insulin: 1 ml in EDTA tube on ice, centrifuge under refrigeration, store at −20 °C		
Cortisol and ACTH: 1 ml in plastic, heparin-coated syringe (keep on ice, centrifuge under refrigeration in plastic tube, store at −20 °C)		
Growth hormone: 1 ml (centrifuge under refrigeration after clotting, store at −20 °C)		
Glucagon: 3 ml in heparin tube (centrifuge under refrigeration, store in plastic vial at −20 °C)		
DNA extraction: 5 ml in EDTA tube		

AcAc acetoacetate, *ACTH* adrenocorticotrophic hormone, *EDTA* ethylenediaminetetraacetic acid, *3OHB* 3-hydroxybutyrate

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Emergency Treatments

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Contents

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4

Introduction

As soon as the diagnosis of a metabolic disorder is suspected, a plan for its emergency management should be made. As stated in ► Chap. 1, both the presentation and the management depend mainly on the pathophysiology involved. This chapter focuses on the main clinical presentations in neonates, children and adults with those inborn errors of metabolism for which emergency treatment may be life-saving and outlines the first steps of such treatment up to when the exact diagnosis is known. The subsequent management of patients is addressed in the specific chapters.

In neonates, the main clinical presentations are as follows:

1. Neurological deterioration (metabolic encephalopathy): This is the most common presentation and the main causes are maple syrup urine disease (MSUD), branched-chain organic acidurias (BCOAs) and urea cycle defects (UCDs). Treatment must be started immediately to avoid severe cerebral sequelae.
2. Liver failure: Galactosaemia, and tyrosinaemia type I (though the latter is rare before 2–3 weeks of life) are amenable to specific emergency treatment.
3. Hypoglycaemia: Blood glucose levels must be corrected immediately. The three groups of disorders usually implicated are hyperinsulinism, glycogen storage diseases (GSD) and mitochondrial fatty acid oxidation (FAO) defects.
4. Cardiac failure: In neonates treatable disorders include FAO defects, infantile Pompe disease and TMEM70 deficiency.
5. Primary hyperlactataemia: This is associated with a general lack of cellular energy and may be due to various enzymatic defects. Some patients may benefit from high-dose vitamin treatment.
6. Intractable seizures: Vitamin responsiveness (pyridoxine, pyridoxal phosphate, folinic acid, biotin) must be assessed systematically.

In older children all these clinical situations can also arise whereas, in adults, emergencies mainly comprise non-epileptic or epileptic encephalopathy and rhabdomyolysis. In particular, any type of coma, refractory seizures or acute psychiatric symptoms can be the presenting sign of a metabolic disorder. In addition, recurrent attacks of unexplained dehydration, abdominal pain, muscle pain and myolysis or peripheral neuropathy can also be indications of a metabolic disease.

Such situations require careful and urgent biochemical investigation. Emergency treatment should be started concurrently and subsequently modified when necessary. Good collaboration with the metabolic laboratory is essential. The results of all laboratory investigations relevant to the diagnosis of metabolic disorders and for which specific emergency therapy exists should be available within 24 h.

4.1 General Principles

4.1.1 Supportive Care

Many patients, especially newborn infants, will require ventilatory and circulatory support. Most will need rehydration and correction of electrolyte, calcium and phosphate imbalance, but such treatments, despite their importance, should not delay the start of specific therapeutic measures. Patients with a metabolic crisis frequently suffer from septicaemia, which can result in persistent catabolism and lead to therapeutic failure. Consequently, infections must be prevented: patients must be thoroughly investigated for infection, and any infection must be actively treated.

4.1.2 Nutrition

Whatever the disease, nutrition is extremely important and both the method of administration and the composition of feeds must be rapidly determined. Briefly, four types of diet can be considered: normal, low-protein, carbohydrate-restricted, and high-glucose, with or without lipid restriction. To promote anabolism, the age-related recommended daily energy should be provided [1–3]. In some situations, the anabolic effect of insulin may reduce energy requirements [4].

The mode of administration will depend on the disorder and the clinical status. Oral (enteral) nutrition is preferable if the condition and clinical status allow it. Continuous enteral tube feeding can be temporarily useful in many patients whose initial condition is poor. Total parenteral nutrition (TPN) is the method of choice in those cases where effective enteral nutrition is precluded (e.g. by intestinal intolerance, high energy or high glucose requirements or invasive techniques required for toxin removal).

4.1.3 Specific Therapies

Specific therapies can be used for some disorders. These mainly comprise substrates that enable the excretion of ammonia by alternate pathways (► Chap. 19), carnitine and vitamin supplementation and administration of additional specific drugs [5] (► Chap. 45). All intensive care units should ensure that these are readily available.

4.1.4 Extracorporeal Procedures for Toxin Removal

For those disorders associated with acute metabolic toxicity, such as UCDs and BCOAs, extracorporeal procedures to remove toxins are necessary when less invasive methods are insufficient. Of the available techniques continuous veno-venous haemodiafiltration (CVVHDF) and haemodialysis (HD) are more efficient than peritoneal dialysis (PD) [6]. However, the choice of the technique is highly influenced by local facilities and experience.

4.2 Emergency Management of Particular Clinical Presentations

As previously stated, the management depends on the pathophysiology and the main clinical presentation. In addition to the management detailed below there are a number of national guidelines for the emergency management of different inborn errors available online. These include those in English from the British Inherited Metabolic Disease Group (BIMDG) (► http://www.bimdg.org.uk/site/guidelines.asp), in Spanish 'Enfermedades Raras Metabólicas Procedimientos de Urgencias y de Situaciones de Riesgo' (► https://ae3com.eu/wp-content/uploads/2020/03/PROTOCOLO-DE-URGENCIAS.pdf) and an initiative by MetabERN currently in progress to provide guidelines in various languages (► https://www.emergencyprotocol.net/).

4.2.1 Neurological Deterioration

The most common treatable disorders causing an acute toxic encephalopathy are MSUD, BCOAs and UCDs, and these should always be considered, particularly in a newborn infant who presents with a sepsis-like illness following an asymptomatic period. In caring for neonates with BCOAs and UCDs there are three main risks: overhydration, cerebral oedema and acute protein malnutrition [7–9]. The management of children and adults (late-onset coma) is essentially similar to that of neonates [8, 9]. In adults, clinical symptoms may however often precede and/or predominate over biochemical abnormalities.

4.2.1.1 Supportive Care

- **Neurological Deterioration with Ketoacidosis**

This is the usual clinical presentation in infants with BCOAs including MSUD. There is, however, no significant metabolic acidosis in MSUD, while BCOAS, especially in neonates, are almost invariably characterized by hyperammonemia [10]. In general, in addition to disease-specific therapies, patients require supportive care, procedures for the removal of toxins, and high-energy protein-free nutrition.

From a practical point of view, two situations should be considered:

- Some patients may not initially appear seriously unwell and may have only a mild acidosis (pH >7.20, HCO_3 >15 mmol/L), mild to moderate dehydration (<10% of birth weight) and normal or moderately raised blood ammonia (<400 μmol/L). Blood glucose, lactate, calcium and cell count are normal. This is frequently the early presentation in MSUD (absence of ketoacidosis) and in methylmalonic, propionic and isovaleric acidurias when recognised early or diagnosed by newborn screening.
- In other patients, the situation appears more severe. This is especially the case for patients with organic acidurias whose diagnosis has been delayed for a few days. They present with severe ketoacidosis (pH <7.10, HCO_3 <10 mmol/L), are seriously dehydrated (by >10% of birth weight), and may have overt hyperammonaemia (>400 μmol/L), mild hyperlactatemia (<5 mmol/L), hypo- or hyperglycaemia, hypocalcaemia, leukopenia and thrombocytopenia.

- **Neurological Deterioration with Hyperammonaemia**

This is most commonly due to primary disorders of the urea cycle (see ► Chap. 19). Affected neonates have acute neurological deterioration with vasomotor instability, apnoeas, fits and hypertension. Biochemically, they exhibit respiratory alkalosis, with plasma ammonia levels above 400 μmol/L, and often very much higher. All other routine laboratory tests are normal and, in particular, ketonuria is not usually present. As a general rule, the treatment is similar to that in the previous group [11]. However, newborn infants with UCDs and severe untreated or persistent hyperammonaemia have in most cases a poor outlook, and even among those who receive the most aggressive treatment the majority of survivors will be handicapped [6]. Infants treated prospectively do better, but there may still be significant complications [12, 13]. Thus, the wisdom of starting treatment should be carefully considered. Some children with organic acidurias diagnosed late may have a similar presentation with severe hyperammonaemia without ketoacidosis [14] (► Chap. 18). The need for urgent management, and unfortunately the poor prognosis, are the same as for UCDs [6, 11]. Rarely, long-chain fatty acid oxidation defects may also present with overwhelming hyperammonaemia (► Chap. 12).

4

Mildly Affected Infants

These neonates should be hydrated over a 24-h period, while a procedure for toxin removal is prepared. Hydration can be performed using a standard 5–10% glucose solution containing 75 mmol/l of Na + (4.5 g/l of NaCl) and 20 mmol/l of K+ (1.5 g/l of KCl). High-calorie, protein-free nutrition should be started in parallel, using carbohydrates and lipids to provide 100–120 kcal/kg/day. Initially, for the 24- to 36-h period needed to test gastric tolerance, parenteral and enteral nutrition are used together. The requirement for toxin removal is dependent on the diagnosis, the levels of metabolites and the short-term clinical and biochemical course. To prevent acute protein malnutrition, the protein-free diet must not be used for more than 2 days. Once the levels of toxic metabolites have decreased, natural proteins are introduced using measured amounts of infant formula (► Sect. "Enteral Nutrition").

Severely Affected Infants

Neonates with severe ketoacidosis present with intracellular dehydration that is often underestimated. In this situation, aggressive rehydration with hypotonic fluids and alkalinisation may cause or exacerbate pre-existing cerebral oedema. Therefore, after ensuring adequate tissue perfusion with an initial intravenous bolus or 10–20 ml/kg of normal saline, rehydration should be planned over a 48-h period, with an infusion of less than 70–150 ml/kg/24 h (according to age) that contains an average concentration of 75 to 150 mmol/l of Na + (4.5 to 9 g/l of NaCl), 30–40 mmol/l of K+ (2–3 g/l of KCl) and 5% glucose. Acidosis can be partially corrected with IV bicarbonate, especially if it does not improve with the first measures applied for toxin removal. However, aggressive therapy with repeated boluses of i.v. bicarbonate may induce hypernatraemia, cerebral oedema, and even cerebral haemorrhage. In order to compensate for bicarbonate consumption, sodium bicarbonate may be substituted for one-quarter to one-half of the sodium requirements during the first 6–12 h of rehydration. To prevent precipitation with calcium, the bicarbonate solution should be connected to the infusion line with a Y-connector. These supportive measures are applied in parallel with a procedure for toxin removal that, in addition to the dialysis of the toxic organic acids, can compensate for some of the fluid and electrolytic imbalance and allow for nutritional support.

Presentation and Management in Adults

Behavioural changes (irritability, anxiety) and confusion are the most common presenting symptoms in adults with hyperammonaemia. During the first 48 h, hydration and parenteral nutrition using a central i.v. line is the method of choice while starting the administration of nitrogen scavengers. Total daily energy intake shall exceed 2000 kcal in women and 2500 kcal in men. In the setting of hyperammonaemia >100 μmol/L, the presence of neurological symptoms (behavioural changes, confusion, ataxia, seizures, drowsiness, coma) is a clear indication for prompt initiation of haemodialysis. However, haemodialysis should only be performed during short intervals to lower the risk of central pontine myelinolysis as the adult brain is very sensitive to osmolarity changes. After 48 h, proteins should be reintroduced, ideally using enteral feeding.

4.2.1.2Nutrition

Parenteral Feeding

Total parenteral nutrition (TPN) is the method of choice in infants with severe illness who are at high risk of gastric intolerance. The amino acid-free TPN solution is suitable for the first 48 h; protein must then be added using a commercially available amino acid solution. Initially, amino acids are introduced in amounts sufficient to meet the minimal daily requirements, and then titrated according to biochemical monitoring. The method is safe if the amino acid solution is evenly distributed over the whole day [15–17]. The minimal isoleucine requirement in neonates is at least equal to that of valine. However, many IV amino acid solutions provide less of the former than the latter. Consequently, when the TPN solution only provides the minimal requirement for L-valine, additional oral supplementation of L-isoleucine (25–100 mg/day) is often necessary. Vitamins, mineral and micronutrients must always be provided to prevent deficiencies.

Enteral Feeding

As soon as the enteral feed is available the switch from parenteral nutrition is scheduled over a 4- to 5-day period, using continuous nasogastric tube feeding [3, 18]. To ensure gastric tolerance, small volumes, e.g. 60 ml/day, are given initially and then increased every 24 h until the full fluid requirement is met. As enteral feeds are increased, the parenteral infusion rate is decreased reciprocally. Ondansetron (0.15 mg/kg in 15 min i.v., up to three times daily) may be tried if there is persistent vomiting. In terms of the formulation of feeds, the first step is to progressively increase the amount of protein given to reach the desired daily requirements using human milk or infant formula. Urinary urea excretion assessment is a simple and useful tool for guiding the reintroduction of natural proteins [19]. Next, calories are slowly added using either glucose polymer and lipids or a commercially available protein-free powder. Minerals, vitamins and micronutrients are also given. Addition of an amino acid mixture, if necessary, is the final step, since it increases the osmolarity

of the solution and can induce diarrhoea. However, in MSUD, a branched-chain-free amino acid mixture is always required as early as possible. During this process, the volume of water is increased to cover the requirement for age and weight.

In mild decompensation, enteral nutrition may be sufficient to result in a rapid clinical and biochemical recovery [18]. In this situation the composition of the enteral formula is initially based on a glucose-lipid mixture. However, to prevent acute protein malnutrition, a protein-free diet should not be used for more than 2 days. In infants, the diet should provide 120–140 kcal/kg/day. Micronutrients, osmolarity, and renal solute load must be assessed to make it possible to provide the recommended dietary allowance (RDA) and prevent diarrhoea and dehydration. Depending on the disorder, an appropriate amino acid mixture can be added to cover the protein requirement. The latter is an absolute requirement in MSUD [3, 15, 17, 20]. Once the toxic metabolites have normalised, natural proteins are introduced using measured amounts of infant formula. Attention must be paid to both the total protein and essential amino acid requirements. For patients with an inborn error blocking an amino acid catabolic pathway, intake of natural protein and essential amino acids must provide the minimal safe requirements (protein accretion + non urinary losses), which are 50–60% below the normal requirements (protein accretion + non urinary losses + urinary losses) and consequently less than the RDA [21]. These minimal requirements represent the basis for initiation of a protein-controlled diet. Next, the natural protein and amino acid intakes are adjusted for growth and according to the specific biochemical control. The final step is transition to appropriate long-term dietary treatment.

4.2.1.3 Specific Therapies

- **Enhancing Anabolism: Insulin**

Owing to its anabolic effect insulin is used to suppress severe catabolism; however, this will only be achieved if dehydration and acidosis are also corrected. Infusion of insulin in high doses (0.05–0.2 IU/kg/h) used in association with large amounts of glucose provided by TPN may be useful [4, 22, 23]. The dose of insulin must be adjusted frequently to control glycaemia. Sustained normalisation of the blood glucose level, which is an indirect marker of effective anabolism, allows for insulin withdrawal. Human growth hormone has been useful in promoting anabolism in a variety of organic acidurias, but is unlikely to be effective in the acute situation.

- **Alternative Pathways**

Neurological damage is primarily related to the duration and the severity of hyperammonaemia; consequently, ammonia must be removed as rapidly as possible [11]. In acute situations, L-arginine is an essential amino acid in all disorders of the urea cycle (except arginase deficiency) and is administered together with sodium benzoate and/or sodium phenylbutyrate, the latter providing alternative pathways for nitrogen excretion by conjugation with glycine and glutamine, respectively [11, 24]. There has been some debate as to whether sodium benzoate or sodium phenylbutyrate should be used for detoxification of ammonia before the diagnosis is known in organic acidurias, as there is the theoretical risk of additional intramitochondrial coenzyme A depletion [25, 26]. However, sodium benzoate is now regularly used, without apparent adverse effects [5, 11, 27, 28]. Sodium phenylbutyrate is given as ammonia scavenger because, following its conversion to phenylacetate, it binds to glutamine to form phenylacetyl-glutamine, which is rapidly excreted. Its use is not recommended in organic acidurias in which glutamine levels are normal or low [28–30]. Enteral sodium phenylbutyrate is used to provide a source of phenylacetate. The i.v. combination of both sodium benzoate and sodium phenylbutyrate may also be used, exclusively by a central line. However, the use of phenylbutyrate containing-drugs must be limited before a precise diagnosis indicating hyperammonaemia is obtained, since glutamine is elevated only in urea cycle defects and is in the low-normal range in organic acidurias. In N-acetylglutamate synthetase deficiency, N-carbamoylglutamate has become available as the treatment of choice. It may also be efficacious in hyperammonaemia attributable to CPS deficiency or N-acetylglutamate synthetase inhibition by acyl-coenzyme A in organic acidurias [10, 28, 31, 32].

L-Carnitine is given to compensate for secondary carnitine deficiency caused by urinary excretion of carnitine-bound organic acids [33, 34]. As a rule, L-carnitine supplementation is never contraindicated in these disorders. Only if a long-chain FAO defect is suspected should the administration of carnitine be limited, at least as a bolus, because of the potential risk of cardiac arrhythmia induced by acute accumulation of toxic long-chain acylcarnitines (▶ Chap. 12).

- **Vitamin Therapy**

Megadoses of specific vitamins should be systematically tested in each case of a potentially vitamin-dependent disorder. Vitamin responsiveness is more likely in late-onset forms than in those presenting in the newborn period. As the response may be masked by the simultaneous use of other therapies, the trial should be repeated later in a stable metabolic period and the results compared with those of in vitro studies.

Biotin is essential in the treatment of both holocarboxylase synthetase and biotinidase

deficiency (► Chap. 27). Hydroxocobalamin should be tried in all cases of methylmalonic aciduria (► Chap. 18), riboflavin in glutaric aciduria type II (MADD) (► Chap. 12). In any severe metabolic decompensation accompanied by insufficient nutritional intake and severe lactic acidaemia a trial with thiamine should also be performed [35, 36] (► Chap. 29).

In adults especially, a short trial of high doses of B vitamins (thiamine, riboflavin, pyridoxine, biotin, folate and cobalamin) is recommended as abnormal biochemical markers may be missing and transporter deficiencies may manifest with non-specific encephalopathies (► Chap. 2).

Additional Drugs

In methylmalonic aciduria, forced diuresis or at least careful maintenance of an appropriate hydration, and sometimes alkalinisation of urine with sodium bicarbonate help to eliminate methylmalonic acid because of its high urinary clearance. In isovaleric aciduria, glycine can be used in combination with carnitine to promote the excretion of glycine conjugates and is particularly useful for long-term treatment. In the emergency treatment, carnitine alone is adequate and essential to correct secondary carnitine deficiency [33, 37].

Extracorporeal Toxin-Removal Procedures

In some cases, the situation deteriorates so rapidly that extracorporeal toxin-removal becomes necessary. Such treatment should be considered if the ammonia concentration exceeds 300 to 400 µmol/l (neonates and infants and young children) and/or if ammonia levels do not decrease adequately within the first 4–6 h with conservative treatment whatever the age of the patient and/or in the presence of neurological symptoms in adolescents and adults. This is often the case in multiorgan failure, as alternative pathway therapy requires intact hepatic and renal function for the formation and excretion of conjugates. In all cases of neonatal hyperammonaemic coma, the dialysis team should be informed immediately. In MSUD, extracorporeal detoxification should be initiated in any neonate, in older patients in the presence of neurological symptoms and/or if leucine levels exceed 15 mg/dl (1100 µmol/l).

The choice of the technique is highly influenced by local facilities and experience. Haemodialysis (HD) continuous veno-venous haemofiltration (CVVHF) and hemodiafiltration (CVVHDF) have been shown to be more effective than peritoneal dialysis (PD). Extracorporeal membrane oxygenation has been used in driving HD and haemofiltration (HF) [38]. A delayed extracorporeal treatment was not superior to PD in improving the short-term outcome in a large series of hyperammonemic neonates [39]. Therefore, the main determinant of neonatal outcome is an early initiation of medical and dialysis treatment according to local facilities, regardless of the dialysis modality. If such management is unavailable locally, the patient should be transferred to a specialist centre. Recent consensus was published on dialysis techniques in pediatric hyperammonemic patients [40]. The advantages and disadvantages of the respective techniques in the emergency treatment of various acute metabolic disorders are as follows.

Peritoneal Dialysis

Manual PD requires minimal technical expertise, can be rapidly initiated in any paediatric intensive care unit and can be effective in newborns [41]. The main cause of failure is poor splanchnic blood flow secondary to shock and septicaemia. It appears that PD is far less effective in older children owing to a smaller peritoneal area relative to body weight.

Continuous Haemofiltration

CVVHF consists in a low-resistance extracorporeal circuit connected to a small-fibre haemofilter that is permeable to water and non-protein-bound small solutes. The ultrafiltrate of plasma is concurrently replaced by an electrolyte and TPN solution. CVVHDF increases solute removal by the addition of diffusive transport from a dialysis solution flowing upstream through the ultrafiltrate compartment of the haemofilter. The advantages of CVVHF and CVVHDF are logistical simplicity, good tolerance in neonates or infants who present with haemodynamic instability, multiorgan failure and a hypercatabolic state, and the ability to use a large volume of TPN without the risk of overhydration. Nevertheless, these procedures should not be applied except in a paediatric intensive care unit by staff trained in the techniques of extracorporeal circulation [40].

Haemodialysis

HD is a very effective and rapid method for removing small solutes [6]. However, multiple dialysis sessions are most often necessary, owing to a rebound in the circulation of toxic metabolites. In addition, clearance is hampered by vascular instability [6].

4.2.1.4 Assessment of Biochemical Progress

In order to evaluate the efficiency of toxin removal it is necessary to undertake regular biochemical monitoring in blood, urine and dialysate or ultrafiltrate within set timed periods. Blood glucose, plasma electrolytes and calcium should be corrected when necessary. Regular blood cell counts are also important since, in organic acidurias, neutropenia and thrombocytopenia may be present or may develop after

the initiation of therapy and may require specific treatment (▶ Chap. 18). Urinary urea excretion and plasma uric acid concentration provide readily available information on catabolism. Repeated assessments for septicaemia must be undertaken and treatment initiated as soon as there is any suspicion of infection. It has been reported that the BCAAs, particularly valine are necessary for the proliferation and maintenance of hematopoietic stem cells [42].

4.2.2 Liver Failure

Liver failure is a predominant finding in children with galactosaemia, hereditary fructose intolerance (HFI), tyrosinaemia type I, and the recently identified disorders of cellular trafficking (see ▶ Chap. 44) NBAS and SCYL1 deficiencies and requires urgent and specific treatment. Neonatal and late-onset forms of these disorders may present with acute deterioration, vomiting, seizures, dehydration, hypoglycaemia, liver failure and tubulopathy. A number of abnormalities are associated with advanced liver disease, including mellituria, hyperammonaemia, hyperlactataemia, hypoglycaemia, hypertyrosinaemia and hypermethionaemia. Tyrosinaemia type I rarely presents before the second or third week of life (▶ Chap. 17). Galactosaemia usually presents in the newborn period, but HFI should not become manifest until after weaning, since fructose is not normally part of infant formulas. As soon as these disorders are considered, galactose, fructose and protein must be excluded from the diet (with normal intake of all other nutrients) pending confirmation of the diagnosis. When galactosaemia (▶ Chap. 14) or HFI is confirmed (▶ Chap. 6), protein can be reintroduced. When tyrosinaemia type I is suspected, treatment with NTBC, along with a low-phenylalanine and low-tyrosine diet must be started urgently, in order to prevent production of toxic metabolites and to promote rapid recovery from acute liver failure (▶ Chap. 17). In NBAS and SCYL1 deficiencies, fever is the trigger that indices recurrent attacks of acute liver failure (RALF).

Of note, patients with urea cycle defects may also present with acute liver failure, including in adults [43]. The following observations should prompt hepatologists to consider a primary UCD and search for hyperammonaemia: the severity of the neurological associated symptoms and/or the discrepancy between severe liver dysfunction and relatively mild cytolysis (▶ Chap. 19). Liver failure may also be observed in mitochondrial FAO defects (▶ Chap. 12), mitochondrial respiratory chain disorders (▶ Chap. 10), transaldolase deficiency (▶ Chap. 7), Wolman disease (▶ Chap. 36) and Wilson disease (▶ Chap. 34).

4.2.3 Neonatal Hypoglycaemia

Whatever the cause of hypoglycaemia, blood glucose levels must be corrected immediately with a glucose bolus (0.2–0.5 g/kg) followed by a continuous infusion. However, because abnormal metabolites may quickly become normal with therapy, adequate samples for metabolic studies (acylcarnitines, glucose, insulin, free fatty acids, ammonia and ketone bodies) should be obtained first. Glucose should then be started via a peripheral i.v. line, with a 10% glucose solution with electrolytes (~10 mg/kg/min). Observation of the patient's glucose requirement to maintain normoglycaemia is useful for both diagnosis and management (▶ Sect. 1.4.14). A glucose supply at a rate equivalent to hepatic glucose production (8 mg/kg/min in the newborn) is usually sufficient for disorders such as GSD I and disorders of gluconeogenesis. Patients with congenital hyperinsulinism will require much higher rates (10–20 mg/kg/min) and promptly respond to parenteral glucagon (▶ Chap. 6).

▪ Glycogen Storage Disease Type I and Fructose-1,6-Bisphosphatase Deficiency

In these disorders, fasting hypoglycaemia is associated with hyperlactataemia and metabolic acidosis. In Fructose-1,6-bisphosphatase deficiency, there may be glyceroluria which strongly indicates a defect of gluconeogenesis (▶ Chap. 15) [44]. As soon as the blood glucose values have returned to normal, continuous enteral feeding is substituted for the glucose infusion. At first a lactose-free milk-based formula containing maltodextrin as the source of carbohydrate is used. Giving a normal energy intake for age in which 50–60% of the energy is supplied by carbohydrates, this allows for a glucose infusion of 10–12 mg/kg/min. This diet can subsequently be changed according to the final diagnosis (▶ Chaps. 5 and 15).

▪ Neonatal Hyperinsulinism

This disorder presents with recurrent hypoglycaemia without ketoacidosis. The newborn requires a continuous supply of glucose that exceeds the capacities of the peripheral i.v. route and continuous enteral feeding. Consequently, central venous line is unavoidable. In cases of persistent hypoglycaemia, treatment with glucagon and/or diazoxide can be started. The emergency treatment of neonatal hyperinsulinism is discussed in ▶ Chap. 6.

▪ Fatty Acid Oxidation Defects

FAO defects cause severe energy deprivation and can be suspected in both newborns and children who present with fasting hypoglycaemia and/or acute deterioration associated with lethargy, hepatomegaly and liver failure,

cardiac dysrhythmia, and high blood creatine-kinase, lactate and uric acid levels. These are serious disorders that may require resuscitation. In order to suppress lipolysis it is at first necessary to give an i.v. solution providing 10–12 mg/kg/min of glucose (120–150 ml/kg/day of a 12–15% glucose solution), preferably in combination with insulin. The initial diet should be fat free. Medium-chain triglycerides (2–3 g/kg/day) can be of advantage in long-chain FAO defects as a fuel for the compromised energy metabolism especially in the heart. However, supplementation should be postponed until the exact site of the defect is known. Hypocarnitinaemia is usually present. The efficacy and safety of carnitine supplementation is still controversial, except in carnitine transporter defect, where it is life-saving (► Chap. 12).

4.2.4 Cardiac Failure

One of the treatable disorders that lead to presentation with cardiac failure in the neonatal period is the group of mitochondrial FAO defects associated with cardiomyopathy or conduction abnormalities. In addition to the usual cardiac drugs and symptomatic treatment of cardiac failure, specific emergency treatments are as discussed above (► Chap. 12). Triheptanoin may also be considered in the management of acute cardiomyopathy associated with long chain-fatty acid oxidation disorders [45]. Infantile Pompe disease should also be considered in the setting of severe neonatal hypotonia with cardiac failure and short PR interval on the EKG (► Chap. 5).

4.2.5 Primary Hyperlactataemia

Whatever the enzyme defect, most newborns with primary hyperlactataemia present with acute metabolic acidosis with increased anion gap (with or without ketosis) (► Sect. 1.4.13) and dehydration requiring supportive care similar to that described for the branched-chain organic acidurias. Usually, this treatment is sufficient to reduce the lactate to a level that does not cause severe metabolic acidosis. In some cases, sustained hyperlactataemia is due to a high-glucose infusion and can be corrected by using a 5% or even a 2.5% i.v. glucose solution. Thus, none of these patients require any procedure for toxin removal except in TMEM70 deficiency (see below).

Few strategies are of proven efficacy in congenital lactic acidaemia. A trial should be performed with thiamine (cofactor for the pyruvate dehydrogenase [PDH] complex), riboflavin (cofactor for complex II) and biotin (cofactor for pyruvate carboxylase). Secondary carnitine deficiency is treated with L-carnitine. Dichloroacetate (50 mg/kg/day in one or two divided doses), an inhibitor of PDH kinase, can be an effective mean for lowering lactate accumulation, and hence correction of acidosis, in both PDH and respiratory chain disorders [46, 47]. However, it has little effect on the clinical status except for reducing tachypnoea. Reversible sensory-motor peripheral neuropathy is a clinically limiting adverse effect of chronic DCA treatment (► Chap. 7). In one patient with the French phenotype of pyruvate carboxylase deficiency, the early administration of triheptanoin allowed survival for several months but this was not confirmed in 2 further patients. (► Chap. 11). The only congenital lactic acidosis in which extracorporeal depuration should be considered for the treatment of severe hyperammonaemia and lactic acidosis is TMEM70 deficiency, a disorder presenting in the neonatal period or early infancy with poor feeding, hypotonia, lethargy, respiratory and heart failure, cardiomyopathy, with or without pulmonary hypertension accompanied by severe lactic acidosis, 3-methylglutaconic aciduria and hyperammonaemia [48].

Additionally severe thiamine deficiency, as can occur in Shoshin beriberi, in premature babies treated with TPN lacking B vitamins or in patients with leukemia, can present with acute cardiac failure, severe lactic acidosis, and life-threatening electrolyte abnormalities (findings similar to those of refeeding syndrome [49]) and for which treatment with an IV infusion of thiamine (100-200 mg) may be life saving (► Chap. 29).

4.2.6 Intractable Seizures

When neonatal seizures are the preponderant or presenting sign, pyridoxine, pyridoxal phosphate [50], biotin and folinic acid [51] must be systematically tested. Familial hypomagnesaemia with secondary hypocalcaemia should be considered, and if present treated with enteral magnesium supplementation (► Chap. 34). Disorders of methyl group transfer (including methylenetetrahydrofolate reductase deficiency and disorders of cobalamin metabolism) may require treatment with hydroxocobalamin, folinic acid, pyridoxine, betaine and/or methionine, depending on the underlying enzymatic defect. Intractable seizures related to GLUT1 deficiency (► Chap. 8) can be efficiently treated with a ketogenic diet (► Sect. 13.5). A ketogenic diet may also be efficient in some mitochondrial disorders.

In suspected metabolic disorders those drugs that may inhibit mitochondrial function should be used only in acute emergencies where no other effective treatment is available. These include the antiepileptic drugs sodium valproate and chloral hydrate.

4.3 Final Considerations

Once the patient is discharged from hospital, precautions must be taken to avoid further episodes of decompensation. Parents must be aware of possible causes and be taught to recognise the early signs and when to initiate the first steps of the emergency treatment at home [52]. Every patient should be supplied with an emergency card detailing their particular management scheme to be followed both at home and in the primary care hospital. If there are recurrent episodes of decompensation, insertion of a gastrostomy and/or a portacath system should be considered.

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Disorders of Energy Metabolism

Contents

The Glycogen Storage Diseases and Related Disorders

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Contents

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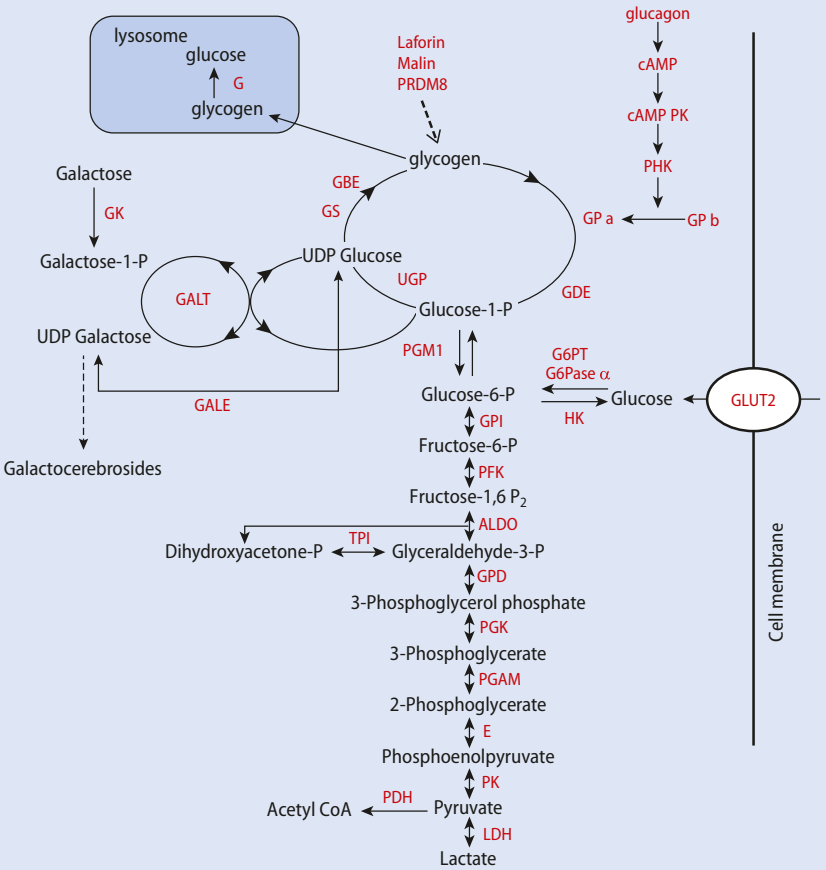
5

Glycogen Metabolism

Glucose is a key fuel for energy production and an absolute requirement for the central nervous system. In view of the importance of glucose homeostasis to prevent cerebral energy deficiency evolution has ensured that the mechanisms for the maintenance of a satisfactory blood glucose concentration are as robust as possible. In the immediate post prandial state carbohydrate absorbed from the gut can be released into the circulation. Glucose sensors in the pancreatic beta cells trigger a release of insulin in response to an increasing blood sugar. Insulin increases glucose uptake into cells where it can be catabolised by aerobic and anaerobic metabolism to produce ATP (■ Fig. 5.1) or be stored as glycogen primarily in the liver but also in muscle. Glycogen is a polysaccharide polymer consisting of glucose molecules in straight chains but with numerous branch points and a molecular weight of between 1 and 20 million. It is structurally similar to animal starch (amylopectin) but has more branch points and a higher MW. At the centre of each glycogen macromolecule is a protein known as glycogenin. Glycogenin 1 is ubiquitously expressed whereas glycogenin 2 is mainly expressed in liver. The role of glycogenin 2 is unclear since a relatively common deletion on the X chromosome that includes its gene *GYG2* appears not to cause any phenotype [1]. Glycogen can form up to 8% of the wet weight of the liver after a meal. In muscle there is approximately 2% by weight and much smaller amounts are also present in the kidneys, in glial cells in the nervous system, and in leucocytes. Only liver glycogen is available for glucose release into the circulation. In muscle, glycogen is used as a source of energy.



■ **Fig. 5.1** Glycogen, Glucose and Galactose metabolism. ALDO aldolase A, cAMP PK cyclic AMP protein kinase, E enolase, G lysosomal a-1,4-glucosidase, G6Pase-α glucose 6 phosphatase-α, G6PT glucose-6-phosphate transporter, GALE galactose epimerase, GALT galactose 1 phosphate uridyl transferase, GBE glycogen brancher, GDE glycogen debrancher, GK galactokinase, GLUT2 solute carrier family 2, GP glycogen phosphorylase, GPD glyceraldehyde phosphate dehydrogenase, GPI glucose phosphate isomerise, GS glycogen synthase, LDH lactate dehydrogenase, PDH pyruvate dehydrogenase, PFK muscle phosphofructokinase, PGK phosphoglycerate kinase, PGAM phosphoglyceratemutase, PGM1 phosphoglucomutase, PHK phosphorylase kinase, PK, pyruvate kinase, TPI triosephosphate isomerase, UGP UDP-glucose/galactose pyrophosphorylase

Glycogen Synthesis

Glycogen synthesis is an energy requiring process. The initial step involves the auto-glucosylation of apoglycogenin by UDPglucose. The same catalytic site of the glycogenin molecule adds a number of glucose residues with an alpha 1,4 linkage. Further elongation of the chain requires the enzymes glycogen synthase for alpha 1,4 linkage and glycogen branching enzyme for alpha 1,6 branch points. The final macromolecule, consisting of up to 30,000 glucose molecules, forms a β particle which can be linked together to form larger α particles, appearing as granules in the cytoplasm.

Glycogenolysis

In the post absorptive state with decreasing glucose concentrations there is a fall in the insulin/glucagon ratio, activation of adenylate cyclase and an increase in cAMP. The initial changes in the cAMP results in a cascade effect to stimulate glycogenolysis. This amplification of this stimulatory process using a number of enzymes (cyclic AMP – dependent protein kinase, glycogen phosphorylase kinase and glycogen phosphorylase) enables very large quantities of glucose to be released from hepatic glycogen rapidly. In addition to glucagon the 'stress' hormones (adrenaline, cortisol and growth hormone) inhibit glycogenesis and stimulate glycogenolysis. The effect of growth hormone on glycogenolysis is limited to muscle. Thyroxine does not have a direct effect on glycogen metabolism but increases sympathetic activity.

Introduction

Disorders of glycogen metabolism primarily involve liver and/or muscle although there are rare neurological phenotypes associated with some enzyme deficiencies (Glycogen Metabolism; Table 5.1). Most are referred to by a roman numeral or by the specific enzyme that is deficient. The use of eponyms is now largely historical. The hepatic glycogenosis generally cause hepatomegaly (apart from glycogen synthase 2 deficiency) and fasting hypoglycaemia whereas the muscle disorders are associated with skeletal myopathy and/or cardiomyopathy. The clinical phenotypes are extremely heterogeneous.

5.1 Hepatic Glycogenoses

5.1.1 Glycogen Synthase 2 Deficiency (GSD 0a)

Despite being a disorder of liver glycogen synthesis and not a cause of glycogen storage, glycogen synthase 2 deficiency is commonly referred to as glycogen storage disease type 0 (GSD 0a).

Clinical Presentation

Individuals with GSD 0a present with symptomatic fasting ketotic hypoglycaemia or are found incidentally with post prandial hyperglycaemia and glycosuria. Symptoms generally become apparent in late infancy after weaning or in childhood. Physical examination is normal although some children may have poor growth, short stature and osteopenia; unlike other GSDs there is no hepatomegaly. For review see [2].

Metabolic Derangement

Deficiency of hepatic glycogen synthase (GS2) results in very low levels of glycogen in liver and consequently limited post prandial and fasting hepatic glucose release. Overnight fasting or fasting related to intercurrent infections are associated with ketosis. Hyperglycaemia and moderate hyperlactataemia occur post-prandially due to reduced uptake of glucose by the liver. Hyperlipidaemia and raised liver transaminases may be found.

Genetics

GSD 0a is an autosomal recessive disorder caused by mutations in *GYS2* which codes for the liver isoform of glycogen synthase. It has 70% homology with *GYS1* which codes for the muscle isoform. A number of mutations have been described. One (p.R246X) is most common in patients of Italian descent. The disorder appears rare but is likely to be underdiagnosed.

Diagnostic Tests

GSD 0a should be considered in patients with ketotic hypoglycaemia, particularly if they are also found to have postprandial hyperglycaemia or glycosuria. GS2 can be assayed in liver tissue but the diagnosis is now best confirmed by mutation analysis.

5

Table 5.1 Phenotypes associated with enzyme deficiencies

Number	Enzyme deficiency	Gene(s)	Eponym	Main phenotype(s)
0a	Liver glycogen synthase	*GYS2*		Ketotic hypoglycaemia
0b	Muscle glycogen synthase	*GYS1*		Cardiac & skeletal myopathy
Ia	Glucose-6-phosphatase-α	*GSPC1*	von Gierke	Hypoglycaemia, hepatomegaly, lactic acidosis
Ib	Glucose-6-phosphate transporter	*SLC37A4*		As Ia + neutrophil dysfunction, infections/ colitis
II	Lysosomal α-1,4-glucosidase	*GAA*	Pompe	Cardiac & skeletal myopathy
III	Glycogen debrancher	*AGL*	Cori	Hypoglycaemia, hepatomegaly
IV	Glycogen brancher	*GBE1*	Andersen	Cirrhosis; Myopathy/cardiomyopathy; Adult polyglucosan body disease
V	Muscle glycogen phosphorylase	*PYGM*	McArdle	Skeletal myopathy
VI	Liver glycogen phosphorylase	*PYGL*	Hers	Hepatomegaly, hypoglycaemia, growth delay
VII	Muscle phosphofructokinase	*PFKM*	Tarui	Skeletal myopathy
IXa	Phosphorylase kinase (various subunits ◘ Table 5.2)	*PHKA2*		Hepatomegaly, hypoglycaemia, growth delay
IXb		*PHKB*		
IXc		*PHKG2*		
IXd		*PHKA1*		Skeletal myopathy
X	Muscle phosphoglycerate mutase	*PGAM2*		Skeletal myopathy
XI	GLUT2	*SLC2A2*	Fanconi-Bickel	Hepatomegaly, hypoglycaemia, renal tubular disease, growth delay
XII	Aldolase A	*ALDOA*		Skeletal myopathy and anaemia
XIII	β-Enolase	*ENO3*		Skeletal myopathy
XIV	Phosphoglucomutase	*PGM1*		Myopathy; PGM1-CDG
XV	Muscle glycogenin deficiency	*GYG1*		Skeletal / cardiomyopathy
	AMP-activated protein kinase	*PRKAG2*		Fatal congenital cardiomyopathy; WPW syndrome with or without cardiomyopathy
	Lysosomal-associated membrane protein 2 (LAMP2)	*LAMP2*	Danon	Cardiomyopathy, skeletal myopathy, mental retardation, maculopathy
	Laforin deficiency Malin deficiency PRDM8 deficiency	*EPM2A* *EPM2B* *PRDM8*	Lafora body disease	Epilepsy, dementia

Treatment and Prognosis

Treatment consists of preventing fasting hypoglycaemia, hyperketosis and hyperlactataemia. This is achieved by regular meals using a diet with increased protein to stimulate gluconeogenesis and complex carbohydrates to provide slow release of glucose. Daytime snacks may be required between meals. Uncooked cornstarch (1.5 to 2 gm/kg) may be necessary before bedtime to prevent morning hypoglycaemia and also regularly during intercurrent infections. The prognosis for GSD 0a is good. Treatment allows for normal growth and the disorder is not associated with long term hepatic or other complications. Significant hypoglycaemia is less frequent in older children and adults but continued dietary treatment may be required to prevent fasting ketosis.

5.1.2 Glycogen Storage Disease Type I (GSD I)

Clinical Presentation

There are two genetically distinct forms of GSD I: GSD Ia caused by deficiency in glucose-6-phosphatase-α and GSD Ib by deficiency in glucose-6-phosphate (G6P) translocase (also known as G6P transporter). Both disorders cause severe fasting hypoglycaemia, lactic acidosis and hepatomegaly. In GSD Ib there is additionally immunological disease due to neutrophil dysfunction.

Patients with GSD I generally appear normal at birth although hepatomegaly and hypoglycaemia can be present in the newborn period. From early infancy there is poor growth, abdominal distension, episodes of tachypnoea, and irritability. Some children become extremely unwell with severe acidosis. Those with GSD Ib may present with frequent and often severe bacterial infections.

On examination infants are generally miserable with a 'dolls like' face, a large abdomen and thin arms and legs. Even though the liver is large, it may not be so easily palpable. On investigations there is fasting hypoglycaemia and lactic acidosis. Fasting tolerance is often very short with blood sugar falling after only 2 to 3 hours. Other abnormalities include hyperlipidaemia and hyperuricaemia. Liver functions test are usually unremarkable without the severe transaminitis seen in GSDIII. In GSD Ib neutropenia may or may not be evident in infancy.

Long term complications are frequent in GSD I and include liver tumours, renal disease, osteoporosis, anaemia, and gastrointestinal disease. They and their treatment are considered in more detail below.

Metabolic Derangement

GSD I is both a disorder of glycogen metabolism and of gluconeogenesis. The failure of glucose dephosphorylation to release free glucose from G6P severely inhibits hepatic glycogen breakdown resulting in excessive glycogen storage and hepatomegly. Hyperlactataemia occurs as a consequence of the disruption to gluconeogenesis and increases with fasting. Since lactate may be used as alternative energy substrate by the brain, untreated patients are, to some extent, protected from the adverse effects of hypoglycaemia on the CNS.

Secondary abnormalities include hyperlipidaemia, particularly hypertriglyceridaemia, and hyperuricaemia. Both occur as a consequence of increased levels of G6P with increased de novo lipogenesis and increased flux through the pentose phosphate pathway to form uric acid from ribose-5-phosphate. Additionally, there appears also to be a reduction in lipid clearance [3] and disturbed sphingolipid metabolism [4].

GSD1a is associated with down regulation of sirtuin 1 (SIRT1) signaling leading to impaired autophagy, mitochondrial and oxidative DNA damage [5], factors which may be important in the development of hepatocellular adenoma and carcinoma.

G6P translocase is necessary for normal neutrophil function. Individuals with GSD Ib have both abnormally functioning neutrophils and low neutrophil numbers, the latter caused by increased neutrophil apoptosis. Neutropenia, however, is not always evident in infants. Bacterial infections are common and may be severe. The neutropenia in GSD1b appears to be a disorder of metabolite repair (as also occurs in deficiency of G6PC3, an endoplasmic reticulum phosphatase homologous to glucose-6-phosphatase) with failure to remove 1,5-anhydroglucitol-6-phosphate (1,5AG6P), a structural analog of glucose-6-phosphate and an inhibitor of low-Km hexokinases which catalyze the first step in glycolysis. For discussion of the molecular mechanisms involved see [6] and [7].

Genetics

GSD Ia is caused by homozygous or compound heterozygous mutations in *G6PC1* and GSD Ib by those in *SLC37A4*. Both disorders are panethnic. GSD I has a frequency of approximately 1 in 100,000 with GSD Ia accounting for 80% of cases. Numerous mutations, mostly missense, have been described in both disorders. Despite some being associated with a complete loss of activity and others with varying degrees of residual function no genotype-phenotype correlation has been established.

Diagnostic Tests

The diagnosis of GSD I and its differentiation into 1a and 1b was previously confirmed by assay of glucose 6 phosphatase-α activity in intact (fresh) and disrupted hepatocytes. However, the diagnosis is now made on the basis of mutation analysis thus avoiding the need for liver biopsy. If a liver biopsy is performed the histology shows excess storage of cytoplasmic glycogen with large lipid vacuoles but no fibrosis. Prenatal diagnosis can be performed by mutation analysis.

Treatment, Complications and Prognosis

Prior to effective dietary treatment most patients with GSD I died during childhood. Now children survive into adulthood. Cognitive development is normal providing there have not been episodes of severe hypoglycaemic encephalopathy. There remain, however, a

number of long term complications that significantly impinge on their health and quality of life.

The aim of treatment is to correct the metabolic abnormalities as far as is possible and to prevent or manage any complications. Consensus guidelines for the management of GSD I have been published in 2002 [8] and in 2014 [9].

Dietary Treatment

(For a practical review see [10].)

The major requirement is to maintain blood sugar within the normal range by frequent feeds. Carbohydrate should make up 60–70% of total calories, fat 20–25% and protein 10–15%. The glucose requirement is based on normal, age related, hepatic glucose production (8–9 mg/kg/min in infants, 5–7 mg/kg/min in children and 2–4 mg/kg/min in adolescents and adults) but must be adjusted according to biochemical control. In the most severely affected patients feeding is required every 60 to 90 minutes. Such a regimen is extremely onerous, particularly at night. Continuous pumped overnight feeds given via a nasogastric tube or gastrostomy are often employed. However, there are risks with such treatment. Nasogastric tubes can become displaced leading a disruption to the supply of feeds or to inhalation pneumonia. Since patients on treatment have reduced hyperlactataemia a disruption in the supply of glucose can cause severe symptomatic hypoglycaemia in the absence of lactate as alternative fuel. Gastrostomies are generally contraindicated in GSD Ib as, except in the case of very mild disease, invariably become chronically infected. Uncooked cornstarch prolongs carbohydrate absorption from the gut and can increase the period of normoglycaemia between feeds. Commercial extended release cornstarch preparations which prolong the period of normoglycaemia are available [11, 12]. It is poorly tolerated in infants but can be introduced gradually in children over the age of 10 months to 2 years. It has been advocated as an alternative to continuous overnight feeding with better blood sugar control [13] although other factors need also be considered [14]. The recommended dose in young children is 1.6 g/kg 3 to 4 hourly and 1.7–2.5 g/kg 4–5 hourly thereafter. In adults carbohydrate requirements are less and decrease further with ageing; overtreatment should be avoided as it can lead to relative hyperinsulism, an increase in hepatomegaly and excessive weight gain [15].

In the absence of glucose 6 phosphatase-α neither fructose nor galactose can be converted to glucose and consequently can lead to a worsening of hyperlactataemia. It is proposed that both sucrose and lactose be restricted in GSD I but there is no agreement on how strict this should be.

Assessing the efficacy of dietary treatment to prevent hypoglycaemia is best achieved by the use of a portable continuous glucose monitor over several days [16].

Hepatic Tumors

Benign hepatocellular adenoma (HCA) occur commonly in older patients with GSD I and were more frequent in those who had portacaval shunts [17]. HCA are evident on liver ultrasound, CT or MR imaging. The adenomas are generally asymptomatic but if large can cause abdominal pain, with bleeding or rupture. Increased production of tumour derived hepcidin can cause a severe refractory anaemia. Adenomas may regress with improved metabolic control [18]. Malignant tumours arising from adenomas are uncommon but have occurred in a number of adult patients. Patients with adenomas need to be monitored for any evidence of malignant change. Consensus recommendations for the surveillance of hepatic adenomas after the onset of puberty in GSD have been published [9]. In summary these are as follows:

- Blood tests, 6 monthly to include liver transaminases, albumin, bilirubin, serum creatinine, international normalized ratio (prothrombin time/partial thromboplastin time), α-fetoprotein (AFP) and chorionic embryonic antigen (CEA) levels. However, AFP and CEA can be normal in HCC in GSD I and are not a reliable marker of malignant transformation.
- Abdominal ultrasound at baseline and then every 12–24 months in children.
- Abdominal computed tomography/magnetic resonance imaging with contrast in older patients or in paediatric patients once adenomas are detected on ultrasound. A rapid increase in size or number of adenomas or an increase in their vascularity of adenomas may be an indication of malignant change.

Treatment of adenomas that are considered worrying include percutaneous ethanol injections, radiofrequency ablation, and partial liver resection. Liver transplant should be considered for patients with multiple adenomas that are growing and in hepatocellular carcinoma (HCC) where there is no evidence of metastatic spread.

Although the aetiology of adenomatous formation and malignant change in GSD I are not fully understood a number of molecular and cellular abnormalities which may be implicated have been demonstrated in HCA from patients, including alterations in chromosome 6, a lack of HNF1A activation, a reduction in IGF2R and LATS1 and deregulation of microRNAs [19–21].

Gastrointestinal Disease

Inflammatory bowel disease occurs frequently in patients with GSD Ib with histology similar to Crohn disease and is thought to be related to neutrophil dysfunction. The enterocolitis may improve on treatment with 5-aminosalicylic acid and granulocyte colony stimulating factor (GCSF). Adalimumab, a monoclonal antibody, has been of benefit in one patient unresponsive to aminosalicylate, GCSF, and antibiotic therapy [22]. More recently, treatment with empagliflozin, an SGLT2 inhibitor, has been reported as effective (see Neutrophil dysfunction below) [23, 24]. Although clinical symptoms of colitis appear to be infrequent in GSD Ia, a number of adult patients have now been reported with this complication. In contrast to patients with GSD1b, the large bowel is involved. The aetiology is not clear but may be due to changes in gut flora as a consequence of longstanding treatment with uncooked cornstarch. Most patients have gone into remission with treatment with aminosalicylic acid [25]. Pancreatitis has been reported in several patients with GSD I. It is assumed that the increase risk is related to hypertriglyceridaemia.

Renal Disease

The kidneys may be large on imaging due to glycogen deposition. Renal disease starts in childhood and effects both proximal and distal renal tubular and glomerular function. In older patients glomerular hyperfiltration, microalbuminuria, proteinuria, renal cysts (and rarely renal carcinoma [26]), nephrocalcinosis, and nephrolithiasis can occur, the latter associated with reduced citrate and increased calcium excretion that predisposes to calcium oxalate precipitation. End stage renal failure can occur in the most severely affected patients requiring renal replacement therapy or transplantation. Renal function should be regularly monitored from childhood. The progression of renal disease may be delayed by good metabolic control, oral citrate supplements in those with low urinary citrate, thiazide diuretics if there is hypercalciuria, and an angiotensin-converting enzyme (ACE) inhibitor or an angiotensin receptor blocker (ARB) medication started at the onset of glomerular hyperfiltration [9]. Hyperlipidaemia has been reported to be correlated with the severity of renal disease and severe hyperlipidaemia to effect the efficacy of ACE inhibitors [27]. Hyperuricaemia may increase the risk of developing renal disease and developing gout. Plasma uric acid should be monitored regularly and hyperuricaemia treated with allopurinol. Renal failure is associated with decreased erythropoietin (EPO) production by the kidney and hence anaemia. Treatment with EPO is advised in children if the haemoglobin falls to less that 100 g/l and in adults to prevent transfusion dependency.

Haematological Disease

Anaemia

Anaemia may result from iron deficiency, colitis in GSD Ib, associated with large adenomas or from chronic renal disease. Treatment is dependent on the cause and is discussed in the relevant sections.

Coagulopathy

Clotting abnormalities in GSD I may manifest as frequent nose bleeds, menorrhagia or increased bruising. The aetiology is not fully understood but is attributed to abnormal platelet function. In some patients there is reduced or dysfunctional von Willebrand factor antigen. The coagulopathy may improve with improved metabolic control otherwise conventional treatment for platelet & factor VIII abnormalities is indicated. This is of particular importance before any surgical procedure.

Neutrophil dysfunction

Infections are a serious, potentially life threatening complication in GSD Ib. Mouth ulcers, chronic and severe periodontits and inflammatory bowel disease are common. Treatment with GCSF improves both neutrophil numbers and function and reduces the frequency and severity of infections and improves the colitis in most patients. Treatment is well tolerated in the short term but patients on treatment develop splenomegaly. Acute myeloid leukaemia has been reported after continuous treatment over many years [28, 29]. Giant cell tumour of the mandible has been reported in 3 patients with GSD1b, all of whom were being treated with GCSF [30].

Published guidelines recommend using GCSF in GSD Ib only if any of the following occur: a neutrophil count below 0.2×10^9/l, a single life-threatening infection requiring intravenous antibiotics, serious enterocolitis documented by abnormal colonoscopy and biopsies or severe diarrhoea requiring hospitalization [31]. Further guidelines recommend using the lowest effective dose, starting at 0.5–1.0 μg/kg given daily or every other day and increasing at 2 weekly intervals until the neutrophil count has increased to 0.5 to 1.0×10^9/l [9]. A prospective trial of vitamin E supplementation in GSD Ib has been reported to improve the neutrophil count and function and reduce the frequency of infections thus allowing the dose GCSF to be reduced with a reduction of GCSF related side effects [32].

Recently, neutropenia and neutrophil dysfunction have been shown to be improved by reducing the intracellular concentration of 1,5-anhydroglucitol-6-phosphate with empagliflozin, an inhibitor of the renal glucose co-transporter SGLT2, that is widely used in the managment of type 2 diabetes [33]. Such treatment is reported to be of significant clinical benefit in reducing the complications associated with the neutrophil abnormalities [23, 24].

Cardiovascular Disease

Systemic hypertension, secondary to renal disease may affect older children and adults and should be managed conventionally. There remains debate as to whether hyperlipidaemia in GSD I may cause premature atherosclerosis. Lipid levels are lowered with improved metabolic but there are no specific published guidelines as to the use of statins or other pharmacological treatments. Pulmonary arterial hypertension has been described in a few patients with GSD I, the majority of whom had other concomitant risk factors.

Bone Disease

Low levels of vitamin D, which are common in GSD I, should be routinely monitored and treated. Osteopenia and osteoporosis are common in adults. Monitoring of bone density every 2 years is recommended.

Endocrine Disorders

Patients with GSD 1a appear to be at increased risk of insulin resistance and metabolic syndrome. It is suggested that this is caused by accumulation of microsomal G6P leading to an increase in the activity of 11β- hydroxysteroid dehydrogenase, an ER bound enzyme that plays a role in the development of metabolic syndrome. This is in contrast to GSD1b where there is a reduction in G6P in the ER [34]. Hypogonadotropic hypogonadism, possibly caused by elevated levels of cortisol suppressing the release of gonadotrophin- releasing hormone, has been reported in 3 unrelated male patients with GSD1b and one with GSD1a [35].

Liver Transplant

Liver transplant is not solely indicated to treat or prevent HCC in GSD I but has the potential to significantly improve the quality of life for patients and families. Fasting tolerance and other metabolic disturbances become normal following successful transplant and most patients show catch up growth. Of those post transplant complications that were unrelated to the procedure, the most frequent have been renal disease in GSD Ia and a persistent neutropenia in GSD Ib. It is not known whether renal disease might be a progression of pre-existing damage and/or caused by immunosuppressive treatment or ongoing disease still effecting the kidney (a kidney-specific G6PC knockout mouse, has been shown to develop nephropathy despite normal liver enzyme activity [36]). In GSD Ib liver transplant has improved glucose tolerance but also reduced infectious complications. Neutropenia has been found to improve in some patients with a reduction in the dose of GCSF required [37]. Complications associated with the transplantation procedure itself have been relatively common occurring in 18 of the 58 patients reviewed by Boers et al. [38].

Other Treatments

Potentially, to avoid disease or treatment complications, gene therapy or cell-based therapies, such as hepatocyte transplantations and liver stem cell transplantations may have role in the future if these can be shown to be efficacious [39]. Research into new treatments, including liver-targeted gene therapy, are ongoing [40].

Contraception and Pregnancy

Progesterone only oral contraception is recommended since those containing oestrogen increase the risk of hepatic adenomas. Intrauterine devices (IUDs) should be avoided in GSD Ib in view of the risk of infection.

A number of successful pregnancies have been reported both in women with GSD Ia and GSD Ib [41, 42]. Despite a high frequency of polycystic ovaries fertility appears to be normal [43]. An increase in glucose requirements has been observed during the first trimester, worsening of renal function during the pregnancy, and in 3 out of 15 patients with GSD Ia, lactic acidosis during delivery [41]. ACE inhibitors must be stopped during pregnancies in women with GSD I.

5.1.3 Glycogen Storage Disease Type III (GSD III)

GSD III is caused by deficiency in glycogen debrancher enzyme (GDE). The disorder is both a hepatic glycogenosis and (in most cases) also a myopathic disorder.

Clinical Presentation

The clinical presentation is generally not as severe as in GSD I. Children present in the first year with poor growth, delayed motor milestones and abdominal distension.

Fasting hypoglycaemia occurs but the fasting tolerance is usually longer than in GSD I and may not be symptomatic at initial presentation [44]. Unlike GSD I, fasting ketosis is prominent. Since gluconeogenesis is normal there is no fasting hyperlactataemia although there is a moderate post prandial increase in lactate. The majority of patients have a myopathy. The liver is often very large and since it can cross the midline may be misdiagnosed as hepatosplemegaly. On initial investigation, in addition to low glucose levels and ketosis with fasting, there is hyperlipidaemia and markedly raised liver transaminases. Creatine kinase levels are raised in the myopathic form, and frequently observed in children before onset of muscle symptoms. Uric acid levels are not raised. If a liver biopsy is undertaken it shows the

accumulation of cytoplasmic glycogen in non membrane bound vacuoles and peri-portal fibrosis.

The skeletal myopathy may worsen with age, presenting with exercise intolerance in young patients and permanent muscle weakness in adults. Left ventricular hypertrophy is common but symptomatic cardiomyopathy is rare. Unlike GSD I, renal disease is not a long term complication. Hepatic adenomas may occur although these are smaller and less frequent than in GSD I. Liver fibrosis may develop into cirrhosis and there is an increased risk of malignant change. Insulin resistance with maturity onset diabetes may occur in some older patients.

Metabolic Derrangement

GDE has both glucosidase and transferase activity; it cleaves α 1,4 glucose linkages of the terminal glucose molecules and then breaks α 1,6 linkage to remove branch point. Most individuals with GSD III have a defect in both liver and muscle (IIIa) but about 15% have only liver involvement (IIIb) and do not develop a myopathy.

GDE deficiency leads to the accumulation of abnormal glycogen (limit dextran). Since some, albeit limited, glucose release is possible from glycogen and there is no defect in gluconeogenesis, hypoglycaemia is usually less severe than in GSD I and lactic acidosis does not develop. Raised liver transaminases indicate chronic hepatocellular damage (even though the increase in aspartate aminotransferase may also in part be related to myopathy). Hyperlipidaemia is common in children but less severe than in GSD I. Hypertriglyceridaemia had been found to improve with age [45].

Genetics

GSD III is an autosomal recessive disorder caused by mutations in *AGL*. Mutations associated with GSD IIIa, occur throughout *AGL* whereas two specific mutations in exon 3 are associated with the GSD IIIb. In the the International Study on Glycogen Storage Disease (ISGSDIII) 86% of identified mutations were non-missense resulting in a truncated protein [44].

Diagnostic Tests

The diagnosis can be made by the assay of GDE activity in leucocytes or by mutation analysis. Prenatal diagnosis is possible by mutation analysis.

Treatment and Prognosis

Expert derived detailed guidelines for the clinical management of GSD III have been published [44, 46]. The aim of treatment is to maintain normoglycaemia, reduce the hyperlipidaemia and ketosis and ensure adequate growth. This is achieved by regular meals and the use of uncooked cornstarch. Overnight continuous feeding is less commonly needed in GSD III than in GSD I. It is possible that the use of intensive high carbohydrate diets worsens skeletal myopathy and increases the risk of cardiomyopathy by encouraging excessive tissue glycogen deposition. Reducing carbohydrate as a proportion of total calories by increasing protein and fat, including (modified) ketogenic diets and the use of oral D, L-3-hydroxybutyrate have been advocated (for reviews see [47, 48]). Acute nutritional ketosis, induced by an oral ketone ester, has recently been reported to improve muscle energy balance in adults with a severe muscle phenotype, whereas no effect was seen in those without overt myopathy [49]. Bone health may be affected with reduced bone mineral density and there is an increased incidence of bone fractures in children [44, 50]; it is important to encourage physical activity and ensure that the diet contains sufficient calcium and vitamin D.

The long-term outcome for individuals with GSD III is generally good with survival into adulthood. Growth may continue into the third decade so that a satisfactory final height is reached. The hepatomegaly may lessen or resolve after puberty. Although polycystic ovaries are common, fertility appears normal and successful pregnancies reported [51]. However, life threatening complications can occur in older patients including worsening of skeletal and or cardiomyopathy (with a risk of sudden death from arrhythmia), end stage liver failure from cirrhosis and hepatic malignancy. As a consequence, it is important that patients remain under follow up throughout life.

5.1.4 Glycogen Storage Disease Type IV (GSD IV)

Glycogen disease type IV is caused by deficiency in glycogen brancher enzyme (GBE).

Clinical Presentation

There are a number of phenotypes associated with GBE deficiency ranging from death in utero to adult presentation. The following clinical disorders are described although these subtypes may overlap:

Liver disease

- Progressive liver disease in infancy. This presents within the first few months of life with failure to thrive and hepatomegaly. Cirrhosis develops with eventual end stage liver disease and portal hypertension. Death is usual by 5 years of age.
- Non-progressive liver disease in childhood. Patients present with hepatomegaly and liver dysfunction, hypotonia and myopathy. However, the liver disease does not progress and survival is into adulthood.

Neuromuscular disease

- Congenital onset – including fetal loss in pregnancy, fetal akinesia deformation sequence (FADS) with arthrogryposis, hydrops and perinatal death, or a severe congenital myopathy similar to spinal muscular atrophy but which may also be associated with cardiomyopathy.
- Juvenile onset – with a myopathy and/or cardiomyopathy [52].
- Adult onset – adult polyglucosan body disease (APBD) or more rarely, myopathy. APBD is described in ▶ Sect. 5.3.2

Metabolic Derrangement

GBE is responsible for transferring short glucosyl chains to form branch points with an alpha 1,6 linkage. Deficiency of GBE results in an abnormal poorly soluble glycogen with fewer branch points (polyglucosan) that leads to tissue damage. This abnormal glycogen accumulates to a varying degree in various organs, including liver, muscle, heart, nervous system and skin. The different clinical manifestations are likely to be related to the degree of enzyme deficiency, with an almost complete absence of GBE in the congenital forms of the disease but significant residual activity in APBD. Liver transaminases are raised in those with hepatic involvement. Fasting hypoglycaemia is uncommon except in end stage liver failure. Liver and muscle histology show swollen hepatocytes that contain periodic acid-Schiff (PAS)-positive and diastase resistance inclusions and evidence of interstitial fibrosis.

Genetics

GBE deficiency is a rare autosomal recessive disorder caused by mutations in *GBE1* (for review see [53]). A common mutation found in the Ashkenazi Jewish population is associated with APBD. There appears to be some degree of genotype-phenotype correlation; none of the mutations found in patients with the congenital form have been found in adult onset patients. More severe disease is most often associated with two null mutations or large deletions rather than missense mutations.

Diagnosis

Although enzyme analysis can be undertaken in liver tissue, cultured skin fibroblasts, peripheral lymphocytes and muscle, the diagnosis is now most often made by *GBE1* mutation analysis. It may also follow genetic testing by gene panels or whole exome sequencing in patients with unexplained neuromuscular of hepatic phenotypes whose diagnosis may otherwise be missed [54].

Prenatal diagnosis is possible by enzyme analysis in cultured amniocytes or chorionic villi cells and by *GBE1* mutation analysis.

Treatment and Outcome

Liver transplant is the only treatment for the progressive liver form [55]. Although following transplant most children have done well with no progressive skeletal myopathy or cardiomyopathy, a few have had a fatal progression of the disease affecting other organs, particularly the heart [56]. Heart transplant may be considered in those with heart failure caused by cardiomyopathy. There is no specific treatment for the other forms of the disease.

5.1.5 Glycogen Storage Disease Type VI (GSD VI)

GSD VI is caused by deficiency in hepatic glycogen phosphorylase.

Clinical Presentation

GSD VI is generally a relatively mild disorder often diagnosed following an incidental finding of hepatomegaly. However, it may also present with symptomatic ketotic hypoglycaemia and growth retardation without obvious hepatomegaly. During adolescence symptoms and signs normally resolve and most adults are asymptomatic. Liver adenoma, liver fibrosis, mild cardiomyopathy are rare complications [57]. Hepatocellular carcinoma has been reported in a single patient [58].

Metabolic Derrangement

Hepatic glycogen phosphorylase catalyses the release and phosphorylation of terminal glucosyl units from glycogen, forming glucose-1-phosphate. Ketosis with or without hypoglycaemia may occur with fasting [59]. Although plasma lipids may be raised, uric acid and creatine kinase are normal. In those with more severe variants recurrent hypoglycaemia and post prandial hyperlactataemia can occur.

Genetics

GSD VI is an autosomal recessive disorder caused by mutations in *PGYL* which codes for the liver isoform of glycogen phosphorylase. A high frequency of missense mutations have been reported [60].

Diagnosis

The diagnosis can be confirmed by mutation analysis or by finding enzyme deficiency in hepatic tissue, erythrocytes, and leukocytes. However, enzyme activity may not always be reduced in blood and even in liver tissue may be difficult to interpret due to residual activity and the

effect of other factors. For example, deficiency of glycogen phosphorylase kinase will cause low activity of glycogen phosphorylase.

Treatment and Outcome

Treatment for asymptomatic children may not be required but those with growth failure or fasting ketosis may benefit from regular meals and snacks and uncooked cornstarch (1.5–2 g/kg). The outcome for individuals with GSD VI is generally excellent with catch up growth occurring for those with short stature in childhood. Hypertriglyceridemia may persist [61].

5.1.6 Glycogen Storage Disease Type IX (GSD IX)

GSD IX is caused by deficiency in hepatic glycogen phosphorylase kinase (PHK) [62]. The enzyme is a multi-enzyme complex consisting of four homotetramers, each made up of an α, β, γ and δ subunit. The γ subunit is catalytic and the other subunits regulatory. There are tissue specific isoforms of the α and γ subunits. The δ subunit, calmodulin, is ubiquitous. The various subtypes of GSD IX are listed in ◘ Table 5.2.

Clinical Presentation

Most patients with GSD IX have a relatively benign disorder, with hepatomegaly often detected incidentally but there may be short stature, fasting hypoglycaemia and ketosis, with raised liver transaminases, cholesterol and triglycerides. Blood lactate and uric acid are normal. There is usually resolution of signs and symptoms by adulthood. GSD IXc can be more severe with an increased risk of hepatic fibrosis and cirrhosis.

Metabolic Derangement

PHK phosphorylates glycogen phosphatase leading to a conformational change to form the active ›a‹ form. Decreased PHK activity results in more glycogen phosphatase remaining in the inactive ›b‹ form with a subsequent reduction in glucose-1-phosphate release from glycogen.

Genetics

The most common form, GSD IXa is an X-linked recessive disorder. The other, rarer hepatic variants are autosomal recessive. The various sub-units and their respective genes involved are listed in ◘ Table 5.2.

Diagnosis

The diagnosis should be considered in children with unexplained hepatomegaly and in those with ketotic hypoglycaemia. PHK can be measured in liver, erythrocytes and leukocytes. However, in view of variable tissue expression enzyme assays may be difficult to interpret. Diagnosis is best achieved by mutation analysis using a DNA panel.

Treatment and Outcome

Asymptomatic patients may not need treatment. For those with growth failure, symptomatic hypoglycaemia

◘ **Table 5.2** PHK deficiency

	Gene	Location	Subunit	Inheritance	Tissue involved
GSD IXa	*PHKA2*	Xp22.13	α_2	XLR	GSD IXa1 (XLG1): Liver & blood GSDI Xa2 (XLG2): Liver[a]
GSD IXb	*PHKB*	16q12.1	β	AR	Liver & muscle
GSD IXc	*PHKG2*	16p11.2	γTL (liver/testis isoform)	AR	Liver[b]
GSD IXd	*PHKA1*	Xq13.1	α_1	XLR	Muscle[c]

[a]mutations causing GSD IXa2 are missense, small in-frame deletions, or insertions with some residual enzyme activity in erythrocytes [60]

[b]In a review of mutations in GSD IXc, missense mutations were the most common (39%) followed by nonsense mutation (23%) [63]

[c]GSD IXd is a cause of, cramps and exercise intolerance, middle age onset and raised CK, with normal red cell & liver PHK activity. There is a variable lactate response with the non-ischaemic exercise test

No disorder has yet been described caused by mutations in *PHKAG1* (at 7p11.2) which codes for the muscle isoform of the γ subunit

A fatal autosomal dominant congenital heart glycogenosis is now known not to be caused by PHK deficiency but by mutations in *PRKAG2* which codes for the $\gamma_2$subunit of AMP-activated protein kinase [64] (see below ► Sect. 5.2.6)

For review see [62]

or chronic ketosis, frequent meals and uncooked cornstarch may be used [65]. Protein can be increased to 15 to 20% of calories to provide a gluconeogenesis substrate. The outcome for most patients is good with resolution of hepatomegaly and catch up growth by adulthood. A patient with IXb has been reported with mild cardiomyopathy [57]. Patients with GSD IXc may have a more severe disease with an increased risk of hepatic fibrosis and cirrhosis [66].

5

5.1.7 Fanconi-Bickel Syndrome

Fanconi-Bickel syndrome, a hepatic glycogen storage disease associated with renal tubular disease, is caused by deficiency in solute carrier family 2 protein (GLUT2) due to mutations in *SLC2A2*. The disorder is discussed in ▶ Chap. 8.

5.2 Muscle and Cardiac Glycogenoses

At rest, muscle predominantly utilizes fatty acids. During submaximal exercise, it additionally uses energy from blood glucose, mostly derived from liver glycogen. In contrast, during very intense exercise, the main source of energy is anaerobic glycolysis following breakdown of muscle glycogen. When the latter is exhausted, fatigue ensues. Enzyme defects within the pathway affect muscle function. GSD III is most often both a myopathic and hepatic glycogenosis. It is described in ▶ Sect. 5.1.3.

5.2.1 Glycogen Storage Disease Type V (Myophosphorylase Deficiency, McArdle Disease)

▪ Clinical Presentation

GSD V, the most common muscle glycogenosis is characterized by exercise intolerance with myalgia and stiffness of exercising muscles, which are relieved by rest. Onset of the disease occurs during childhood, but diagnosis is frequently missed at an early age because affected children are often considered to be just lazy. Two types of effort are more likely to cause symptoms: brief intense isometric exercise, such as lifting heavy weights, or less intense but sustained dynamic exercise, such as running or climbing a hill. Moderate exercise, such as walking on level ground, is usually well tolerated. All patients experience a constant phenomenon, named the 'second wind': if they rest briefly after the onset of exercise-induced myalgia, they are then able to continue to exercise with a lower level of pain and fatigue. This phenomenon is considered to be related to the ability to metabolize free glucose that is mobilized in the bloodstream. Myoglobinuria is the major complication, and occurs in about half of the patients. Creatine kinase (CK) can increase to more than 100,000–1,000,000 IU/L during episodes of rhabdomyolysis, leading to a risk of developing acute renal failure. With carnitine palmitoyl transferase II (CPT II) deficiency, GSD V is the second most common disorder leading to episodes of recurrent myoglobinuria, although lipin1 deficiency is now also recognised as a relatively frequent cause in children (▶ Chap. 35). Clinical examination is usually normal between crises, but proximal muscle weakness and wasting occur in approximately 35% of the patients over 40 years of age [67]. Two patterns of muscle weakness may be observed: (1) proximal and symmetrical, or (2) scapulo-humeral and asymmetrical. Resting serum CK is consistently elevated in McArdle patients. Electromyography (EMG) can be normal or show non-specific myopathic features at rest but documents electrical silence in contracted muscles.

▪ Metabolic Derangement

There are three isoforms of glycogen phosphorylase: brain/heart, liver and muscle, all encoded by different genes. GSD V is caused by deficient myophosphorylase activity.

▪ Genetics

GSD V is an autosomal recessive disorder caused by mutations in *PYGM*. The number of known pathogenic mutations has rapidly increased to over 190 (▶ http://databases.lovd.nl/shared/variants/PYGM). By far the most common mutation in Caucasians is the p.R50X (c.148C > T) mutation, which accounts for 81% of the alleles in British patients and 63% of alleles in US patients [68].

No genotype-phenotype correlations have been detected. In addition, an angiotensin-converting enzyme (ACE) insertion/deletion polymorphism might play a significant role as a phenotype modulator in individuals with GSD V [69].

An exception to this homogeneous phenotype associated to recessively inherited *PYGM* mutations, is the recent description of a French family with dominant *PYGM* mutation transmission leading to abnormal conformation and aggregation of myophosphorylase, and permanent muscle proximo-distal muscle weakness at adult age [70].

▪ Diagnosis

The ischaemic forearm exercise test (IFET) was first used by McArdle to describe the absence of elevation of lactate during exercise, but its main drawbacks are muscle pain with possible rhabdomyolysis (▶ Chap. 3).

Consequently the ischaemic test should be abandoned and replaced by the standardized non-ischaemic FET, which has a sensitivity of 100% in McArdle disease [71]. Ammonia levels should be also assessed in parallel with lactate, as an abnormal increase in ammonia is always observed in GSD V. This measurement of ammonia also allows discrimination of patients with disorders of glycogenolysis from those with nonorganic muscle symptoms, because in the latter the lack of an increase in both lactate and ammonia indicates insufficient effort due to lack of cooperation. Alternative diagnostic tests include (1) a cycling test at a moderate and constant workload, during which patients with GSD V show a consistent decrease in heart rate between the seventh and the 15th minutes of exercise, indicating the second wind phenomenon or (2) ^{31}P-magnetic resonance spectroscopy to demonstrate abnormal alkalinisation after exercise [72]. Muscle biopsy shows vacuoles and subsarcolemmal accumulation of glycogen that is normally digested by diastase. Negative staining using a specific myophosphorylase confirms the diagnosis, but muscle biopsy should always be performed several weeks after an episode of rhabdomyolysis, as the histochemical abnormalities may be overshadowed by the intensity of the necrotic process. However, muscle biopsy should be avoided when the diagnostic is suspected, as sequencing of *PYGM* is now available.

Treatment

There is no pharmacological treatment, but exercise intolerance may be alleviated by aerobic conditioning programs [73] or by ingestion of oral sucrose (37 g), which may have a prophylactic effect when taken 5 to 15 minutes before planned activity [74]. This effect is explained by the fact that sucrose is rapidly split into glucose and fructose; both bypass the metabolic block in GSD V and hence contribute to glycolysis [75]. It has been reported that work capacity and exercise tolerance are improved after a carbohydrate-rich diet, an effect that needs to be explored in larger controlled trials [76]. Patients should also avoid strenuous efforts and leisure activities that put them at risk, such as swimming far from the shore and mountaineering.

5.2.2 Disorders of Glycolysis

Several other enzyme deficiencies affecting the glycolytic pathway have been reported and are described fully in ▶ Chap. 7 (◘ Table 5.1). They all present with exercise intolerance and possibly also with episodes of rhabdomyolysis similar to those in GSD V. Additional clinical, biological and morphological features may allow these very rare disorders to be distinguished from GSD V: in particular hemolytic anemia is frequently associated with rhabdomyolysis episodes in PFK and PGK deficiencies, and multisystemic manifestations are major diagnostic clues of PGK or PGM deficiencies including neurological symptoms) [77, 78].

When a disorder of glycolysis is suspected, the first step in the evaluation of patient should be a forearm exercise test for measurements of lactate and ammonia levels. Absent or blunted lactate production with an abnormal rise of ammonia levels is a characteristic feature, which should always be followed by search for *PYGM* mutations, before performing a muscle biopsy. ^{31}P-NMR spectroscopy, which is available only in highly specialized centers, allows detection of an abnormal increase in the phosphomonoester peak in PFK and PGK deficiencies, a useful criterion for distinguishing these enzyme deficiencies [72].

Muscle histology shows inconstant subsarcolemmal vacuoles and glycogen accumulation on PAS staining. This glycogen is normally digested by diastase, except in PFK deficiency, which can also lead to accumulation of abnormally branched glycogen (polyglucosan) with a hyaline aspect on standard haematein-eosin stain and resistance to diastase digestion. Specific anomalies such as tubular aggregates may be observed in PGAM deficiency. A specific histochemical reaction is also available for PFK and may help to confirm the diagnosis of GSD VII. The diagnosis is made from the biochemical analysis of enzyme deficiencies either on muscle biopsy for all enzymes, or in erythrocytes for PFK, PGK and aldolase A and from molecular analysis.

5.2.3 Glycogen Storage Disease Type II (Pompe Disease)

GSD II, also named Pompe disease, acid α-glucosidase deficiency or acid maltase deficiency, is caused by deficiency of the lysosomal enzyme acid α-glucosidase. It is the second most common cause of muscle glycogenosis after GSD V. In contrast to other GSDs, in GSD II glycogen accumulates primarily within the lysosomes, not in the cytoplasm.

Clinical Presentation

Pompe disease presents as a spectrum, with infantile, juvenile and adult forms named according to the age at onset, rate of progression and extent of organ involvement.

The classic infantile form usually presents within the first months of life with hypotonia (floppy infant syndrome) and hypertrophic cardiomyopathy, which can be detected on chest X-ray and electrocardiogram. Additional clinical features can be dysphagia, smooth

muscle dysfunction, enlargement of the tongue and liver and failure to achieve major motor milestones. Most untreated infantile onset patients die from cardiopulmonary failure or aspiration pneumonia prior to one year of age [79]. The juvenile forms are characterized by predominant skeletal muscle dysfunction, with motor and respiratory problems. Calf hypertrophy can be present, mimicking Duchenne muscular dystrophy in boys. Myopathy and respiratory insufficiency deteriorate gradually, and patients may become dependent on a ventilator or wheelchair.

The adult form develops in the third or fourth decade and affects the trunk and proximal limb muscles, mimicking inherited limb-girdle muscle dystrophies [80]. Involvement of the diaphragm is frequent, and acute respiratory failure may be the initial symptom in some patients. Therefore, the presence of a severe respiratory insufficiency in a patient with moderate limb-girdle muscle weakness is a major clue in the diagnosis of adult-onset Pompe disease. By contrast with the infantile form, the heart is generally not affected. The major cause of death in adults is respiratory insufficiency. Pulmonary function tests should be undertaken annually and respiratory support started when necessary, as in some patients this can prolong life for decades. Rarer causes of death are strokes related to intracranial aneurysm or arteriopathy due to accumulation of glycogen in vascular smooth-muscle cells [81].

Metabolic Derangement

The enzyme defect results in the accumulation of glycogen within the lysosomes, with different critical thresholds depending on the organ, explaining why the heart is unaffected in adults who have significant residual enzyme activity. Intermediary metabolism is unaffected. Autophagy probably also has a major role in the pathogenic process, with studies showing an autophagic build-up due to dysfunction of endocytic and autophagic pathways in the muscle fibres [82]. A failure to digest glycogen could result in local starvation, inducing autophagy with a pathological cycle due to lysosomal dysfunction.

Genetics

Over 580 mutations have been reported in *GAA* encoding acid α-glucosidase, missense variants are the most frequent type followed by small deletions. There is some degree of genotype-phenotype correlation with severe mutations (such as del exon18) associated with the infantile form and 'leaky' mutations associated with the adult variant. The most common mutation in adults and children with a slowly progressive course is c.-32-13 T > G (approximately 80% of Caucasian patients) [83].

Diagnosis

The diagnosis always relies on demonstrating acid α-glucosidase deficiency; infants with the classic infantile form have less than 1% residual activity, whereas children and adults have residual activity of no more than 30% of normal values. Sensitivity and specificity of enzymatic assays performed in various tissues may be altered by interference with neutral α-glucosidase activities, and skin fibroblasts are the best tissue for diagnosis owing to lower biochemical interferences. Screening methods for acid α-glucosidase deficiency (together with Fabry disease, Gaucher disease, mucopolysaccharidosis type I) have been developed and used for newborn screening [84]. Enzymatic prenatal diagnosis is also possible on chorionic villi, but DNA analysis is a far better procedure in this context if mutations have already been detected in the parents or a previously affected child.

Muscle biopsy shows a severe vacuolar myopathy with accumulation of both lysosomal and free glycogen in the infantile form, but this procedure is not recommended in babies because of the anaesthetic risks. Conversely, the diagnosis is frequently established in adults from the result of a muscle biopsy performed in the context of diagnostic work-up of a muscle dystrophy. A vacuolar myopathy with PAS-positive material is present in approximately two thirds of adults, but in one third of cases the muscle biopsy may be normal or show nonspecific changes, potentially leading to a mistaken diagnosis [85]. Electromyography may also help in establishing the diagnosis in the myopathic forms of the disease, showing pseudo-myotonic discharges, more frequently in paraspinal muscles, in addition to the myopathic features.

Treatment and Outcome

Palliative therapy relies on prevention of cardiorespiratory failure, with the possibility of long-lasting survival in adults with ventilatory support. A major step towards treatment of Pompe disease has been achieved with the large-scale production of recombinant acid α-glucosidase (rhGAA), initially in milk from transgenic rabbits and subsequently from CHO cells (alglucosidase alfa). Alglucosidase alfa (Myozyme or Lumizyme) has been commercially approved since 2006, studies in infants showed major beneficial effects on cardiomyopathy and muscle weakness, with increased survival. Doses of 20 mg/kg by infusion every other week are recommended though higher does have been found more effective [86]. However, only about one third of children on enzyme replacement therapy (ERT) gain normal motor function status and become ventilator free although the outcome appears better for those started on treatment following identification by newborn screening (for review see [87]).

In long term survivors on ERT involvement of disease affecting the CNS can become apparent [88]. Several factors may limit the efficacy of ERT in children, including the severity and extent of muscle damage at the start of treatment and the appearance of high levels of IgG antibodies to rhGAA. However, immune modulation with rituximab, methotrexate, and intravenous immunoglobulin (IVIG) can induce immune tolerance in those who are ERT-naive [89].

In adults, a double-blind placebo-controlled trial showed improvement of the walking distance and stabilisation of vital capacity after 18 months of treatment [90]. Several open studies of adults series have confirmed that ERT may improve or stabilize limb muscle strength and respiratory function, on the basis of the distance walked during the 6-minute walk test and respiratory functional tests or duration of ventilation. Recently published long-term follow-up data in adults with treatment durations up to 10 years, showed that the majority of adult patients with Pompe disease benefit from long-term ERT, with a ceiling effect after 3 to 5 years. Many patients experience secondary decline with considerable individual variation [91, 92].

Gene therapy treatments are in development [93].

5.2.4 Danon Disease (LAMP-2 Deficiency)

Danon disease is a rare X-linked disorder caused by a primary deficiency of lysosomal-associated membrane protein 2 (LAMP2) .

Clinical Presentation

The disease presents after the first decade, and the characteristic clinical features in male patients include cardiomyopathy in all cases and mild skeletal myopathy and mental retardation in over 70%. Eye fundus examination may detect either retinopathy or maculopathy, and these visual abnormalities may be important clues to this diagnosis in patients with unexplained hypertrophic cardiomyopathy [94]. Hemizygous females can also be affected, with a later age at onset and either hypertrophic or dilated cardiomyopathy but with slower progression.

Metabolic Derangement

This disease has been classified with glycogenoses because of the appearance on muscle biopsy, in most cases, of small cytoplasmic vacuoles containing autophagic material and glycogen in muscle fibres.

Genetics

Danon disease is caused by mutations in *LAMP2* on Xq24.

Diagnosis

The diagnosis may be confirmed with muscle biopsy, by the absence of LAMP-2 staining on immunohistochemistry and detection of mutations in *LAMP2*.

Treatment

No specific therapy is currently available. Outcome in males is poor with a rapid progression to heart failure [95]. Prevention or treatment of arrhythmias is frequently required; cardiac transplantation should be considered.

5.2.5 Glycogen Depletion Syndromes: Muscle Glycogen Synthase Deficiency (Muscle GSD Type 0, GSD 0b) and Glycogenin 1 Deficiency

Two muscular glycogenosis, due to deficiencies of enzymes involved in the initial steps of glycogen synthesis, namely glycogenin and glycogen synthase, have recently been identified [96, 97].

Clinical Presentation

Both disorders present with myopathy and cardiomyopathy.

Metabolic Derangement

In both diseases the major pathological hallmark is a profound depletion of glycogen in muscle on PAS staining, associated with a marked predominance of oxidative (type 1) muscle fibres and mitochondrial proliferation. However, there is an unexplained difference between them in the cardiac pathology, with an absence of glycogen in cardiomyocytes in GSD 0b, whilst PAS-positive material lacking the normal ultrastructural appearance of glycogen was present in the patient with glycogenin 1 deficiency.

Genetics

Glycogenin 1 deficiency

Glycogenin is an autoglycosylated glycosyltransferase that catalyses the formation of a short glucose polymer of approximately ten glucose residues. There are two glycogenin isoforms: glycogenin-1, encoded by *GYG1*, is the muscle isoform, but is also expressed in other tissues to a minor degree; glycogenin-2, encoded by *GYG2*, is the liver isoform and is also expressed in cardiac muscle and other tissues, but not in skeletal muscle. Recessively inherited mutations of *GYG1*, leading to inactivation of autoglycosylation of glycogenin-1, have been detected in adults with severe cardiomyopathy [96, 98]. This disorder has been named GSD XV. Different *GYG1* mutations have also been found in a number of

patients with a polyglucosan skeletal myopathy without cardiomyopathy (see below).

Muscle glycogen synthase deficiency (Muscle GSD Type 0, GSD 0b)

Muscle glycogen synthase is ubiquitously expressed and encoded by *GYS1*, whereas *GYS2,* encoding for hepatic glycogen synthase, is only expressed in the liver. A recessively inherited stop mutation in *GYS1* has been reported in three siblings with muscle fatigability and hypertrophic cardiomyopathy. Epilepsy was observed in the oldest child, who died of cardiac arrest at the age of 10 years. Glucose tolerance was investigated in the two younger siblings and was found to be normal [97].

Treatment

No specific treatment has been reported apart from selective β_1-receptor blockade for cardiac protection (Table 5.3).

5

Table 5.3 Polyglucosan storage diseases

Gene(s)	Enzyme/protein	Tissue	Disease	Clinical phenotype(s)
GBE1	Glycogen branching enzyme	Liver, nervous system, muscle, heart, skin	GSD IV	– Fetal akinesia deformation sequence – Liver disease in infancy – Cardiomyopathy – Skeletal myopathy
			Adult polyglucosan body disease (APBD)	– Progressive central and peripheral nervous system disease
PKFM	Phosphofructokinase	Muscle	GSD VII	– Skeletal myopathy
RBCK1	Ring-box protein C-kinase (ubiquitin ligase)	Heart, muscle, liver	Polyglucosan body myopathy 1 with or without immunodeficiency	– Early onset fatal immunodeficiency and a hyperinflammatory disease with severe dilated cardiomyopathy and myopathy – Progressive proximal muscle weakness with dilated cardiomyopathy
GYG1	Glycogenin 1	Muscle & heart	GSD15	– Juvenile onset myopathy with severe cardiomyopathy
		Muscle	Polyglucosan body myopathy type 2 (PGBM2)	– Late onset slowly progressive myopathy
PRKAG2	PR kinase AMP-activated γ-subunit	Heart, muscle	Hypertrophic cardiomyopathy type 6 (HCM6)	– Late adolescence /early adult onset, ventricular pre-excitation, cardiomyopathy
EPM2A	Laforin (glycogen phosphatase)	Brain and nervous system	Lafora disease	– Childhood onset intractable epilepsy, myoclonus, dysarthria, ataxia and dementia
NHLRC1 (EPM2B)	NHL repeat-containing protein 1 (Malin, ubiquitin ligase)			
PRDM8	PR domain containing protein-8			

Polyglucosan (PG) is an abnormal, insoluble, form of glycogen with fewer branching points that can accumulate in deposits known as PG bodies. Depending on the condition PG bodies can be found in various tissues including the brain, nerve, muscle, skin, liver and retina. The mechanism that causes the accumulation of PG bodies is not fully understood but is thought to be due to dysfunction of either the ubiquitin proteasomal system and/or the lysosomal autophagic pathway. The known genetic causes and clinical phenotypes are summarised in this table and in the text. For review see [99]

5.2.6 Muscle and Cardiac Glycogenosis with Polyglucosan Bodies Due to *RBCK1* and *GYG1* Mutations

Polyglucosan storage myopathies are a group of glycogen storage diseases with accumulation of polysaccharides that are less branched than normal glycogen, and resistant to α-amylase digestion. Polyglucosan may aggregate into dense inclusions known as polyglucosan bodies which are the pathological hallmark of these diseases (Table 5.3).

Two new forms of glycogenosis have been described, characterised by accumulation of polyglucosan in skeletal muscle or heart [98, 100, 101].

Clinical Presentation

Patients with *RBCK1* mutations present with progressive muscle weakness and also often develop a rapidly progressive cardiomyopathy which may require cardiac transplantation. Patients with polyglucosan accumulation in skeletal muscle due to *GYG1* mutations, in contrast to the patient with glycogen depletion in skeletal muscle and *GYG1* mutations, described above, rarely develop cardiomyopathy. The clinical phenotype of patients with *GYG1* mutations is characterized by a slowly progressive proximo-distal and asymmetric myopathy, starting at adult age.

Genetics

Glycogenin 1 deficiency

GYG1 mutations which have been identified initially in 7 patients with polyglucosan body myopathy are missense, nonsense, or frameshift pathogenic variants, distributed all over the gene. There was either reduced or complete absence of glycogenin 1 protein, in accordance with the deleterious effects of the variants. The most frequent variant was c.14313G > C, identified in patients from different ethnic backgrounds. This common splice site variant caused a complete or nearly complete alternative splicing, with profound reduction of wild-type glycogenin 1 [101]. Mutated abnormal glycogenin 1 may be the cause of the cardiomyopathy which does not occur in those who completely lack glycogenin 1 [102].

***RBCK1* mutations**

All patients are homozygous or compound heterozygous for missense or truncating mutations in this gene which encodes an E3 ubiquitin ligase. How *RBCK1* is involved in glycogen metabolism remains unknown. Furthermore, children with loss-of-function mutations in the same gene had a distinct phenotype characterised by failure to thrive, autoinflammation, and recurrent episodes of sepsis [103].

5.2.7 AMP-Activated Protein Kinase (AMPK) Deficiency

Clinical Presentation

Symptoms start typically in late adolescence with ventricular pre-excitation (Wolff-Parkinson-White syndrome) predisposing to supraventricular arrhythmias. There is a progressive mild to severe cardiac hypertrophy and an increased risk of sudden cardiac death. The disorder is usually described as familial hypertrophic cardiomyopathy with Wolff-Parkinson-White syndrome. Although glycogen storage typically affects only the heart, a skeletal muscle involvement with myalgias or muscle weakness may also occur in some patients, and a skeletal muscle glycogenoses has been reported in a patient with exercise intolerance, high CK levels and a forearm exercise test showing a blunted lactate increase [104].

Metabolic Derangement

AMPK is a cellular energy sensor that is activated by exercise in muscle and an increase in the AMP/ATP ratio, stimulating fatty acid oxidation, glycolysis and glucose oxidation. This enzyme forms a heterotrimeric complex comprising a catalytic subunit (α) and two regulatory subunits (β and γ). Three isoforms of the gamma subunits are known (γ1, γ2 and γ3) with different tissue expression, and each contains four repeats of a structural module known as a cystathionine β–synthase (CBS) domain [105]. Pathological examinations of the hearts from affected patients revealed vacuoles containing polysaccharide.

Genetics

The *PRKAG2* gene coding for the γ-subunit of AMPK is located on chromosome 7q36. Mutations in the γ2-subunit of AMPK are transmitted as an autosomal dominant trait with full penetrance [106]. Interestingly, molecular analysis performed in babies who had died of cardiac congenital glycogenoses, which had been attributed to a heart-specific variant of phosphorylase b-kinase deficiency, revealed a recurrent activating mutation in *PRKAG2*. Therefore, it appears that the low PHK activities that were determined in the hearts of these patients were either artefacts or secondary to a down-regulation induced by AMPK dysfunction or cardiac glycogen deposition [64].

Diagnosis

The diagnosis, if clinically suspected, is based on ECG, echocardiography and molecular genetics. The differential diagnosis includes Pompe, Danon (LAMP2) and Fabry diseases.

Treatment

Treatment requires a pacemaker/defibrillator and heart transplant.

5.3 Brain Glycogenoses

In the brain branching enzyme, glycogen synthase, debranching enzyme and phosphorylase are present in both astrocytes and neurons. In neurons, however, there is no glycogen synthesis, since glycogen synthase is directed toward glycogen degradation in the proteasome system by the laforin-malin complex. In astrocytes glycogen is degraded to supply energy during brief episodes of hypoglycaemia and hypoxia. Glycogenolysis in astrocytes produces lactate, which is exported by a monocarboxylic transporter to neurons, where it is oxidised in the mitochondria [107] (► Chap. 8). Brain GSDs present with adult neurodegeneration/epilepsy syndromes associated with accumulation of polyglucosan bodies. Polyglucosan deposition in the nervous system is characteristic of Lafora disease and adult polyglucosan body disease, but can also occur in normal ageing.

5.3.1 Lafora Disease (Neuronal Laforin/Malin Defects)

Clinical Presentation

Lafora disease is an autosomal recessive form of myoclonic epilepsy that typically manifests during adolescence and is characterised by tonic-clonic, myoclonic and absence seizures, or focal seizures frequently associated with visual symptoms. As the disease progresses, affected individuals develop a rapidly progressive dementia with visual loss, apraxia and aphasia, leading to a vegetative state and death within a decade of disease onset.

Metabolic Derangement

The hallmark of Lafora disease is the presence of large inclusions (Lafora bodies) composed of abnormal glycogen molecules in the axons and dendrites of neurons, especially in the cerebral cortex, substantia nigra, thalamus, globus pallidus and dentate nucleus. The abnormal glycogen has an elevated phosphate content and reduced branching. Polyglucosan bodies are also seen in muscle, liver, heart, skin and retina, showing that Lafora disease is a generalised glycogenoses. The mechanisms by which accumulation of abnormally branched glycogen triggers neuronal apoptosis are undetermined [107, 108].

Genetics

Lafora disease has been found associated with mutations in two genes: Epilepsy, Progressive Myoclonus 2a (*EPM2A*) and Epilepsy, Progressive Myoclonus 2b (*EPM2B*)). *EPM2A* is mutated in about 50% of individuals and encodes laforin; *EPM2B* is mutated in 30–40% and encodes malin. These two mutations share an identical phenotype, as these two proteins operate through a common physiological pathway. An early onset form of the disorder has been described in a single family with mutations in *PRDM8*. The PRDM8 protein is of unknown function but has been shown to interact with laforin and malin [109].

Diagnosis

A skin biopsy will reveal the pathognomonic Lafora bodies in most patients. Mutation analysis will confirm the diagnosis.

Treatment

No treatment is available.

5.3.2 Adult Polyglucosan Body Disease

Clinical Presentation

Adult polyglucosan body disease (APBD) is the adult-onset form of branching enzyme deficiency, and this rare disorder is characterised by progressive central and peripheral nervous system involvement. The first symptoms of APBD usually occur between the fourth and fifth decade of life, with early onset bladder dysfunction followed by progressive spastic paraplegia, and to a lesser extent, peripheral neuropathy and cognitive impairment occurring in about 50% of cases in the later stages of the disease. An earlier relapsing, remitting course is also described [110]. Several clinical variants, mimicking spinocerebellar ataxia, extrapyramidal disorders, or motor neuron disease, have been described [111–113]. Brain MRI most often show atrophy of the cervical spine and white matter abnormalities on T2 sequences predominantly in the periventricular regions, pyramidal tracts and the medial lemniscus of the pons and medulla [114].

Genetics

Most of the patients with APBD are of Ashkenazi Jewish ancestry, with a prevalent mutation in *GBE1* (p.Tyr329Ser) in this population. However, in a significant number of patients, only one heterozygous mutation has been found despite similar residual enzymatic activity than patients with two identified mutations. In fewer cases, no mutation has been found in *GBE1*, suggesting a genetic heterogeneity [114].

Diagnosis

Although the presence of polyglucosan bodies in skin, muscle or nerve tissues can further orientate the diagnosis, APBD is primarily confirmed by a reduction of GBE enzymatic activity to 10–20% normal in patients' leukocytes or fibroblasts.

Treatment

No treatment is currently available. A recent clinical placebo-controlled trial of triheptanoin did not show benefit in a cohort of 23 patients [115].

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Congenital Hyperinsulinism and Genetic Disorders of Insulin Resistance and Signalling

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Contents

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Glucose-Induced Insulin Secretion and Insulin Receptor Pathway

When glucose is transported into the pancreatic β-cell it is phosphorylated to glucose-6-phosphate by glucokinase (GCK), a highly regulated enzyme. GCK has a Km for glucose close to its concentration in blood and functions as a glucose sensor; any rise in blood sugar is followed by a proportional increase in the rate of glycolysis with phosphorylation of ADP to ATP by the glycolytic pathway and the mitochondrial respiratory chain. At the plasma membrane, this increased ATP/ADP ratio is detected by ATP/ADP – sensitive potassium channels (K_{ATP}), leading to their closure, and subsequently to the depolarization of the plasma membrane of the pancreatic β-cell. This depolarization opens voltage-sensitive Ca^{2+} channels, and the influx of extracellular Ca^{2+} stimulates insulin secretion by exocytosis from storage granules.

In addition to this glucose-stimulated insulin secretion (GSIS; ◘ Fig. 6.1), other mechanisms regulate the release of insulin to meet all physiological circumstances: (1) transcription factors, such as *HNF1A* and *HNF4A*, (2) metabolic factors which modulate ATP production, namely leucine (through the activation of the glutamate dehydrogenase (GDH, *GLUD1*), short-chain L-3-hydroxyacyl-CoA dehydrogenase (SCHAD, *HADH*), the monocarboxylate transporter (MCT1, *SLC16A1*), and the mitochondrial uncoupling protein 2 (*UCP2*), (3) receptors for various hormones and neuropeptides including somatostatin, insulin, GLP1, GIP, etc.

Insulin, released into the blood, will act on the insulin receptors of insulin-sensitive cells. The downstream signalling pathway of the insulin receptor includes phosphatidyl inositol 3 kinase, AKT serine/threonine kinases and mTOR. Its activation will lead to the translocation of GLUT4 glucose transporter to the plasma membrane, thus causing an influx of glucose into the cells, and will turn the cell metabolism to glucose utilization and cellular growth.

Of note, cerebral cells are poorly insulin-sensitive and are highly dependent on circulating glucose to support brain metabolism. Consequently, in congenital hyperinsulinism, there is a significant risk of brain damage from neuroglucopenia, while insulin-sensitive cells are generously fed.

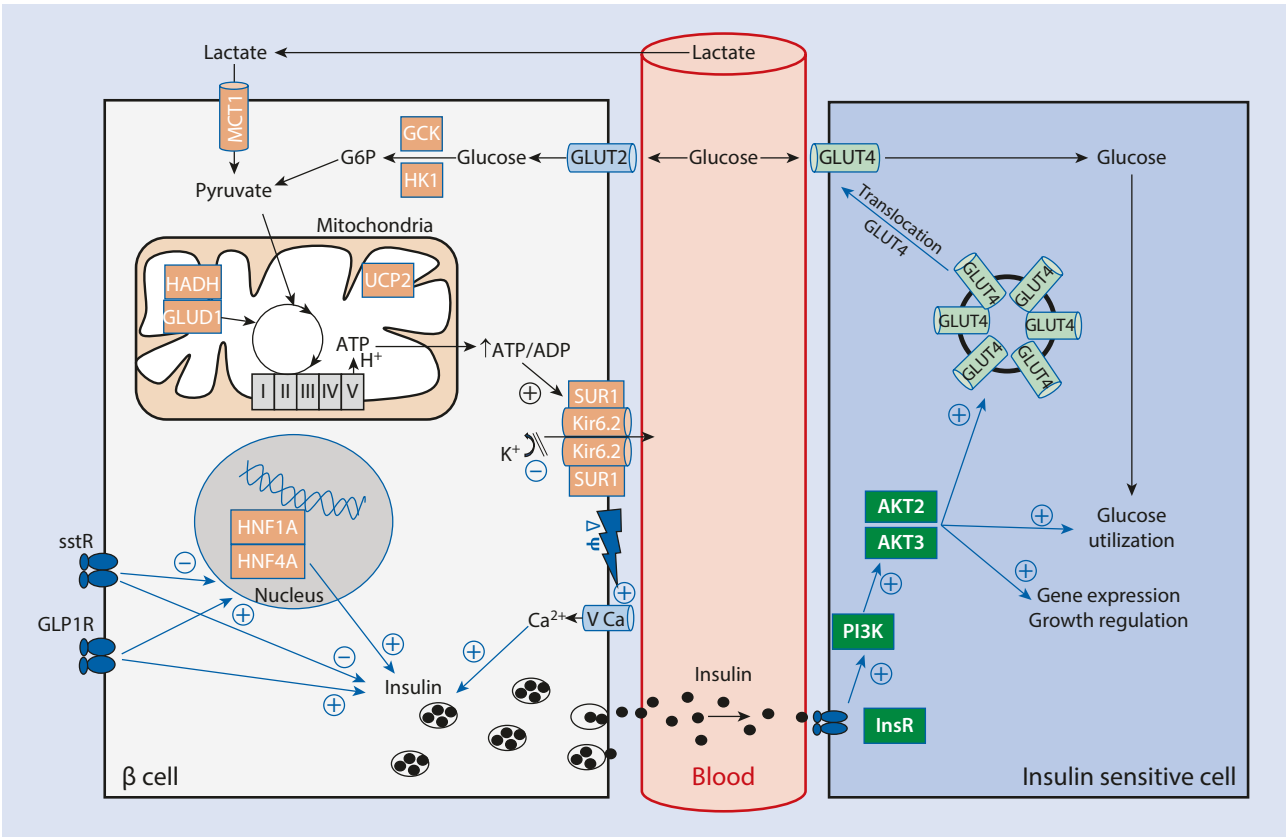

Fig. 6.1 Glucose-induced Insulin Secretion and Insulin receptor pathway

Introduction

Congenital hyperinsulinism (CHI) comprises all genetic causes leading to hyperinsulinaemic hypoglycaemia (HI) due to a primary defect of the pancreatic ß-cell. CHI can present at any age but is most common in infancy [1]. Severe CHI is responsible for recurrent severe hypoglycaemia in neonates, in whom a delayed diagnosis or inappropriate medical management is responsible for brain damage in more than 30%, underlining the importance of an early diagnosis and of an urgent care. Most CHI patients are responsive to a first line oral treatment with diazoxide. If unresponsive, somatostatin analogues and/or frequent/continuous feeds, may be required with further exploration in order to determine the histopathological form of the CHI: in diffuse forms, all ß-cells throughout the pancreas are involved, while in focal forms, hypersecreting ß-cells are restricted to a small region of the pancreas, allowing a cure after a targeted partial pancreatectomy.

Alternative strategies for diffuse forms of CHI include subtotal pancreatectomy (leading to insulin-dependent diabetes in most cases) or an intensive medical treatment for years. The severity of CHI improves within a few years, allowing a progressive discontinuation of all therapies. Moreover, because surgery has not shown to improve the long-term neurologic outcome, several new treatments are in development in order to avoid the need for subtotal pancreatectomy.

While CHI are diseases of the insulin-producing ß cells, some other hypoglycaemia-leading diseases are explained by genetic disorders of the insulin-sensitive cells [2, 3]. The defect is located either on the insulin receptor or on its downstream pathway, defining 2 groups: respectively insulin resistance syndrome (IRS) and insulin signalling disorders (ISD). The main biological difference is that, during hypoglycaemia, insulinemia is elevated (IRS) or suppressed (ISD). Dysmorphic features might not be obvious from birth; thus, the first apparent symptoms may be hypoglycaemia.

6.1 Clinical Presentation

In adults and children, hypoglycaemia produces glucopenic symptoms (drowsiness, faints, seizures, hallucinations, or any other neurological symptoms) and adrenergic symptoms (pallor, sweating, tachycardia). However, adrenergic symptoms can be nonspecific in neonates and toddlers, or attenuated in patients with daily hypoglycaemia, leading to a delay in both diagnosis and management. Hypoglycaemia may occur after a meal, following a fast, several times a day or exceptionally. In CHI, there may be a family history of hypoglycaemia, of MODY diabetes, or both. Rarely, the HI can be induced by strenuous physical exercise (*SLC16A1* mutations) (► Chap. 10). In syndromic CHI and in ISD, the dysmorphic features can be the presenting symptom before hypoglycaemia.

In order to reduce the risk of severe hypoglycaemia induced brain damage, the indication for glycaemia screening in new-borns is large: prematurity, post maturity, low or large birth weight, macrosomia, perinatal stress (pre-eclampsia, birth asphyxia), malformation, and a family history of a genetic cause of hypoglycaemias [4]. Still, about half of CHI patients, present with hypoglycaemic seizure or coma, because most don't meet the screening criteria (e.g. mean birth weight is 3.7Kg) [5]. In severely affected patients, hypoglycaemia is almost permanent and a high rate of continuous glucose (mean 16 mg/Kg/min) is required to normalize glycaemia. Associated features may be a transient hypertrophic cardiomyopathy, and hypocortisolism.

In infants, three clinical pictures exist [1, 6]:

1. Transient (perinatal stress) neonatal HI can occur in new-borns from diabetic mothers, those who are small for gestational age or as a consequence of a perinatal stress such as foetal distress or following birth asphyxia. Hypoglycaemia can be severe, but usually resolves within a few days, or by 4 months of age at the latest. In this latter case, infants may require transient treatment with glucose enriched feeds and/or diazoxide, to which patients are always responsive. The genetic screening for mutation in genes associated with CHI is normal [7].
2. "Isolated" congenital HI is inherited but occurs primarily as a seemingly isolated abnormality. Hypoglycaemia can reveal the disease from all ages. The familial history may disclose members with hypoglycaemia or neonatal and/or maturity-onset diabetes of youth (*ABCC8, KCNJ11, HNF1A, HNF4A, GCK* mutations), or with intellectual disability or epilepsy (*GLUD1* mutations). Macrosomia is not constantly observed. The age at presentation appears earlier in *HNF4A* mutations (1 day), variable in K_{ATP} gene mutations (1 day to 1 year, mean: 27.4 days), and delayed in *GLUD1* and *HADH* mutations (157 days & 125 days) [8].

The genetic study on blood leukocytes identifies the genetic cause of the CHI in about 50% of patients responsive to diazoxide and 90% in those unresponsive [9]. Indeed, some CHI causing genes are still to be dis-

covered and an undetermined portion of patients might bear somatic mutations in CHI-causing-genes in their pancreas. All patients harbouring mutations in a CHI causing gene can be responsive to the first line oral treatment, diazoxide. However, some mutations in *ABCC8*, *KCNJ11* and *GCK* are resistant.

The disease may involve either the whole pancreas (diffuse form, CHI-D), or a portion (focal form, CHI-F and atypical/mosaic form, CHI-A). For neonates with diazoxide unresponsive CHI, the determination of the histopathological form is particularly relevant, because CHI-F and CHI-A may be cured after an elective partial pancreatectomy. These three forms cannot be distinguished based on clinical criteria, however genetic testing and ^{18}F-DOPA-PET imaging accurately predict the diagnosis in most cases.

Despite isolated CHI being described as non-syndromic, patients may present with additional features:

- *GLUD1* mutations lead to hyperammonemia-hyperinsulinism syndrome (HIHA). About half of patients may also present with various neurological symptoms including mild to moderate intellectual disability, epilepsy (atypical, absence, & myoclonic seizures), pyramidal syndrome or dystonia.
- *HNF1A* and *HNF4A* mutations can down-regulate *GLUT2* thus leading to features of Fanconi-Bickel Syndrome (FBS) (▶ Chap. 8). *HNF1A* mutations are also associated with liver adenomatosis.

3. Syndromic HI, occurs when HI is part of a developmental syndrome. Syndromic patients represent up to 10% of all CHI patients [10]. Hypoglycaemia can be their initial manifestation and they are usually diazoxide responsive. At birth, dysmorphic features may be either absent or discreet so that the syndrome remains inapparent. HI may be severe during the neonatal period but can resolve within it (Sotos syndrome), or during the first year of life Beckwith Wiedemann Syndrome (BWS), or within a few years (CDG, Kabuki, Turner). BWS, Kabuki syndrome, Turner syndrome are the most frequent [11, 12]. HI is also described in: congenital disorders of glycosylation (CDG) syndrome type 1 (*PMM2*-CDG, *PMI*-CDG, *ALG3*-CDG, *ALG6*-CDG), Sotos (*NSD1* / 5q35 deletion / NFIX), Timothy (*CACNA1C*), PASNA (*CACNA1D*), MSSGM1 (*TRMT10A*), Costello (HRAS), Perlman (*DIS3L2*), Simpson-Golabi-Behmel (*GPC3*), *FOXA2* mutations (alternative name: *HNF3B*), *EIF2S3* mutations, Ondine (*PHOX2B*), adenosine kinase deficiency (*ADK*) (▶ Chap. 32) and Rubinstein-Taybi (*CREBBP, EP300*). HI was also reported in patients with Moebius, Cowden, Nager, Usher type C syndrome and various chromosomal abnormalities.

The clinical spectrum of IRS is large, with some patients otherwise almost asymptomatic, while others present with Leprechaunism or Donohue syndrome, revealed by elfin facies with large ears, decreased subcutaneous fat and muscle mass, hypertrichosis and acanthosis nigricans.

In ISD, dysmorphia might be discreet (hemihypertrophy in *AKT2* mutations), while some children are severely impaired from birth. Thus, *AKT3* and *PI3KCA* mutations were associated with various syndromes such as megalencephaly-polymicrogyria-polydactyly-hydrocephalus syndrome (MPPH) or megalencephaly-capillary malformation (MCAP) [13, 14].

6.2 Metabolic Derangement

CHI is the consequence of a primary functional defect of the pancreatic ß-cells. The inappropriate secretion of insulin will lead to neuroglucopenia both by inhibiting hepatic glucose release from glycogen and gluconeogenesis, and by increasing glucose uptake into muscular and fatty tissues. CHI is heterogeneous and caused by various defects in the regulation of insulin secretion by the pancreatic β-cell. These include (1) channelopathies affecting the K_{ATP} channel (*ABCC8* and *KCNJ11* mutations); (2) metabolic defects: enzymes deficiencies, such as glucokinase (*GCK* mutations), glutamate dehydrogenase (*GLUD1* mutations), or short-chain l-3-hydroxyacyl-CoA dehydrogenase (*HADHSC* mutations); transporter deficiencies such as the monocarboxylate transporter 1 (*SLC16A1* mutations) and the mitochondrial uncoupling protein 2 (UCP2); and (3) transcription factors impairment, such as HNF1A and HNF4A [15].

Exceptionally, hypoglycaemia can be the consequence of a defect in the insulin-sensitive cells leading to an impaired insulin clearance and/or an over-activation of the insulin signalling pathway. Mutations in the insulin receptor, or in its signalling pathways (*PI3KCA*, *AKT2* or *AKT3*) will promote glucose uptake and utilization, as well as cell growth.

6.3 Genetics

The estimated incidence of CHI ranges between 1:27,000 and 1:50,000 live births in outbred western population, but in countries with substantial

consanguinity it may be as high as 1 in 2,500 [16]. To date, mutations in 10 genes are known to cause isolated CHI-D, 20 genes to cause syndromic CHI, 1 IRS and 3 ISD. The pattern of inheritance can be dominant or recessive, but the genetic abnormality sometimes occurs de novo and with a variable degree of mosaicism. In isolated CHI, the inheritance is autosomal recessive for *ABCC8, KCNJ11* and *HADH* mutations; autosomal dominant or de novo for *GLUD1, GCK, UCP2, SLC16A1, HNF1A, HNF4A* mutations and in some cases for *ABCC8* and *KCNJ11* mutations. In syndromic CHI, the mode of inheritance depends on the diagnosis.

- In diazoxide-responsive patients, a genetic abnormality is found in up to 47% of patients. *ABCC8* and *GLUD1* mutations are predominant (about 40% each), the other cases are equally distributed between *HNF4A, HNF1A, HADH, KCNJ11* and *UCP2* mutations [8, 9].
- In diazoxide-unresponsive patients, 47% of patients have a CHI-D and 53% a CHI-F. A genetic cause is found from blood leukocytes in about 80–90% of cases. In CHI-D, *ABCC8* and *KCNJ11* mutations represent most cases (47–69% with a recessive inheritance and 20–34% with a dominant inheritance), and *GCK* only 2%. Finally, 10 to 20% of cases are genetically unexplained by leukocytes DNA studies [8, 9]. This ratio might decrease by genetic studies on pancreatic tissues, which may uncover mosaicism for some mutations.

CHI-F is the consequence of an isodisomy of a paternally inherited recessive mutation in *ABCC8* (88%) or *KCNJ11* (11%), both located on the 11p15.5 chromosomal region, associated with a loss of the corresponding maternal allele, thus leading to an imbalance expression of imprinted growth factors and tumour suppressor genes (e.g. IGF2, H19). This somatic event, which is sporadic and spontaneous, probably occurs during the foetal period in a clone of ß-cells. Rarely, some individuals with CHI-F have been reported without the paternally inherited K_{ATP} gene mutation (1% of cases) and thus were consistent with a diagnosis of BWS [17].

The finding in blood leukocytes of a single paternally inherited mutation in a K_{ATP} gene (*ABCC8* and *KCNJ11*) is evocative of CHI-F, but this mutation might also be a dominantly inherited mutation responsible for CHI-D. The challenge is to foresee the dominant or recessive effect of a specific mutation. While stop mutations are certainly recessive, missense mutations remain hazardous to classify, unless already observed in a CHI-F patient (thus certainly recessive). Electrophysiology and immunostaining studies were shown able to determine the effect and inheritance of a specific mutation. Despite data are still scarce however, when available, it brings valuable information to adapt accurately the patients care [18].

The pathomechanism behind CHI-A appears various but consist in a mosaic somatic event within the abnormal pancreatic region: somatic mosaic dominant mutations in *ABCC8* or *GCK* genes, 11p15 paternal disomy, other chromosomal abnormalities [19–21].

6.4 Diagnostic Tests

The investigation of CHI is sequential [15]:

1. Diagnosis of HI. Regardless of the cause, the diagnosis of HI relies on:
   - Fasting and/or post-prandial hypoglycaemia (<2.5–3 mmol/L).
   - Inappropriate plasma insulin levels (≥1μUI/mL) and c-peptide (≥0.2 ng/mL) at the time of hypoglycaemia (potentially missed by a single sample because of the pulsatile secretion of insulin). However insulin levels are low to undetectable in ISD which present with hypoketotic hypoglycaemia (14).
   - Absent/low blood & urines ketones bodies (blood: <2 μmol/L) and non-esterified fatty acids (NEFA, <1,5 μmol/L).
   - An increase in blood glucose greater than 1.7 mmol/L (30 mg/dL) within 30–40 minutes after SQ/IM or IV administration of 1 mg glucagon during hypoglycaemia [15].
   - The need for a high glucose infusion rate (GIR) to keep blood glycaemia >3 mmol/L is characteristic of an insulin related hypoglycaemia (e.g. >8 mg/kg/min in neonates, >10 in infants, >6 during childhood, and >3 in adults) [22].

2. Diagnosis of CHI. Once the diagnosis of HI has been made, some anamnestic, clinical and biological features can indicate the likely aetiology: a family history of MODY diabetes (mutations in *GCK, HNF1A,* or *HNF4A*) or if hypoglycaemia occurs after strenuous physical exercise (mutations in *SLC16A1*); Hyperammonaemia (usually between 80 and 180 μmol/L in HIHA syndrome); urine organic acids and plasma acylcarnitines (high 3-OH-glutarate in urine and C4 -OH-carnitine in plasma, in *HADH* mutations), serum transferrin isoelectrofocusing (CDG syndromes type I); Clinical syndromic features (hemihypertrophy, overgrowth, fat pads, cardiomyopathy or heart or vertebral malforma-

tions…); diazoxide-unresponsiveness (*ABCC8, KCNJ11, GCK* mutations). Finally, in some cases, the differential diagnosis of non-genetic HI should be considered, such as insulinoma, auto-immune syndromes and Münchhausen syndrome by proxy (fabricated or induced illness).

3. ^{18}F-fluoro-L-DOPA positron emission tomography combined with a contrast-enhanced CT angiography (^{18}F-DOPA-PET/CTA) has an 89% sensitivity and 98% specificity to diagnose and localize a CHI-F [23, 24]. It should be performed in diazoxide-unresponsive patients, when genetic analysis has not found a cause for a CHI-D.
4. Pathology. If a partial (CHI-F, CHI-A) or a subtotal (CHI-D) pancreatectomy is considered, the pancreatic biopsies sampled at the beginning of the surgery, will confirm the diagnosis intraoperatively. Three histopathological categories exist [15]:
   (a) CHI-D. The pancreatic architecture is preserved, as well as the islets pattern. However, the latter contain very active ß-cells with very abundant cytoplasm and highly abnormal nuclei (3 to 4 times the size of acinar nuclei) [25].
   (b) CHI-F (a consequence of a paternal uniparental disomy of the 11p15 associated with a paternally inherited K_{ATP} gene mutation, in a subset of ß-cells). Focal forms are small, 2 to 7 mm within a normal pancreatic tissue. The focal form contains focal adenomatous hyperplasia of islets composed of very large islets containing a heterogeneous population of endocrine cells of various sizes. Some of these cells have large cytoplasm and large nuclei of irregular shape. By contrast, islets observed outside the lesion are normal and contain endocrine cells of usual (or shrunken) size without enlarged nuclei. The area of abnormal pancreatic development is multilobular and can have satellites in nearby pancreas tissue [25].
   (c) CHI-A (atypical focal CHI, also called mosaic CHI or LINE (localized islet cells nuclear enlargement)), is rare (up to 10% of cases) [17, 26]. It consists of a morphological mosaicism of the pancreatic ß-cells. While most of the pancreas is normal (with endocrine cells of usual or shrunken size), one or some adjacent lobules contain abnormal ß-cells showing evidence of hyper-activity (large nuclei and cytoplasm). There is no 11p15 involvement and no mutation in CHI genes in leucocytes. However, in some cases, either an excessive and unexplained expression of hexokinase I or a somatic dominant mutation in *GCK* or *ABCC8*, were found in the abnormal ß-cells [20, 26].

6.5 Treatment

Initial Medical treatment [15]

In case of severe hypoglycaemia in neonates, the blood sugar must be normalized urgently by using 10% dextrose IV or orally 2 mL/Kg every 5 to 10 minutes, and a continuous glucose infusion considered. Glucagon 0.3 mg/Kg (max 1 mg) IM, IV or SQ can also be used as an emergency treatment. In severe HI, the glucose infusion rate (GIR) needed to normalize glycaemia may exceed the gastrointestinal tolerance of neonates, thus a central IV line is often urgently required. The proposed initial GIR is 8 mg/Kg/min, from where the rate should be titrated up every 15–30 min until glycaemia get normalized within the normal range. Where glucose requirements becomes extreme (e.g. >16 mg/Kg/min), continuous SC or IV glucagon (1-2 mg/24 h) allows a clinical stabilization with a reduction of glucose intake by about 40% [27].

In neonates, when no significant and spontaneous improvement in the GIR is observed after several days (e.g. 7–15 days), a specific treatment for HI must be considered. The first line treatment is oral diazoxide 10–15 mg/Kg/d in three divided doses per day. Pulmonary hypertension (PHT) has been reported in about 2.4% of infants treated with diazoxide, usually before 4 months old. Affected children were born at earlier gestational age and more frequently had potential PHT risk factors, including respiratory failure and structural heart disease [28]. Therefore, an echocardiography is recommended before initiating this treatment and during follow up. Fluid retention may cause oedema or reopening of patent ductus arteriosus, especially in premature or low birth weight babies, thus thiazide diuretics can be prescribed alongside diazoxide at initiation. Hypertrichosis is a frequent long-term side effect but resolves after the discontinuation of this treatment. Diazoxide-responsiveness is assessed during a 5 days trial: absence of hypoglycaemia (threshold ≥3–3.8 mmol/L) under a normal diet and during a fast (neonates: 6 hours fast; children 12 hours).

In case of a diazoxide-unresponsive HI, further treatments (octreotide +/– feeding) are needed, and genetic studies +/– ^{18}F-DOPA-PET/CT will screen patients for surgically accessible pancreatic lesion (CHI-F and CHI-A).

Octreotide is started at an initial dose of 5–10 µg/Kg/d either continuously (IV or SQ) or in SQ every 8 hours. The responsiveness is reassessed every 48-72 h because a phenomenon of tachyphylaxis may limit its efficiency after some hours. When unresponsive, the dose can be progressively titrated every 48 hours up to 30 µg/Kg/d. The criteria for efficiency are the same than for diazoxide. Octreotide is usually well tolerated, but

put neonates at risk of necrotizing enterocolitis, especially those with hemodynamic instability (sepsis, heart failure ...) and those with pre-existing bowel disease [29].

Some neonates may be resistant to all medications, leading physicians to increase carbohydrates in meals, or to resort to continuous enteral feeding.

Surgery

Surgery is recommended when CHI-F & A are suspected based on genetics or PET-CT results. It might, however, also be required in CHI-D when hypoglycemia still persists despite maximal medical management. The beginning of the surgical procedure is dedicated to confirming a diagnosis by intraoperative examination of pancreatic biopsies. In case of CHI-F & A, the pancreatic area corresponding to the focal uptake of radiotracer on the PET imaging will be removed. Additional pancreatic resections might be performed if abnormal ß-cells are still detected on the margins by intraoperative histology.

In case of CHI-F unresponsive to all medical therapies, a subtotal (95–98%) pancreatectomy might be proposed [30]. However, because insulin requiring diabetes occurs almost always after this surgery, this procedure should be avoided when possible [31].

The post-operative workup must comprise a Doppler ultrasound to check the integrity of the splenic vein and artery, which, from unintentional intraoperative injury can lead to splenic insufficiency.

Long-Term Medical Management

When the patient requires long-term treatment (surgery not performed or failed), the goal consists in maintaining normoglycaemia with the least impact possible on the quality of life of patients and their families. Daily subcutaneous octreotide injections can be changed to a long-acting somatostatin analogue: lanreotide or long acting release (LAR) octreotide (given by injection every 4 weeks initially) [32, 33]. Beside the usual side effects of somatostatin analogues, these long-term injections may be complicated by biliary stones, transient diarrhoea, and by sub-cutaneous nodules at the site of injection which disappear after a few months. Some other medical treatments are currently under evaluation (mTor inhibitors, soluble glucagon, exendin (9–39), insulin receptor inhibitors, other somatostatin agonists etc.) [15]. Some patients may need long-term dietetic treatment, which encompasses frequent carbohydrate feeding, uncooked cornstarch (from age 9 months old), enteral feeding through a G-tube (continuously 24H a day, or only at night).

This long-term treatment must be evaluated on a regular basis. Because of the progressive spontaneous remission of CHI, this treatment will be gradually reduced and then stopped in most cases during childhood.

6.6 Prognosis

Outside of syndromic HI and HIHA syndrome, developmental delay is observed in up to 50% of patients, unrelated to the underlying genetic defect [34, 35]. Severe brain damage is the consequence of profound and prolonged hypoglycaemias presenting with coma and/or status epilepticus in neonates, and typically result in bilateral cavitating occipital lesions on brain MRI. The subsequent treatment strategy (conservative medical management vs. surgery) seems to have less influence on the neurological outcome.

Surgery will immediately and definitively cure most patients with a CHI-F, with a limited risk of complications (limited risk of exocrine pancreatic insufficiency in the case of pancreatic head resection with Roux-en-Y pancreaticojejunostomy) [36]. However, CHI-D patients who have undergone a subtotal pancreatectomy, have an unpredictable short-term outcome with normo-glycaemic, diabetes, or persistence of hypoglycaemic all possible but generally patients are more easily managed than before surgery. Thereafter there is an almost inevitable evolution to insulin-requiring diabetes, affecting 91% of patients within 14 years after the surgery [31].

Without surgery, hypoglycaemia, in most patients (CHI-F and D), will resolve slowly and spontaneously over several months to years, allowing the progressive withdrawal of most or all treatments, usually during childhood. However, because some genes are responsible for both CHI-D and monogenic diabetes (*HNF1A, HNF4A, GCK, ABCC8, KCNJ11*), we recommend lifelong glycaemic follow-up, in order to screen for diabetes in patients cured from CHI-D [37].

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Disorders of Glycolysis and the Pentose Phosphate Pathway

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Contents

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Glycolysis and the Pentose Phosphate Pathway

Glycolysis which converts each molecule of glucose to two of pyruvate (◘ Fig. 7.1), consists of two phases and ten steps. The first five steps are the preparatory phase, consuming energy (ATP) to convert glucose into two, three-carbon sugar phosphate molecules. In the other five steps, the second 'pay-off' phase, ATP and NADH are produced.

The pentose phosphate pathway (PPP; ◘ Fig. 7.1) consists of two distinct parts: the first part, an oxidative, non-reversible pathway, produces NADPH, and the second part, a non-oxidative, reversible pathway, produces ribose for nucleotide and nucleic acid synthesis and connects intermediates to glycolysis.

7

Introduction

Because glycolysis is the most important source of energy in erythrocytes and in some types of skeletal muscle fibres, inherited diseases of glycolysis are mainly characterized by haemolytic anaemia and/or metabolic myopathy. Ten inborn errors of the glycolytic pathway are known, all inherited as an autosomal recessive trait except the X-linked phosphoglycerate kinase and glycerol kinase deficiencies. Hexokinase (HK), glucose-6-phosphate isomerase (GPI) and pyruvate kinase (PKD) deficiencies cause severe haemolytic anaemia. As these are exclusively haematological disorders they are not discussed further. Muscle phosphofructokinase (PFKM), aldolase A, triosephosphate isomerase (TPI) and phosphoglycerate kinase (PGK) deficiencies are characterized by haemolytic anaemia alone or coupled with neurological disease and/or myopathy. Phosphoglycerate mutase (PGAM), enolase and lactate dehydrogenase (LDH) deficiencies present with a purely myopathic syndrome characterized by exercise induced cramps and myoglobinuria. Glycerol kinase deficiency (GKD) is an X-linked disorder that is either an isolated condition presenting with hypoglycaemia and acidosis or part of a contiguous gene deletion where it is also associated with congenital adrenal hypoplasia and/or Duchenne muscular dystrophy. Glucose-6-phosphate can also be formed by the conversion of the glycogen derived glucose-1-phosphate, a reaction catalysed by phosphoglucomutase (PGM). PGM1 deficiency is a congenital disorder of glycosylation (▶ Chap. 43).

Four inborn errors in the pentose phosphate pathway (PPP) are known. Glucose-6-phosphate dehydrogenase deficiency is an X-linked defect in the first, irreversible step of the pathway. As this is an exclusive haematological disorder it is not discussed further. Ribose-5-phosphate isomerase (RPI) deficiency has been described in four patients, who presented with developmental delay and a slowly progressive leukoencephalopathy. Transaldolase (TALDO) deficiency often presents in the neonatal or antenatal period with hepatosplenomegaly, liver dysfunction, hepatic fibrosis and anaemia. In transketolase (TKT) deficiency, the main clinical symptoms are short stature, developmental delay and congenital heart defects. Sedoheptulokinase (SHPK) deficiency, a defect related to the PPP has been described as an isolated disorder and also as part of a 57-kb deletion in nephropathic cystinosis (▶ Chap. 26). Essential pentosuria is the result of a partial deficiency of l-xylulose reductase (xylitol dehydrogenase) an enzyme of the glucuronic acid pathway. Affected individuals excrete large amounts of l-xylulose in urine. Pentosuria, a benign disorder that occurs almost exclusively in Jewish people, is not discussed further here [1].

7.1 Muscle Phosphofructokinase (PFKM) Deficiency

7.1.1 Clinical Presentation

In its typical presentation, PFKM deficiency (GSD VII or Tarui Disease) is clinically indistinguishable from McArdle's disease (▶ Chap. 5). So far more than one hundred patients have been described with prominent clinical symptoms characterized by muscle cramps, exercise intolerance, rhabdomyolysis and myoglobinuria, often associated with haemolytic anaemia and hyperuricemia. The onset of the classic form is usually in childhood but an infantile and a late onset form have also been reported. Patients with infantile onset may manifest as 'floppy babies'. Symptoms include myopathy, psychomotor retardation, cataract, joint contractures, with death during early childhood. Curiously, none of the infantile cases had evidence of haemolytic anaemia [2].

7.1.2 Metabolic Derangement

PFK catalyses the ATP-dependent conversion of fructose-6-phosphate to fructose-1,6-bisphosphate. Tissues deficient in PFK cannot use free or glycogen derived glucose as a fuel source and accumulate glycogen. Human PFK is a tetramer made up of various combinations of 3 subunits: muscle (M), liver (L) and platelet (P). Muscle and liver PFK are a homotetramers of 4 M and 4 L subunits, respectively. Erythrocytes contain both L and M subunits, which randomly tetramerize to form the various combinations.

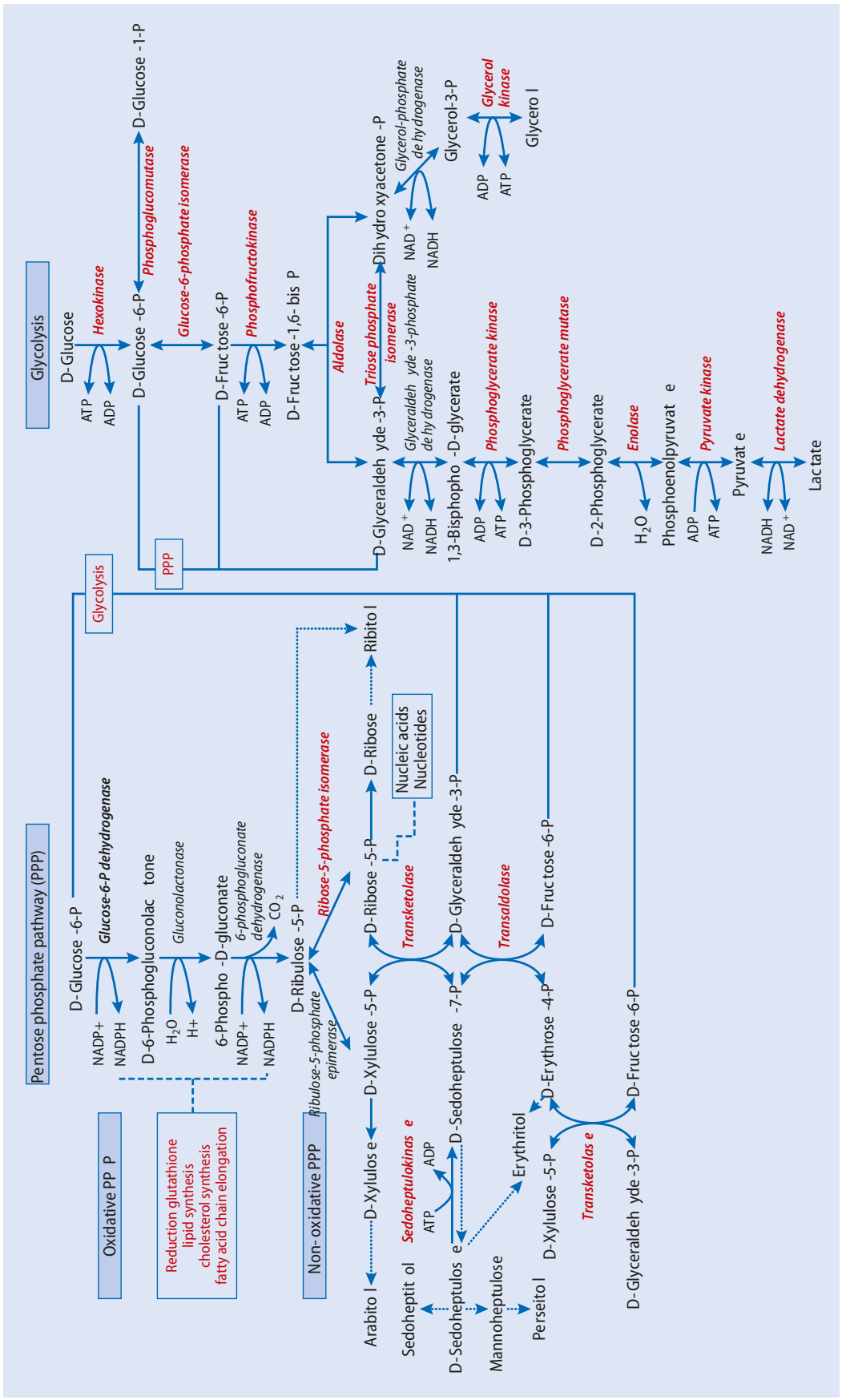

Fig. 7.1 The pentose phosphate pathway and glycolysis. The conversion of the sugar phosphates into their corresponding sugars and polyols (*dotted arrows*) has not been proven in humans. (ATP adenosine triphosphate, ADP adenosine diphosphate, NADP nicotinamide adenine dinucleotide phosphate, *NADPH* reduced form)

7.1.3 Genetics

PFKM deficiency is an autosomal recessive disease with a predominance of cases among Ashkenazi American Jews and Japanese populations. About 25 distinct mutations in *PFKM* have been identified in patients of different ethnic origins. *PFKM* encodes the M subunit. It spans 30 kb and contains 24 exons and at least 3 promoter regions. Among the detected mutations missense and splicing mutations are the most frequent [3].

7.1.4 Diagnostic Tests

A few simple laboratory test results, such as an increased bilirubin concentration and reticulocyte count, reflecting compensated haemolytic anaemia, are useful in distinguishing PFKM disease from McArdle disease. The forearm non ischemic exercise test is characterized by a flat lactate curve but with a normal increase of ammonia (► Chap. 3).

Definitive diagnosis requires biochemical documentation of PFK enzyme deficiency in muscle with a histochemical and/or biochemical assay and by sequence analysis of *PFKM* [2].

7.1.5 Treatment and Prognosis

No specific treatment or cure exists. Management primarily consists of avoiding strenuous exercise. Symptoms normally resolve with rest. Patients should avoid high-carbohydrate meals since glucose is not an alternative substrate in PFKM deficiency. Some patients have been helped by a high protein diet. A ketogenic diet has been proposed in children with the more severe variant of PFKM deficiency. A 5 year follow-up study with ketogenic diet in an adult patient showed an alleviation of muscles symptoms and improvement in exercise performance and oxygen uptake [4]. Except for the very rare fatal infantile form, the disease does not progress to severe disability [2].

7.2 Aldolase A (ALDOA) Deficiency

7.2.1 Clinical Presentation

ALDOA deficiency (GSD XII) is a rare cause of haemolytic anaemia alone or in combination with neurological abnormalities and myopathy. Only 8 patients from five families have been reported so far [5]. Non-spherocytic haemolytic anaemia was present in five patients, with myopathy in two of them. Three siblings from a Moroccan family were reported with isolated episodic rhabdomyolysis triggered by fever without haemolytic anaemia. Mental retardation was present in two patients. The onset is generally within the first months of life. If not promptly recognized, death from severe rhabdomyolysis can occur in myopathic patients.

7.2.2 Metabolic Derangement

Aldolase converts fructose-1,6-bisphosphate to dihydroxyacetone phosphate and glyceraldehyde-3-phosphate. In mammals there are three tissue-specific aldolases encoded by distinct genes: ALDOA in erythrocytes and muscle, aldolase B in the liver, small intestine, and kidney (*Aldolase B deficiency*, ► Chap. 15) and aldolase C in the brain. Since ALDOA is the sole isozyme in erythrocytes and muscle it is predicted to affect these tissues more severely than others. The downstream effects of this enzyme block are the inhibition of glucose production and reduced regeneration of ATP.

7.2.3 Genetics

ALDOA deficiency is an autosomal recessive disease due to mutations in *ALDOA*. So far, four missense mutations (p.Asp128Gly, p.Glu206Lys, p.Cys338Tyr, p.Ala279Val) and a nonsense mutation (p.Arg303*) have been reported. Interestingly, functional studies of the p.Cys338Tyr and p.Ala279Val mutations associated with the myopathic phenotype showed that these mutations disrupt the enzyme in a temperature-sensitive fashion according the crucial role of fever as the trigger of rhabdomyolysis [5].

7.2.4 Diagnostic Tests

The diagnosis of ALDOA deficiency is made by measuring the enzymatic activity in erythrocytes or muscle biopsy. Notably, in erythrocytes ALDOA activity is dramatically reduced even in patients with myopathic symptoms without haemolytic anaemia. Genetic diagnosis is made by sequencing *ALDOA*.

7.2.5 Treatment and Prognosis

There is no specific treatment. Management primarily consists of avoiding strenuous exercise that may cause rhabdomyolysis. In patients with severe chronic anaemia, regular blood transfusions are required.

7.3 Triosephosphate Isomerase (TPI) Deficiency

7.3.1 Clinical Presentation

TPI deficiency is the most severe glycolytic enzymopathy and apart from the infantile form of PFK deficiency, is the only one that is lethal, with death occurring in childhood from respiratory distress. It is characterized by severe congenital haemolytic anaemia from birth, coupled with neurological dysfunction and progressive neuromuscular impairment, most often beginning in the first months of life. Neurological complications include dystonia, tremor, pyramidal tract signs, spinal motor neuron involvement, axonal neuropathy, episodic seizures and psychomotor delay [6, 7]. Cardiomyopathy and recurrent infections can occur [8].

7.3.2 Metabolic Derangement

TPI catalyses the interconversion of dihydroxyacetone phosphate (DHAP) and glyceraldehyde-3-phosphate, with the reaction favouring formation of DHAP by a ratio of 20:1. The triosephosphates, produced by aldolase, are interconnected to lipid metabolism, the glycerol-3-phosphate shuttle and to the pentose phosphate pathway (PPP). TPI deficiency causes elevation of DHAP concentration, particularly in red blood cells, which lack the capacity to metabolize DHAP in the glycerophosphate shuttle via a glycerophosphate dehydrogenase. Patients show a 20- to 60-fold increased red cell DHAP concentration. DHAP is converted non-enzymatically to methylglyoxal, a toxic compound that causes oxidative stress, DNA damage and apoptosis [8]. TPI impairs the synaptic vesicle cycle leading to neurological problems [9]. Therefore, TPI deficiency is also a cellular trafficking disorder involved in synaptic vesicle cycle and neurotransmission (► Chap. 30).

7.3.3 Genetics

TPI deficiency is an autosomal recessive disorder due to mutations in *TPI*. The Glu104Asp mutation accounts for approximately 80% mutant alleles within patients. This mutation causes the most severe symptoms. Some additional missense or nonsense mutations have been identified so far, mostly in compound heterozygotes, coupled with Glu104Asp. The recombinant mutant Glu104Asp has been produced and characterized, showing that it causes instability of the TPI dimer and dissociation of the enzyme into inactive monomers. The unstable TPI monomers are thought to display high aggregation ability, further contributing to the formation of toxic aggregates from them is folded protein particularly in the brain, triggering neuronal dysfunction. Interestingly those patients bearing a null mutation (with no TPI protein) display a very severe haemolytic anaemia without any neurological signs. Studies on cellular and animal models suggest that this enzymopathy may be a conformational rather than a metabolic disease. [8, 10]. These findings can impact drug development, which could focus on stabilizing the TPI enzyme structure and not necessarily restore TPI enzymatic activity.

7.3.4 Diagnostic Tests

The diagnosis is made by enzymatic assay of TPI activity in tissues (red blood cells, leucocytes, fibroblasts and muscle) and by sequence analysis of *TPI*.

7.3.5 Treatment and Prognosis

No effective therapy is available, but aggressive supportive care, especially assisted respiration, has appeared to prolong life in some instances. Usually death occurs in childhood.

7.4 Phosphoglycerate Kinase (PGK) Deficiency

7.4.1 Clinical Presentation

PGK deficiency is an X-linked disorder and has three main clinical presentations: non-spherocytic haemolytic anaemia alone, myopathy alone, or the association of anaemia and central nervous system (CNS) involvement. The combined involvement of CNS and muscle or of all three tissues is much less frequent. Symptoms of CNS involvement can be mental retardation, behavioural abnormalities, seizures, strokes or early or juvenile-onset of parkinsonism. Parkinsonism may be responsive to levodopa [11]. The myopathic form is clinically indistinguishable from PFK deficiency and is characterised by recurrent episodes of exercise-induced cramps and myoglobinuria. The onset is generally in childhood but an infantile onset has been also reported [12].

7.4.2 Metabolic Derangement

PGK catalyses the transfer of the high-energy phosphate from 1,3-diphosphoglycerate (1,3-DPG) to ATP, converting the 1,3-DPG to 3-phosphoglyceric acid.

There are two human PGK isozymes: PGK1 and PGK2 encoded by two distinct genes. While PGK2 is expressed only in testis, PGK1 is expressed in all somatic cells and plays an important role in the generation of ATP during glycolysis. Although PGK1 is ubiquitously expressed, only tissues dependent on high-energy requirement are affected.

7.4.3 Genetics

PGK deficiency is usually fully expressed in male hemizygotes, heterozygous females may have a variable degree of haemolytic anaemia. Recently, a heterozygous female with an early-onset parkinsonism was reported [13]. So far 27 mutations in *PGK1* have been reported, most of which are of a missense type but frameshift and splicing mutations have been found. The relationship between the molecular alterations and the highly heterogeneous clinical features in PGK deficiency is still unclear. Mutations associated with haemolytic anaemia and CNS involvement are spread all along the gene, whereas the mutations causing isolated myopathy tend to cluster in the C terminal domain. Functional studies demonstrated that patients with unstable variants but only mildly affected in catalytic properties present haemolysis associated with neurological dysfunctions. Conversely, myopathy without haemolytic or neurological symptoms is mainly present in patients with variants heavily affected in both catalytic properties and protein stability [14].

7.4.4 Diagnostic Tests

The muscle form is characterized by a flat lactate curve with the forearm non ischemic exercise test (► Chap. 3). Muscle pathology is of little diagnostic help because glycogen storage is either undetectable or very mild. The differential diagnosis should include other causes of hereditary nonspherocytic haemolytic anaemia.

Definitive diagnosis requires biochemical assay of the PGK enzyme activity in muscle and/or erythrocytes and identification of *PGK1* mutations by molecular analysis.

7.4.5 Treatment and Prognosis

No specific treatment or cure exists. Management primarily consists of avoiding strenuous exercise. Symptoms normally resolve with rest. Some patients have been helped by a high protein diet. Except for the very rare fatal infantile onset form, the disease does not progress to severe disability. In patients with severe chronic anaemia, regular blood transfusions are required. Splenectomy has been shown to be beneficial in some cases. The prognosis is variable, depending on the severity of the anaemia and on the presence of the other manifestations [12].

7.5 Phosphoglycerate Mutase (PGAM) Deficiency

7.5.1 Clinical Presentation

To date, 16 patients with PGAM deficiency (GSD X) have been reported. Clinical symptoms are characterized by muscle cramps, exercise intolerance, and myoglobinuria. The onset of the disease is generally in adolescence but in two patients the onset was reported in the adulthood with muscle cramps triggered only by hard exercise. An unusual pathological feature of PGAM deficiency, described in about 36% of patients, is the association with tubular aggregates in muscle. The disease is more common in African-Americans [15].

7.5.2 Metabolic Derangement

PGAM catalyses the interconversion of 2-phosphoglycerate and 3-phosphoglycerate, by using 2,3-bisphosphoglycerate as a cofactor. Human PGAM is a dimeric enzyme, with normal mature muscle containing predominantly the muscle-specific M homodimer (PGAM-M) and the brain specific subunit (PGAM-B) that accounts for the residual activity (usually less than 7%) observed in PGAM deficient patients. Patients affected by PGAM deficiency have a genetic defect involving the muscle PGAM-M.

7.5.3 Genetics

PGAM deficiency is due to mutations in *PGAM*. *PGAM* contains 3 exons. The most common mutation is a nonsense mutation in exon 1 (c.233G > A, p.Trp78*) described exclusively in African-American patients. The p.Trp78* mutation was found homozygote in 7 cases, in association with Glu89Ala and Gly93Glu in two cases and heterozygote in a patient with an asymptomatic hyper CKemia and only a partial reduction of PGAM activity. Two missense mutations (Arg90Trp, Arg10Gln) and a frameshift mutation (p.Gly178fs30*) have been described in four Italian patients. A nonsense mutation (Arg180*) was found homozygote in a patient of Pakistani ethnicity [15].

7.5.4 Diagnostic Tests

The forearm non ischemic test generally causes abnormally low, but not absent, venous lactate responses with normal increase of ammonia.

Definitive diagnosis requires biochemical documentation of PGAM enzyme defect in muscle and by sequence analysis of *PGAM*.

7.5.5 Treatment and Prognosis

No specific treatment or cure of the enzyme defect exists. Management primarily consists of avoiding strenuous exercise. The disease does not progress to severe disability.

7.6 Enolase Deficiency

7.6.1 Clinical Presentation

Enolase deficiency is a very rare inherited metabolic myopathy caused by enzymatic defect of distal glycolysis with only five patients described so far. All patients presented typical signs of myopathy with exercise intolerance, myalgia, cramps, and severe episodes of rhabdomyolysis followed by acute renal insufficiency with anuria in two of them [16, 17].

7.6.2 Metabolic Derangement

Enolase catalyses the interconversion of 2-phosphoglyceric acid (PGA) and phosphoenolpyruvate. In mammals, enolase is composed of three subunits (α, β and γ) encoded by three different genes; the subunits associate to form five different isozymes as homo or heterodimers (αα, αβ, αγ, ββ, and γγ). The α-subunit is expressed in many tissues, γ primarily in neurons whereas β is prevalent in muscle. Enolase deficiency is due to reduction of β-enolase activity.

7.6.3 Genetics

Enolase deficiency is an autosomal recessive disorder due to mutations in *ENO3*, encoding β-enolase. All reported mutations are missense mutations and change highly conserved amino acid residues [16, 17].

7.6.4 Diagnostic Tests

The forearm non ischemic test demonstrates no or a very mild increase of venous lactate with a normal increase of ammonia. Definitive diagnosis is made by enzymatic assay of enolase activity in muscle, which is less than 10% of normal values in the patients. Genetic diagnosis is made by sequencing *ENO3* or by NGS custom panel for genes associated with rhabdomyolysis [17].

7.6.5 Treatment and Prognosis

Management primarily consists of avoiding strenuous exercise. The disease does not progress to severe disability.

7.7 Lactate Dehydrogenase (LDH) Deficiency

7.7.1 Clinical Presentation

LDH deficiency has been reported in five Japanese families and two Caucasian families. Clinical features are characterized by impaired ability to sustain exercise due to muscle pain and stiffness during heavy exercise. Rhabdomyolysis can occur [2]. In addition to muscle symptoms, several types of skin lesions have been documented. It is speculated that LDH deficiency might affect keratinocyte metabolism via impaired ATP production in the anaerobic stage [18].

7.7.2 Metabolic Derangement

LDH converts pyruvate to lactate when oxygen is absent or in short supply. In muscles, LDH is involved in the breakdown of stored glycogen during anaerobic exercise. In human somatic tissues, five isozymes of tetrameric LDH are formed by a combination of the LDH-A (muscle) and LDH-B (heart) subunits encoded by two different genes (*LDH-A* and *LDH-B*). LDH-A4 is located primarily in skeletal muscle and LDH-B4 is located primarily in the myocardium. There is a third LDH-C subunit expressed only in mature testis and sperm. In patients with LDH-A deficiency, insufficient interconversion between pyruvate and lactate following exercise causes pyruvate and NADH accumulation and ATP reduction.

7.7.3 Genetics

To date, only patients with *LDH-A* mutations have been reported. The disease is inherited with an autosomal recessive mode. In the majority of patients, a common 20-bp deletion has been reported [19].

7.7.4 Diagnostic Tests

In patients with myoglobinuria, LDH deficiency can be suspected in the presence of high values of serum CK but extremely low values of LDH. Electrophoretic analysis of serum LDH isozymes demonstrates only one activity band of LDH-B4 and complete lack of the LDH-A subunit. The definitive diagnosis is made by enzymatic assay of LDH activity in muscle and by sequence analysis of *LDH-A*.

7.7.5 Treatment and Prognosis

Treatment primarily consists of avoiding strenuous exercise. The disease does not progress to severe disability.

7.8 Glycerol Kinase Deficiency (GKD)

7.8.1 Clinical Presentation

GKD (see also ▶ Chap. 35) is a rarely diagnosed X-linked recessive disorder, which occurs either together with congenital adrenal hypoplasia (CAH) and/or with Duchenne muscular dystrophy (DMD) or as an isolated form, either symptomatic or asymptomatic. More than 100 male patients have been reported so far, while only a few cases of symptomatic female carriers are described. The first descriptions noted that there are 3 clinically distinct forms of GKD: infantile, juvenile, and adult. The infantile form is associated with severe developmental delay. Those with the adult form have no symptoms and are often detected fortuitously. Juvenile patients are often symptomatic [20].

Complex GKD presents in infants and is caused in general by partial deletion of Xp21, which includes the genes of glycerol kinase, congenital adrenal hypoplasia, Duchenne muscular dystrophy and intellectual disability (*IL1RAPL*). Symptoms depend on the size of deletion and appear almost exclusively in males. Usually the first and most severe are due to the adrenal hypoplasia, which, if untreated, may be rapidly fatal. The symptoms of GKD also occur early in life, but they may be masked by the mineralocorticoid deficiency. Duchenne muscular dystrophy appears in childhood and is always symptomatic. Developmental impairment and intellectual disability occur often with complex GKD. The reasons for it are heterogeneous, but usually, there is a connection with the deletion of *DMD* or *IL1RAPL* genes [21].

Patients with isolated GKD are at risk from hypoglycaemia and hyperketonaemia. Juvenile patients present with episodic vomiting, metabolic acidosis, stupor, and coma. Patients with the adult form have no apparent clinical problems. They are usually identified through testing for hyperlipidemia when they are found to have pseudotriglyceridemia as a result of the increased glycerol in their blood being falsely identified as triglyceride [20].

7.8.2 Metabolic Derangement

GKD catalyses the phosphorylation of glycerol to glycerol phosphate and is essential for its further utilization as a gluconeogenetic precursor and for re-esterification of free fatty acids (FFA).

GKD is usually suspected in the laboratory from two findings: (a) high glycerol excretion in the urine found by gas chromatography-mass spectrometry (GC-MS) in the course of investigation of urinary organic acids and (b) falsely high serum triglyceride concentration as a result of the co-determination of glycerol in the method used to measure triglyceride. Further studies of glycerol metabolism are needed when urine and serum glycerol are elevated. In complex GKD, biological abnormalities may be due to the other genes involved, i.e. signs of adrenal failure with sodium loss in urine, hypoglycaemia and hyperkalaemia; and elevated CK. In isolated GKD, ketoacidotic attacks with or without hypoglycemia have been reported.

7.8.3 Genetics

GKD is caused by mutations in *GK* alone or as part of a contiguous gene deletion syndrome [20, 21]. The latter is due to microdeletions in the Xp21.2–p21.3 region often involving *GK*, *DAX1* (dosage-sensitive sex reversal locus and the adrenal hypoplasia congenita (AHC) locus on the X chromosome, gene 1) and/or part of the *DMD* (Duchenne muscular dystrophy) genes [20]. Point mutations or deletions in *DAX1* are responsible for AHC and hypogonadotropic hypogonadism. *DAX1* is closely linked to *GK* and a deletion often affects both loci [20]. Even though numerous aberrations in the *GK* gene have been reported, there does not seem to exist an apparent correlation between genotype and phenotype.

7.8.4 Diagnostic Tests

Determination of glycerol in urine is a rapid and simple way to diagnose GKD. Glycerol can also be detected by GC-MS when screening for organic acidurias. A few cases of hyperglycerolaemia not due to GKD have been reported and, if in doubt, a mutation in *GK* should be looked for [22]. This will then also make it possible

to identify female carriers and symptomless males as required for family counselling. Both specific and indirect assays of GK activity in fibroblasts are available and are adequate to detect complete GKD (enzyme activity <1–5% of reference) [23]. Provocation with a ketogenic diet, fasting or an exercise test are unhelpful in making a diagnosis and dangerous since they may cause severe complications [22].

7.8.5 Treatment and Prognosis

Treatment for isolated or complex GKD is based on symptomatic therapies and prevention. Both forms of GKD carry a risk of acute acidosis, hypoglycemic episodes associated with impaired consciousness and neurological symptoms, triggered by fasting and catabolism. Metabolic crises should be avoided by providing an adequate supply of fluid, calories and glucose during intercurrent illness [22]. In acute situations, IV glucose, in adequate amounts to block lipolysis and avoidance of lipid intake, is indicated. IV solutions containing glycerol or lipids (e.g some anaesthetic drugs) should be avoided. In complex GKD, adrenal insufficiency symptoms must be recognized and treated promptly with glucocorticoid and mineralocorticoid replacement therapy. Steroid replacement doses need to be adjusted in patients with CAH during intercurrent infections. Patients displaying Duchenne myopathy should be managed and supported accordingly. Patients are at risk of intellectual impairment and should be monitored to allow early intervention.

In isolated GKD, pseudo-hypertriglyceridemia responds poorly to conventional therapy which consequently should be avoided. A low-fat diet has reportedly been of value [24], alcohol should be avoided. In younger children, parents should be advised to give frequent complex carbohydrate-rich meals, and during infections and before intense physical activities, extra food or glucose. Since ketosis has been an early sign in many episodes, home testing for ketonuria can be useful. Most patients after puberty do not require dietary treatment but they should avoid situations that may cause extreme metabolic stress. In addition, there appears to be an increased risk of glucose intolerance and for which patients need to be monitored [22]. Special precautions must be taken during anaesthesia.

Prognosis is related to the early recognition of the disease, especially in complex GKD and to prompt treatment of associated adrenal insufficiency [24]. The frequency of metabolic decompensation generally declines after puberty [22]. With appropriate management the long-term prognosis for isolated GKD is very good [22].

7.9 Ribose-5-Phosphate Isomerase (RPI) Deficiency

7.9.1 Clinical Presentation

RPI deficiency has now been diagnosed in 4 male patients [25–28], it is a rare leukoencephalopathy and was first reported in 2004. Patients present neonatally or in infancy with hypotonia and/or developmental and speech delay. Seizures, spasticity, ataxia, neuroregression with gradual loss of vision and speech have been observed in 3 of the 4 patients [27]. The youngest patient described by Brooks et al., presenting with neonatal onset leukoencephalopathy and psychomotor delays did not show signs of regression or epilepsy at the age of 6 years [28]. One patient displayed polyneuropathy [29].

Ophthalmological examination revealed retinitis pigmentosa in two patients and optic atrophy in one. Progressive and multifocal cerebral white matter abnormalities on magnetic resonance imaging (MRI) of the brain were seen in all individuals. In two patients who had magnetic resonance spectroscopy (MRS) of the brain, highly elevated peaks in the 3.5–4.0 ppm region (the sugar and polyol region of the spectrum) were detected and were identified as representing ribitol and d-arabitol. EEG showed progressive background slowing and increased epileptic activity in three patients.

7.9.2 Metabolic Derangement

RPI is an enzyme of the reversible part of the PPP. In theory, this defect leads to a decreased capacity to interconvert ribulose-5-phosphate and ribose-5-phosphate and results in the accumulation of sugars and polyols: ribose and ribitol from ribose-5-phosphate or ribulose-5-phosphate, and xylulose and arabitol from ribulose-5-phosphate via xylulose-5-phosphate. The concentrations of ribitol and arabitol displayed a steep descending brain/CSF/plasma gradient.

7.9.3 Genetics

Human *RPIA* consists of a monomer of 311 amino acids. The mode of inheritance is autosomal recessive. Mutations detected in *RPIA* so far include missense mutations, a small deletion and a splice site mutation, suggesting loss of function as the underlying mechanism.

7.9.4 Diagnostic Tests

The diagnosis of RPI deficiency can be made by the analysis of sugars and polyols in urine, plasma or CSF. Urinary ribitol and arabitol, are more than 10 times elevated. Extremely high concentrations of these pentitols are also found in CSF. The myo-inositol concentration in CSF was decreased in one patient. In vivo brain MRS reveals elevated peaks in the 3.5- to 4.0-ppm region, corresponding to arabitol and ribitol and could be further used to suspect diagnosis in patients presenting with unexplained leukoencephalopathy.

The diagnosis can be confirmed by an enzyme assay in fibroblasts or lymphoblasts, and by sequence analysis of *RPIA*.

7.9.5 Treatment and Prognosis

Therapeutic options for RPI deficiency are not available. Supportive therapy and rehabilitation for the neurological complications are indicated. The prognosis is unclear, given that only four patients have been described so far. RPI deficiency seems to be a (slowly) progressive disease with loss of motor and cognitive milestones and progression of cerebral white matter abnormalities.

7.10 Transaldolase (TALDO) Deficiency

7.10.1 Clinical Presentation

TALDO deficiency has now been diagnosed in more than 40 patients from more than 30 different families [30, 31].

Wide phenotypic variability has been reported [30]. Most patients display the first symptoms of the disease in the neonatal or antenatal period while others may have milder or no symptoms at birth and may be diagnosed only later in life due to liver manifestations.

Among the early onset symptoms intrauterine growth retardation, oligohydramnios and hydrops fetalis have been described [25]. Neonates present with hepatomegaly and splenomegaly with portal hypertension, coagulopathy, abnormal liver function tests, cholestatic jaundice and elevated liver enzymes. Anaemia and thrombocytopenia are almost always present in the early onset forms of the disease and most showed dysmorphic features (antimongoloid slant, low-set ears, hypertrichosis and skin abnormalities (cutis laxa, spider telangiectasies and multiple haemangiomas), neonatal oedema and congenital heart defects (septal defects, cardiomyopathy, tetralogy of Fallot).

Hepatic fibrosis and cirrhosis are the histological liver hallmarks later in life and in late-onset patients. Early onset hepatocellular carcinoma was present as the only symptom of TALDO deficiency in one of the late onset patients [32]. One asymptomatic patient of 8 years old was diagnosed after molecular testing because of an affected sibling [32]. Given the variability in phenotype and outcome, TALDO deficiency could be considered in adults with cirrhosis of unknown aetiology.

Renal manifestations (tubulopathy, renal failure, nephrocalcinosis) and endocrine disorders have frequently been reported, leading to transient hypoglycaemia, and testicular or ovarian insufficiency leading to cryptorchidism, clitoris enlargement, secondary amenorrhea and bone development abnormalities with rickets.

Mild transient hypotonia was described in several patients, but mental and motor development were normal in the majority in whom assessment was possible (ten patients died before the age of 6 months). In contrast to those with RPI deficiency, brain MRI and MRS did not reveal abnormalities in patients with TALDO deficiency.

7.10.2 Metabolic Derangement

TALDO is located in the reversible part of the PPP and recycles pentose phosphates into glycolytic intermediates in concerted action with transketolase. Its deficiency results in the accumulation of polyols (erythritol, arabitol, ribitol, sedoheptitol and perseitol), erythronic acid and seven-carbon sugars derived from the pathway intermediates [30].

7.10.3 Genetics

TALDO is encoded by *TALDO1*. The mode of inheritance is autosomal recessive. Mutations detected in *TALDO1* include missense mutations, in frame deletions, frameshift mutations and a duplication. Most patients are homozygous for a mutation in *TALDO1* which is in line with the high frequency of consanguinity [30]. A possible dominant effect of *TALDO1* haplo-insufficiency has been described which possibly predisposes to acetaminophen induced liver injury [31]. This study also showed a high prevalence of *TALDO1* variants.

7.10.4 Diagnostic Tests

Diagnosis of TALDO deficiency is achieved by detecting elevated urine concentrations of the seven-carbon sugars sedoheptulose, mannoheptulose, sedoheptitol, perseitol and sedoheptulose-7-phosphate and the polyols erythritol, arabitol and ribitol [30]. Elevations are most

striking in the neonatal period and are more subtle in older patients. In plasma and CSF, there are only minor elevations of polyols or none at all. Elevated concentrations of sedoheptulose-7-phosphate can be detected in blood spots, suggesting that newborn screening may be feasible [25]. MRS is not informative, and the gold standard of diagnosis is measurement of TALDO activity in fibroblasts, lymphoblasts, erythrocytes or a liver biopsy and sequence analysis of *TALDO1*.

Prenatal diagnosis is possible by sequence analysis of *TALDO1* in chorionic villi and amniocytes. In amniotic fluid from an affected fetus, increased concentrations of sedoheptulose and ribitol were detected [25]. Prenatal diagnosis may therefore also be possible by measuring sedoheptulose and ribitol in amniotic fluid supernatant.

7.10.5 Treatment and Prognosis

For most patients the outcome seems to be correlated to the severity of the liver impairment. Nine patients died of acute liver failure at the onset of the disease or of chronic liver failure-related complications including bleeding and respiratory distress. However, a few patients with an initially severe liver disease associated with fibrosis and cirrhosis are currently stable. In our current knowledge of the disease, patients who present at birth with liver failure and who do not recover before the first month of life, and those who have deteriorating liver function within the first 6 months of life, have a high mortality close to 100%. These patients should be carefully monitored and considered promptly for liver transplantation. However, in one patient with neonatal liver failure, all liver symptoms, including hepatomegaly resolved by age 15 ([25], patient 5).

There is no specific treatment for TALDO deficiency. In general, patients should receive standard symptomatic care, e.g., optimal nutrition and vitamin supplementation for the presenting symptoms (liver and renal) and in severe cases transfusion support and monitoring for bleeding and thrombocytopenia. Liver manifestations may be treated symptomatically or require liver transplantation. Two patients have been reported in the literature to have received successfully liver transplants at age 1 year [33] and 16 months [32] before the diagnosis was known. One other patient age 6 months [30] died in the course of liver transplantation.

Hormone disturbances are in general transient and can be addressed by specific replacement therapy.

Emerging pathophysiological insights into TALDO deficiency, namely the role of oxidative stress in liver pathology, have been described in the *Taldo1*−/− mouse model and seem to respond well to common antioxidant therapies such as N-acetylcysteine (NAC) [34]. A single specific experimental therapy in a TALDO patient has been reported using NAC, over a 6-month period, which was well tolerated and was associated with a sustained normalization of alpha fetoprotein (AFP) levels and stable clinical course [35]. It has recently been reported that heterozygous TALDO deficiency might predispose to acetominophen induced liver injury, which is preventable by treatment with NAC [31].

7.11 Transketolase (TKT) Deficiency

7.11.1 Clinical Presentation

TKT deficiency has been detected through whole exome sequencing in five affected individuals from three unrelated families, all of whom have proportional short stature and developmental delay [36, 37]. Congenital heart disease (including atrium septal defects, ventricular septal defects, patent foramen ovale and patent ductus arteriosus) was present in 4 of the 5 patients. Their age at diagnosis varied between 5 and 24 years old. In the older patients, bilateral cataracts and psychiatric symptoms (ADHD, obsessive compulsive disorder, stereotypic behaviour and self-injury) were present. In more than one patient hypotonia and ocular abnormalities (including juvenile bilateral cataract and inflammation in the eye) were described. Three patients also had facial dysmorphism.

7.11.2 Metabolic Derangement

Transketolase is a reversible, thiamine-dependent enzyme in the pentose phosphate pathway (PPP). It catalyses the transfer of a two-carbon unit from xylulose-5-phosphate to one of two substrates: ribose-5-phosphate producing glyceraldehyde- 3-phosphate and sedoheptulose-7-phosphate or erythrose- 4-phosphate producing glyceraldehyde-3-phosphate and fructose-6-phosphate. Its deficiency results in the accumulation of polyols (erythritol, arabitol and ribitol) in urine and plasma and accumulation of pent(ul)ose-5-phosphates in urine.

7.11.3 Genetics

Human *TKT* consists of 16 exons. The mode of inheritance is autosomal recessive. In two families from Ashekenazi heritage the c.769_770delins18 mutation was detected [37]. TKT deficiency may be more common in the Ashkenazi Jewish community.

7.11.4 Diagnostic Tests

Diagnosis of TKT deficiency is achieved by detecting elevated urine or plasma concentrations of erythritol, arabitol and ribitol. In urine also elevation of ribose-5-phosphate and ribulose+xylulose-5-phosphate can be detected. Confirmation is done by enzymatic analysis in fibroblasts, lymphoblasts or erythrocytes or DNA analysis.

7.11.5 Treatment and Prognosis

Therapeutic options for TKT deficiency have not yet been identified. Thiamine or benfotiamine (a synthetic derivative of thiamine) might in theory, be of benefit in patients with some residual TKT activity and should be investigated. Due to the low number of patients the prognosis in TKT deficiency is unclear.

7.12 Sedoheptulokinase (SHPK) Deficiency

7.12.1 Clinical Presentation

SHPK deficiency was first described as part of a large 57-kb-deletion in infantile nephropathic cystinosis [25]. This deletion extends from exon 10 of *CTNS*, upstream through *CARKL/SHPK*, to intron 2 of *TRPV1*. In a previous small study, no difference in the clinical phenotype between patients with other mutations causing the severe infantile nephropathic type or patients with the deletion could be found, suggesting that isolated SHPK deficiency would result in a benign or mild phenotype. In 2015, two patients were identified with isolated SHPK deficiency [38]. Clinical presentation in patient 1 showed neonatal cholestasis, hypoglycaemia, anaemia and dysmorphism, while patient 2 presented with congenital arthrogryposis multiplex, multiple contractures and dysmorphisms. Since both patients presented very differently and without a clear clinical overlap with cystinosis patients (caused by the 57-kb deletion), the biochemical defect of SHPK deficiency seems to be either unrelated to the clinical phenotypes or might have a broad phenotypic presentation, which is dependent on external factors and/or the genetic backgrounds of the individuals. Three additional patients have been detected with mild phenotypes and one even asymptomatic, further suggesting that SHPK deficiency is a benign disorder (M.Wamelink, personal communication).

7.12.2 Metabolic Derangement

Strongly elevated excretion of sedoheptulose and erythritol were detected in the isolated SHPK deficient patients and the cystinosis patients with the homozygous 57-kb deletion. Kardon et al. indicated that the accumulation of erythritol is likely derived from sedoheptulose through convertion to sedoheptulose-1-phosphate by fructokinase and to erythrose and dihydroxyacetone-phosphate by aldolase B. Erythrose would then finally be reduced to erythritol [39].

7.12.3 Genetics

The mode of inheritance is autosomal recessive. Mutations detected in *SHPK* include stop mutations and a large 57-kb deletion.

7.12.4 Diagnostic Tests

Diagnosis of SHPK deficiency is achieved by detecting elevated urine concentrations of sedoheptulose and erythritol. With low-normal excretion of sedoheptulose-7-phosphate [25]. Confirmation is done by enzymatic analysis in fibroblasts or DNA analysis.

7.12.5 Treatment and Prognosis

Therapeutic options for SHPK deficiency have not yet been identified. SHPK deficiency is likely a benign disorder.

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Disorders of Glucose and Monocarboxylate Transporters

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Contents

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Glucose Transporters

D-Glucose and other monosaccharides are hydrophilic substances that cannot easily cross the lipophilic bilayer of the cell membrane. Since these carbohydrates are most important for the energy supply of essentially all cell types, specific transport mechanisms have had to evolve: proteins embedded in the cell membrane function as hydrophilic pores that allow cellular uptake and release, transcellular transport, and sensing (generation of a membrane potential without transport) of these sugars.

Glucose transporter proteins can be divided into several groups. Sodium-dependent glucose transporters (SGLTs, symporter systems, secondary 'active' transporters encoded by members of the *SLC5* family) typically couple sugar transport to the electrochemical gradient of sodium and hence can transport glucose against its own concentration gradient. Only recently, a first metabolic disorder due to the defect of a proton-associated sugar transporter (PAST, encoded by the *SLC45* family) has been described. Facilitative glucose transporters (GLUTs, uniporter systems, 'passive' transporters encoded by members of the *SLC2* family) can transport monosaccharides only along an existing gradient (Fig. 8.1).

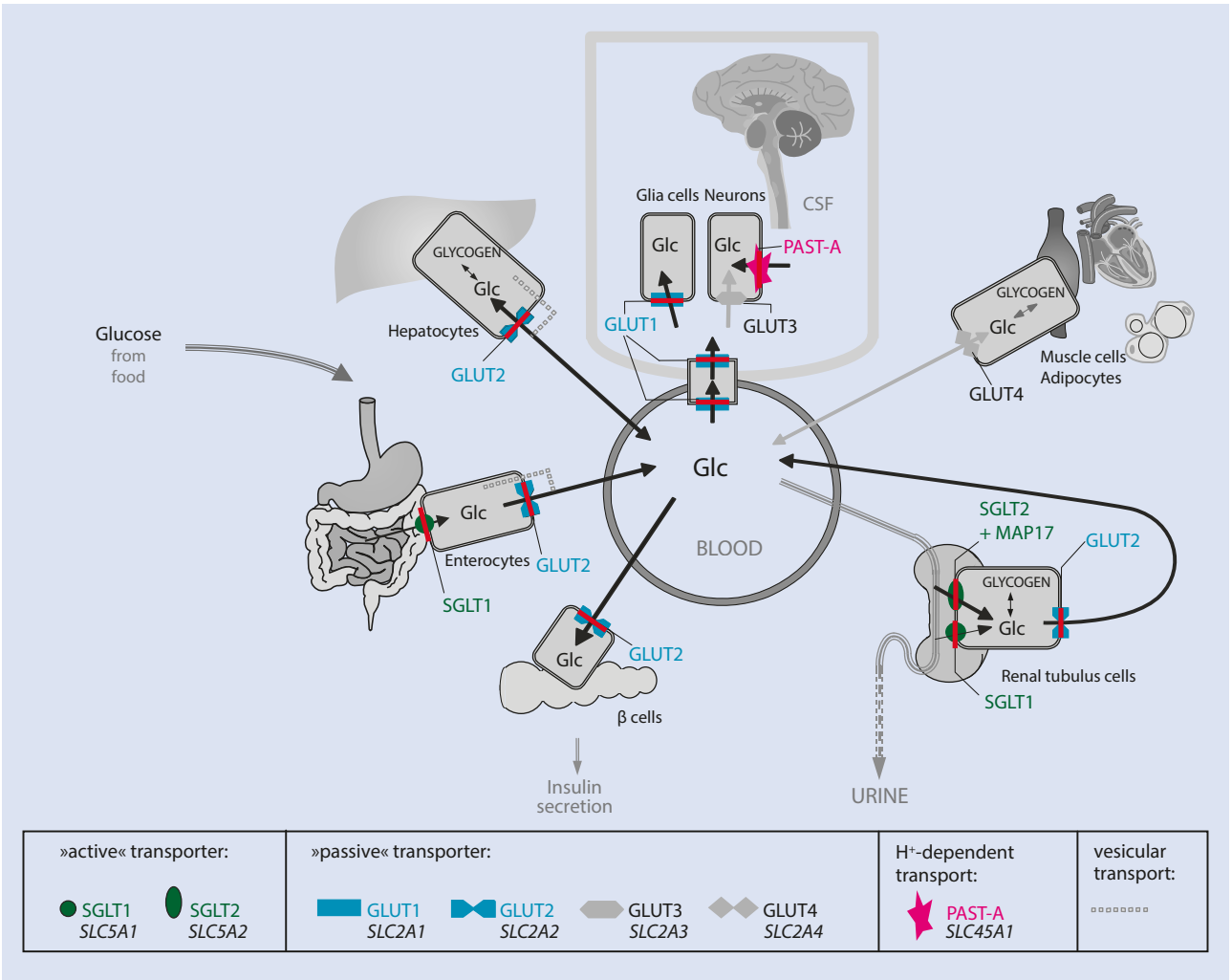

Fig. 8.1 Major routes of glucose transport. Transport across cell membranes is depicted by arrows, and specific transporters by symbols: round for sodium-dependent, secondary 'active' transporters (SGLTs, encoded by members of the *SLC5* family), and angular for facilitative, 'passive' transporters (GLUTs, encoded by members of the *SLC2* family), the star-shaped symbol represents the protein-associated sugar transporter-A (PAST-A, encoded by a member of the *SLC45* family) for which a defect was only recently described. Known defects are shown by coloured (bright blue, green and pink, respectively) instead of grey transporter symbols. Vesicular transport is depicted by small bubbles. *For details see text.* Glc glucose, CSF cerebrospinal fluid

Introduction

To date, five congenital defects of glucose transporters are known (Fig. 8.1). The clinical picture depends on tissue-specific expression and substrate specificity of the affected transporter. SGLT1 deficiency causes intestinal glucose-galactose malabsorption, a condition that presents with severe osmotic diarrhoea and dehydration soon after birth. In renal glucosuria, a harmless renal transport defect characterised by glucosuria at normal blood glucose concentrations as well as the absence of any other signs of renal tubular dysfunction, SGLT2, or very rarely, a membrane-associated protein (MAP17) is affected. In GLUT1 deficiency syndrome, clinical symptoms such as microcephaly, epileptic encephalopathy, and paroxysmal movement disorders are caused by impaired glucose transport at the blood-brain barrier and into astrocytes. A defect in a proton-associated sugar transporter (PAST-A) of neurons can also result in neurologic but also in psychiatric symptoms. Fanconi-Bickel syndrome is the result of a deficiency of GLUT2, an important glucose and galactose carrier of hepatocytes, renal tubular and pancreatic β-cells. Patients typically present with a combination of increased hepatic glycogen storage and generalised renal tubular dysfunction with glucosuria as a pronounced feature.

Monocarboxylate transporters (MCTs) can be a therapeutic target in cases when glucose as a major fuel of cells cannot be taken up (as in GLUT1 deficiency) or cannot be completely metabolised (as in PDH deficiency). MCTs may allow uptake of alternative substrates (lactate, ketones) in such disorders but MCTs themselves may be affected in some metabolic diseases. Examples are MCT1 deficiency (as a cause of severe ketoacidotic crises), MCT1 overexpression in β-cells (the metabolic basis of exercise-induced hyperinsulinism), the Allan-Herndon-Dudley syndrome, a rare X-linked inherited disorder of brain development due to a defect of MCT8 (a monocarboxylate transporter involved in cellular thyroid hormone uptake), and MCT12 deficiency, a rare cause of familial cataracts due to impaired creatine transport.

8.1 Congenital Glucose/Galactose Malabsorption (SGLT1 Deficiency)

8.1.1 Clinical Presentation

Typically, children with congenital glucose-galactose malabsorption (GGM) caused by SGLT1 deficiency present with bloating and profuse watery diarrhoea within days after a normal birth and a normal pregnancy (with no polyhydramnios). Stools are very loose and may be mistaken for urine. Both breast- and bottle-fed infants are affected, but if newborns are given tea sweetened with glucose or polymers of glucose then symptoms may start even before milk feeds. As a result of the diarrhoea, patients develop severe hypertonic dehydration, often with fever, which may be misinterpreted as a sign of a gastrointestinal infection. If the correct diagnosis is missed and glucose and galactose are not eliminated from the diet and if parenteral fluid administration is not available patients die from hypovolaemic shock. In typical cases, the diagnosis is considered after repeated frustrating attempts to switch from parenteral fluids to oral feeds [1]. The finding of an acidic stool pH and the detection of reducing substances in the stool are clues to the diagnosis, and most patients have mild intermittent glucosuria [2]. Chronic dehydration might be responsible for the nephrolithiasis and nephrocalcinosis that develop in a number of cases [3].

8.1.2 Metabolic Derangement

Congenital deficiency of SGLT1 is the basic defect in this disorder [4] which demonstrates the pivotal role of this protein in intestinal transepithelial transport of glucose and galactose. SGLT1 is a high-affinity, low-capacity sodium-dependent transporter of the two monosaccharides at the brush border of enterocytes. At the basolateral membrane, glucose transport is mediated by facilitative diffusion and/or by a membrane vesicle-associated transport process [5]. Fructose is not a substrate for SGLT1 and is considered to be mainly absorbed by GLUT5 (although genetic defects of this transporter have never been detected in individuals with intestinal fructose malabsorption; ▶ Chap. 15). The fact that patients with glucose-galactose malabsorption show mild glucosuria points to an additional physiological role of this transporter in renal glucose reabsorption [2].

8.1.3 Genetics

In most populations, GGM is a relatively rare autosomal recessive disorder. Its exact prevalence is unknown, but the fact that approximately 65% of reported patients are homozygous (in contrast to compound heterozygosity in the remainder) confirms its rarity [1]. A high carrier rate for pathogenic *SLC5A1* variants (1:20) was reported for the Amish population in the United States [6]. To date, approximately 70 different genetic variants have been reported [4, 6], scattered all over the gene; the existence of a mutational hot spot is controversial.

8.1.4 Diagnostic Tests

Owing to its life-threatening character, GGM must be suspected clinically and treatment started before the diagnosis can be confirmed. The clinical stabilisation of patients on parenteral nutrition with no foods given orally or those on a fructose-based formula, are in favour of the diagnosis. Oral monosaccharide tolerance tests (measuring stool pH, reducing substances and blood glucose) combined with a hydrogen breath test can be performed, but some of the test parameters may be unreliable owing to antibiotics, which are frequently given to sick neonates. In these tests, glucose and galactose, but not fructose, may induce severe clinical symptoms in affected infants. Glucose and galactose uptake studies on intestinal biopsies are possible but invasive. Molecular genetic studies are recommended, particularly if prenatal diagnosis is likely to be requested for in a future pregnancy.

8.1.5 Treatment and Prognosis

Whenever GGM is considered, glucose and galactose should be omitted from the diet. A formula containing fructose as the only carbohydrate is well tolerated by infants with GGM. Such a formula is easily prepared by addition of this monosaccharide to commercially available carbohydrate-free dietary products. The preparation of the diet becomes more complicated when additional foods are introduced. It has repeatedly been observed that glucose tolerance improves with age by an, as yet, unknown mechanism [7]. To date, there are no long-term studies on the outcome of patients with GGM and it is not clear how strict the adherence to the glucose- and galactose-restricted diet must be for the patients not to have an increased risk of nephrolithiasis. Similarly, there is no information on long-term sequelae of a high-fructose diet on liver function (► Chap. 15).

8.2 Renal Glucosuria (SGLT2 Deficiency)

8.2.1 Clinical Presentation

Most individuals with renal glucosuria, a congenital defect of SGLT2, are detected during a routine urine examination. Only a small number present with polyuria and/or enuresis. Thus, renal glucosuria is an important differential diagnosis when diabetes mellitus is considered, but is easily excluded by the presence of normal blood glucose concentrations. Renal glucosuria is an isolated defect of tubular glucose reabsorption at the proximal tubules and, in general, does not affect other glomerular or tubular kidney functions [8].

Mild renal glucosuria (0.4–5 g/1.73 m^2/d) is relatively common. Individuals with a higher glucose excretion or a virtual absence of tubular glucose reabsorption (termed renal glucosuria type 0) are extremely rare.

8.2.2 Metabolic Derangement

In most cases renal glucosuria is a non-disease. Only individuals with massive glucose excretion may have a propensity to hypovolaemia and (ketotic) hypoglycaemia (with an activation of counter regulatory hormones [9]); they can present with a delay of somatic maturation [10]. In patients with massive glucosuria mild secondary hyperaminoaciduria has been described [11].

In recent years SGLT2 has become an important therapeutic target. SGLT2 inhibitors such as empagliflozin or dapagliflozin are used in type-2 diabetes (to enhance elimination of glucose and stabilise glucose homeostasis) and in glycogen storage disease type-1b (to eliminate accumulating 1,5-anhydroglucocitol which is responsible for neutropenia ► Chap. 5) [12].

8.2.3 Genetics

Most individuals with renal glucosuria have been found to carry genetic variants of *SLC5A2* [13]. Its product is a low-affinity carrier that transports glucose but not galactose. Homozygosity or compound heterozygosity for *SLC5A2* variants results in the severe types of renal glucosuria whereas heterozygosity is associated with mild glucose excretion, albeit not in all of the carriers [13]. To date, approximately 100 functionally relevant variants have been described which are scattered all over *SLC5A2* [13]. It has been shown that a small membrane-associated protein, MAP17, is necessary for proper SGLT2 function. Its deficiency is another, very rare cause of renal glucosuria and homozygosity for a functionally relevant variant within the MAP17 gene (*PDZK1IP1*) was detected in a single case [14].

8.2.4 Diagnostic Tests

Diagnosis is straight forward in patients with glucosuria and normoglycaemia who do not show any other evidence of renal tubular dysfunction.

8.2.5 Treatment and Prognosis

For most cases dietary treatment is not indicated, and the prognosis, even in individuals with a complete loss of renal tubular glucose reabsorption, is excellent [11].

8.3 Glucose Transporter-1 Deficiency Syndrome (GLUT1DS)

8.3.1 Clinical Presentation

GLUT1 deficiency syndrome (GLUT1DS) in its classic form presents as an early-onset epileptic encephalopathy with three cardinal features: severe epilepsy, a complex movement disorder, and developmental delay [15].

Following an uneventful fetal and neonatal period (persumably because of immature tight junctions of endothelial cells at the blood brain barrier allowing paracellular glucose transport at that stage of development), distinctive paroxysmal eye-head movements or an isolated tremor may be the initial manifestation of GLUT1DS [16]. Seizures occur within the first year of life, are aggravated by fasting and often present as cyanotic spells or peculiar eye movements. In later childhood, they can be of various types and frequency, often refractory to anticonvulsants. GLUT1DS has been reported to account for up to 10% of cases with early-onset absence epilepsy [17] and for approximately 5% of patients with myoclonic astatic epilepsy [18].

With increasing age, a complex motor disorder becomes apparent, including dystonia, ataxia, chorea, and spasticity. Patients may develop an ataxic-spastic gait, action limb dystonia, mild chorea, and cerebellar action or dystonic tremor [19]. In addition, patients often develop non-epileptic paroxysmal events with episodes of ataxia, weakness, Parkinsonism, alternating hemiplegia, and non-kinesogenic dyskinesias. These episodes may be triggered by poor dietary intake [15].

Global developmental delay becomes apparent in almost all patients with GLUT1DS. Impaired language development is often the most prominent feature. Severe cases may develop secondary microcephaly [15, 19].

Several GLUT1DS variants have been recognised in recent years. Such patients present with only one or two of the cardinal features as described above, for instance isolated early-onset absence epilepsy or an isolated movement disorder without epilepsy. Additional features may be myoclonus and dyspraxia. Manifestations with only minimal symptoms in adults have also been described [15]. Paroxysmal exertion-induced dystonia (PED) has been found to be allelic to classic GLUT1DS. PED is characterised by onset beyond childhood, a normal head circumference, a normal interictal neurologic examination, and a less prominent decrease of CSF glucose concentration when compared with classic GLUT1DS [15, 20].

8.3.2 Metabolic Derangement

GLUT1 is a membrane-spanning, glycosylated protein that facilitates transport of glucose, the principal fuel for brain energy metabolism, across the blood-brain barrier. Impaired GLUT1 function results in a diminished CSF glucose concentration (hypoglycorrhachia). Hence, GLUT1DS impairs glucose supply to both neurons and glia cells, leading to acute and chronic clinical symptoms, deceleration of brain growth, and cerebral and retinal microvasculature defects [21, 22]. GLUT1 is also highly expressed in erythrocytes where 5% of the membrane protein is GLUT1. This explains why an exercise induced energy deficit can be accompanied by haemolytic anaemia which may result from alterations in intracellular electrolytes caused by a cation leak through mutant GLUT1 [20].

8.3.3 Genetics

Approximately 80% of patients are heterozygous for functionally relevant variants within *SLC2A1*. Both autosomal dominant and autosomal recessive inheritance have been described [23–25]. Variants are mostly de novo, of various character (missense, nonsense, and splice-site, haploinsufficiency, compound heterozygosity, and paternal mosaicism), and randomly distributed [15, 25]. Genetic variants have been studied on the molecular level [26] and often correlates with phenotypic severity [19]. In approx. 10–15% of cases with a GLUT1DS-like presentation no *SLC2A1* variant can be detected, even with additional use of MLPA analysis to detect copy number variations [27]. Some may be explained by impaired transcriptional regulation of *SLC2A1* as suggested for defects of *PURA*, the gene for the purine-rich element-binding protein A [28].

8.3.4 Diagnostic Tests

GLUT1DS illustrates the importance of CSF evaluation in children with undiagnosed epilepsy and/or movement disorders. The diagnostic lumbar puncture should be done in a metabolic steady state, e.g., following a 4–6 hour fast. GLUT1DS should be suspected in any child with a CSF glucose concentration below 2.5 mmol/l (normal >3.3 mmol/l). Values are age-specific and may vary considerably (range 0.9–2.9 mmol/l) [29]. A CSF to blood glucose ratio, which normally is >0.6, should be obtained in a non-ictal, metabolic steady state. A ratio

of <0.5 (range 0.19–0.52) in the absence of hypoglycaemia or a CNS infection is diagnostic. Typically, CSF cell count, protein and lactate concentrations are normal [19, 29].

Other routine laboratory analyses are unremarkable and interictal EEGs are often normal. If abnormal, an improvement in the EEG with glucose intake may be of diagnostic value. Ictal EEGs may show focal slowing or epileptiform discharges in infants and a generalised 2.5- to 4-Hz spike-wave pattern in older children. Neuroimaging is generally uninformative but PET studies may demonstrate a diminished cortical glucose uptake [30]. Recently, a blood test detecting reduced red blood cell GLUT1 expression by flow cytometry analysis has been reported to be of diagnostic value [31]. Western blot analysis and glucose uptake into erythrocytes may confirm GLUT1DS but are not commercially available [32].

8.3.5 Treatment and Prognosis

In the fasted state, ketone bodies provide an alternative fuel to the brain. This metabolic situation can be mimicked by different types of a high-fat, low-carbohydrate ('ketogenic') dietary treatment (KDT) (► Chap. 13). These diets may restore brain energy metabolism in GLUT1DS since ketone body transport at the blood brain barrier is mediated by MCT1 (► Sect. 8.7) and not dependent on GLUT1. Classic ketogenic diets (3:1 and 4:1 ratios of fat and non-fat sources [in grams], respectively) and the modified Atkins diet may effectively control seizures and improve movement disorders and development [33]. Exogenous ketone salts and ketoesters may serve as a supplemental fuel for the brain without dietary restriction. Both can be administered orally and result in ketone plasma concentrations in a similar range as observed in a KDT. However, oral ketone salts are expensive, have an unpleasant taste and may cause sodium overload. In contrast to KDT experience is limited. Triheptanoin is another artificial energy source that may provide ketones as well as anaplerotic substances for the citric cycle (► Chap. 12). Despite encouraging initial reports, clinical trials of triheptanoin (UX007) for the treatment of seizures and movement disorders in GLUT1DS did not show beneficial effects (Clinical ► Trials.gov Identifier: NCT01993186; NCT02960217). In any type of dietary modification, multivitamin and calcium supplements are required [15]. Treatment should be maintained throughout childhood and into adolescence. In adults, the indication for KDT is less clear, but also in this age group the modified Atkins diet is increasingly recommended in GLUT1DS.

Substances known to inhibit GLUT1, such as anticonvulsive drugs (phenobarbital, chloralhydrate, diazepam), methylxanthines (theophyllin, caffeine), alcohol, and green tea, should be avoided. If an antiepileptic medication is required, one has to be aware that no data on the specific effectiveness of anti-epileptic drugs in this condition is available. Valproate interferes with GLUT1 function in vitro, but may be used in GLUT1DS [34]. Ethosuximide should be considered in early-onset absence epilepsy.

GLUT1DS treated early with KDT has a favourable prognosis: patients continue to make progress, acquire speech and mobility, and the disease generally stabilises after puberty. However, with increasing age seizures typically decrease but impaired speech and movement abnormalities, in particular paroxysmal dystonia, become the predominant problems, often poorly controlled by KDT. In some individuals seizures and a variable degree of impairment may persist despite adequate treatment, and a sheltered environment is often required. To date, the first patients diagnosed with GLUT1DS have now reached adulthood but experience is still limited [35].

Very recent therapeutic approaches aim at restoring the GLUT1 protein content and function via upregulation of the normal *SLC2A1* allele using small molecule or gene transfer strategies [15, 36].

8.4 Intellectual Developmental Disorder with Neuropsychiatric Features (PAST-A Deficiency)

8.4.1 Clinical Presentation

Another defect of a cerebral glucose transporter has only recently been described. Patients with a dysfunctional proton-associated sugar transporter (PAST-A) were found to suffer from neurodevelopmental disability associated with epilepsy and variable neuropsychiatric features. Facial dysmorphism was consistently found in the few published cases. Microcephaly is not part of the syndromic presentation. Stereotyped hand movements were repeatedly observed and a wide range of behavioural problems including anxiety and autism were described [37].

8.4.2 Metabolic Derangement

Since the 1980s, GLUT3 has been considered the brain-type glucose transporter, one that unlike GLUT1 is expressed in neuronal cells and not in glia cells, but no

congenital defect has ever been reported. PAST-A is a glucose transporter expressed in neurons that links glucose transport to the gradient of protons allowing regulatory effects, e.g., the pH dependency of glucose transport with a decrease at low blood pH value [38]. Impaired uptake of glucose into neurons by a dysfunctional transporter results in the reported symptoms.

8.4.3 Genetics

PAST-A is encoded by *SLC45A1*, a member of the SLC45 family of proton/glucose symporters [38]. All patients described to date were homozygous for missense variants which could mean that complete loss of function of this transporter is not compatible with life. The two *SLC45A1* variants described, c.526C > T [p.Arg176Trp] and c.629C > T [p.Ala210Val], were found in functional expression studies to have a reduced but not abolished transport activity [37].

8.4.4 Diagnostic Tests

Laboratory tests are not helpful, both plasma and CSF glucose are within normal limits. MRI scans have been reported normal even in the third decade of life. Whether PET studies are helpful has not been elucidated.

8.4.5 Treatment and Prognosis

A ketogenic diet did not control seizure activity when used in the two children of the first publication. The principle behind warrants further evaluation of the ketogenic diet or a modified Atkins diet in such patients.

8.5 Fanconi-Bickel Syndrome (GLUT2 Deficiency)

8.5.1 Clinical Presentation

Patients with Fanconi-Bickel syndrome (FBS), which is caused by GLUT2 deficiency, typically present at 3–10 months of age with a combination of hepatomegaly, a Fanconi-type nephropathy with severe glucosuria, a propensity to hypoglycaemia in the fasted state, and glucose and galactose intolerance in the fed state [39]. A few patients have come to attention because of neonatal diabetes mellitus [40], others owing to the finding of hypergalactosaemia on newborn screening [41], and occasionally because of the identification of early cataracts [42]. Initially, hepatomegaly, which is caused by massive accumulation of glycogen, may not be present, and non-specific symptoms such as fever, vomiting, chronic diarrhoea, renal tubular acidosis, and failure to thrive may predominate. With increasing age, the clinical presentation with a protuberant abdomen, moon-shaped face, and short stature becomes increasingly similar to that of patients with other hepatic glycogen storage diseases (► Chap. 5). The kidneys also accumulate glycogen and their enlargement can be detected by ultrasound. Hypophosphataemic rickets is the major manifestation of tubular dysfunction, resulting in joint swelling, bowing of legs and pathological fractures. Patients with FBS have entirely normal mental development but growth and puberty are severely retarded [39]. GLUT2 deficiency has been reported in patients with very mild clinical signs and symptoms, including cases presenting with isolated glucosuria [43].

8.5.2 Metabolic Derangement

Fanconi-Bickel syndrome is caused by congenital deficiency or variably impaired function of GLUT2, a high-K_m monosaccharide carrier that can transport both glucose and galactose [44]. This facilitative glucose carrier is expressed in hepatocytes and at the basolateral membrane of reabsorbing cells of the proximal tubule and of enterocytes. GLUT2 has been detected within the cell membrane of pancreatic β-cells, however, in humans the major facilitative transporter in β-cells has been shown to be GLUT1 and the rate-limiting step in sensing glucose concentration is glucokinase (► Chap. 6).

Intestinal uptake of glucose and galactose appear unimpaired in FBS; this has been explained by additional transport systems for glucose, SGLT1 in the apical membrane and a membrane vesicle-associated pathway at the basolateral membrane [5]. Neverless, patients may show intestinal symptoms due to very high fluid intake to compensate for renal losses. Postprandial hyperglycaemia and hypergalactosaemia are caused by impaired hepatic uptake of the two sugars. To what extent hyperglycaemia is further exaggerated by a diminished insulin response caused by an impairment of glucose sensing of β-cells has remained controversial [45]. In FBS hepatocytes, GLUT2 has the effect of a malfunctioning glucose sensor: in the fasted state, when extracellular glucose concentration declines, the concentrations of glucose and glucose-6-phosphate within hepatocytes are inappropriately high in FBS patients. This stimulates glycogen synthesis, inhibits gluconeogenesis and glycogenolysis, and ultimately predisposes to hypoglycaemia and hepatic glycogen accumulation [39].

Impaired transport of glucose out of renal tubular cells results in the accumulation of glycogen and free glucose within these cells. This impairs other transport functions, resulting in a generalised tubulopathy with disproportionately severe glucosuria. The extreme amounts of glucose lost with the urine (even at times when blood glucose is low) may contribute to the propensity to develop hypoglycaemia and dehydration.

8.5.3 Genetics

FBS is a very rare autosomal recessive condition caused by pathogenic variants in *SLC2A2*. More than 70% of cases come from consanguineous families [46]. *SLC2A2* codes for a 524 amino acid protein with 55% amino acid identity to GLUT1. The genomic structure of *GLUT2* encompasses 11 exons and to date, more than 100 different genetic variants scattered throughout the gene have been detected [39, 46].

8.5.4 Diagnostic Tests

Diagnosis of FBS is suggested by the characteristic combination of altered glucose homeostasis, hepatic glycogen accumulation, and the typical features of a Fanconi-type tubulopathy. Elevated biotinidase activity in serum has been found to be a useful screening test for hepatic glycogen storage disorders including FBS [47]. Fasting hypoglycaemia and impaired glucose and galactose tolerance may be documented during oral loading tests. Laboratory signs may include mildly elevated transaminases without signs of an impaired hepatic protein synthesis or a diminished secretory function. Plasma lipids, uric acid and lactate are elevated. If a liver biopsy is performed, both histologic and biochemical methods show an increased glycogen content; enzymatic studies of all glycogenolytic enzymes, however, give normal results. Hyperaminoaciduria, hyperphosphaturia, hypercalciuria, renal tubular acidosis, mild tubular proteinuria and polyuria are indicative for a generalised proximal tubular dysfunction. A hallmark of the diagnosis of FBS is the relatively severe glucosuria. Calculated tubular glucose reabsorption is dramatically reduced or even zero in most patients [39].

The diagnosis of FBS is ultimately confirmed by the detection of homozygosity or compound heterozygosity for *SLC2A2* variants [46] which can be functionally characterized regarding membrane expression and residual transport activity [48].

8.5.5 Treatment and Prognosis

Only symptomatic treatment is available. Measures are directed towards an improvement of glucose homeostasis and an amelioration of the consequences of renal tubulopathy. FBS patients should receive a diet with adequate caloric intake compensating for the renal glucose losses. Frequent feeds using slowly absorbed carbohydrates are recommended. Continuous carbohydrate supply by tube feeding of oligosaccharide solutions during the night may be indicated. The administration of uncooked corn starch has been demonstrated to have a beneficial effect on metabolic control, particularly on growth [49] but nocturnal enteral feeding seems to be superior [50].

Regarding tubulopathy, water and electrolytes must be replaced in appropriate amounts. Administration of alkali may be necessary to compensate for renal tubular acidosis. Hypophosphataemic rickets requires supplementation with phosphate and vitamin D preparations. With these measures, prognosis is fairly good and some of the originally described paediatric patients have reached adulthood. The main subjective problem for these adult patients are short stature and orthopaedic problems from hypophosphataemic rickets and osteomalacia. Hepatic adenomas, as described for other glycogen storage diseases, have not been observed in FBS, and adenoma formation did not precede the development of hepatocellular carcinoma reported in a single case [51]. Metabolic decompensation with severe acidosis and renal insufficiency similar to that seen in diabetic glomerulosclerosis have been exceptional complications causing death already in childhood [39].

8.6 Other Defects of Glucose Transporters

Renal hypouricaemia (GLUT9 deficiency) is a realtively common autosomal recessive disorder characterised by impaired renal urate reabsorption on both sides of tubular cells. It may be associated with severe complications such as exercise-induced acute renal failure and nephrolithiasis. Genetic studies in some of these patients detected aberrations in *SLC2A9* as the underlying cause [52]. Expression studies demonstrated that GLUT9 is considerably more active as a urate transporter than as a facilitative glucose transporter.

Arterial tortuosity syndrome (GLUT10 deficiency), characterised by generalised tortuosity and elongation of all major arteries, has originally been listed among disorders of glucose transport. It could be demonstrated, however, that not glucose but a structurally related substance is transported by GLUT10. This

transporter facilitates dehydroascorbic acid import into mitochondria of smooth muscle cells and insulin-stimulated adipocytes, protects cells against oxidative stress and connects mitochondrial function to TGF-beta signaling [53]. This might explain clinical similarities to individuals with pathogenic variants of the TGF-β receptor gene.

Monocarboxylate Transporters

Monocarboxylate transporters (MCTs) are a family of 14 members of plasma membrane proteins encoded by the *SLC16* family of solute load carriers. They were designated according to the first 4 and still best studied members, MCT1–4, whose substrates can be characterised by the presence of one carboxylate group such as occur in lactate, pyruvate, short-chain fatty acids, β-hydroxybutyrate and acetoacetate, and in monocarboxylate drugs [54, 55]. In vitro studies with human MCTs have shown that they are involved in the transport of e.g., γ-hydroxybutyrate, phenylbutyrate, valproate, salicylate, certain β-lactam antibiotics and statins. In inborn errors of metabolism they play an important role in situations when ketone bodies or a ketogenic diet are used to provide an alternative metabolic substrate to glucose, such as in GLUT1DS (► Sect. 8.3) or PDH deficiency (► Chap. 11).

MCT1–4 have been extensively studied and were shown to be L-lactate/proton symporters. They are unglycosylated but form heterodimers with the glycoproteins basigin (CD147) or embigin (GP70) which are responsible for proper trafficking to the membrane. MCT1–4 help to maintain an energy balance and pH homeostasis and allow metabolic cooperation between different tissues with distinct energetic profiles. In particular, the tissue distribution of MCT1–4 with different kinetic characteristics enables a metabolic coupling in which lactate, pyruvate, or ketone bodies supplied by one tissue can be taken up and metabolised by another. Thus, MCTs are important in liver gluconeogenesis from lactate produced in muscle (Cori cycle) or in liver ketone production for other tissues. In addition to shuttling substrates between different tissues with particular energy demands, MCT1–4 are attributed a major role in lactate shuttling between different cell types within the same tissue. This concept has been described for skeletal muscle, in which highly glycolytic fast-twitch white muscle fibers release lactate via MCT4, which is taken up by oxidative slow-twitch red muscle fibers by MCT1. A rapid exchange of lactate has also been proposed for different cell types of the brain, known as the astrocyte-neuron lactate shuttle hypothesis. In this case, glycolytic astrocytes provide energy by lactate release through MCT1 and MCT4 for neurons which express the high affinity uptake transporter MCT2. Similar cooperation between cell types of the same tissue has been demonstrated to be important for visual function and spermatogenesis [56].

MCTs with numbers beyond 1–4 are less well characterised. They have a distantly related amino acid sequence but are not necessarily protein-coupled symporters and may have a specificity for quite different substrates. While, for example, MCT7 (encoded by *SLC16A6*)[1] releases ketone bodies from the liver, MCT8 (*SLC16A2*) transports thyroid hormones T3 and T4, MCT9 (*SLC16A9*) is a carnitine efflux transporter, MCT10 (*SLC16A10*) transports aromatic amino acids, and MCT12 (*SLC16A12*) is a creatine transporter [54, 55].

8.7 Monocarboxylate Transporter-1 Deficiency (MCT1 Deficiency)

8.7.1 Clinical Presentation

MCT1 deficiency should be considered if recurrent ketoacidotic episodes with hyperventilation or cyclic vomiting occur. Such episodes typically start in the first years of life and can be accompanied by severe acid base imbalances typically induced by infections or fasting and a normal or borderline blood glucose concentration [57]. A few patients have been observed with exercise-induced muscle cramping, stiffness, or fatigue, but also crises with CK elevation and rhabdomyolysis. In between attacks, electromyographic studies and muscle histology is normal [58].

8.7.2 Metabolic Derangement

Loss of MCT1 function results in an impaired uptake of ketone bodies produced by the liver in peripheral tissues and an accumulation in blood. This occurs particularly in metabolic conditions that stimulate ketone production, i.e., catabolic situations with hormonal counter regulation. Since gluconeogenesis is not impaired, hypoglycaemia is not typical; it can occur however, due to activation of the glycolytic pathway and increased consumption. Thus, this presentation is similar to ketolysis defects (► Chap. 13).

1 *Note* that numbering in MCT and *SLC16* nomenclature do not match.

MCT1 is ubiquitously expressed and the most abundant lactate transporter in glia cells of the brain. Since it has been demonstrated in animal and cell culture models that down-regulation of this transporter prevents learning and long-term memory formation [59], it is not surprising that mild to moderate intellectual disability has been reported in homo- or compound heterozygous individuals. Exercise-induced symptoms have been explained by decreased lactate clearance from muscle which in few heterozygous patients was documented to be reduced to approx. 50% [58].

8.7.3 Genetics

Both autosomal recessive as well as dominant traits have been described for cases with ketoacidosis which means that patients were found to be either homozygous or heterozygous for pathogenic variants of *SLC16A1*. However, heterozygous family members who carry the same variant as a heterozygous index case have repeatedly been found asymptomatic which means that additional factors accompanying heterozygous MCT1 variants are expected to contribute to clinical manifestation. The myopathic form has only been reported with dominant inheritance and it has remained unclear why there is limited overlap regarding the two types of manifestation.

8.7.4 Diagnostic Tests

Recurrent severe metabolic acidosis with an anion gap due to ketone accumulation detectable in blood and urine is the key finding in MCT1 deficiency. Diagnosis relies on targeted or untargeted genetic testing.

8.7.5 Treatment and Prognosis

Treatment is based on an interruption of catabolism by the infusion of high amounts of glucose and early preventive measures to forstall metabolic deterioration. If the diagnosis is established early, prognosis is generally favourable.

8.8 Exercise-Induced Hyperinsulinism (β-Cell MCT1 Overexpression)

In this rare condition patients present with signs and symptoms of non-ketotic hypoglycaemia, the characteristic manifestation of congenital hyperinsulinism (► Chap. 6).

8.9 Allan-Herndon-Dudley Syndrome (MCT8 Deficiency)

8.9.1 Clinical Presentation

Patients with this type of MCT deficiency present with severe neurological problems. They may have microcephaly and biparietal narrowing. Hypotonia is a leading sign and patients may already come to attention as a floppy infant at birth. Despite a relatively normal life span, patients have a moderately or severely impaired intellectual development, paroxismal dyskinesia with spastic or athetoid movements, and in the later course development of contractures. If severely affected, they never learn to talk or walk, and around a half are provided with a feeding tube. A delayed myelination or white matter changes on brain MRI are typical. Furthermore are there signs of chronic thyrotoxicosis with deterioration of body weight, muscle wasting, and tachycardia and characteristic changes of serum concentrations of thyroid hormones [60, 61].

8.9.2 Metabolic Derangement

Triiodinethyronine is important for the developing brain. Its uptake at the apical side of the blood brain barrier (together with the organic ion transporter-14 (SLCO1C1) on the basolateral membrane) and organic ion transporter-14 (SLCO1C1) organic ion transporter-14 (SLCO1C1) into neurons is mediated by MCT8 and a disruption of this uptake results in the aforementioned neurologic symptoms [62]. High peripheral concentrations of thyroid hormone are responsible for the hypermetabolic state.

8.9.3 Genetics

MCT8 deficiency is a rare X-linked disorder. MCT8 is encoded by *SLC16A2* and hemizygosity for missense, nonsense, small and large deletions, as well as chromosomal rearrangement interrupting this gene have been described in affected males. Heterozygous females may have a milder thyroid phenotype but are free of neurological symptoms [60].

8.9.4 Diagnostic Tests

Male patients with this condition have pathognomonic thyroid function test results with elevated serum fT3, low rT3, low or low-normal fT4, and an increased fT3/

fT4 ratio of >0.75 mmol/mmol. TSH concentration is normal or slightly elevated [60].

8.9.5 Treatment and Prognosis

Only symptomatic measures are available for the neurologic manifestations. Prenatal treatment in an affected pregnancy with intraamniotic instillation of high doses of levothyroxine resulted in an improved outcome [63]. Only recently, results of a multicenter study using the T3 analogue TRIAC (triiodothyroacetique) showed that features of peripheral thyrotoxicosis were alleviated in paediatric and adult patients with MCT8 deficiency [64].

8.10 Familial Cataract, Microcornea Syndrome (MCT12 Deficiency)

In a large Swiss pedigree with numerous patients with juvenile cataract, microcornea and mild renal glucosuria exome studies were performed which resulted in detection of a nonsense variant of *SLC16A12* [65]. The corresponding protein, MCT12, is a creatine carrier, therefore also termed creatine transporter-2 (CRT2). The suggested role in the lens may be related to energy provision, antioxidant activity, and protection against UV radiation, a risk factor for cataract development. MCT12 deficiency is a dominant condition. MCT12 p.Q215* was detected in the index family and other functionally characterised missense variants of this gene have been found in patients with age-ralated cataract [66]. Renal glucosuria was later explained by heterozygosity for SGLT2 p.A89T [67] which means that the mutated monocarboxylate transporter is only responsible for the ophthalmologic manifestations. Since the molecular chaperone basigin improved creatine transport activity in missense variants [66], there is hope that such a type of treatment will become available for patients.

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Disorders of Creatine Metabolism

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Contents

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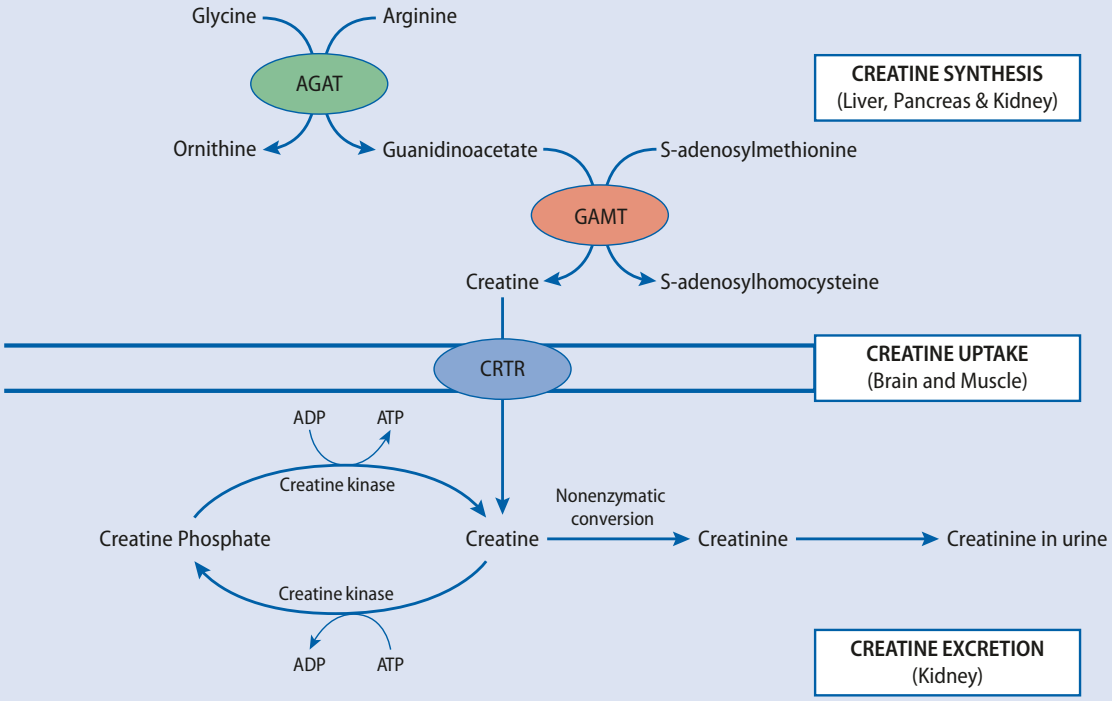
Creatine Synthesis and Transport

Creatine is synthesized by two enzymatic reactions: (1) L-arginine:glycine amidinotransferase (AGAT, encoded by *GATM*) catalyses the transfer of an amidino group from arginine to glycine, yielding guanidinoacetate; (2) S-adenosyl-L-methionine:N-guanidinoacetate methyltransferase (GAMT, encoded by *GAMT*) catalyses the methylation of the amidino group in the guanidinoacetate molecule, yielding creatine. Creatine synthesis occurs mainly, but not exclusively, in the kidney, in the liver and in the pancreas. The kidney and pancreas have high AGAT activity, and the liver has high GAMT activity. Circulating endogenously synthetized and dietary creatine is taken up by a sodium and chloride dependent creatine transporter (CRTR, encoded by *SLC6A8*) into high energy demanding organs such as muscle and brain. The cerebral creatine pool is partly maintained by the CRTR expressed at the blood-brain barrier and potentially also at the synaptic cleft.

Intracellular creatine phosphate is a phosphorylated creatine molecule and serves as high-energy phosphate reserve in muscle and brain, which can be rapidly converted into creatine by creatine kinase (CK) to recycle adenosine triphosphate. The reverse reaction to form creatine-phosphate and ADP is also catalyzed by CK. There are three cytosolic isoforms of CK (brain type CK-BB, muscle type CK-MM and the heart type CK-MB), and two mitochondrial isoforms of CK, ubiquitous mtCK and sarcomeric mtCK. Creatine and creatine-phosphate are converted into creatinine by a non-enzymatic conversion. Creatinine is excreted in urine. Its constant daily turnover is 1.5% of body creatine and depends on the total body creatine, and in particular on the muscle mass (i.e. 20–25 mg/kg/24 h in children and adults, which is lower in infants younger than 2 years of age; ◘ Fig. 9.1).



◘ **Fig. 9.1** Metabolic pathway of creatine / creatine phosphate that mainly but not exclusively occurs in the organs indicated. (AGAT arginine:glycine amidinotransferase, GAMT guanidinoacetate methyltransferase, CRTR creatine transporter (SLC6A8), CK creatine kinase)

Introduction

Primary disorders of creatine metabolism are a group of inborn errors of creatine synthesis (arginine:glycine amidinotransferase (AGAT, encoded by *GATM*), guanidinoacetate methyltransferase (GAMT, encoded by *GAMT*) deficiencies), and the X-linked creatine transporter (CRTR, encoded by *SLC6A8*) deficiency [1]. They typically present with systemic and / or cerebral creatine deficiency and global developmental delay, cognitive dysfunction or intellectual disability along with epilepsy, movement disorders and behavioural problems. Diagnostic markers include high guanidinoacetate concentrations in body fluids in GAMT and low levels in AGAT deficiency in both sexes and increased urine creatine to creatinine ratio in CRTR deficiency in males and rarely in females. Oral creatine supplementation leads to near complete restoration of cerebral creatine in creatine synthesis defects: In GAMT deficiency, reduction of guanidinoacetate is achieved by ornithine supplementation and / or dietary protein or arginine restriction. In CRTR deficiency, creatine, arginine and glycine supplementation does not significantly improve outcomes, although partial clinical improvement has been reported in few patients. Normal neurodevelopmental outcomes have been reported in early treated patients with creatine synthesis defects.

Secondary changes in creatine metabolism have been described in disorders affecting arginine and ornithine metabolism such as ornithine aminotransferase (OAT) deficiency, urea cycle defects, hyperammonemia, hyperornithinemia, homocitrullinuria syndrome, Δ(1)-pyrroline-5-carboxylate synthase deficiency.

Another type of disorder affecting AGAT, but without (cerebral) creatine deficiency is caused by heterozygous autosomal dominant missense variants in *GATM*. These particular variants result in intramitochondrial aggregates that cause Fanconi syndrome and kidney failure.

9.1 Clinical Presentation

The common clinical hallmark of primary disorders of creatine metabolism (AGAT, GAMT, and CRTR deficiencies) is global developmental delay (GDD) with prominent speech and language delay or intellectual disability (ID). GDD and ID range from mild to severe and are characteristically associated with behavioural problems such as hyperactivity and autism spectrum disorder. Epilepsy occurs in GAMT and CRTR deficiencies. Movement disorders with or without basal ganglia changes in brain magnetic resonance imaging (MRI) are additional features of GAMT deficiency. Myopathy is an additional feature of AGAT deficiency.

9.1.1 Arginine: Glycine Amidinotransferase (AGAT) Deficiency

Less than 20 patients have been diagnosed worldwide. In a recent study of 16 patients (age 3 weeks – 25 years) from 8 families and 8 different ethnic backgrounds [2], non-syndromic ID with speech and language delay was the most common clinical feature. Microcephaly and hand stereotypies have also been observed. Half of the patients developed clinical, electrophysiological and histopathological signs of myopathy secondary to creatine- and guanidinoacetate-phosphate deficiency and lack of ATP production.

9.1.2 Guanidinoacetate Methyltransferase (GAMT) Deficiency

About 130 patients have been diagnosed worldwide. In a study of 48 patients (age 1 week – 34 years) from 38 families [3], ID with speech and language delay, behavioural problems and epilepsy were the most common clinical features, followed by movement disorder with or without basal ganglia changes in brain MRI. ID tends to be severe and associated with disruptive and self injurious behaviour. Overall there seems to be a positive relationship between the degree of ID and early initiation of the treatment. Epilepsy varies from occasional to drug resistant seizures. Movement disorder is mostly hyperkinetic and dystonic. Presentations masquerading as Leigh like syndrome and mitochondrial disease [4], late onset ballistic and dystonic movement disorder [5] and intermittent fever induced ataxia [3] have been reported in single patients.

9.1.3 Creatine Transporter (CRTR) Deficiency

More than 180 male patients from 150 families have been diagnosed (► www.LOVD.nl/SLC6A8). In a study of 101 patients from 85 families [6] non-syndromic ID was the most common clinical feature (from mild to severe). Less than one third of patients were able to speak in sentences. Behavioural problems (autistic features, hyperactivity and disruptive behaviours), seizures, movement disorders and gastrointestinal problems (vomiting, reflux, feeding difficulties) were frequently reported. Additional features included muscular hypotonia, low muscle mass, hyperextensible joints, short stature, and dysmorphic features (broad/prominent forehead, mid-face hypoplasia, myopathic facies, ptosis, short nose, simple/unfolded/large ears).

Brain atrophy and cardiac arrhythmia were also seen. Neurological and psychiatric problems appear to be progressive in the few adults reported in the literature [7]. Heterozygous females for the familial pathogenic or likely pathogenic variant in *SLC6A8* may have learning disability or mild ID [8]. A few female cases with severe phenotype of CRTR deficiency have been reported previously [9].

9.1.4 Autosomal Dominant Renal Fanconi syndrome and Kidney Failure Due to Partial AGAT Deficiency

This is a newly described genetic cause of renal Fanconi syndrome and kidney failure caused by mitochondrial aggregation of fully penetrant heterozygous *GATM* missense variants (Fanconi renotubular syndrome type 1). Patients with this autosomal dominant disorder develop renal Fanconi syndrome with glucosuria, hyperphosphaturia, generalized aminoaciduria, low molecular weight proteinuria and metabolic acidosis. Debilitating rickets or bone deformities have not been described in these patients. Neither central nervous system (CNS) involvement nor cerebral creatine deficiency were reported [10].

9.2 Metabolic Derangement

Systemic and cerebral creatine deficiency is caused by reduced synthesis of creatine in AGAT and GAMT deficiencies and by impaired creatine uptake / recycling into the brain in CRTR deficiency. Reduced muscle creatine levels have been described in AGAT [11] and in GAMT deficiencies [12, 13]. Low intracellular creatine and creatine-phosphate results in reduced production of creatinine. Thus, plasma creatinine concentration and urinary creatinine excretion are low in patients with disorders of creatine metabolism [14]. Guanidinoacetate is depleted in AGAT deficiency and accumulates in GAMT deficiency. Guanidinoacetate is an alternative substrate for CK. High or normal levels of guanidinoacetate-phosphate in GAMT and CRTR deficiency might serve as a second high-energy phosphate carrier to compensate creatine phosphate deficiency. In AGAT deficiency, guanidinoacetate and creatine are not synthetized. Deficiency of creatine-phosphate and guanidinoacetate-phosphate likely leads to myopathy. The neurotoxic effects of high guanidinoacetate levels in the CNS might explain why patients with GAMT deficiency have more common and severe epilepsy compared to the other two disorders of creatine metabolism. S-adenosylmethionine is required as a methyl group donor for the methylation of guanidinoacetate to form creatine. Although up to 75% of the body's methyl group transfer is utilized for the formation of creatine from guanidinoacetate, no major alterations of S-adenosylmethionine and metabolites of the methylation and remethylation cycle have been found in AGAT and GAMT deficiencies.

9.3 Genetics

Both AGAT (encoded by *GATM*) and GAMT (encoded by *GAMT*) deficiency, show autosomal recessive inheritance. Nine different pathogenic *GATM* variants (nonsense, splice, frameshift, missense) have been identified in 8 families from different ethnic backgrounds with AGAT deficiency [2].

Four likely pathogenic missense variants affecting evolutionary conserved amino acid residues are reported in *GATM* that cause autosomal dominant renal Fanconi syndrome and kidney failure. These variants were detected in five families (36 individuals) and obviously have a negative dominant effect, leading to aggregation of the GATM protein with main location in the proximal tubular cells [10].

About 60 different pathogenic variants in *GAMT* have been identified. More than 60% are missense variants. The c.327G > A and c.59G > A have been reported in 24% and in 21% of alleles. While c.327G > A occurs in all ethnicities, c.59G > A has been found in patients from Spain, Portugal and Turkey [3, 15–17]. Carrier frequency of GAMT deficiency was between 1 in 250 in Dutch newborns and 1 in 812 individuals in the general population [18, 19].

At present, more than 130 pathogenic variants in *SLC6A8* have been identified (► www.LOVD.nl/SLC6A8). The majority of pathogenic variants are missense variants followed by single nucleotide deletions and insertions. A minority of these variants have been described in multiple unrelated individuals. Among 101 males from 85 families [6], one third of patients had a de novo pathogenic variant. The possibility of low level somatic or germ line mosaicism [20] should be taken into account when counselling mothers of boys with presumed de novo variants. Pathogenic missense variants with residual CRTR activity might be associated with a milder phenotype [6], whereas large deletions extending beyond the 3′ end of *SLC6A8* were associated with a more severe phenotype [21].

The prevalence of CRTR deficiency is relatively high compared to AGAT and GAMT deficiencies. Combined analysis of all studies published, including cohorts of males with ID and autism and ID and neurological disease, yielded a prevalence of 0.4% (CI 0.2–0.5) [22, 23].

9.4 Diagnostic Tests

GAMT, AGAT and CRTR deficiencies present with non-specific GDD with prominent speech and language delay. In children and adults with unexplained developmental disability and/or ID, biochemical and molecular genetic investigations of cerebral creatine deficiency disorders should be included. Patients undergoing brain MRI as part of the diagnostic evaluation should also have brain magnetic resonance spectroscopy (MRS) to screen for cerebral creatine deficiency disorders.

9.4.1 In Vivo Brain MRS and MRI

Profound cerebral creatine deficiency is identified by in vivo brain MRS in patients with GAMT and AGAT deficiencies and in males with CRTR deficiency. Females with CRTR deficiency can have normal or mildly decreased creatine in brain MRS. In patients with low brain creatine, further diagnostic tests including urine guanidinoacetate and creatine to creatinine ratio measurements as well as targeted molecular genetic investigations of *GAMT, GATM* and *SLC6A8* are required for the differentiation of the three cerebral creatine deficiency disorder. Brain MRS is not only applied for diagnostic purposes, but also for monitoring of treatment in GAMT and AGAT deficiencies. Besides brain MRS, in GAMT deficiency brain MRI may show isolated globus pallidus involvement with T2 prolongation.

9.4.2 Metabolite Analysis

Analysis of urine guanidinoacetate, creatine and creatinine is an important screening test for AGAT, GAMT and CRTR deficiencies [14]. Low guanidinoacetate level is characteristic of AGAT deficiency. In the majority of patients, urine and plasma guanidinoacetate levels are undetectable or <10% of lower reference range. Elevated guanidinoacetate in urine is a sensitive marker for GAMT deficiency. In untreated patients, urine and plasma guanidinoacetate levels are often more than 10 times elevated; in CSF, more than hundredfold elevations are reported. Plasma creatine levels are largely influenced by nutritional factors [24]. Thus, normal plasma creatine levels do not exclude the presence of CDS. In CRTR deficiency impaired renal tubular [re]uptake results in urinary loss of creatine; consequently there is a high urine creatine to creatinine ratio which serves as a diagnostic marker in males [6, 13]. Symptomatic and asymptomatic heterozygous females have normal or mildly elevated urine creatine to creatinine ratio [8, 9]. False positive values may be due to creatine rich diet or neuromuscular disorders [23]. Reference values have been established for urine creatine and creatinine excretion [24] showing a strong dependence on age and sex. As a consequence of low creatinine excretion, an increase in the concentration of urine amino acids and organic acids, when expressed as a ratio to creatinine may suggest a cerebral creatine deficiency disorder. There is no specific biomarker for AGAT aggregation syndrome.

9.4.3 Molecular Genetic Investigations

Direct sequencing of *GATM, GAMT* and *SLC6A8* is required for the confirmation of the diagnosis. All three genes are included in next generation panels for epilepsy and intellectual disability. Whole exome or genome sequencing might identify more patients with cerebral creatine deficiency disorders.

9.4.4 Biochemical Functional Investigations

Enzyme analyses of GAMT and AGAT in cultured fibroblasts or lymphoblasts and creatine uptake in fibroblasts are necessary if 1) variants of unknown significance in *GAMT*, *GATM* and *SLC6A8*; or 2) strong clinical suspicion with a heterozygous variant in *GAMT* and *GATM*. Recently a method has been published enabling a fast analysis of GAMT activity in lymphocytes [25]. Overexpression of missense variants for functional characterization may be necessary to confirm their pathogenicity [16, 20].

9.4.5 Prenatal Diagnosis

Prenatal diagnosis and preimplantation genetic diagnosis for at-risk pregnancies require prior identification of the disease-causing variant(s) in the family. In GAMT deficiency prenatal diagnosis is established by determination of guanidinoacetate in amniotic fluid [26].

9.4.6 Newborn Screening

Elevated guanidinoacetate levels have been confirmed in blood spots of affected newborns with GAMT defi-

ciency using tandem mass spectrometry [27]. Two- and three- tier algorithms including the addition of a chromatographic step to remove the interfering chemicals and GAMT sequencing have been successful in reducing the false positive rate [28]. Despite the implementation of pilot GAMT newborn screening in a handful newborn screening programs, only 2 individuals have been identified in more than one million screened newborns as in early 2022. Newborn screening for AGAT deficiency and CRTR deficiency is not currently feasible since there is no suitable biomarker detectable in blood spots.

9.5 Treatment and Prognosis

9.5.1 AGAT Deficiency

The aim of treatment is to restore cerebral and muscular creatine levels. Treatment with creatine monohydrate (100–800 mg/kg/d) results in almost complete restoration of brain creatine levels, and significant improvement of myopathy in most patients [2]. Early diagnosis and treatment may prevent ID and myopathy in patients [2].

9.5.2 GAMT Deficiency

The aim of the treatment is to restore cerebral creatine levels and to decrease accumulation of guanidinoacetate. Creatine-monohydrate supplementation (400–800 mg/kg) results in correction of reduced cerebral creatine levels [3]. Suppression of guanidinoacetate production is achieved by additional L-ornithine supplementation (by competitive inhibition of AGAT activity) and dietary arginine restriction (substrate deprivation). The guanidinoacetate reducing effect of L-ornithine is best achieved by dosages of 400–800 mg/kg/d. Dietary arginine restriction (0.2–0.3 grams / kg / day natural protein intake) and supplementation of arginine-free essential amino acid formula has been shown reduce, but not normalize, plasma and CSF guanidinoacetate levels [3, 17, 29]. Sodium benzoate has been recommended as an additional guanidinoacetate lowering approach via its ability to conjugate with glycine and thus reduce its availability for guanidinoacetate synthesis [13]. In one patient treated with L-ornithine and arginine restricted diet, additional sodium benzoate resulted in only 12% additional reduction of plasma guanidinoacetate levels [29]. In recent studies long-term treatment outcomes of 60 patients with GAMT deficiency were reported; early initiation of combined treatments favourably impacts neurodevelopmental outcomes and normal neurodevelopment has been achieved in very early treated patients [3, 30].

9.5.3 CRTR Deficiency

CRTR deficiency appears to be the most difficult to treat disorder as cerebral creatine restoration has not been achieved thus far [22]. Besides high dose creatine supplementation, arginine, glycine and S-adenosylmethionine supplementation (substrates for creatine synthesis) have been employed with the aim to enhance intracerebral creatine synthesis. Improvements mainly in muscle mass, behaviour, communication, gross motor abilities, and epilepsy have been observed [9, 31, 32]. S-adenosylmethionine, in addition to creatine, arginine and glycine supplementation [33] or with supplementation of creatine ethylester [34], did not increase cerebral creatine levels.

High levels of homocysteine and reduced levels of folate have been reported during arginine supplementation and may be prevented by simultaneous supplementation of folate and/or creatine [35]. In a recent treatment outcome study, none of the males showed either deterioration or improvements in their disease associated symptoms, whereas two females did show improvement. Creatine monotherapy resulted in deterioration of disease associated symptoms in one male. Arginine and glycine are precursors of intracerebral creatine synthesis and their transport across the blood brain barrier is unaffected in individuals with creatine transporter deficiency. Therefore combined creatine, arginine and glycine therapy has been considered as an additional option which might stop disease progression in males and improve clinical features in females [36].

9.5.4 Autosomal Dominant Renal Fanconi syndrome and Kidney Failure Due to Dominant *GATM* Variants

There is no specific treatment for this condition. However, it is suggested that creatine supplementation could serve as a pharmacologic intervention by suppressing the expression the mutated *GATM* allele [10].

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Disorders of Oxidative Phosphorylation

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Contents

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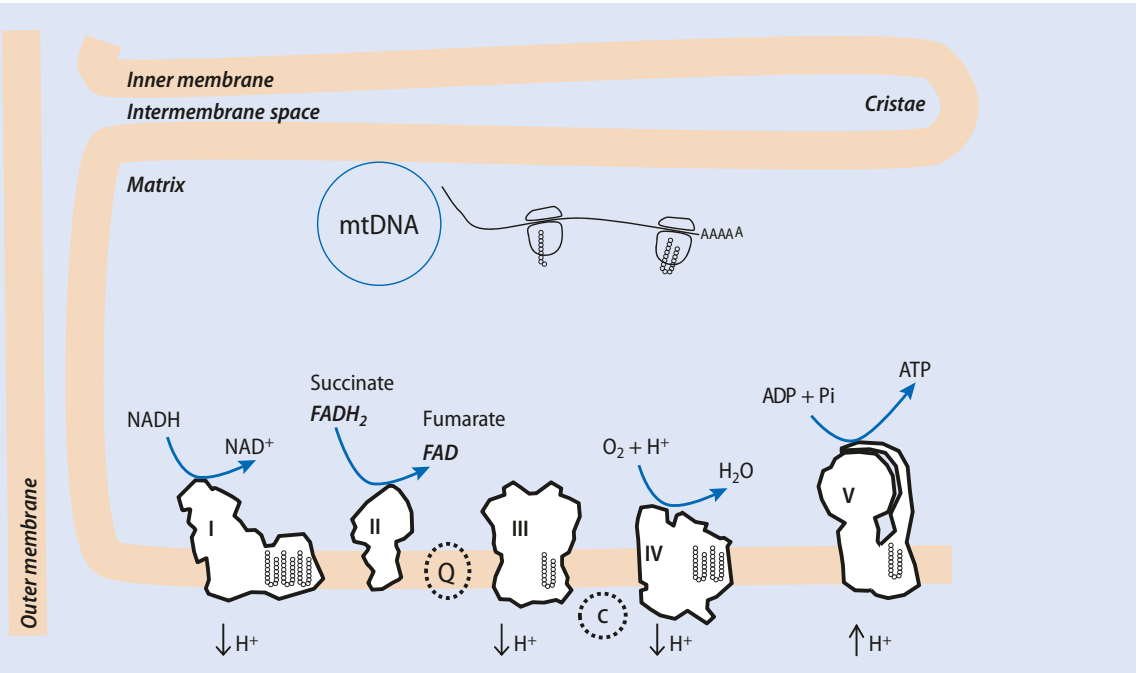
Respiratory Chain and Oxidative Phosphorylation System

The respiratory chain (complexes I–IV) and oxidative phosphorylation (OXPHOS) system (complexes I–V) are embedded in the inner mitochondrial membrane and are responsible for ATP production by aerobic metabolism (◘ Fig. 10.1). Complex I (NADH-ubiquinone oxidoreductase) contains 44 different polypeptide subunits, seven of which are encoded by mitochondrial DNA (mtDNA). Complex II (succinate-ubiquinone oxidoreductase) has only four subunits, all encoded by nuclear genes. Complex II includes succinate dehydrogenase (SDH) which catalyses the oxidation of succinate to fumarate and is a component of the Krebs cycle. (► Chap. 11) Complex III (ubiquinol-cytochrome *c* oxidoreductase) is composed of 11 subunits only one of which (cytochrome *b*) is encoded by mtDNA. Complex IV (cytochrome *c* oxidase, COX) comprises 14 polypeptides including 3 encoded by mtDNA. Complex V (ATP synthase or F_1F_0 ATPase) has 16 subunits, of which two (ATP6 and 8) are encoded by mtDNA.

Reducing equivalents generated by the oxidation of pyruvate, fatty acids and the Krebs cycle are transferred to the respiratory chain via NADH (reduced nicotinamide dinucleotide) and $FADH_2$ (reduced flavin adenine dinucleotide) . Electrons derived from oxidation of pyruvate and fatty acids are transferred via NADH to complex I, whilst electrons from succinate in the Krebs cycle are transferred to complex II via $FADH_2$. The electron carriers in the respiratory chain are flavins, iron-sulphur (FeS) complexes, quinones and the haem groups of cytochromes (◘ Fig. 10.2). Ubiquinone (Coenzyme Q_{10}, CoQ) and cytochrome *c* (cyt *c*) function as mobile carriers of electrons between OXPHOS complexes. From complexes I and II, electrons are transferred to ubiquinone and then to complex III, and via cyt *c* to cytochrome *c* oxidase (COX), before finally reducing molecular oxygen to water. The energy released during this sequential electron transfer is used to generate an electrochemical gradient, by translocating protons from the matrix to the intermembrane space. Protons are pumped at three coupling sites (complexes I, III and IV) and the resulting membrane potential (proton motive force, ~150 mV) is used by complex V (ATP synthase or F_1F_0 ATPase) to drive ATP synthesis from ADP and inorganic phosphate by free energy transduction. ATP synthase is an enzyme complex that can either hydrolyse or synthesise ATP, according to the energy status of the cell.



◘ **Fig. 10.1** Oxidative phosphorylation (OXPHOS) is the final part of aerobic energy metabolism. It takes place in mitochondria involving respiratory chain enzyme complex I (NADH dehydrogenase, I), complex II (succinate dehydrogenase), complex III (ubiquinol-cytochrome *c* oxidoreductase, III), complex IV (cytochrome *c* oxidase, IV), and the ATP synthase (complex V). These five enzymes contain multiple subunits, 13 subunits are encoded on the mitochondrial genome (mtDNA) and are produced by mitochondrial protein synthesis. Several cofactors including coenzyme Q_{10} (Q) and cytochrome *c* (*c*) are involved

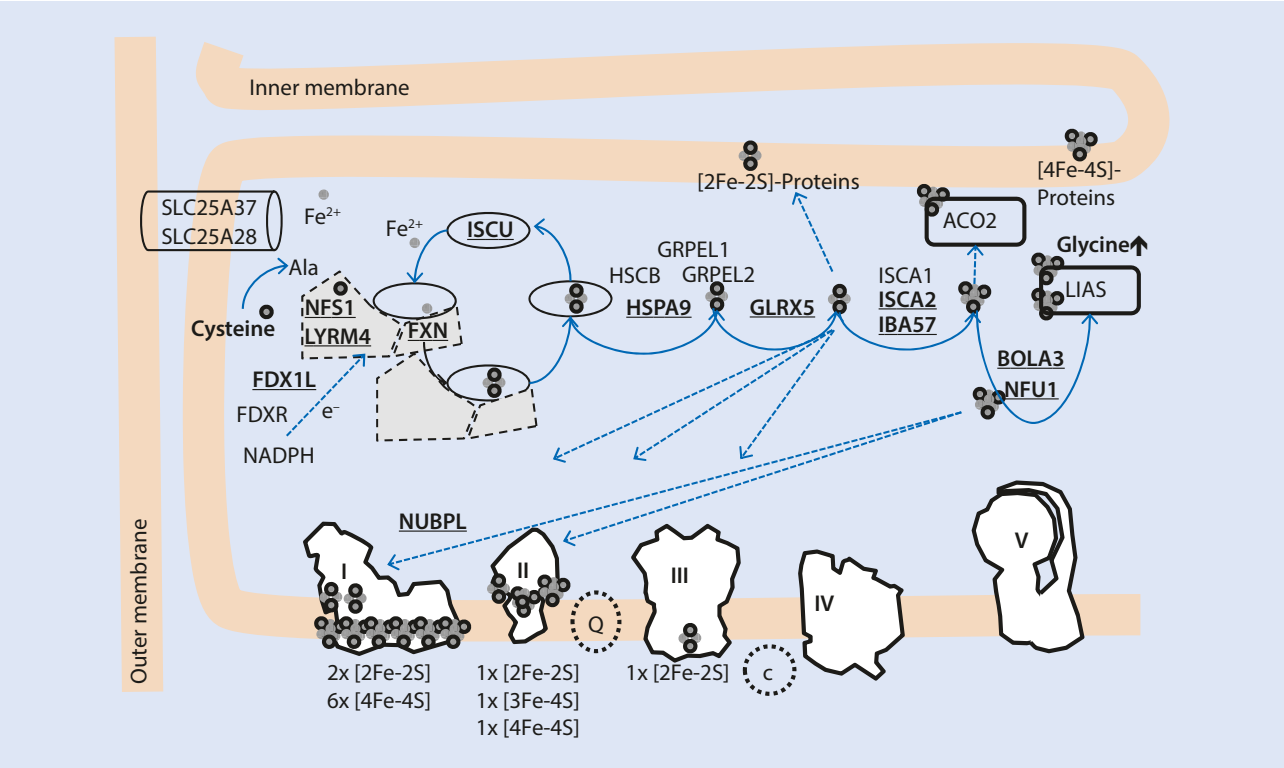


Fig. 10.2 The respiratory chain enzyme complexes I, II, and III depend on iron-sulphur (FeS) cluster cofactors, either [2Fe-2S], [3Fe-4S] or [4Fe-4S]. The formation of FeS clusters uses sulphur from cysteine, Fe^{2+}, NADPH, and ATP as substrates. A series of enzymatic steps are involved and mutations in 12 proteins (in bold and underlined) have been identified that cause human disease. Since FeS clusters are also required for lipoate synthesis, elevated glycine – due to glycine cleavage deficiency – can be indicative for FeS cluster defects (► Chap. 23). ISCU iron sulfur scaffold complex protein, FXN frataxin, ACO aconitase, LIAS lipoic acid synthetase, Ala alanine, FDXR ferredoxin reductase, HSP heat-shock protein, ISCA Iron-sulfur cluster assembly

Introduction

Disorders of oxidative phosphorylation encompass heterogeneous infantile, childhood and adult onset diseases characterised by variable involvement of high energy requiring organs. The central and peripheral nervous systems, skeletal and cardiac muscle, eyes, ears, kidneys and liver are frequently involved. Some well-characterised mitochondrial syndromes are recognised, but many patients have overlapping features not corresponding to a specific syndrome. Theoretically any organ or tissue or combination of organs may be affected, with onset at any age. Owing to the involvement of two distinct genomes, the nuclear genome and that located within the mitochondrion itself, a range of modes of inheritance of mitochondrial disease has been observed: maternal (mitochondrial DNA), autosomal recessive, autosomal dominant, X-linked and sporadic. Disease mechanisms include mutations affecting OXPHOS subunits and assembly factors, and disorders of mitochondrial DNA maintenance, protein synthesis, cofactor biosynthesis and lipid metabolism. Mitochondrial trafficking and organelle communication at membrane contact sites are discussed in ► Chap. 44. The complexity of underlying disease mechanisms, together with clinical, biochemical and genetic heterogeneity, creates enormous diagnostic challenges. Most mitochondrial diseases, especially childhood-onset forms, are characterised by relentless progression. Specific treatments are available for some extremely rare forms of mitochondrial disease, but the majority of cases lack curative treatments, and the mainstay of treatment is supportive.

10.1 Clinical Presentation

Clinical diagnosis of mitochondrial disease is extremely challenging since the presence of mitochondria in virtually all cells (except mature erythrocytes) means that mitochondrial dysfunction can affect any organ or combination of organ systems [1]. Moreover, clinical presentation can occur at any time, ranging from antenatal presentations (e.g. intrauterine growth restriction, birth defects) to isolated myopathy in the elderly. The spec-

trum of clinical features associated with mitochondrial disease is listed in Table 10.1.

Traditionally, clinical suspicion of a mitochondrial disorder usually arose in one of three scenarios:

1. A constellation of symptoms and signs that falls within a recognised mitochondrial 'syndrome'.
2. A complex multisystem presentation involving two/more unrelated organ systems that can best be explained by an underlying disorder of energy generation.
3. The presence of lactic acidosis, or other investigation result leading to suspicion of a mitochondrial disease (e.g. characteristic neuro-imaging, 3-methylglutaconic aciduria, ragged red fibre myopathy).

Table 10.1 Clinical features of mitochondrial disease

Organ/tissue	Recognised symptoms/signs in mitochondrial diseases
Brain	Stroke-like episodes, seizures, ataxia, encephalopathy, hypotonia, spasticity, dystonia, extrapyramidal movement disorder, parkinsonism, developmental delay and/or regression, cognitive decline
Eye	Ptosis, progressive external ophthalmoplegia, corneal opacification, cataracts, pigmentary retinopathy, optic neuropathy and atrophy
Ear	Sensorineural hearing loss, auditory neuropathy
Heart	Hypertrophic cardiomyopathy, dilated cardiomyopathy, conduction defects (Wolff-Parkinson-White, complete heart block)
Lungs	Pulmonary hypertension
Kidney	Fanconi-type tubulopathy, nephritis (focal segmental glomerulosclerosis), steroid-resistant nephrotic syndrome
Liver	Acute hepatic dysfunction/failure, hepatomegaly, hypoglycemia
Gut	Enteropathy, dysmotility, pseudo-obstruction, pancreatic exocrine insufficiency
Endocrine organs	Diabetes mellitus, hypo/hyperthyroidism, growth hormone deficiency, hypoparathyroidism, adrenal insufficiency
Gonads	Primary ovarian failure, hypo/hypergonadotrophic hypogonadism
Bone marrow	Sideroblastic anaemia, neutropaenia, pancytopaenia, dyserythropoiesis
Immunity	B cell immune deficiency
Skeletal muscle	Myopathy (often proximal, but may be distal or generalised), exercise intolerance, rhabdomyolysis
Peripheral nerve	Axonal sensorimotor or demyelinating neuropathy
Skin and hair	Hypertrichosis, cutis laxa, pili torti, alopecia

Nowadays, however, an increasingly common scenario is the identification of pathogenic variants in a known mitochondrial disease gene revealed by a genome-wide next generation sequencing (NGS) approach, in a patient in whom clinical suspicion of a mitochondrial disorder was low prior to the genetic testing.

In the following, the clinical features of some of the more well-recognised mitochondrial syndromes are described, grouped by age at presentation. Other syndromic mitochondrial presentations are summarised in Table 10.2. However, it should be noted that many patients do not have clinical presentations fitting neatly into recognised syndromes, but instead have overlapping constellations of symptoms, so the reality is that mitochondrial disorders comprise hundreds of different clinical entities. Furthermore, the range of mitochondrial phenotypes is continuing to expand at a very rapid pace as more and more gene defects are being linked to mitochondrial dysfunction by NGS technologies.

10.1.1 Neonatal and Infantile Presentations

Congenital lactic acidosis typically presents in the newborn period with tachypnoea and sepsis-like nonspecific illness, and is a relatively frequent manifestation of mitochondrial disease [1]. Lactic acidosis may be associated with features of Leigh syndrome (see below) and/or multisystem involvement including cardiomyopathy or renal tubulopathy. Congenital lactic acidosis may be caused by deficiency of pyruvate dehydrogenase, pyruvate carboxylase and biotinidase, as well as isolated or combined OXPHOS deficiencies. Lactate elevation may also be observed in patients with Krebs cycle enzyme deficiencies, gluconeogenesis defects, carbonic anhydrase VA deficiency, long chain fatty acid oxidation defects and organic acidurias (▶ Chap. 1).

Leigh syndrome is the most frequent clinical presentation of mitochondrial disease in childhood. It was originally a neuropathological diagnosis, consisting of bilateral symmetrical focal lesions in the basal ganglia and brainstem characterised by spongiform change, vacuolation of the neuropil, demyelination, gliosis, necrosis, capillary proliferation and relative preservation of neurons. However since neuropathology is rarely performed nowadays, diagnosis is currently usually based on a characteristic clinical history associated with typical brain magnetic resonance imaging (MRI) features (T2-weighted bilateral symmetrical hyperintense lesions

Table 10.2 Recognised mitochondrial syndromes

Syndrome	Clinical features	Associated gene defect(s)
ADOA	Dominant optic atrophy with progressive vision loss starting in childhood, variably associated with SNHL (*OPA1*) or retinal degeneration (*SSBP1*)	*OPA1*, *SSBP1* (AD)
Alpers-Huttenlocher	Infantile/early childhood onset of developmental delay/regression, intractable epilepsy, +/− liver failure	*POLG*, *CARS2*, *FARS2*, *NARS2*, *PARS2, TWNK* (AR)
Amish lethal microcephaly	Severe microcephaly, micrognathia, hepatomegaly	*SLC25A19* (AR)
ARSACS	Autosomal recessive spastic ataxia of Charlevoix-Saguenay: spasticity, ataxia, muscle wasting, nystagmus, dysarthria	*SACS* (AR)
Ataxia neuropathy spectrum	Ataxia, epilepsy, cognitive impairment, psychiatric symptoms, eye movement disorders, involuntary movements, peripheral neuropathy	*POLG*, *OPA1, TWNK* (AR)
Barth	Cardiomyopathy, skeletal myopathy, short stature, (cyclical) neutropaenia	*TAZ* (X-linked)
Bjornstad	Pili torti, congenital sensorineural hearing loss	*BCS1L* (AR)
Cowchock	Early childhood onset slowly progressive axonal sensorimotor neuropathy +/− SNHL and learning difficulties	*AIFM1*, *COX7B* (X-linked)
DCMA	Dilated cardiomyopathy with ataxia syndrome	*DNAJC19* (AR)
Ethylmalonic encephalopathy	Hypotonia, seizures, abnormal movements, petechiae, acrocyanosis, chronic diarrhoea	*ETHE1* (AR)
Friedreich ataxia	Progressive ataxia, muscle weakness, dysarthria, sensory neuropathy, cardiomyopathy, diabetes mellitus	*FXN* (AR)
GRACILE	Growth retardation, aminoaciduria, cholestasis, iron overload, lactic acidosis, early death	*BCS1L* (AR)
HUPRA	Hyperuricaemia, pulmonary hypertension, renal failure, alkalosis	*SARS2* (AR)
IOSCA	Infantile onset spinocerebellar ataxia	*TWNK* (AR)
Kearns-Sayre syndrome (KSS)	Onset <20y of progressive external ophthalmoplegia, pigmentary retinopathy, cardiac conduction defects, ataxia, high CSF protein	mtDNA deletion (sporadic)
LBSC	Progressive leukoencephalopathy with brainstem and spinal cord calcifications (LBSC) and hearing loss	*KARS1* (AR)
LBSL	Leukoencephalopathy with brainstem and spinal cord involvement and lactate elevation (LBSL): movement disorder starting in childhood/adolescence, spasticity, ataxia	*DARS2* (AR)
Leigh	Subacute necrotising encephalomyelopathy: infantile/early childhood onset of vomiting/feeding difficulties, stepwise neurodevelopmental regression following infection/metabolic stress, dysphagia, hypotonia, dystonia, ataxia, ophthalmoparesis, nystagmus, optic atrophy; bilateral symmetrical MRI lesions involving basal ganglia and/or brainstem	>100 genes (maternal, AR, X-linked, de novo dominant)
LHON	Leber hereditary optic neuropathy: subacute painless vision loss affecting both eyes sequentially, onset teens/20s, male preponderance	*MT-ND1, MT-ND4, MT-ND6* (maternal)
LSFC	Leigh syndrome French Canadian variant: hypotonia, developmental delay, mild facial dysmorphism, chronic well-compensated metabolic acidosis, episodes of severe acidosis and coma associated with high mortality	*LRPPRC* (AR)
LTBL	Leukoencephalopathy with thalamus and brainstem involvement and high lactate (LBTL): infantile onset hypotonia, developmental delay/regression, characteristic MRI brain changes, some cases have milder course with improvement after 2y	*EARS2* (AR)

(continued)

Table 10.2 (continued)

Syndrome	Clinical features	Associated gene defect(s)
MDDS	Mitochondrial DNA depletion syndromes (hepatocerebral, myopathic and encephalopathic variants)	*POLG*, *DGUOK*, *MPV17*, *TWNK*, *TK2*, *SUCLA2*, *SUCLG1*, *RRM2B*, *MGME1* (AR), *SSBP1* (AD or AR)
MEGDEL	3-methylglutaconic aciduria, deafness (SNHL), encephalopathy, Leigh-like disease: hypotonia, feeding difficulties and hepatopathy in infancy, later dystonia and spasticity	*SERAC1* (AR)
MELAS	Mitochondrial encephalomyopathy, lactic acidosis, stroke-like episodes: childhood-onset muscle weakness, migraine headache, vomiting and seizures; stroke-like episodes before 40y (seizures, altered consciousness, hemiparesis, hemianopia); cognitive decline	*MT-TL1* m.3243A>G (80% cases) and other mtDNA point mutations (maternal)
MEMSA	Myoclonic epilepsy, myopathy, sensory ataxia	*POLG* (AR)
MERRF	Myoclonic epilepsy with ragged-red fibres: myoclonus, myopathy, spasticity, epilepsy, ataxia, peripheral neuropathy, cognitive decline, multiple symmetrical lipomata	*MT-TK* and other mtDNA point mutations (maternal), *POLG* (AR)
MIDD	Maternally inherited diabetes and deafness: adult onset SNHL, insulin-dependent diabetes mellitus, macular retinal dystrophy	*MT-TL1* m.3243A>G (maternal)
MIRAS	Mitochondrial Recessive Ataxia Syndrome: Ataxia neuropathy spectrum	*POLG* (AR)
MLASA	Myopathy, lactic acidosis, sideroblastic anaemia	*PUS1*, *YARS2* (AR)
MNGIE	Mitochondrial neurogastrointestinal encephalopathy: adolescent/early adult onset of gastrointestinal dysmotility, peripheral neuropathy, leukoencephalopathy	*TYMP* (AR), may be mimicked by *POLG, RRM2B* (AR) and *MT-TL1* and *MT-TV* (maternal)
Mohr-Tranebjærg	Deafness (SNHL), dystonia, optic neuronopathy (DDON), cognitive decline, psychiatric symptoms	*TIMM8A* (X-linked)
NARP	Neuropathy, ataxia, and retinitis pigmentosa; childhood onset sensory neuropathy, muscle weakness, learning difficulties, visual impairment	*MT-ATP6* (maternal)
PCH6	Pontocerebellar hypoplasia type 6: neonatal onset seizures, hypotonia, severe lactic acidosis at birth (later resolves), progressive microcephaly, developmental stasis from birth	*RARS2* (AR)
Pearson	Infantile onset transfusion-dependent sideroblastic anaemia (later recovers), variably associated with neutropaenia and thrombocytopaenia, exocrine/endocrine pancreatic failure, severe lactic acidosis and hepatic impairment; those who survive early childhood subsequently develop KSS	mtDNA deletion (sporadic)
PEO	Progressive external ophthalmoplegia +/– skeletal myopathy	mtDNA deletion (sporadic), mtDNA point mutations (maternal), *POLG*, *TWNK*, *RRM2B*, *SLC25A4* (AD)
Perrault	Premature ovarian failure, SNHL	*CLPP*, *ERAL1, HARS2*, *LARS2*, *TWNK, HSD17B4* (AR)

Table 10.2 (continued)

Syndrome	Clinical features	Associated gene defect(s)
RIRCD	Reversible infantile respiratory chain deficiency: 'Benign reversible' mitochondrial myopathy causing hypotonia, severe muscle weakness leading to feeding difficulties or respiratory failure, recovery by 12–18 months or	*MT-TE* (maternal inheritance, incomplete penetrance)
	Acute liver failure	*TRMU* (AR)
SANDO	Sensory ataxia, neuropathy, dysarthria, ophthalmoplegia: see Ataxia neuropathy spectrum	*POLG*, *TWNK*, *OPA1* (AR)
SCAE	Spinocerebellar ataxia with epilepsy: see MEMSA	*POLG* (AR)
Sengers	Congenital cataract, hypertrophic cardiomyopathy, muscle weakness, lactic acidosis	*AGK* (AR)
SIFD	Sideroblastic anaemia, B cell immune deficiency, periodic fevers and developmental delay	*TRNT1* (AR)
Wolfram	Diabetes insipidus, diabetes mellitus, optic atrophy, deafness (DIDMOAD)	*WFS1* (AR)

affecting the basal ganglia and/or brainstem) and compatible biochemical findings (lactate elevation in blood and/or cerebrospinal fluid (CSF) and/or documented respiratory chain enzyme deficiency) [2]. Onset is typically in infancy or early childhood with neurodevelopmental regression following an intercurrent viral illness (which may be mild) or other metabolic stress. There is frequently a preceding history of feeding difficulties and vomiting. Neurological findings include bouts of hyper- or hypo-ventilation, hypotonia, spasticity, dystonia, ataxia, tremor, ophthalmoparesis and optic atrophy. Multisystem involvement may include cardiomyopathy, renal tubulopathy and gastro-intestinal dysfunction (vomiting, diarrhoea, constipation, faltering growth). Periods of stability are interspersed by episodes of further neurodevelopmental regression, often without obvious triggers. Progressive brainstem involvement eventually leads to death from central respiratory failure. Late onset may occur, including in adulthood in rare cases. Leigh syndrome is biochemically and genetically heterogeneous and can be caused by mutations in more than 100 genes (on two genomes, mitochondrial and nuclear) encoding mitochondrial proteins. The neurodegenerative changes are frequently accompanied by multisystem features, which can be helpful in differential diagnosis [3]. Some geographical isolates may allow a more rapid diagnosis in some communities, such as the Leigh syndrome French Canadian variant originating from the Saguenay-Lac-Saint-Jean region of Quebec [4]. Disease mechanisms leading to Leigh syndrome include defects of OXPHOS subunits and assembly, mtDNA maintenance and gene expression, cofactor biosynthesis and mitochondrial membrane lipid remodeling, quality control and dynamics [5].

3-Methylglutaconic aciduria, deafness, encephalopathy and Leigh-like disease (MEGDEL) is a form of Leigh syndrome caused by recessive *SERAC1* mutations leading to defective phosphatidylglycerol remodelling in the mitochondrial membrane [6]. A full description is given in ▶ Chap. 35.

The **Pearson marrow-pancreas syndrome** typically presents shortly after birth with lactic acidosis and a severe transfusion-dependent sideroblastic anaemia, variably associated with neutropaenia and/or thrombocytopaenia. Transfusion requirement usually resolves by 2 years of age, reflecting clearance of the responsible large-scale mtDNA deletion from rapidly dividing blood cells. There is a 'common' ~4.9 kb heteroplasmic mtDNA deletion but many other mtDNA deletion species have been reported. A high mortality in the first 5 years of life is related to liver failure and/or overwhelming acidosis. Those who survive have a progressive multisystem disease course (associated with accumulation of mtDNA deletions in non dividing tissues), including renal tubulopathy (leading to severe electrolyte losses and sometimes progressing to end-stage kidney disease), cardiomyopathy, cardiac conduction defects (complete heart block), pancreatic exocrine and/or endocrine insufficiency, hypothyroidism, hypoparathyroidism and adrenal insufficiency, and eventually develop the neurological features of Kearns-Sayre syndrome (see below) [7].

The **mitochondrial DNA depletion syndromes (MDDS)** are a group of encephalomyopathic, myopathic and hepatocerebral syndromes which usually present in infancy or early childhood and in most cases are rapidly progressive, leading to death in infancy or childhood. These are autosomal recessive disorders of mtDNA maintenance caused by mutations in genes

involved directly in mtDNA replication or in mitochondrial nucleoside salvage, resulting in progressive mtDNA depletion in affected tissues [8, 9]. Infants with **hepatocerebral MDDS** (caused by mutations in *DGUOK* (► Chap. 36, *POLG*, *TWNK, MPV17* or *SUCLG1*) present with hepatic dysfunction manifesting as persistent vomiting, hypoglycaemia and sometimes hepatomegaly, with associated lactic acidosis. Neurological involvement may present as roving eye movements or developmental delay and/or regression. Alpers-Huttenlocher syndrome (see below) is a form of hepatocerebral MDDS. **Myopathic MDDS** (caused by *TK2* mutations leading to thymidine kinase deficiency, ► Chap. 36) presents in infancy with hypotonia and muscle weakness, often with involvement of the bulbar musculature leading to feeding difficulties. Affected infants also have lactic acidosis and markedly elevated creatine kinase. Death from respiratory failure occurs in early childhood owing to progressive respiratory muscle weakness, although survival to teenage years has been reported. **Encephalomyopathic MDDS** (caused by mutations in *SUCLA2*, *SUCLG1*, *RRM2B*, *ABAT* or *MGME1*) presents with global developmental delay, hypotonia and muscle weakness in infancy, variably associated with sensorineural hearing loss (SNHL), dystonia, Leigh-like MRI lesions and methylmalonic aciduria (*SUCLA2*), fatal infantile lactic acidosis with methylmalonic aciduria (*SUCLG1*) (► Chap. 18), or prominent renal involvement (*RRM2B*). The most recently reported cause of MDDS involved dominant or recessive mutations in *SSBP1* encoding the single stranded DNA binding protein, which were associated with optic atrophy and variable retinopathy or a multisystem disorder including retinal dystrophy, deafness, hypertrophic cardiomyopathy, nephropathy, ataxia and growth retardation [10, 11].

Alpers-Huttenlocher syndrome (progressive neuronal degeneration of childhood with epilepsy, PNDE) is a form of hepatocerebral MDDS usually caused by recessive *POLG* mutations [12], and rarely by mutations in the Twinkle helicase (encoded by *TWNK*) or the mitochondrial cysteinyl-, phenylalanyl-, asparaginyl- and prolyl-tRNA synthetases encoded by *CARS2*, *FARS2, NARS2* and *PARS2* respectively [13–15] (► Chap. 37). There is a classical clinical triad of intractable seizures often resistant to multiple antiepileptic drugs, developmental regression and (terminally) liver failure. Seizures may be focal, multifocal or generalised. Disease progression may be rapid, with intractable seizures or liver failure leading to death within weeks or months, or may follow a more indolent course. Rarely there may be no further progression for several years. Liver failure may be triggered by sodium valproate treatment, or occur spontaneously. EEG in the early stages of disease may be pathognomonic, showing unilateral occipital rhythmic high-amplitude delta with superimposed (poly) spikes (RHADS) [16].

Reversible infantile respiratory chain deficiency (RIRCD) may present as a myopathy or a hepatopathy. Infants with so-called 'benign reversible' mitochondrial myopathy develop a rapidly progressive myopathy associated with hypotonia, profound muscle weakness and severe lactic acidosis at a few weeks of age. Nasogastric or gastrostomy feeding is usually required, and some affected infants need ventilatory support for up to 12–18 months. Gradual recovery starts from ~6 months. Two maternally inherited homoplasmic mtDNA point mutations, m.14674T>C/G in the *MT-TE* gene, have been linked to benign reversible mitochondrial myopathy [17]. In other infants transient acute liver failure is caused by recessive mutations in *TRMU*, encoding an enzyme responsible for 2-thiolation of uridine on the wobble positions of the mitochondrial tRNAs for lysine, glutamate and glutamine, an essential post-transcriptional modification needed for accurate and efficient synthesis of the 13 mtDNA-encoded OXPHOS proteins [18]. Spontaneous recovery has been noted in some infants with *TRMU* mutations following supportive care, but others need liver transplantation. The underlying molecular mechanisms responsible for spontaneous remission of RIRCD have not yet been unravelled.

Infantile onset coenzyme Q_{10} (CoQ_{10}) biosynthesis deficiency has now been linked to 10 gene defects and typically presents with multisystem disease including a rapidly progressive nephropathy (frequently progressing to end-stage kidney disease) variably associated with SNHL, optic atrophy, ataxia, dystonia, weakness and stroke-like episodes [19]. Other children with CoQ_{10} deficiency may present with steroid-resistant nephrotic syndrome, either in isolation or associated with seizures and/or learning difficulties. Primary CoQ_{10} deficiency is clinically heterogeneous and other reported phenotypes in later childhood include encephalomyopathy with seizures and recurrent myoglobinuria, cerebellar ataxia and isolated myopathy. Prompt diagnosis and treatment of disorders of CoQ_{10} biosynthesis with high-dose exogenous CoQ_{10} supplementation may result in a good outcome, although some cases have antenatal onset and a poor response to treatment.

Barth syndrome [20] and **Sengers syndrome** [21] are two syndromic cardiomyopathies presenting in infancy with methylglutaconic aciduria. Both are described in ► Chap. 35.

Isolated organ involvement

Not all infants with mitochondrial disease present with classical syndromes or multisystem disease. Some have isolated organ involvement, e.g. epileptic encephalopathy [22], hypertrophic cardiomyopathy [23] or acute

liver failure [24]. Patients with mutations in *LARS1* (encoding a cytoplasmic leucyl-tRNA synthetase) also present with acute liver failure in the first few months of life, which may be mistaken for mitochondrial disease. Additional symptoms include anaemia, renal tubulopathy, developmental delay, seizures, failure to thrive and deterioration of liver function with minor illness [25] (▶ Chap. 37). Another increasingly recognized phenotype of mitochondrial disease is early-onset leukoencephalopathy. Causes include deficiencies of mitochondrial aminoacyl tRNA synthetases (DARS2, EARS2, AARS2, MARS2 and KARS1), iron-sulfur cluster assembly 2 (ISCA2) and other proteins involved in lipoic acid metabolism, complex IV assembly factors (COA7 and COA8), and complex I subunits and assembly factors (■ Table 10.3).

10.1.2 Presentation in Childhood and Adolescence

Kearns-Sayre syndrome (KSS) is usually sporadic and caused by single large-scale rearrangements of mtDNA (most commonly the ≈4.9 kb 'common' deletion that is also frequently seen in Pearson syndrome) [7], but may occasionally be autosomal recessive when it is caused by nuclear-encoded defects of mtDNA maintenance (e.g. *RRM2B* mutations) associated with multiple mtDNA deletions [26]. Clinically there is a triad of progressive external ophthalmoplegia (PEO), pigmentary retinopathy and onset <20 years, with at least one of the following: heart block, cerebellar ataxia or raised CSF protein (>1 g/l). Multisystem disease manifestations include car-

■ **Table 10.3** Specific MRI brain findings in mitochondrial disorders

Syndrome	Characteristic MRI changes
Cavitating leukoencephalopathy	Cystic leukoencephalopathy (*NDUFV1, COA8, IBA57, ISCA2, NFU1, LYRM7*); with tigroid-like changes (*NDUFA2*); with posterior predominance (COA8); with spinal cord hypotrophy (*COA7*); with succinate peak on MRS (*SDHA, SDHAF1*)
Kearns-Sayre syndrome	Bilateral high-signal lesions in subcortical white matter, globus pallidus, thalamus and brain stem; cerebral, cerebellar and brainstem atrophy; basal ganglia calcification (CT)
LBSC	Leukoencephalopathy with brainstem and spinal cord calcifications (*KARS1*)
LBSL	T2-weighted and FLAIR high signal intensity in cerebral subcortical, periventricular and deep white matter, posterior limbs of internal capsules, centrum semiovale, medulla oblongata, intraparenchymal trajectory of trigeminal nerves, deep cerebellar white matter, and spinal cord dorsal column and lateral cortico-spinal tracts (*DARS2*)
Leigh	T2-weighted focal symmetrical hyperintensities affecting basal ganglia, variably extending into midbrain and brainstem
LTBL	T2-weighted symmetrical hyperintensities in deep cerebral white matter (sparing periventricular rim), thalami, midbrain, pons, medulla oblongata and cerebellar white matter; increased lactate on proton MRS (*EARS2*)
MEGDEL	Bilateral basal ganglia involvement, especially putamina, but early sparing of dorsal putamina leading to a characteristic putaminal 'eye' without signal alteration; later progressive putaminal involvement (*SERAC1*)
MELAS	T2-weighted hyperintense lesions in grey and subcortical white matter of temporal, parietal, and occipital lobes, sparing deep white matter and crossing vascular boundaries, +/− basal ganglia calcification, generalised cerebral atrophy (*MT-TL1*)
MNGIE	Asymptomatic leukoencephalopathy (demyelination) (*TYMP*)
NUBPL deficiency	Complex leukoencephalopathy involving deep cerebral white matter, basal ganglia, thalami and corpus callosum, with progressive cerebellar atrophy (*NUBPL*)
PCH6	Severe progressive atrophy of cerebellum and pons; cerebral cortex may also be affected (*RARS2*)
SDH deficiency	Succinate peak on ^{1}H-magnetic resonance spectroscopy

Where changes are associated with a specific gene, this is given in brackets. *LBSC* leukoencephalopathy with brainstem and spinal cord calcifications, *LBSL* leukoencephalopathy with brainstem and spinal cord involvement and lactate elevation, *LTBL* leukoencephalopathy with thalamus and brainstem involvement and high lactate, *MEGDEL* 3-methylglutaconic aciduria, deafness, encephalopathy and Leigh-like disease, *MELAS* mitochondrial encephalomyopathy with lactic acidosis and stroke-like episodes, *MNGIE* mitochondrial neurogastrointestinal encephalomyopathy, *PCH6* pontocerebellar hypoplasia type 6, *SDH* succinate dehydrogenase

diac conduction defects, short stature, renal tubulopathy, dysphagia, gastrointestinal dysmotility, pancreatitis, diabetes mellitus, SNHL and cognitive deficits (learning difficulties or dementia) [7].

Mitochondrial encephalomyopathy with lactic acidosis and stroke-like episodes (MELAS) is a maternally inherited multisystem disorder usually manifesting in mid-late childhood [27]. Most (≈80%) cases have the common m.3243A>G mtDNA point mutation in *MT-TL1*, although most individuals with this mutation (which is present in ≈1 in 400 of the White European population) never develop symptoms of MELAS [28]. Stroke-like episodes typically first occur in late childhood or adolescence but may commence in adult life. Migraine-like headache with vomiting and seizures may herald the stroke-like episodes, which may be associated with hemianopia or cortical blindness. Other clinical features include myopathy, cognitive decline, myoclonus, ataxia, episodic coma, optic atrophy, short stature, SNHL and hypertrophic cardiomyopathy. Intrafamilial variability is well recognised [27].

Myoclonic epilepsy with ragged red fibres (MERRF) is a maternally inherited disorder frequently causing myoclonus and/or generalised seizures and ataxia, with onset usually in childhood or adolescence. Approximately 80% of cases have a 'common' mtDNA mutation, m.8344A>G in *MT-TK*. There is enormous clinical heterogeneity, even within families, and other features include SNHL, optic atrophy, pigmentary retinopathy, nystagmus, ophthalmoparesis, dysarthria, exercise intolerance, cardiomyopathy, multiple symmetrical lipomas and psychiatric disturbance [29].

Neuropathy, ataxia and retinitis pigmentosa (NARP) is a maternally inherited disorder caused by m.8993T>G/C mutations in *MT-ATP6* also associated with maternally inherited Leigh syndrome [30]. Symptoms of sensory neuropathy, muscle weakness, epilepsy and ataxia usually start in late childhood or early adult life. Later features include retinitis pigmentosa and cognitive decline. Short stature, SNHL, progressive external ophthalmoplegia and cardiac conduction defects may occur. The severity and extent of disease depend (at least partly) on the percentage and distribution of mutant mtDNA, as discussed below. Other mtDNA point mutations in *MT-ATP6* and *MT-ATP8* may also cause NARP, and the clinical spectrum extends to isolated peripheral neuropathies indistinguishable from Charcot-Marie-Tooth disease [31].

Leber hereditary optic neuropathy (LHON) presents in adolescence or early adulthood with bilateral painless subacute loss of central vision due to optic neuropathy [32]. LHON is maternally inherited with incomplete penetrance and an extreme male preponderance. In most cases optic neuropathy is the only clinical feature, but in rare 'LHON-plus' families associated symptoms may include dystonia, tremor, cardiac conduction defects or psychiatric disturbance. Three usually homoplasmic mtDNA mutations (m.11778G>A, m.3460G>A and m.14484T>C) in complex I subunit genes account for greater than 90% of cases, but other mtDNA mutations have also been reported (▶ www.mitomap.org).

Myoclonic epilepsy, myopathy, sensory ataxia (MEMSA) includes recessive *POLG*-related epilepsy syndromes previously known as spinocerebellar ataxia with epilepsy (SCAE) and mitochondrial recessive ataxia syndrome (MIRAS) [12]. Clinical features include ataxia and epilepsy, with or without myoclonus, and epilepsia partialis continua. Seizures are difficult to treat and episodes of acute encephalopathy or status epilepticus may occur.

Mitochondrial neurogastrointestinal encephalomyopathy (MNGIE) typically presents in adolescence or early adult life with gastrointestinal dysmotility (dysphagia, early satiety, post-prandial nausea and vomiting, bloating, constipation, diarrhoea or repeated episodes of pseudo-obstruction), severe cachexia, ptosis and/or ophthalmoplegia, proximal myopathy and demyelinating peripheral neuropathy [33]. A florid leukoencephalopathy revealed by brain MRI is usually relatively asymptomatic. The main underlying cause is thymidine phosphorylase deficiency (▶ Chap. 36) leading to impaired mtDNA maintenance because of intramitochondrial nucleoside imbalance, resulting in accumulation of multiple mtDNA deletions and point mutations and progressive mtDNA depletion. Mutations in *POLG*, *RRM2B*, *MT-TL1* and *MT-TV* have also been reported to present with a MNGIE-like phenotype including chronic intestinal pseudo-obstruction and neuropathy.

Isolated organ involvement in childhood and adolescence may include skeletal myopathy (exercise intolerance +/− rhabdomyolysis) and steroid-resistant nephrotic syndrome (in disorders of coenzyme Q_{10} biosynthesis).

10.1.3 Adult-Onset Disorders

Adult-onset mitochondrial disorders are frequently (in >50% of cases) caused by mtDNA mutations. Many of the disorders which typically present in late childhood or adolescence (including KSS, MELAS, MERRF, MEMSA, MNGIE, NARP and LHON) may have onset in early adult life. The full MELAS syndrome is seen in fewer than 10% of patients with the m.3243A>G point mutation [28]. The most frequent presentation associated with this mutation is **maternally inherited diabetes and deafness (MIDD)**. Diabetes (often progressing to insulin requirement) and SNHL usually start in the fourth decade [34]. Other frequent problems experienced by individuals with the m.3243A>G mutation include cardiomyopathy (which may be a cause of sudden unexpected death), nephropathy (focal segmental glomeru-

losclerosis), gastrointestinal dysmotility, short stature and macular retinal dystrophy (frequently diagnosed in the diabetic retinopathy screening clinic in patients with undiagnosed MIDD) [28].

One of the most frequent adult presentations of mitochondrial myopathy is **progressive external ophthalmoplegia (PEO)**, which may or may not be accompanied by a proximal skeletal myopathy or extend to complete KSS. PEO may be caused by sporadic mtDNA deletions, be maternally inherited as a result of mtDNA point mutations, or be dominantly inherited (due to mutations in one of several nuclear genes involved in mtDNA maintenance, most commonly *POLG*). Patients with *POLG* mutations with adult onset may present with dominant PEO, recessive epilepsy syndromes or with **sensory ataxic neuropathy, dysarthria and ophthalmoparesis (SANDO)** also known as **ataxia neuropathy syndrome (ANS)** [12, 35].

Isolated organ involvement in adults with mitochondrial disease includes myopathy (rarely rhabdomyolysis), epilepsy (especially myoclonic or epilepsia partialis continua) [36] and peripheral neuropathy (e.g. Charcot-Marie-Tooth hereditary neuropathy type 2A2, an autosomal dominant axonal peripheral sensorimotor neuropathy caused by mutations in *MFN2* encoding an outer mitochondrial membrane GTPase essential for mitochondrial fusion) [37]. Mutations in two dually localised aminoacyl tRNA synthetases KARS1 and GARS1, which act in both the mitochondria and the cytosol, as well as four cytosolic aminoacyl tRNA synthetases AARS1, HARS1, MARS1 and YARS1, have also been reported to cause peripheral neuropathies, mostly axonal and affecting both motor and sensory function, whilst defects of WARS1 cause hereditary motor neuropathy [38]. In addition, two cytosolic aminoacyl tRNA synthetases (DARS1 and RARS1) have been linked to hypomyelination and spasticity (▶ Chap. 39). Sometimes the presence of additional clinical features such as acute epileptic encephalopathy (e.g. in QARS1 deficiency) may lead to confusion with primary mitochondrial disorders.

10.2 Metabolic Derangement

Oxidative phosphorylation (OXPHOS) is a central and essential part of mitochondrial energy metabolism [39]. One function of OXPHOS is the oxidation of reduced REDOX molecules NADH and $FADH_2$ by using molecular oxygen (O_2) as electron acceptor. These REDOX molecules are formed by numerous oxidation reactions in the cell including glycolysis, but predominantly by the degradation of pyruvate, fatty acids and amino acids in mitochondria. By harnessing a proton gradient that is generated by respiratory chain enzyme complexes I, III, and IV, mitochondrial ATP synthase (complex V) produces the majority of the cellular energy molecule ATP (◘ Fig. 10.1). Insufficient ATP supply affects highly energy dependent tissues most severely. Owing to the importance of mitochondrial energy metabolism in multicellular organisms, a complete loss of OXPHOS is not observed in systemic human disease; there is always some residual function left. The amount of residual activity is often variable in different tissues, which can complicate the diagnosis of OXPHOS diseases.

In the absence of oxidative mitochondrial metabolism, human cells can survive by using ATP from anaerobic glycolysis. This fermentative pathway has two major disadvantages: it is approximately 20-fold less efficient and it generates the reduced molecule lactate as a final product, which has to be excreted from the cell. Depending on glutamate availability, pyruvate can be metabolised to alanine by a specific transaminase and secreted as well. Lactate, pyruvate and alanine are the typical products of anaerobic glycolysis and elevation of these compounds in body fluids (e.g. blood, urine and CSF) is a typical but not a specific finding in OXPHOS defects. (▶ Sect. 1.4.13).

Each OXPHOS enzyme consists of multiple polypeptide subunits, in total approximately 90. OXPHOS complexes I, III, IV, and V also contain subunits that are encoded by the mitochondrial DNA, which is a unique feature of the mitochondrial organelle within the cell. The formation of these 13 protein subunits depends on a complex machinery required for replication and transcription of mitochondrial DNA and translation on mitochondrial ribosomes [40]. These processes necessitate in total more than 200 enzymes, ribosomal RNAs (rRNAs) and transfer RNAs (tRNAs) (▶ Chap. 39). Furthermore, OXPHOS depends on numerous cofactors, e.g. coenzyme Q_{10}, iron-sulphur clusters that are also involved in lipoic acid synthesis (◘ Fig. 10.2 and ▶ Chap. 23), haem and copper, that all need to be synthesised and/or transported to the OXPHOS system. Furthermore specialised membrane lipids such as mitochondrion-specific cardiolipin also play an essential role in mitochondrial health, being important for the formation of cristae structure and activation of OXPHOS enzymes [41] (▶ Chap. 35). In addition, the formation of all of the OXPHOS enzymes depends on numerous assembly factors. Proper maintenance of the organelle including import of the cytosolically formed subunits, protein turnover, fission, and fusion are prerequisites for normal OXPHOS function. Last but not least, OXPHOS is sensitive to toxic metabolites, which might be formed by other mitochondrial pathways or perturbations of mitochondrial reactive oxygen species (ROS)-defence [42, 43].

The list of factors that influence OXPHOS is continuously expanding and the final number can only be estimated at present. Taking into account the number of more than 1500 mitochondrial proteins in the human mitochondrial proteome and the expanding list of other relevant factors, up to 10% of the human proteome might be involved in this metabolism [44]. In summary, mitochondria are complex organelles with multitudinous functions, and consequently mitochondrial disease may be associated with diverse metabolic derangements.

10.3 Genetics

OXPHOS disorders comprise defects of two genomes, the maternally inherited mitochondrial ~16.6 kb genome with known mutations in all 37 encoded genes, and currently 260 nuclear encoded genes, a number that is increasing exponentially. Mitochondrial disease may be inherited by any mode of inheritance: maternal (mtDNA), autosomal recessive, autosomal dominant, X-linked or sporadic (de novo mutations). Furthermore, somatic mutations may occur in both genomes.

10.3.1 Mitochondrial DNA Mutations

Any type of mutation can occur in mtDNA, including point mutations, small rearrangements, large-scale rearrangements (single deletions and duplications, multiple deletions), or copy number reduction known as mtDNA depletion. Several hundred different pathogenic mtDNA mutations have been reported and catalogued in the MITOMAP online database (► www.mitomap.org). Mutations can either affect single OXPHOS subunits, tRNAs, rRNAs, or a combination of these. In contrast to single OXPHOS subunit defects, mutations affecting the tRNA and rRNA genes usually result in a decrease of multiple OXPHOS enzymes.

Peculiarities of mitochondrial genetics include the presence of hundreds or even thousands of copies of mtDNA molecules in individual cells, leading to the phenomena of heteroplasmy (co-existence of mutant and wild-type mtDNA in variable percentages) and homoplasmy (100% wild-type or mutant mtDNA). Pathogenic relevance of a mtDNA mutation depends on the proportion of mutated DNA. A minimal amount of wild-type mtDNA is necessary to maintain OXPHOS function in cells in a particular tissue (threshold effect). This minimal amount varies between different mtDNA mutations. Some mutations can be found in a homoplasmic or nearly homoplasmic state (e.g. LHON mutations). For many of the more common mtDNA mutations the threshold is typically between 80–95% mutation load (e.g. MELAS, MERRF mutations), while for large deletions the level is often between 50–70% deleted mtDNA. For a few mutations, e.g. anticodon mutations of mitochondrial tRNAs, a dominant-negative effect has been observed, with pathogenic relevance at a mutation load of less than 20% [45].

10.3.2 Nuclear Gene Defects

Mutations in at least 260 nuclear genes have been identified that result in either single or combined OXPHOS defects [1]. A classification of nuclear gene defects causing mitochondrial disease is presented in ◘ Table 10.4.

Mutations of OXPHOS structural subunits or OXPHOS assembly factors typically result in single OXPHOS enzyme defects (◘ Table 10.4). Mutations of complex I structural subunits and complex IV assembly factors appear to be relatively frequent causes of isolated complex I or complex IV deficiency, respectively.

Defects of mtDNA maintenance include mutations in factors needed for mtDNA replication and enzymes involved in the metabolism of nucleotides necessary for mtDNA replication [8]. Defects in this group of genes result either in mtDNA depletion (*SUCLA2*, *SUCLG1*, *SSBP1*) or in mtDNA depletion and/or multiple deletions of mtDNA (*POLG*, *POLG2*, *TWNK*, *MGME1*, *DNA2*, *RNASEH1*, *DGUOK*, *TYMP*, *MPV17*, *SLC25A4*, *RRM2B*, *TK2*, *MFN2*, *OPA1*, *SPG7*, *AFG3L2*, *CHCHD10*, *SAMHD1*, *C1QBP*) (◘ Table 10.4). The consequence of depletion of mtDNA or accumulation of multiple mtDNA deletions is usually a reduction of OXPHOS complexes I, III, IV and V, although in the early stages of disease there may be an isolated deficiency of complex I or complex IV.

Defects of mitochondrial gene expression (RNA processing, mRNA and tRNA modification) also lead to reduction of OXPHOS enzyme complexes I, III, IV and V, as do the large group of **defects of mitochondrial translation,** including mutations in mitochondrial ribosomal proteins, aminoacyl tRNA synthetases and factors needed for initiation, elongation and regulation of translation (◘ Table 10.4) [40].

Defects of cofactors and their biosynthesis comprise a fast growing subgroup of OXPHOS defects, including mutations in factors required for biosynthesis and/or transport of CoQ_{10}, Fe-S clusters (◘ Fig. 10.2 and ► Chap. 23), haem, riboflavin (► Chap. 12), copper and iron (► Chap. 34) [1] (◘ Table 10.4).

Defects of mitochondrial membrane lipids constitute another expanding disease group that includes *TAZ*, *AGK* and *SERAC1* mutations, leading to Barth, Sengers and MEGDEL syndromes respectively (◘ Table 10.4 and ► Chap. 35) [41].

Table 10.4 Genetic defects resulting in OXPHOS deficiency (37 mitochondrial and 260 nuclear genes)

Type	Subtype	Inheritance (affected genes)	OXPHOS defect (typical)
OXPHOS subunit	Complex I	**AR** *(NDUFA2, NDUFA6, NDUFA8, NDUFA9, NDUFA10, NDUFA11, NDUFA12, NDUFA13, NDUFB3, NDUFB8, NDUFB9, NDUFB10, NDUFS1, NDUFS2, NDUFS3, NDUFS4, NDUFS6, NDUFS7, NDUFS8, NDUFV1, NDUFV2);* **mtDNA** *(MT-ND1, MT-ND2, MT-ND3, MT-ND4, MT-ND4L, MT-ND5, MT-ND6);* **X-linked** *(NDUFA1, NDUFB11)*	CI
	Complex II	**AR** *(SDHA, SDHB, SDHD);* **somatic in tumours** *(SDHC)*	CII
	Complex III	**AR** *(CYC1, UQCRB, UQCRC2, UQCRFS1, UQCRQ);* **mtDNA** *(MT-CYB)*	CIII
	Complex IV	**AR** *(COX4I1, COX4I2, COX5A, COX6A1, COX6A2, COX6B1, COX8A, NDUFA4);* **mtDNA** *(MT-CO1, MT-CO2, MT-CO3;* **X-linked** *(COX7B)*	CIV
	Complex V	**AR** *(ATP5F1A, ATP5F1E, ATP5F1D);* **mtDNA** *(MT-ATP6, MT-ATP8)*	CV
Assembly factor	Complex I	**AR** *(ACAD9, FOXRED1, NDUFAF1, NDUFAF2, NDUFAF3, NDUFAF4, NDUFAF5 (=C20orf7), NDUFAF6 (=C8orf38), NDUFAF8, SFXN4, TIMMDC1, TMEM126A, TMEM126B)*	CI
	Complex II	**AR** *(SDHAF1);* **somatic in tumours** *(SDHAF2)*	CII
	Complex III	**AR** *(BCS1L, LYRM7, TTC19, UQCC2, UQCC3)*	CIII
	Complex IV	**AR** *(CEP89, COA3, COA5, COA7, COA8, COX14 (=C12orf62), COX20 (=FAM36A), FASTKD2, PET100, PET117, SURF1)*	CIV
	Complex V	**AR** *(ATPAF2, ATP5MD (=USMG5), TMEM70)*	CV
	Multiple complexes	**AR** *(OXA1L)*	CI, CIV, CV
mtDNA replication	Nucleotide metabolism	**AR** *(***ABAT, *DGUOK, *MPV17, **SAMHD1, ***SUCLA2, ***SUCLG1, *TK2, *TYMP);* **AR and AD** *(*RRM2B, *SLC25A4)*	CI, CIII, CIV, CV
	mtDNA replication	**AD** *(**DNA2, **POLG2);* **AR** *(*MGME1, *RNASEH1);* **AR and AD** *(*POLG, ***SSBP1, *TWNK,)*	CI, CIII, CIV, CV
mtDNA transcription	Regulation	**AR** *(LRPPRC)*	CIV
	RNA processing	**AR** *(MTPAP, ELAC2);* **X-linked** *(HSD17B10)*	CI, CIII, CIV, CV
Mitochondrial translation	Transfer RNA	**mtDNA** *(MT-TA, MT-TC, MT-TD, MT-TE, MT-TF, MT-TG, MT-TH, MT-TI, MT-TK, MT-TL1, MT-TL2, MT-TM, MT-TN, MT-TP, MT-TQ, MT-TR, MT-TS1, MT-TS2, MT-TT, MT-TV, MT-TW, MT-TY)*	CI, CIII, CIV, CV
	tRNA modification	**AR** *(ERAL1, GTPBP3, MRM2, MTO1, NSUN3, PNPT1, PUS1, THG1L, TRIT1, TRMT5, TRMT10C, TRMU, TRNT1)*	CI, CIII, CIV, CV
	tRNA loading	**AR** *(AARS2, CARS2, DARS2, EARS2, FARS2, GATB, GATC, GARS1, HARS2, IARS2, KARS1, LARS2, MARS2, NARS2, QRSL1, PARS2, RARS2, SARS2, TARS2, VARS2, WARS2, YARS2)*	CI, CIII, CIV, CV
	Start-tRNA	**AR** *(MTFMT)*	CI, CIII, CIV, CV
	Ribosomal RNA	**mtDNA** *(MT-RNR1, MT-RNR2)*	CI, CIII, CIV, CV
	Ribosomal protein	**AR** *(MRPL3, MRPL12, MRPL24, MRPL44, MRPS2, MRPS7, MRPS14, MRPS16, MRPS22, MRPS23, MRPS25, MRPS28, MRPS34, PTCD3)*	CI, CIII, CIV, CV
	Regulation	**AR** *(C12orf65, GFM1, GFM2, GUF1, RMND1, TACO1, TSFM, TUFM)*	CI, CIII, CIV, CV

(continued)

Table 10.4 (continued)

Type	Subtype	Inheritance (affected genes)	OXPHOS defect (typical)
Cofactor	Coenzyme A	**AR** *(SLC25A42)*	
	Coenzyme Q_{10}	**AR** *(COQ2, COQ4, COQ5, COQ6, COQ7, COQ8A, COQ8B, COQ9, PDSS1, PDSS2)*	CI + III, CII + III
	Copper	**AR** *(COA6, COX10, COX15, SCO1, SCO2)*	CIV
	Haem	**AD** *(CYCS, PPOX)*; **AR** *(SLC25A38)*; ***X*-linked** *(HCCS)*	CII, CIII, CIV
	Iron-sulphur cluster	**AR** *(BOLA3, FDX1L, FDXR, FXN, GLRX5, HSCB, HSPA9, IBA57, ISCA1, ISCA2, ISCU, LYRM4, NFS1, NFU1, NUBPL)*	CI, CII, (CIII)
	Lipoic acid	**AR** *(LIAS, LIPT1, LIPT2, MECR)*	CI, CII
	Niacin	**AR** *(NADK2, NAXD, NAXE)*	CI-CV
	Riboflavin	**AR** *(FLAD1, SLC25A32)*	CI, CII
	S-Adenosyl methionine	**AR** *(SLC25A26)*	CI, CIII, CIV, CV
Mitochondrial homeostasis	Fission	**AD and AR** *(DNM1L, GDAP1)*; **AR** *(MIEF2, MFF, STAT2)*	CI-CV
	Fusion	**AR** *(MSTO1, NME3, SLC25A46, YME1L1)*; **AR and AD** *(**MFN2, **OPA1)*	CI, CIII, CIV, CV
	Lipid	**AR** *(AGK, PISD, SERAC1)*; **X-linked** *(TAZ)*	CI, CIII, CIV, CV, ANT
	Lipid?/Protein import?	**AR** *(DNAJC19)*	CI-CV
	Protein import	**AR** *(GFER, PAM16, PMPCA, TIMM22, TIMM50, TOMM70, XPNPEP3)*; **X-linked** *(AIFM1, TIMM8A)*	CI-CV
	Calcium import	**AR** *(MICU1, MICU2)*	
	Quality control	**AR** *(CLPP, HTRA2, LONP1, MIPEP, PMPCB, PITRM1, SACS)*; **AR and AD** *(**AFG3L2, CLPB, HSPD1, **SPG7)*	CI-CV
	MICOS assembly	**AR** *(MICOS13 (=QIL1 = C19orf70))*	
Inhibitor	Inhibitor of mitochondrial protein import	**AD** *(HTT)*	CI-CV
	Toxic metabolite	**AR** *(D2HGDH, L2HGDH, ECHS1, ETHE1, HIBCH, SLC25A1, SQOR, TXN2, TXNIP)*; **somatic in tumours** *(IDH2)*	CI-CV
Unknown	–	**AD** *(**CHCHD10)*; **AR** *(C1QBP, FBXL4, RTN4IP1, TMEM65)*; **AR and AD** *(ATAD3A, OPA3)*	CI, CIII, CIV, CV

Mutations in these genes are associated with *mtDNA depletion and multiple mtDNA deletions; **multiple mtDNA deletions; ***mtDNA depletion

AD autosomal dominant, *AR* autosomal recessive, *CI-CV* complexes I, II, III, IV, and V, *ANT* adenine nucleotide translocator

An increasing number of defects are related to the maintenance of mitochondrial organelles, which is a prerequisite for OXPHOS function. This group includes defects of the mitochondrial protein import machinery, mitochondrial solute import, mitochondrial dynamics, and quality control (Table 10.4). It is anticipated that next generation sequencing will lead to a rapid increase in recognition of this group of disorders, since several of these defects mainly affect the central nervous system, which is difficult to assess using conventional functional studies.

Toxic metabolites of several mitochondrial metabolic pathways can cause either isolated or combined OXPHOS deficiency (Table 10.4) [42, 43].

X-chromosomal OXPHOS defects reported to date include mutations in eight X-chromosomal genes (*NDUFA1, NDUFB11, COX7B, HSD17B10, HCCS, TAZ, TIMM8A, AIFM1*). Remarkably, X-chromosomal OXPHOS defects group into three patterns of disease manifestation: (i) only heterozygous females are affected and presumably disease is embryonic lethal in affected males (*NDUFB11*, *COX7B*, *HCCS*); (ii) defects occur in both sexes (*HSD17B10*); or (iii) disease only reported in males or with very mild phenotype in females (*NDUFA1*, *TAZ*, *TIMM8A*, *AIFM1*).

10.3.3 Frequency of Mutations

Precise data on the frequency of individual OXPHOS defects are not available since patient registries often contain incomplete information and the genetic diagnosis is missing in numerous patients. Several studies have indicated that mtDNA mutations account for only 15–30% of OXPHOS defects in childhood. In adult patients the proportion of patients with mtDNA mutations is much higher. Among mtDNA mutations, the 'common' ~4.9 kb deletion m.8483_13459del4977 is frequently found in patients with Pearson and Kearns-Sayre syndromes or PEO. The m.3243A>G mutation, which is the most frequent cause of MELAS, is also associated with other clinical features as discussed above and is found at low mutational load in 0.2% of the European population, as is the homoplasmic m.1555A>G mutation that predisposes to profound SNHL following exposure to aminoglycoside antibiotics [46]. The m.8993T>G mutation causing maternally inherited Leigh syndrome and NARP and the m.8344A>G mutation causing MERRF are also relatively frequent. The frequent mutations for LHON (m.3460G>A, m.14484T>C, and m.11778G>A) are detectable in 0.3% of all mitochondrial genomes [47].

The frequency of nuclear DNA mutations is often related to founder events and differs between populations. In our experience mutations in *POLG* and *SURF1* are most prevalent [48, 49], but most gene defects causing primary mitochondrial disease are rare or ultra-rare.

10.4 Diagnostic Tests

The starting point in diagnostics of OXPHOS defects should be a detailed evaluation of the clinical and family history including analysis of a three-generation pedigree. Diagnosis can be extremely challenging because of the heterogeneous clinical presentations associated with many OXPHOS defects, with a wide differential diagnosis. This means that a high index of clinical suspicion is needed. Signs of developmental regression, often associated with infectious disease, are typical findings. Precise clinical investigations, metabolic tests, and screening for multi-system involvement should be performed. Depending on availability, the use of genetic screening tests, especially exome or genome sequencing, should be considered early in the diagnostic workup [50, 51]. Functional studies of OXPHOS enzymes in tissue biopsies could either come later in the diagnostic workup or can be performed in parallel in acutely ill patients.

10.4.1 Screening Tests

Metabolic Investigations

Simple standard tests for categorising a metabolic disorder including full blood count, pH, bicarbonate and lactate should be performed as first line investigations (► Chap. 1).

Interpretation of lactate measurements needs to consider that lactate is the product of anaerobic ATP production via glycolysis, which is a physiological means to provide energy rapidly. Therefore, the patient's exercise load at the time of sample collection needs to be considered, since elevated lactate may be appropriate in a crying child or following epileptic seizure activity (► Chap. 1).

Other metabolic investigations include measurement of pyruvate, the end product of glycolysis. Pyruvate can be increased in patients with OXPHOS defects, but a caveat is that the preanalytical requirements for pyruvate investigation are demanding. Perchloric acid needs to be added to deproteinise the sample, which has to be cooled immediately, otherwise pyruvate will continue to be metabolised in the blood sample leading to a falsely low result. Therefore, pyruvate has not become a widely used metabolite. Alanine correlates to the concentration of pyruvate, since transamination occurs especially during amino acid catabolism and excess glutamate levels. Amino acid analysis can provide helpful data, particularly if ratios between certain amino acid concentra-

tions are analysed, e.g. alanine/lysine (normally <3:1). Glycine may be elevated in defects of lipoic acid biosynthesis [52] (▶ Chap. 23), whilst low levels of citrulline and arginine have been reported in maternally inherited Leigh syndrome, NARP, MELAS and Pearson syndrome [53].

Urinary organic acid analysis frequently reveals elevation of lactate, pyruvate, and Krebs cycle intermediates (succinic, malic, fumaric, 2-oxoglutaric and citric acids), which are all indicative of an OXPHOS defect. Elevation of urinary 3-methylglutaconic acid has been found in a heterogeneous group of OXPHOS disorders, including Barth, Sengers and MEGDEL syndromes (▶ Chaps. 18 and 35), as well as deficiencies of ATP synthase, mitochondrial import, CLPB, ATAD3A and HTRA2, all of which seem to have abnormalities of mitochondrial cristae in common [54–56] . Elevated excretion of ethylmalonic acid is found in ETHE1 deficiency [43] (▶ Chap. 20) but is also present in other conditions. Mild elevation of methylmalonic acid is associated with succinyl-CoA-ligase deficiency (▶ Chap. 18).

Investigation of acylcarnitines can be helpful in defects of flavin cofactor metabolism (e.g. *FLAD1* mutations) and defects of inhibitors originating from the valine degradation pathway (ECHS1 and HIBCH deficiencies) [42, 57] (▶ Chap. 18). Some cases with *MT-ATP6* mutations may present with abnormally elevated propionylcarnitine (C3) or hydroxyisovalerylcarnitine (C5OH) mimicking mutiple carboxylase deficiency (▶ Chap. 27) [58].

Investigation of purines and pyrimidines in the plasma or urine of MNGIE patients show an elevation of thymidine and deoxyuridine [33] (▶ Chap. 32). Specific investigation of coenzyme Q_{10} in peripheral blood mononuclear cells or tissue biopsies is of diagnostic relevance in patients with suspected CoQ_{10} biosynthetic disorders and can also be useful in identifying secondary CoQ_{10} deficiency [59]. In myopathic patients, elevation of the biomarkers FGF-21 and GDF15 correlate well with mitochondrial dysfunction [60, 61] (■ Table 10.5). Multi-omics methods are increasingly being used to reveal metabolic and proteomic signatures of mitochondrial dysfunction [62] (▶ Chap. 3). It is anticipated that similar techniques will be used to study other mitochondrial disorders in the near future.

■ Neuroimaging

Certain mitochondrial syndromes are associated with characteristic lesions on brain magnetic resonance imaging (MRI), for example parieto-occipital stroke-like lesions not corresponding to vascular territories in MELAS, and bilateral focal symmetrical T2 hyperintense lesions in the basal ganglia, variably extending into the midbrain and brainstem, in Leigh syndrome (■ Table 10.3) (▶ Chap. 1) [63, 64]. Some mitochondrial aminoacyl tRNA synthetase deficiencies have been linked to specific brain MRI 'signatures' (■ Table 10.3), and leukoencephalopathies are a feature of some mitochondrial disorders, particularly Leigh syndrome caused by mutations in nuclear-encoded complex I subunits and assembly factors, and disorders of iron-sulphur cluster biosynthesis [1]. Magnetic resonance spectroscopy may be used to visualise increased cerebral lactate (a nonspecific finding) or elevated succinate (deficiencies of succinate dehydrogenase (complex II) subunits or assembly factors).

■ Screening for Multi System Involvement

Involvement of multiple organs is a typical finding in OXPHOS disease, especially in infantile and childhood onset disease. It is important to screen systematically for multisystem involvement for two reasons. Firstly, the pattern of organ involvement may point to a specific syndromic/genetic diagnosis. Secondly, it is imperative to search for organ involvement for which there may be supportive therapy (▶ Sect. 10.5.2). Investigations looking for multisystem disease may include echocardiogram, electrocardiogram, measurement of renal tubular and endocrine (pituitary, thyroid, parathyroid, pancreatic, adrenal) function, and ophthalmological and audiological assessment. Reassessment over time is important since clinical evolution of mitochondrial disorders is typical, with disease progression and involvement of new organ systems.

10.4.2 Muscle and Other Tissue Biopsies

If a biopsy is needed, e.g. for confirmation of a genetic diagnosis or in a critically unwell individual, usually the best tissue to be investigated by biochemical or genetic techniques is a clinically affected tissue. Investigation of a skeletal muscle biopsy is frequently helpful in identifying the underlying OXPHOS defect in patients with neuromuscular symptoms. If results are normal, consideration should be given to whether the correct tissue has been investigated, e.g. patients with *POLG* mutations may have normal OXPHOS results in muscle but a clear biochemical phenotype in liver. The samples and conditions detailed in the following paragraph should be considered.

■ Tissue Sampling and Storage

1. Fresh tissue (muscle, liver) for investigation of mitochondrial respiration, and for cell culturing (skin fibroblasts, lymphocytes, myoblasts). These tissue samples should be sent on water-ice [not necessary for skin] in an appropriate transport medium.

Table 10.5 Metabolites in mitochondrial disease (see also ▶ Sect. 3.2.1)

Metabolite	Sample	Investigation	MRS	OXPHOS defect	Category
Lactate	P, U, CSF	CC, OA	^{a}H	all	all
Pyruvate	P, U, CSF	CC, OA		all	all
Alanine	P, U, CSF	AA		all	all
Glycine +/– lactate, BCAA, 2KG and 2KA	P, U, CSF	AA		CII > CI > CIII	Cofactor/lipoic acid, Fe-S cluster, SAM (*BOLA3*, *GLRX5*, *IBA57*, *ISCA2*, *LIAS*, *LIPT2*, *NFU1*, *SLC25A26*)
Acylcarnitine	P, BS	MS		CII, CI,all	Inhibitors (*ECHS1*, *HIBCH*), Cofactor/flavins (*FLAD1*, *SLC25A32*)
Coenzyme Q_{10}	PBMC, M, F	HPLC, MS		CI + III, CII + III	Cofactor/Coenzyme Q_{10}
Thymidine, deoxyuridine	P, U	PP		CI, CIII, CIV, CV	Replication (*TYMP*)
FGF-21	P	ELISA		all	all
Krebs cycle metabolites	U	OA		all	all
3-Methylglutaconic acid	U	OA		CI, CIII, CIV, CV	Defects affecting IMM integrity (*AGK*, *ATP5F1E*, *ATP5F1D*, *ATAD3A*, *CLPB*, *DNAJC19*, *HTRA2*, *MICOS13*, *OPA3*, *SERAC1*, *TAZ*, *TIMM50*, *TMEM70*)
Methylmalonic acid	U	OA		CI, CIII, CIV, CV	Replication/nucleotide (*SUCLA2*, *SUCLG1*)
Succinate	U	OA	^{a}H	CII, in lower amount for all	Subunit/Assembly/Cofactors of CII
Ethylmalonic acid	U	OA		CIV	Inhibitor (*ETHE1*)
2-Methyl-2,3-dihydroxybutyrate	U	OA		all	Inhibitors (*ECHS1*, *HIBCH*)

^{a}*H* proton. *2KA* 2-ketoacids, *2KG* 2-ketoglutarate, *AA* amino acids, *BCAA* branched chain amino acids, *BS* dried blood spot, *CC* clinical chemistry, *CI-V* complexes, I-V, *CSF* cerebrospinal fluid, *F* fibroblasts, *IMM* inner mitochondrial membrane, *MS* mass spectrometry, *M* muscle, *OA* organic acids, *P* plasma, *PBMC* peripheral blood mononuclear cells, *PP* purines and pyrimidines, *U* urine

2. Snap-frozen tissue (muscle, liver, heart, kidney) for enzymatic investigations, histochemical staining, western blot and blue-native gel electrophoresis (send packed with dry ice).
3. Fixed tissue for electron microscopic investigations.
4. Blood for genetic investigations: EDTA blood, stable for several days (temp 4–25 °C).
5. Other useful samples for mtDNA investigations: urine, buccal swab, hair roots.

Types of Investigations

Numerous biochemical techniques have been established for the analysis of mitochondrial energy metabolism. By investigating intact mitochondria from fresh tissue or cultured cells, all kinds of OXPHOS defects can be identified, including disorders that depend on the integrity of the mitochondrial membranes, for which it is essential to measure the activity of ATP synthase. In frozen tissue it is still possible to measure the activity of OXPHOS complexes I-IV and the hydrolytic activity of complex V.

Histopathology

Histochemical investigation of complex II (succinate dehydrogenase, SDH) and complex IV (cytochrome *c* oxidase, COX) can be performed on frozen tissue sections. Furthermore, Gömöri trichrome staining identifies

cells with mitochondrial accumulation (e.g. 'ragged-red' muscle fibres) and sequential COX/SDH staining can reveal so-called 'ragged-blue' fibres (COX-negative, SDH-positive). A major advantage of histological investigation is the ability to detect a heterogeneously affected tissue, especially muscle fibres with different mutation load due to heteroplasmic mtDNA mutation. Microdissection of single muscle fibres either positive or negative for COX staining, and quantification of the mutation load, can be used to determine the pathogenic relevance of novel mtDNA mutations. Tissue sections from cryo-tissue or formalin-fixed paraffin-embedded (FFPE) samples can also be used for immunohistochemical staining, which may show reduced immuno-staining of subunits in cases with OXPHOS deficiency. Histopathological examination of cardiac or renal tissue may reveal 'giant' mitochondria, although these are not specific for mitochondrial disease. Analysis of bone marrow aspirates in Pearson syndrome demonstrates vacuolation of myeloid precursors and the presence of ringed sideroblasts. Post-mortem brain pathology may reveal characteristic features in Leigh or Alpers-Huttenlocher syndromes.

10

Electron Microscopy

Electron microscopy (EM) can reveal abnormal ultrastructure of mitochondria, e.g. paracrystalline inclusions or concentric cristae, or extremely elongated mitochondria in the case of mitochondrial fission defects. These findings are often helpful in combination with other results but might be misleading if they are interpreted out of context, since subtle ultrastructural abnormalities of mitochondria (e.g. alterations in number or size or abnormal cristal morphology) can also be found in other disease processes.

Investigation of OXPHOS Activity

Spectrophotometric assays have been established for all OXPHOS enzymes: complexes I, II, III, IV and the oligomycin-sensitive ATPase activity of complex V. Furthermore the combined activities of complex I + III (NADH:ferricytochrome *c* oxidoreductase) and complex II + III (succinate:ferricytochrome *c* oxidoreductase) may be measured. These combined activity assays can enable the detection of defects in coenzyme Q_{10}, which is an essential electron carrier in these reactions (Fig. 10.1). These enzyme investigations allow the identification of isolated or combined OXPHOS deficiencies. Combined OXPHOS defects are most frequent, and typical combinations of enzyme deficiencies can point to the underlying genetic defect. For example, combined reduction of complexes I, III, IV and V is a typical pattern of defects in mtDNA-dependent disorders including mtDNA mutations (point mutations and rearrangements involving tRNA or rRNA genes) and nuclear-encoded defects of mtDNA maintenance and mitochondrial gene expression (Table 10.4). Combined decrease of complexes I, II, and eventually III together with PDHc deficiency and glycine elevation is suggestive of a defect of iron-sulphur cluster biosynthesis (Table 10.4 and Fig. 10.2). Such findings allow confirmation of pathogenicity of genetic variants or direction of subsequent genetic investigations to pinpoint the precise genetic defect. Reduced activities of complexes I + III and II + III in muscle biopsy, with normal activities of the individual complexes I, II and III when assayed separately, suggests CoQ_{10} deficiency. However other patterns of respiratory chain enzyme deficiency have been observed in primary CoQ_{10} deficiency, therefore direct measurement of CoQ_{10} in muscle biopsy is the preferred screening test.

Investigation of the oxidation of different substrates by intact mitochondria isolated from fresh tissue biopsies or cultured patient cells provides the best characterisation of mitochondrial function, and allows identification of decreased activity of ATP synthase, pyruvate oxidation, the Krebs cycle, and substrate transport. Available techniques include measurement of oxygen consumption (by polarography or fluorimetry), ATP formation (luciferase-coupled assay), and the use of radiolabelled substrates in flux assays.

Blue or clear native gel electrophoresis allows further characterisation of OXPHOS defects by enabling the separation of intact OXPHOS enzymes and even supercomplexes [65]. Abnormal OXPHOS assembly or absence of single enzymes can be identified by this method.

In a research setting, functional complementation can be used to investigate the pathogenic relevance of novel genetic defects by complementing an OXPHOS defect in patient cells using stable transfection with the wild-type candidate gene, e.g. with a lentiviral vector.

Tissue-Specificity

Defects of OXPHOS enzymes can occur in a tissue-specific manner. There are several reasons for this phenomenon including different threshold for minimal residual activities in different organs but also expression of different isoforms of involved enzymes, different degrees of X-inactivation in X-chromosomal disorders, different percentages of mtDNA heteroplasmy, or somatic mosaicism of an affected gene. Reinvestigation of a clinically affected tissue (usually skeletal muscle, liver or heart) should be considered in cases where genetic investigations in blood do not identify the underlying cause.

10.4.3 Molecular Genetic Investigations

In recent years next-generation sequencing of the whole mitochondrial genome, together with high throughput genome-wide sequencing of the nuclear genome,

has revolutionised genetic testing for mitochondrial disorders. Since OXPHOS defects are an extremely heterogenous and fast expanding group of genetic disorders, the investigation of single mutations or single genes is only useful in a subgroup of patients with a clear clinical picture and few underlying mutations (e.g. LHON, MELAS, MNGIE). Most clinical manifestations of mitochondrial disease have been associated with a large number of different gene defects. Leigh syndrome is a particular example of a genetically heterogeneous disorder (currently linked to >100 genes) [3] where targeted sequencing is indicated in only a few exceptions (e.g. maternally inherited Leigh syndrome due to *MT-ATP6* mutations). For this reason, exome sequencing is currently the state of the art for next-generation genetic screening although some centres use next-generation sequencing of large gene panels such as the 'MitoExome' [50]. Genome sequencing is now increasingly being taken up into routine diagnostics [51], although interpretation of the resulting data remains challenging, with variants of uncertain significance identified in many investigated patients. However, owing to growing numbers of available control and patient sequences, more and more variants will be classified. Screening the mitochondrial genome for large-scale rearrangements and/or depletion requires other techniques, such as long-range, real-time or droplet digital PCR, and it is important to investigate a clinically relevant tissue such as muscle.

10.5 Treatment and Prognosis

10.5.1 Treatable Disorders

Currently no curative treatments correcting the underlying disease process exist for the vast majority of mitochondrial disorders [66, 67]. However, a few remarkable exceptions should be noted. Many patients with *ACAD9* mutations associated with exercise intolerance, lactic acidosis or infantile-onset cardiomyopathy appear to respond to oral riboflavin supplementation (100–400 mg/day) [68]. Disorders of CoQ_{10} biosynthesis may respond to high dose oral CoQ_{10} supplementation (at least 30 mg/kg/day in childhood, up to 3 g/day in adults), although response to treatment is highly variable [59]. Complete prevention of symptoms has been reported in some cases who received early treatment, whilst other cases have persistent ataxia or progressive renal impairment despite treatment. Patients with a mild form of mitochondrial pyrophosphatase (PPA2) deficiency died from sudden cardiac failure after drinking small amounts of alcohol. Siblings with the same *PPA2* genotype did not develop any severe symptoms when strictly avoiding alcohol [69].

Vigilance for reversible infantile respiratory chain deficiency is also associated with good outcomes, although some affected individuals with myopathy may require prolonged ventilatory support (up to 18 months), whilst those with acute liver failure caused by *TRMU* mutations may need liver transplantation [17, 18].

10.5.2 Supportive Management

Supportive measures remain the mainstay of management for most patients and involve screening for and pro-actively treating known complications of mitochondrial disease when they occur. Such interventions may include the use of antiepileptic drugs (AEDs) for seizures (levetiracetam and clobazam appear to the most effective AEDs for mitochondrial epilepsy, and sodium valproate should be avoided, particularly in patients with *POLG* mutations, since its use may precipitate acute liver failure) [22]. Currently there is no evidence to exclude other drugs in the majority of patients affected by primary mitochondrial disease, but decisions regarding drug prescribing should always be tailored to the specific needs and risks of individual patients [70]. Other supportive measures include ptosis surgery; hearing aids or cochlear implants; hormone replacement (growth hormone, thyroxine, insulin or hydrocortisone as needed); blood transfusions for anaemia (especially in Pearson and MLASA syndromes); fluid and electrolyte replacement for renal tubulopathy; medical treatment for cardiomyopathy; pacemaker insertion for cardiac conduction defects; and, occasionally, organ transplantation (heart, kidney, liver) in an appropriate clinical context such as isolated end-stage organ involvement in an otherwise 'healthy' child. L-arginine therapy may ameliorate the frequency and severity of stroke-like episodes in MELAS [27], whilst folinic acid supplementation has been reported to improve seizures and other neurological problems in patients with mitochondrial disease associated with cerebral folate deficiency [71] (► Chap. 28).

10.5.3 Vitamin and Cofactor Cocktails

Some centres (particularly in the United States) advocate 'cocktails' of vitamins and cofactors for patients with mitochondrial disease. Whilst some of these have a logical rationale, e.g. trials of riboflavin for complex I deficiency or thiamine for PDHc deficiency, many are nonspecific antioxidants and currently there is no evidence base to support or refute the use of these supplements [66]. An exhaustive list of the components of such cocktails is not given here, because of the lack of evidence.

10.5.4 Experimental Approaches

Numerous pharmacological and gene therapy approaches are currently under investigation and are beginning to reach clinical trials [67]. The slow rate of progression of therapy development for mitochondrial diseases is partly because of the enormous difficulty in designing and executing effective clinical trials for these extremely heterogeneous multisystem disorders with an unpredictable disease course. Pharmacological strategies currently in or close to clinical trial for mitochondrial disease include the following: (1) antioxidants (e.g. analogues of CoQ_{10} or N-acetylcysteine); (2) agents aiming to stimulate mitochondrial biogenesis (e.g. bezafibrate and vitamin B_3 analogues); (3) molecules that may 'stabilise' lipid components of the mitochondrial membranes; (4) rapamycin and analogues to modulate mitophagy (the selective destruction of damaged mitochondria); (5) nucleoside supplementation for TK2 deficiency; and (6) enzyme replacement therapy for MNGIE. Gene therapy approaches are also under investigation, including AAV and lentiviral approaches for nuclear gene defects and nuclease digestion and allotopic expression for mtDNA mutations [67].

10.5.5 Genetic Counselling and Prenatal and Preimplantation Genetic Diagnosis

Nuclear Gene Defects

Almost all childhood-onset nuclear-encoded mitochondrial disease is inherited in an autosomal recessive manner, with a few exceptions which are X-linked (as listed above). Nuclear gene defects causing mitochondrial disease presenting in adult life are frequently inherited as autosomal dominant traits, although some are recessive. Genetic counselling, prenatal and pre-implantation genetic diagnosis (PGD) are relatively straightforward for patients with nuclear gene defects in whom the underlying mutation/s is/are known and follows the same principles as for other Mendelian disorders.

MtDNA Defects

The situation is more complex for mtDNA mutations, not least because of the phenomenon of heteroplasmy arising from the multiple copy number of mtDNA molecules. Most large-scale rearrangements and some point mutations of mtDNA are sporadic, with a low risk of transmission. Heteroplasmic mtDNA point mutations are generally maternally inherited, but the factors that determine what percentage of mutation will be transmitted to the next generation are poorly understood. The genetic bottleneck for mtDNA means that there may be large shifts in the proportion of mutation transmitted from mother to offspring, and this varies between different mutations. It is therefore difficult to offer women who harbour heteroplasmic disease-causing point mutations accurate recurrence risks, although some mutations are better understood than others. The m.8993T>G mutation in *MT-ATP6* associated with maternally inherited Leigh syndrome and NARP appears to be particularly skewed to extremes of mutation (very low or very high) in oocytes, and this has allowed prenatal diagnosis for this mutation to be performed successfully on a number of occasions. Pre-implantation genetic diagnosis (PGD) has also been used successfully for some mtDNA mutations, particularly the m.8993T>G mutation [72, 73]. The factors that determine clinical expression of homoplasmic point mutations associated with LHON are incompletely understood and so it is difficult to predict the recurrence risk, although it is approaching ten times higher for males than females who harbour LHON mutations.

Transmission of mtDNA point mutations may be avoided by donor egg in vitro fertilisation (IVF). In 2015 the UK government legislated two mitochondrial 'donation' IVF techniques (pronuclear transfer and spindle cell transfer) to be used with the aim of preventing the transmission of serious mitochondrial disease from a mother to her child, although concerns remain about the long-term efficacy and safety of these techniques [74]. Regulation of these procedures in the UK is by the Human Fertilisation and Embryology Authority, which in March 2017 licensed the first UK clinic permitted to perform pronuclear transfer. One report documented the birth, in Mexico, of an apparently healthy baby born to a mother harbouring the m.8993T>G mutation [75]. Mutation load at birth was reported to be low, but longer-term follow up has not been published.

10.5.6 Prognosis

Many early-onset mitochondrial disorders lead to death in infancy and early childhood, but natural history is extremely variable, even for patients with the same genetic defect [76].

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Disorders of Pyruvate Metabolism and the Tricarboxylic Acid Cycle

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Contents

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Pyruvate Metabolism and the Tricarboxylic Acid Cycle

Pyruvate is formed by glycolysis from glucose and other monosaccharides, from lactate and from alanine, a neoglucogenic amino acid (◘ Fig. 11.1). The final stage of glycolysis converts glyceraldehyde-3-phosphate (GAP) into pyruvate generating cytosolic reduced adenine nucleotide (NADH) via GAP dehydrogenase (GAPDH). NADH is shuttled into the mitochondrion for re-oxidation, via the malate-aspartate shuttle (MAS) or via the glycerol-3-phosphate (G3P) shuttle. Two enzymes and two carriers are required for the function of the MAS: the two enzymes, glutamate oxaloacetate transaminase (GOT) and malate dehydrogenase (MDH) are present both in the cytoplasm (GOT1 and MDH1) and in the mitochondrial matrix (GOT2 and MDH2). The two carriers are located in the inner mitochondrial membrane (AGCs and OGC). AGC1 (or Aralar) is highly expressed in central nervous system and in skeletal muscle whereas AGC2 (or citrin) is mainly present in intestine and liver. The calcium sensibility of AGC1 plays a major role in the calcic regulation of substrate supply for the oxidative phosphorylation (OXPHOS) to the mitochondria. In neurons AGC1 is also crucial to supply cytosolic aspartate for N-Acetyl-aspartate formation (▶ Chap. 22). In the presence of high levels of glycolytically produced NADH, the cytosolic MDH1 reduces oxaloacetate into malate. Malate is exported from the cytosol into the mitochondrial matrix by OGC in exchange for 2-ketoglutarate. In the mitochondria, the oxidation of malate into oxaloacetate regenerates NADH, which can be used to pass electrons to the respiratory chain for ATP synthesis. Oxaloacetate and glutamate are then transformed into aspartate and 2-ketoglutarate by GOT2. AGC1 exports aspartate from the matrix to the cytosol in exchange for glutamate plus a proton and aspartate is then converted into oxaloacetate by GOT1 achieving the cycle. The G3P shuttle acts in a way similar to the MAS to re-oxidize the cytosolic pool of NADH but it is mainly involved in lipid biosynthesis (▶ Chap. 35). Dihydroxyacetone phosphate from glycolysis is reduced to G3P by the cytosolic NAD-dependent G3PDH. The mitochondrial G3PDH re-oxidizes G3P to DHAP and transfers two electrons from FAD to the electron transport chain. The mitochondrial pyruvate carrier allows the uptake of pyruvate into mitochondria. Pyruvate can be then converted into acetyl-coenzymeA (acetyl-coA) by the pyruvate dehydrogenase complex (PDHC) for further oxidation in the TCA cycle. Pyruvate can also enter the gluconeogenic pathway by sequential conversion into oxaloacetate by pyruvate carboxylase (PC), followed by conversion to phosphoenolpyruvate by phosphoenolpyruvate carboxykinase (PEPCK). Acetyl-coA is also formed by fatty acid oxidation. One of the primary functions of the TCA cycle is to generate reducing equivalents in the form of NADH and reduced flavin adenine dinucleotide ($FADH_2$), which are utilized to produce energy under the form of ATP in the electron transport chain by the OXPHOS. The MAS senses cytosolic calcium (Ca^{2+}) with respect to its Ca^{2+} sensitive component AGC1 and plays an essential role in the control of substrate supply for OXPHOS. Another important role of the TCA cycle comes from the fact that succinyl-CoA is at the crossroad of several pathways (ketolysis, catabolism of isoleucine,valine, threonine, methionine, odd chain fatty acids and cholesterol) and that succinyl-CoA ligase (SUCL) can yield directly GTP and ATP in the absence of oxygen (a mechanism known as substrate level phosphorylation) and participates to mtDNA maintenance.

Introduction

Owing to the role of pyruvate and the tricarboxylic acid (TCA) cycle in energy metabolism, as well as in gluconeogenesis, lipogenesis and amino acid synthesis, defects in pyruvate metabolism and in the TCA cycle almost invariably affect the central nervous system. The severity and the different clinical phenotypes vary widely among patients and are not always specific, the range of manifestations extending from overwhelming neonatal lactic acidosis with early death to relatively normal adult life and variable effects on systemic functions. Similar clinical manifestations may be caused by other defects of energy metabolism, especially defects of the mitochondrial respiratory chain (▶ Chap. 10). Diagnosis relies primarily on biochemical analysis of accumulated metabolites in body fluids, DNA analysis and, in some instances, confirmation by definitive enzymatic assays in cells or tissues. Prenatal diagnosis is now achieved preferentially by DNA analysis. Pyruvate carboxylase (PC) deficiency constitutes a defect both in the TCA cycle and in gluconeogenesis, but generally presents with severe neurological dysfunction and lactic acidosis rather than with fasting hypoglycaemia. Deficiency of phosphoenolcarboxykinase (PEPCK) is limited to its cytosolic form and affects gluconeogenesis. Deficiency of pyruvate dehydrogenase complex (PDHC) impedes glucose oxidation and aerobic energy production, and ingestion of carbohydrate aggravates lactic acidosis. The defects of mitochondrial pyruvate carrier have the same presentation as PDHC deficiencies. Treatment of disorders of pyruvate metabolism comprises avoidance of fasting (PC

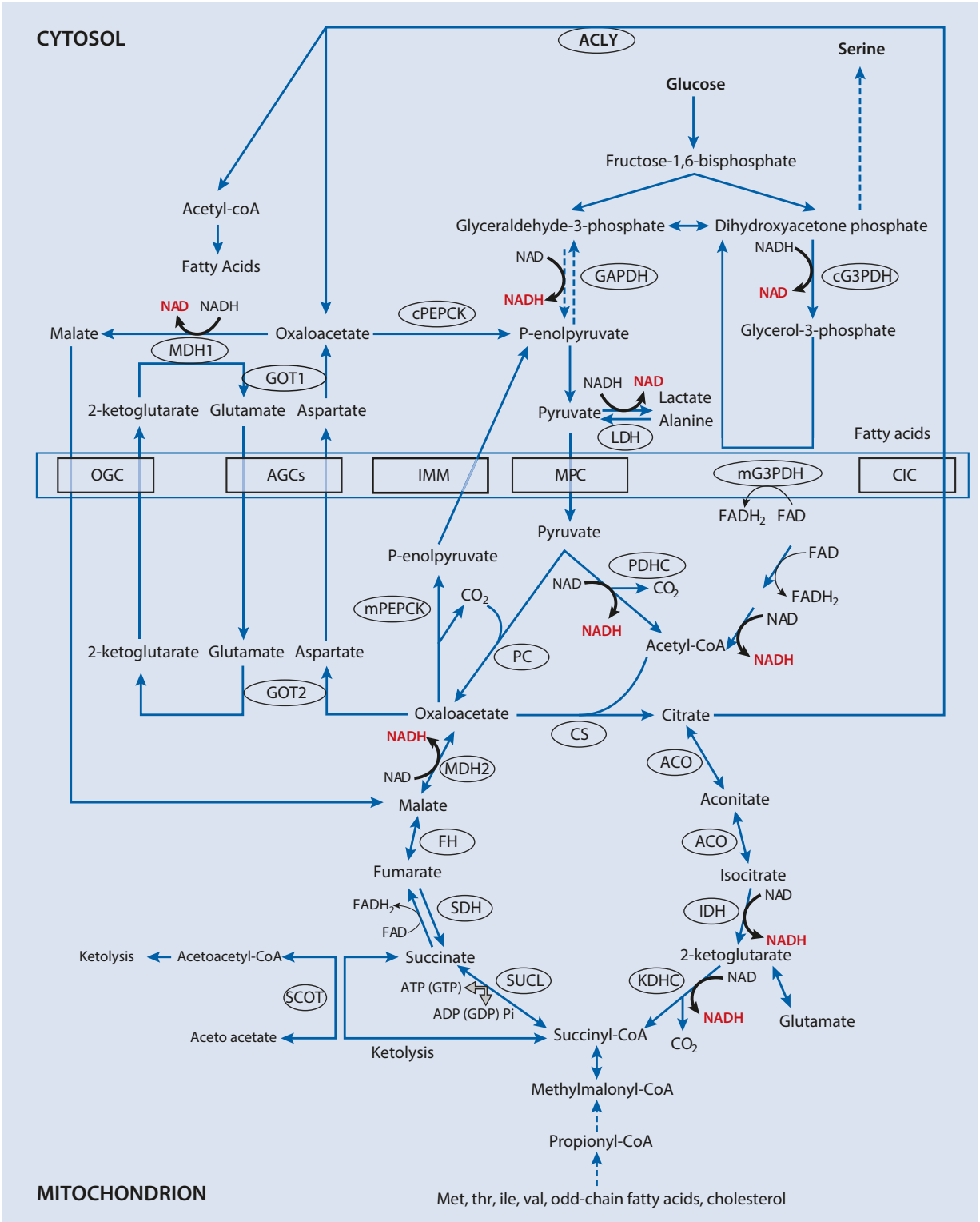

Fig. 11.1 Overview of glucose, pyruvate/lactate, fatty acid and amino acid oxidation by the tricarboxylic acid cycle. ACLY ATP, citrate lyase; ACO, aconitase; AGCs, aspartate glutamate carriers 1 et 2; ATP, adenosine triphosphate; CIC, citrate carrier; CS, citrate synthase; FH, fumarase; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GOT1, GOT2, glutamate oxaloacetate transaminases 1 and 2; cG3PDH, mG3PDH, cytosolic and mitochondrial glycerol-3-phosphate dehydrogenases; GTP, guanosine triphosphate; IDH, isocitrate dehydrogenase; IMM, inner mitochondrial membrane; KDHC, 2-ketoglutarate dehydrogenase complex; LDH, lactate dehydrogenase; MDH1, MDH2, malate dehydrogenases 1 and 2; MPC, mitochondrial pyruvate carrier; OGC, oxoglutarate carrier; PDHC, pyruvate dehydrogenase complex; PC, pyruvate carboxylase; cPEPCK, mPEPCK, cytosolic and mitochondrial phosphoenolpyruvate carboxykinases; SCOT, succinyl-CoA 3-oxoacid CoA transferase; SDH, succinate dehydrogenase; SUCL, succinyl-CoA ligase

deficiency) or minimising dietary carbohydrate intake (PDHC deficiency) and enhancing anaplerosis (restoration of pools of intermediate metabolites). Dihydrolipoamide dehydrogenase (E3) deficiency affects PDHC and also the 2-ketoglutarate dehydrogenase complex (KDHC) and the branched-chain 2-ketoacid dehydrogenase (BCKD) complex (► Chap. 18), with biochemical manifestations of all three disorders. The deficiencies of the TCA cycle enzymes interrupt the cycle but do not always result in accumulation of the corresponding substrates. Succinyl-CoA ligase deficiency causes a mild methylmalonic accumulation. Succinate dehydrogenase deficiency represents a unique disorder affecting both the TCA cycle and the respiratory chain. The defects of the malate-aspartate shuttle (MAS) impede the import of reduced nicotinamide adenine dinucleotide (NADH) from the cytosol to the mitochondrion and the subsequent pyruvate oxidation. *NAXE* and *NAXD* deficiencies disrupt the cellular NAD(P)HX repair system and cause lethal neurometabolic disorders of early childhood.

11.1 Pyruvate Carboxylase (PC) Deficiency

11.1.1 Clinical Presentation

PC deficiency is an autosomal recessive disorder with an incidence of around 1 in 250,000. Several dozen patients have been described in detail, allowing the definition of three overlapping phenotypes that probably constitute a continuum from the most severe (type B) to the less severe form (type C). For a review see [1].

1. Patients with the French phenotype (type B) become acutely ill 3–48 h after birth with hypothermia, hypotonia, lethargy and vomiting [1, 2, 3]. These children exhibit a severe neurological neonatal deterioration with initially a preserved level of consciousness but then rapid deterioration with rigidity, hypokinesia and tremor (resembling infantile parkinsonism) and abnormal ocular movements [2]. Hepatomegaly, seizures and failure to thrive may occur. Most die in the neonatal period in the setting of severe lactic acidosis with intractable tubulopathy and multiorgan (liver) failure. Some survive, but they remain unresponsive and severely hypotonic and finally succumb from respiratory infection before the age of 5 months.
2. Patients with the North American phenotype (type A) become severely ill between 2 and 5 months of age. They develop progressive hypotonia and are unable to smile. Frequent episodes of acute vomiting, dehydration, tachypnoea, facial pallor, cold cyanotic extremities and lactic acidosis, characteristically precipitated by metabolic or infectious stress, occur. Clinical examination reveals pyramidal tract signs, ataxia and nystagmus. All patients are severely mentally retarded and most of them have seizures. Hepatomegaly and renal dysfunction (tubular acidosis) may also be present. Neuroradiological findings (also found in type B) include subdural effusions, severe antenatal ischaemia-like brain lesions and periventricular haemorrhagic cysts, followed by progressive cerebral atrophy and delayed myelination. Leigh syndrome has also been reported but its frequency remains uncertain. The course of the disease is generally progressive, with death in infancy.
3. The Type C phenotype is more benign and has only been reported in a few patients without clear ethnic predilection [1]. The clinical course is dominated by the occurrence of acute episodes of lactic acidosis and ketoacidosis, usually responding rapidly to hydration and bicarbonate therapy. Despite the important enzyme deficiency, the patients have a near-normal cognitive and neuromotor development. However, dystonia, episodic ataxia, dysarthria, transitory hemiparesis, seizures and subcortical leukodystrophy have been described in some cases.

Prenatal features have been reported on a limited number of cases. Ultrasound examination and MRI had revealed frontal horn impairment associated with subependymal and paraventricular cysts in few cases which could be suggestive of a PC deficiency in unknown families [4].

11.1.2 Metabolic Derangement

PC is a biotinylated mitochondrial matrix enzyme that converts pyruvate and CO_2 to oxaloacetate. It plays an important role in gluconeogenesis, anaplerosis and lipogenesis. For gluconeogenesis, pyruvate must first be carboxylated into oxaloacetate, because the last step of glycolysis, conversion of phosphoenolpyruvate to pyruvate is irreversible. Oxaloacetate, which cannot diffuse freely out of the mitochondrion, is translocated into the cytoplasm via the MAS. Once in the cytoplasm, oxaloacetate is converted into phosphoenolpyruvate by PEPCK, which catalyses the first committed step of gluconeogenesis.

The anaplerotic role of PC, i.e. the generation of TCA cycle intermediates from oxaloacetate, is even more important. In severe PC deficiency, the lack of TCA cycle intermediates lowers reducing equivalents in the mitochondrial matrix. This drives the redox equilibrium between 3-OH-butyrate and acetoacetate in the direction of acetoacetate thereby lowering the 3-OH-butyrate/acetoacetate ratio. Aspartate, formed in the mitochondrial matrix from oxaloacetate by transamination, also decreases. As a consequence, the translocation of reducing equivalents between cytoplasm and mitochondrial matrix by the MAS is impaired. This drives

the cytoplasmic redox equilibrium between lactate and pyruvate in the direction of lactate, and the lactate/pyruvate (L/P) ratio increases. Reduced TCA cycle activity also plays a role in the increase of lactate and pyruvate. Since aspartate is required for the urea cycle, plasma ammonia and citrulline can increase. The low 2-ketoglutarate production explains the low plasma level of glutamate. The energy deprivation induced by PC deficiency has been postulated to impair astrocytic buffering capacity against excitotoxic insults and to compromise microvascular morphogenesis and autoregulation, leading to white matter degeneration [1].

The key-role of PC in lipogenesis derives from the condensation of oxaloacetate with intramitochondrially produced acetyl-CoA into citrate. Deficient lipogenesis explains the widespread demyelination of the cerebral and cerebellar white matter and symmetrical periventricular cavities around the frontal and temporal horns of the lateral ventricles, reported in the few detailed neuropathological descriptions of PC deficiency. PC is present in oligodendrocytes, where it plays an anaplerotic role [5]. PC deficiency in the oligodendrocytes should result in insufficient fatty acid synthesis and myelin malformation, whereas the impairment of oxidative metabolism in microglial cells is associated with an inflammatory response possibly contributing to neurodegeneration [6].

In a patient with the type B phenotype, muscle biopsy disclosed nemaline rods that probably occurred due to defective energy metabolism. Mitochondrial accumulation in type 1 fibers raises the possibility that the thin filaments may become the target structures of mitochondrial dysfunction [7].

PC requires biotin as a cofactor. Secondary PC deficiency is thus also observed in biotin-responsive multiple carboxylase (► Chap. 27) and in carbonic anhydrase VA deficiency (► Chap. 19).

11.1.3 Genetics

PC deficiency is an autosomal recessive disorder. PC is a tetramer formed by 4 identical subunits organized in two conformational states over the course of its two steps enzymatic reaction.

In the fatal form, the presence of at least one truncating mutation in the *PC* gene tends to lead to type B presentation while biallelic missense mutations tend to lead to type A, with some exceptions. Structure-function studies showed that missense mutations disturbing the ligands fixation or the balance between the two conformational states seem to be associated to type A presentation. Missense mutations associated to type C presentation destabilize the monomers but do not lead to a misbalance between the conformers [8]. The p. Ala610Thr mutation, frequent in Indo-American patients, is associated with type A.

Mosaicism was reported in a few cases and found to be correlated with prolonged patient survival [9].

11.1.4 Diagnostic Tests

The most severely affected patients typically exhibit an elevated L/P ratio, low hydroxybutyrate/acetoacetate (H/A) ratio with paradoxical postprandial ketosis, hypercitrullinemia and hyperammonemia, with low glutamine, parameters that often remain unaltered in types A and C.

Hence, the possibility of PC deficiency should be considered in any child presenting with lactic acidosis and neurological abnormalities, especially if associated with hypoglycaemia, hyperammonaemia or ketosis. In neonates, a high L/P ratio associated with a low H/A ratio is nearly pathognomonic [2]. Discovery of cystic periventricular leukomalacia at birth associated with lactic acidosis is also highly suggestive. Typically, blood lactate increases in the fasting state and decreases after ingestion of carbohydrate.

In patients with type B, blood lactate concentrations reach 10–20 mM (normal <2.2 mM) with L/P ratios between 50 and 100 (normal lower <15). In patients with type A, blood lactate is 2–10 mM with normal or only moderately increased L/P ratio (less than 50). In patients with type C, lactate can be normal and only increase (usually above 10 mM) during acute episodes. Overnight blood glucose concentrations are usually normal. Hypoglycaemia can occur during acute episodes of metabolic acidosis. The blood H/A ratio is decreased (less than 2, normal 2.5–3).

Hyperammonaemia (100–600 μmol/L, normal <50), and an increase of blood citrulline (100–400 μmol/L, normal <40), lysine and proline, contrasting with low glutamine, are constant findings in patients with type B [2]. Plasma alanine is usually normal in type B, but increased (500–1400 μmol/L, normal <450) in all reported patients with type A. During acute episodes, aspartate can be very low [10]. An elevation of total cholesterol or its precursors (mevalonic acid) may occur in type A and B forms [1].

In cerebrospinal fluid (CSF), lactate, the L/P ratio and alanine are increased and glutamine is decreased. High levels of homovanillic acid (a metabolite of dopamine) in the CSF and low staining of GABAergic markers in the substantia nigra have been found in a type B patient [11].

Mutation analysis is now usually used for confirmation of the diagnosis and prenatal diagnosis.

Measurement of PC activity may be performed on cultured skin fibroblasts using $^{14}CO_3HNa$. Residual PC enzymatic activity is of limited value for the distinction among the 3 phenotypes because enzymatic analysis often yields activities below 5% of normal regardless of PC deficiency type [1]. But measurement of PC activity can prove if an unknown variant impairs PC activity.

11.1.5 Treatment and Prognosis

Outcomes of treated patients exhibiting the severe A and B forms are very poor. Patients should be promptly treated with intravenous 10% glucose with appropriate electrolytes and may require bicarbonate in the setting of severe metabolic acidosis (pH <7.0). In one patient with the French phenotype who was treated with high doses of citrate and aspartate [10], lactate and ketones diminished and plasma amino acids normalized, except for arginine. However, in the CSF, glutamine remained low and lysine elevated. An orthotopic hepatic transplantation completely reversed ketoacidosis and the renal tubular abnormalities and decreased lactic acidaemia in a patient with a severe phenotype, although concentrations of glutamine in CSF remained low [12]. A patient with type B was started on early treatment with triheptanoin, a triglyceride containing three 7-carbon fatty acids (4 g of triheptanoin/kg body weight, providing 35% of total caloric intake) in order to restore anaplerosis by providing the intramitochondrial source of both oxaloacetate and acetyl-CoA [13]. Lactate, the L/P ratio, ammonia, and citrulline decreased rapidly with a progressive increase in glutamine. Although there was a clinical improvement without evidence of neurodegeneration, the patient died during an episode of acute decompensation at 8 months of age. Two further patients with type B have been reported to be unsuccessfully treated with triheptanoin, continuously from 11 and 21 days of age. They also received citrate, aspartate and dichloroacetate. No clinical or biochemical improvement was observed and the patients died at 7 and 8 months of age respectively with a severe neurological impairment [14].

Neither biotin, thiamine, dichloroacetate, high-fat diet nor high-carbohydrate diet has been shown to provide clinical benefit. PC deficiency is a contraindication for a ketogenic diet, since this would increase paradoxical ketosis due to decreased oxaloacetate availability.

Symptomatic treatment of seizures must be undertaken with caution since valproic acid and barbiturates have adverse effects in patients with energy defects. Exacerbation of lactic acidosis and increase of alanine, lysine and leucine have been reported with the use of ACTH in a type B patient with infantile spasms [1].

11.2 Phosphoenolpyruvate Carboxykinase (PEPCK) Deficiency

Six patients with this gluconeogenesis disorder have been described: they all have a deficiency of cytosolic phosphoenolcarboxykinase (PEPCK) confirmed at molecular level. Biallellic mutations in *PCK1* induce recurrent illness or fasting-related hypoglycaemia with ketonuria and inconstant lactacidemia. In one patient, an inaugural episode of liver failure with hyperammonaemia and encephalopathy has been resolved by dextrose administration. The neurological development was not altered [15, 16, 17].

Reduced activity of the mitochondrial PEPCK is now considered as a secondary phenomenon.

Note added in proof: A series of 24 Finnish patients with cytosolic PEPCK deficiency have been recently described; all displayed a very consistent metabolic profile including fasting hypoglycemia, hyperlactataemia, low ketones and high fumarate/succinate excretion. [P. Vieira et al. JIMD 2022, 45:223–234].

Structure and Activation/Deactivation System of the Pyruvate Dehydrogenase Complex

PDHC, and the two other mitochondrial 2-ketoacid dehydrogenases, KDHC and the BCKD complex, are similar in structure and analogous or identical in their specific mechanisms. They are composed of three components: E1, 2-ketoacid dehydrogenase; E2, dihydrolipoamide acyltransferase; and E3, dihydrolipoamide dehydrogenase. E1 is specific for each complex, utilizes thiamine pyrophosphate, and is composed of two different subunits, E1α and E1β. The E1 reaction results in decarboxylation of the specific 2-ketoacid. For the PDHC, the E1 component is the rate-limiting step and is regulated by phosphorylation/dephosphorylation catalysed by two enzymes, E1 kinase (inactivation) and E1 phosphatase (activation). E2 is a transacetylase that utilises covalently bound lipoic acid. E3 is a flavoprotein common to all three 2-ketoacid dehydrogenases. Another important structural component of the PDHC is E3-binding protein (E3BP), formerly protein X. This component has its role in attaching E3 subunits to the core of E2 (Figs. 11.2 and 11.3).

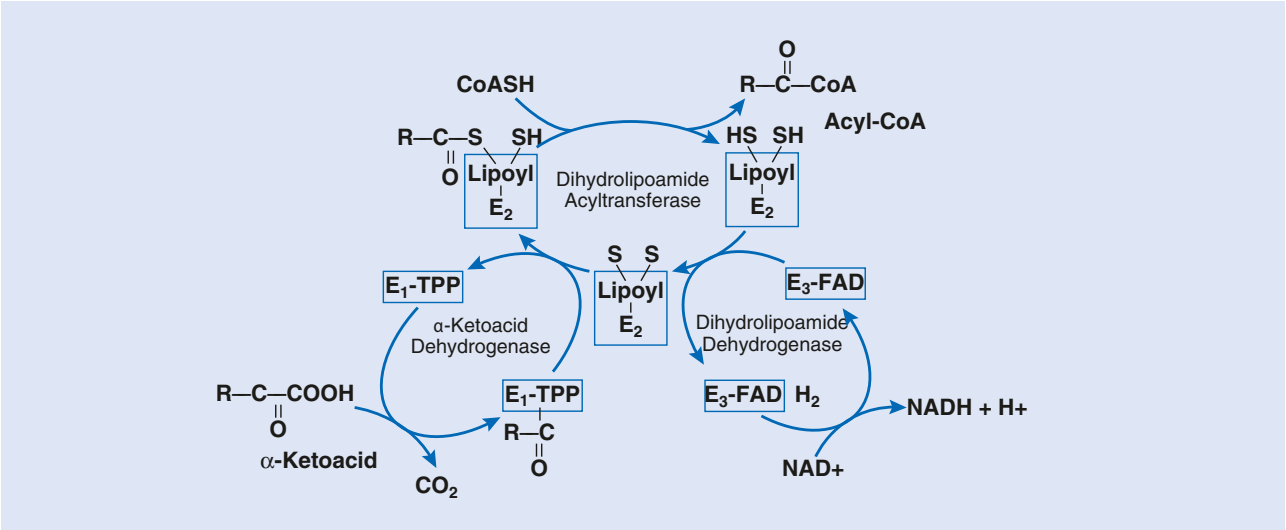

Fig. 11.2 Structure of the 2-ketoacid dehydrogenase complexes, pyruvate dehydrogenase complex (PDHC), 2-ketoglutarate dehydrogenase complex (KDHC) and the branched-chain α-ketoacid dehydrogenase complex (BCKD). CoA, coenzyme A; FAD, flavin adenine dinucleotide; NAD, nicotinamide adenine dinucleotide; R, methyl group (for pyruvate in PDHC) and the corresponding moiety for KDHC and BCKD; TPP, thiamine pyrophosphate

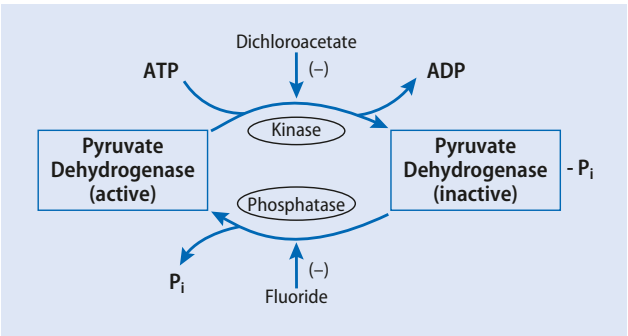

Fig. 11.3 Activation/deactivation of PDHE1 by dephosphorylation/phosphorylation. Dichloroacetate is an inhibitor of E1 kinase, and fluoride inhibits E1 phosphatase. ADP, adenosine diphosphate; P, phosphate

11.3 Pyruvate Dehydrogenase Complex (PDHC) Deficiency

11.3.1 Clinical Presentation

In a recent review, about 466 cases of PDHC deficiency have been reported [18] representing 74.2% of patients with a pyruvate oxidation disorder. Among them, 77% have a defect of the E1α subunit (Fig. 11.2). The most common symptoms of PDHE1α deficiency are delayed development, hypotonia, seizures and ataxia.

In hemizygous males, three presentations are encountered: neonatal lactic acidosis, Leigh's encephalopathy and intermittent ataxia. These correlate with the severity of the biochemical deficiency and the location of the gene mutation. In the first presentation, neonatal lactic acidosis is often associated with brain dysgenesis, such as agenesis of the corpus callosum. In Leigh's encephalopathy, quantitatively the most important group, the initial presentation is usually within the first 5 years of life and includes respiratory disturbances or episodic weakness and ataxia with absence of tendon reflexes. Respiratory disturbances may lead to apnoea, dependence on assisted ventilation or sudden unexpected death. Intermittent dystonic posturing of the lower limbs occurs frequently. A moderate to severe developmental delay may manifest within the next few years. A very small subset of male patients is initially much less severely affected, with intermittent episodic ataxia after carbohydrate-rich meals, progressing slowly over years into mild Leigh's encephalopathy.

A number of patients have developed an acute flaccid neuropathy mimicking Guillain-Barre syndrome during infancy or an acute episodic ataxia [19, 20], without mental retardation. Isolated optic atrophy with acute peripheral neuropathy can also be a late presenting finding [21].

Females with PDHE1α deficiency tend to have a more uniform clinical presentation, although with variable severity, depending on variable X chromosome lyonization [22]. This includes dysmorphic features, microcephaly, moderate to severe mental retardation and spastic di- or quadriplegia, resembling non progressive encephalopathy. Dysmorphism comprises a narrow head with frontal bossing, wide nasal bridge, upturned nose, long philtrum and flared nostrils resembling fetal alcohol syndrome. Other features are low-set ears, short fin-

gers and short proximal limbs, simian creases, hypospadias and an anteriorly placed anus. Seizures are encountered in almost all female patients. These appear within the first 6 months of life and are diagnosed as infantile spasms (flexor and extensor) or severe myoclonic seizures. Brain MRI frequently reveals severe cortical/subcortical atrophy, dilated ventricles, partial to complete corpus callosum agenesis [23]. Severe neonatal lactic acidosis can be present. An isolated paroxysmal exercise dystonia responding to thiamine was also seen in a female [24].

Males and females who are mosaic for PDHE1α deficiency have been reported with an attenuated phenotype [25].

Neuropathology can reveal various degrees of dysgenesis of the corpus callosum usually associated with migration defects, such as the absence of the medullary pyramids, ectopic olivary nuclei, abnormal Purkinje cells in the cerebellum, dysplasia of the dentate nuclei, subcortical heterotopias and pachygyria [26].

In the rare reports of antenatal forms related to PDHE1 deficiency, fetal sonography and MRI displayed brain dysgenesis with enlargement of the lateral ventricles [27]. Postmortem examination showed clastic and neurodevelopmental lesions with a cranial dysmorphism [28].

The phenotype of the PDHE1β deficiency is quite similar to that of the PDHE1α deficiency [18]. About 20 patients were reported either with early-onset lactic acidosis and severe developmental delay or with a moderate clinical course with slowly progressive neurological features reflecting basal ganglia and brain stem involvement associated with typical findings of Leigh syndrome [29].

Seven cases of PDHE2 (dihydrolipoamide transacetylase) deficiency have been described [30, 31, 32]. The phenotypic spectrum encompasses severe developmental delay with lactic acidosis to mild motor disability and moderate intellectual disability. Episodic dystonia is frequently observed whereas other neurological features like hypotonia are often less prominent. Bilateral lesions in the globus pallidus have been found in neuroradiological imaging. Lactate in blood is normal or slightly elevated.

The main clinical manifestations of E3BP (formerly protein X) deficiency are hypotonia, delayed psychomotor development and prolonged survival [18]. Often more slowly progressive, it also comprises early-onset neonatal lactic acidosis associated with subependymal cysts and thin corpus callosum and/or Leigh disease [33].

E1-phosphatase deficiency is very rare (◘ Fig. 11.3). Four cases with molecular identification of variants have been described with hypotonia, feeding difficulties and delayed psychomotor development [34].

11.3.2 Metabolic Derangement

Defects of PDHC provoke conversion of pyruvate into lactate and alanine rather than to acetyl-CoA, the gateway for complete oxidation of carbohydrate via the TCA cycle (◘ Fig. 11.1). The conversion of glucose to lactate yields less than one tenth of the ATP that would be derived from complete oxidation of glucose via the TCA cycle and the respiratory chain. Deficiency of PDHC thus specifically interferes with production of energy from carbohydrate oxidation, and hyperpyruvicaemia, lactic acidaemia and hyperalaninaemia are aggravated by consumption of carbohydrate.

PDHC deficiency impairs production of NADH but, unlike respiratory chain or MAS defects, does not hamper oxidation of NADH. PDHC deficiency thus does not modify the NADH/NAD^+ ratio in the cell cytosol, which is reflected in a normal L/P ratio both in blood and CSF. In contrast, deficiencies of respiratory chain complexes are generally characterised by a high L/P ratio (► Chaps. 1 and 10).

11.3.3 Genetics

All components of PDHC are encoded by nuclear genes, and synthesized in the cytoplasm as precursor proteins that are imported into the mitochondria, where the mature proteins are assembled into the enzyme complex. *PDHA1* coding for E1α-subunit is located on the X chromosome; therefore, most cases of PDHC deficiency are X-linked. To date, over 166 different mutations of *PDHA1* have been characterized [35, 36, 37, 38]. About half of these are small deletions, insertions or frameshift mutations, and the other half are missense mutations. Premature termination codons, mostly resulting from frame-shift insertions or deletions in exons 10 and 11, are frequently noted in females, while missense mutations predominate in males [36]. No null E1α mutations have been identified in males, except in a mosaic state [25], suggesting that such mutations are likely to be lethal. Rare splicing mutations involve exonic [39] or intronic [40] regulatory sequences. A few large rearrangements have been identified, such as a large intragenic 5 kb deletion [41]. In only about 25% of cases was the mother of a child with PDHE1α deficiency a carrier of the mutation. Therefore, since most cases of PDHC deficiency appear to be the consequence of new E1α mutations, the overall rate of recurrence within any one same family is low.

Mutations in *PDHB* are a very rare cause of PDHC deficiency [18, 42]. A deficiency in a patient was due to an increased proteasome-mediated degradation of the ubiquinated E1β subunit [43].

Mutations in the *PDHX* gene encoding the E3BP subunit are the second most frequent molecular cause of PDHC deficiency. All pathogenic mutations are truncating mutations. A founder mutation was found in a group of Roma children with cerebral palsy as clinical picture [44]. Other patients carry private mutations [45, 46]. Two large rearrangements have been identified, one of them due to retrotranspositional insertion of a full-length LINE-1 element [47].

Mutations in *DLAT* encoding E2 subunit [30, 31] and in the pyruvate dehydrogenase phosphatase gene *(PDP1)* [48, 49] have also been identified.

Activating mutations in the pyruvate dehydrogenase kinase isoenzyme 3 (*PDK3*) gene have been found to cause X-linked dominant Charcot-Marie-Tooth disease type 6. Findings suggest a reduced pyruvate flux due to Arg158His mutant PDK3-mediated hyper-phosphorylation of the PDHC as the underlying pathogenic cause of peripheral neuropathy [50, 51].

11.3.4 Diagnostic Tests

The most important laboratory test for initial recognition of PDHC deficiency is the measurement of lactate and pyruvate in blood and CSF. In PDHC deficiency, CSF lactate concentrations are generally raised and in excess of blood lactate [52]. Quantitative analysis of plasma amino acids and urinary organic acids may also be useful. Blood lactate, pyruvate and alanine can be intermittently normal when measured after an overnight fast, but characteristically an increase is observed after an oral carbohydrate load. While the L/P ratio is almost always normal, a high ratio can be found if the patient is acutely ill, if blood is very difficult to obtain, or if the measurement of pyruvate (which is unstable) is not done reliably. The practical solution allowing avoidance of these artefacts is to obtain several samples of blood, including samples collected under different dietary conditions (during an acute illness, after overnight fasting, and postprandially after a high-carbohydrate meal) (▶ Chaps. 1 and 3). Glucose tolerance or carbohydrate loading tests are not necessary for a definite diagnosis. In contrast to PC deficiency, fasting hypoglycaemia is not an expected feature of PDHC deficiency, and blood lactate and pyruvate usually decrease after fasting.

Molecular analysis of PDHC genes is often more efficient than measuring the enzyme activity. The most commonly used material for assay of PDHC activity is cultured skin fibroblasts. PDHC can also be assayed in lymphocytes, separated from EDTA-blood, after less than 2 days. PDHC is commonly assayed by measuring the release of $^{14}CO2$ from [1-^{14}C]pyruvate in cell homogenates and tissues. PDHC activity should be measured at low and high TPP concentrations to detect thiamine-responsive PDHC deficiency [53]. PDHC must also be activated (dephosphorylated; ◘ Fig. 11.3), which can be done by preincubation of whole cells or mitochondria with dichloroacetate (DCA, an inhibitor of the kinase; ◘ Fig. 11.3). In E1-phosphatase deficiency there is a deficiency in native PDH activity, but on activation of the PDH complex with DCA, activity becomes normal. The three catalytic components of PDHC can be assayed separately. Immunoblotting of the components of PDHC can help distinguish whether a particular protein is missing. In females with PDHE1α deficiency, X inactivation can interfere with the biochemical analysis. E3BP, which anchors E3 to the E2 core of the complex, can only be evaluated using immunoblotting, since it has no catalytic activity [45].

11.3.5 Treatment and Prognosis

The prognosis for individuals with PDHC deficiency is generally poor, and treatment is not very effective. Experience with early prospective treatment to prevent irreversible brain injury is lacking. Perhaps the most rational strategy for treating PDHC deficiency is the use of a ketogenic diet [54] (▶ Sect. 13.6). Oxidation of fatty acids, 3-hydroxybutyrate and acetoacetate are providers of alternative sources of acetyl-CoA. In a series of males with PDHC deficiency caused by identical *PDHA1* mutations it was found that the earlier the ketogenic diet was started and the more severe the restriction of carbohydrates, the better was the outcome for mental development and survival [55]. Thiamine has been given in variable doses (500–2,000 mg/day), with lowering of blood lactate and apparent clinical improvement in some patients [21, 56] (▶ Chap. 29).

DCA offers another potential treatment for PDHC deficiency. DCA, a structural analogue of pyruvate, inhibits E1 kinase, thereby keeping any residual E1 activity in its active (dephosphorylated) form (◘ Fig. 11.3). DCA can be administered orally or parenterally usually at doses of 10–50 mg/kg/day. Of note, reversible sensory-motor peripheral neuropathy is a clinically limiting adverse effect of chronic DCA treatment [57]. Chronic DCA treatment was shown to be beneficial in some patients, improving the function of PDHC, and this has been related to specific DCA-sensitive mutations [58]. Sporadic reports have also shown a beneficial effect of concomitant DCA and thiamine. A ketogenic diet and thiamine should thus be tried in each patient. DCA can be added in the setting of severe lactic acidosis, in acute situations. Phenylbutyrate in combination with DCA has shown to

increase PDH activity in mice [59]. Fibroblasts of patients with PDHC deficiency and missense mutations responded with increase of activity when incubated with phenylbutyrate [60].

11.4 Dihydrolipoamide Dehydrogenase (DLD) Deficiency

11.4.1 Clinical Presentation

Approximately 30 cases of DLD (E3) deficiency have been reported. Since this enzyme is common to all the 2-ketoacid dehydrogenases (◘ Fig. 11.2), E3 deficiency results in multiple 2-ketoacid-dehydrogenase deficiency and should be thought of as a combined PDHC and TCA cycle defect. E3 deficiency phenotype is a continuum that ranges from early-onset neurological impairment to late-onset isolated liver disease. The most frequent presentation is severe and progressive hypotonia and failure to thrive, starting in the first months of life. Metabolic decompensations are triggered by infections. Progressively, hypotonia, psychomotor retardation, microcephaly and spasticity occur. Some patients develop a typical picture of Leigh's encephalopathy. In a few cases, the main clinical picture is a Reye-like syndrome characterized by recurrent bouts of liver failure frequently associated with encephalopathy. In two patients, the clinical presentation was primarily myopathic [61, 62].

11.4.2 Metabolic Derangement

DLD (E3) is a flavoprotein shared by all three mitochondrial 2-ketoacid dehydrogenase complexes (PDHC, KDHC and BCKD; ◘ Fig. 11.3). It is also the L component of the Glycine cleavage system (▶ Chap. 23).

The predicted metabolic manifestations for E3 deficiency are the result of the deficiency state for each enzyme: increased blood lactate and pyruvate, elevated plasma alanine, glutamate, glutamine and branched-chain amino acids (leucine, isoleucine, valine and often alloisoleucine), and increased urinary lactic, pyruvic, 2-ketoglutaric acids, branched-chain 2-hydroxy- and 2-ketoacids.

11.4.3 Genetics

DLD mutations are inherited as an autosomal recessive trait. The disease-causing mutations occur at different locations in the enzyme and affect preferentially either the reactive oxygen species generation or the affinity for the multienzyme complex that may explain variable phenotypic expression [63].

The p.Gly229Cys mutation is the major cause of E3 deficiency in Ashkenazi Jewish patients and in patients from the Middle East and the Maghreb and is often associated with the liver phenotype [64].

11.4.4 Diagnostic Tests

The initial diagnostic screening should include analyses of blood lactate and pyruvate, plasma amino acids, and urinary organic acids. However, the pattern of metabolic abnormalities is not seen in all patients or at all times in the same patient, making the diagnosis more difficult [65]. This is especially true for elevation of blood branched-chain amino acids concentrations which are rarely and moderately elevated.

In cultured skin fibroblasts, blood lymphocytes, or other tissues, the E3 component can be assayed using a spectrophotometric method. Clinical severity does not parallel the loss in E3 activity.

11.4.5 Treatment and Prognosis

There is no dietary treatment for E3 deficiency although restriction of dietary branched-chain amino acids was reportedly helpful in one case. dl-Lipoic acid has been tried, but its efficacy remains controversial [66]. Riboflavin was successful in correcting the myopathic phenotype in a patient with DLD deficiency and a chaperone effect was discussed [62].

11.5 2-Ketoglutarate Dehydrogenase Complex (KDHC) Deficiency

11.5.1 Clinical Presentation

Isolated deficiency of KDHC has been reported in several unrelated families. As in PDHC deficiency, the primary clinical manifestations included developmental delay, hypotonia, ataxia, opisthotonos and, less commonly, seizures and extrapyramidal dysfunction. On MRI, bilateral striatal necrosis can be found compatible with Leigh syndrome. In one patient the clinical picture was mild: during the first months of life, he developed opisthotonos and axial hypertonia, which improved with age. All patients presented in the neonatal period and early childhood [67].

11.5.2 Metabolic Derangement

KDHC is a 2-ketoacid dehydrogenase that is analogous to PDHC and BCKD (Fig. 11.2). It catalyzes the oxidation of 2-ketoglutaric acid (2-KGA) to yield CoA and NADH. The E1 component, 2-ketoglutarate dehydrogenase, is a substrate-specific dehydrogenase that utilises thiamine and is composed of two different subunits. In contrast to PDHC, the E1 component is not regulated by phosphorylation/dephosphorylation. The E2 component, dihydrolipoyl succinyl-transferase, is also specific to KDHC and includes covalently bound lipoic acid. The E3 component is the same as for PDHC. An E3-binding protein has not been identified for KDHC. Since KDHC is integral to the TCA cycle, its deficiency has consequences similar to those of other TCA enzyme deficiencies.

11.5.3 Genetics

KDHC deficiency is inherited as an autosomal recessive trait. *OGDH* encodes the E1 component and *DLST* the E2 component. Before the identification of the molecular defects in *OGDH* in 2020, the diagnosis was solely confirmed by an enzymatic spectrophotometric assay in cultured skin fibroblasts.

11.5.4 Diagnostic Tests

Urinary organic acid analysis can show increased excretion of 2-KGA with or without concomitantly increased excretion of other TCA cycle intermediates. However, mildly to moderately increased urinary 2-KGA is a common finding and not a specific marker of KDHC deficiency. Some patients with KDHC deficiency also have increased blood lactate with normal or increased L/P ratio. Plasma glutamate and glutamine may be increased.

11.5.5 Treatment and Prognosis

There is no known effective treatment.

11.6 Succinyl-CoA Ligase (SUCL) Deficiency

Succinyl-CoA ligase (SUCL) converts succinyl-CoA and GDP or ADP to succinate and GTP or ATP in the TCA cycle. This process, known as substrate-level phosphorylation, allows the formation of high energy-phosphates (GTP or ATP) in the absence of oxygen. A complex of SUCL with a nucleotide diphosphate kinase (NDPK) is also involved in mtDNA maintenance. Succinyl-CoA ligase is composed of an α-subunit (encoded by *SUCLG1*) and two β-subunits (encoded by *SUCLA2* and *SUCLG2*). The substrate specificity for ADP or GDP is determined by the β-subunit, whereas the α-subunit is shared. SUCL deficiency causes mild methylmalonic aciduria, variable lactic acidosis, accumulation of succinylcarnitine and mitochondrial DNA depletion (▶ Chaps. 10 and 18). Patients with different genetic backgrounds have been found to have mutations in *SUCLA2*, and in *SUCLG1* [68]. The clinical picture in patients with *SUCLA2* mutations is highly homogeneous and comprises early-onset encephalomyopathy, dystonia, deafness and Leigh-like MRI abnormalities. Patients with *SUCLG1* mutations are clinically heterogeneous, showing either a severe form with neonatal multiorgan failure and early death or a phenotype similar to those with *SUCLA2* mutations. Hypertrophic cardiomyopathy and liver involvement are exclusively found in patients with *SUCLG1* mutations [68].

11.7 Succinate Dehydrogenase (SDH) Deficiency

Succinate dehydrogenase (SDH) is part of both the TCA cycle and the respiratory chain, which explains why SDH deficiency resembles more the phenotypes associated with defects of the respiratory chain (▶ Chap. 10).

The clinical picture of this very rare disorder is highly variable. Some patients have multisystem involvement, whereas others have only isolated cardiac or muscle injury with onset in adulthood and normal development. Neurological findings are the most frequent and include developmental regression following acute illness, initial hypotonia and spasticity. Severe cardiomyopathy (dilated or hypertrophic) is frequently reported. Ophthalmologic features include ophthalmoplegia, retinopathy, nystagmus, optic atrophy, and blindness [69]. Early-onset leukoencephalopathy with spasticity and motor regression involvement were reported in SDHA, SDHB [70, 71] and in deficiency of the assembly factor SDHAF1 [72].

Specific thalamus and brainstem MRI patterns observed in a cohort of 19 patients seem to be unique for the SDH-related leukoencephalopathy and to allow differentiation from other mitochondrial leukoencephalopathies [73]. Another specific but inconstant finding is accumulation of succinate in affected white matter seen at MRS [73].

SDH is part of a larger enzyme unit, complex II (succinate-ubiquinone oxidoreductase) of the respira-

tory chain. Complex II is composed of four subunits: the flavoprotein SDHA catalyzes the oxidation of succinate to fumarate (formally, the 'succinate dehydrogenase' activity) (◘ Fig. 11.1); the iron-sulfur protein SDHB transfers electrons to the ubiquinone pool of the respiratory chain (the 'succinate-coenzyme Q reductase' activity); SDHC and SDHD subunits are anchoring proteins. In addition, SDHAF1 and SDHAF2 factors are required for correct assembly. Complex II is unique among the respiratory chain complexes in that all four of its subunits are nuclearly encoded.

SDHA mutations are the most common causes of isolated complex II deficiency, followed by *SDHAF1* mutations. *SDHB* and *SDHD* mutations have been reported in only ten and two patients respectively [69, 74, 75]. Metabolic presentations have yet to be reported in associations with *SDHC* or *SDHAF2*. A myopathic phenotype observed first in Swedish patients with combined defects of SDH and aconitase is due to defects in separate iron-sulfur cluster encoding genes (► Chap. 10). As SDH plays a role as a tumor suppressor, a germline heterozygous mutation associated with a loss of heterozygosity of the wild type allele in the tumoral tissue induces phaeochromocytoma or paraganglioma [76] (see fumarase deficiency, next section).

Lactic acidosis and elevated CSF lactate are inconstant findings and, in contrast to the other TCA cycle disorders, SDH deficiency does not always lead to a characteristic organic aciduria [69]. Diagnostic confirmation of a suspected SDH deficiency requires mitochondrial respiratory chain complex II activity assay, by spectrophotometric procedures.

As SDHA is a flavoprotein, administration of riboflavin, a precursor of FAD, has been tested in some patients but a few of them have exhibited an improvement of their neurological conditions [69].

11.8 Fumarase (FH) Deficiency

Fewer than 100 patients with fumarase deficiency have been reported [77]. Most of the patients present in infancy with a severe encephalopathy, hypotonia, failure to thrive and seizures, poor neurological outcome and profound mental retardation. Dysmorphic features such as frontal bossing, hypertelorism and depressed nasal bridge were noted. Milder cases with developmental delay and epilepsy resembling non progressive cerebral palsy have also been reported.

Neuropathological changes include mainly ventriculomegaly with hydrocephaly and cerebral atrophy, Cortical malformations, polymicrogyria, have also been reported.

Antenatal manifestations include polyhydramnios, congenital hydrocephalus and premature birth [78].

Fumarase catalyzes the reversible interconversion of fumarate and malate (◘ Fig. 11.1). Its deficiency, like other TCA cycle defects, causes: (1) impaired energy production due to interruption in the flow of the TCA cycle and (2) potential secondary enzyme inhibition associated with accumulation in various amounts of metabolites proximal to the enzyme deficiency, such as fumarate, succinate, 2-KGA and citrate (◘ Fig. 11.1).

FH (the gene encoding for fumarase) mutations are inherited as an autosomal recessive trait. A single gene, encodes alternately translated transcripts to generate a mitochondrial and a cytosolic isoform. Heterozygous germline mutations in the fumarase gene are associated with a predisposition to cutaneous and uterine leiomyomas and to kidney cancers [76]. As for succinate dehydrogenase (SDH) deficiency (see above), fumarase and SDH loss of enzyme activity are solely observed in the tumoral tissues. This is due to the combination of a germline mutation (heterozygosity) with a somatic loss of heterozygosity in the other allele (in the tumour). A similar mechanism is observed in focal forms of hyperinsulinism (► Chap. 6).

The biological key finding is increased urinary fumaric acid, sometimes associated with increased excretion of succinic acid and 2-KGA. Fumarase enzyme activity can be assayed in mononuclear blood leukocytes or cultured skin fibroblasts, using a spectrophotometric method.

There is no specific treatment. A ketogenic diet is usually considered to be contraindicated for treating epilepsy associated with FH deficiency or other enzymatic defects within the TCA cycle, since the reduced TCA activity could impair the condensation of acetyl-CoA produced from fatty acids beta-oxidation with oxaloacetate. However, a milder course has been reported in one patient with early introduction of a high fat/low carbohydrates diet [79].

11.9 Mitochondrial Aconitase (ACO) deficiency

About thirty cases of mitochondrial aconitase deficiency due to biallelic *ACO2* mutations have been reported with a large clinical spectrum ranging from infantile cerebellar and retinal degeneration (ICRD) with progressive microcephaly to isolated late onset optic atrophy. The correlation between the residual activity and the phenotypic severity is not fully consistent [80, 81]. Plasma metabolomics in eight patients identified a metabolic fingerprint of aconitase deficiency (decrease of

cis-aconitate, isocitrate, 2-ketoglutarate, phosphoenol pyruvate and hydroxybutyrate) [82].

11.10 Mitochondrial Isocitrate Dehydrogenase (IDH) deficiency

Biallelic mutations in *IDH3A* and *IDH3B* encoding the A and B subunits of mitochondrial NAD^+-specific isocitrate dehydrogenase (IDH3) have been found to be associated with nonsyndromic retinitis pigmentosa in 3 families [83]. One case of IDH3 deficiency was also described associated with severe encephalopathy, hypotonia and autonomic dysregulation [84]. Patients with somatic mutations in the gene encoding for the cytosolic $NADP^+$-specific IDH1 or in the gene encoding for the mitochondrial $NADP^+$-specific IDH2 have presented with malignant gliomas, acute myeloid leukaemia, or choloangiosarcoma [85]. These somatic point mutations in *IDH1/2* alter the enzymes normal ability to convert isocitrate to 2-KGA and confer to the enzymes a new function: the ability to convert 2-KGA to d-2-hydroxyglutarate (D-2HG) an oncometabolite. Conversely, heterozygous germline mutations in *IDH2* were identified in patients with d-2-hydroxyglutaric aciduria (D-2-HGA), a rare neurometabolic disorder characterized by supraphysiological levels of D-2HG [86]. Cancer has not been reported in these patients (► Chap. 22).

11.11 Malate-Aspartate Shuttle (MAS) defects

Defects in some components of the malate-aspartate shuttle were described, with highly similar neurological phenotypes. Patients all exhibited early infantile progressive epileptic encephalopathies with microcephaly.

A defect of the mitochondrial aspartate glutamate carrier AGC1 encoded by *SLC25A12* was first described [87] and has now been reported in four additional children [88, 89]. Brain MRI revealed hypomyelination, probably due to the lack of N-acetylaspartate. A ketogenic diet was initiated in one patient with a clear beneficial effect, probably by reducing the glycolytically generated NADH that shifts the equilibrium of the MAS [90]. Defects in AGC2 (*SLC25A13*) are involved in type II citrullinemia (► Chap. 19).

A homozygous mutation of *MDH1* encoding the cytosolic MDH was recently described in two related children [91]. The absence of hyperlactataemia and increased blood glycerol-3-phosphate levels in both patients suggested a compensatory effect of the G3P shuttle.

Biallelic mutations in *MDH2* encoding the mitochondrial MDH were described in three unrelated patients with increased lactate in blood and CSF [92]. Despite disruption of the TCA cycle, nearly normal concentrations of urinary organic acids were observed in these patients. A ketogenic diet has appeared to decrease the frequency of epileptic seizures for the two children. *MDH2* was also identified as a pheochromocytoma and paraganglioma susceptibility gene [93]. Loss of heterozygosity of the wild type allele and significant reduction of MDH activity were observed in the tumours.

GOT2 deficiency has been identified in four children with variants of *GOT2*. Hyperlactataemia, mild hyperammonemia and citrullinemia were observed in these patients. The intramitochondrial synthesis of aspartate from oxaloacetate is decreased in GOT2 deficiency leading to lower concentrations of aspartate in the cytosol and reduced arginosuccinate synthesis with citrulline accumulation and hyperammonemia. Serine biosynthesis is also hampered by the impaired cytosolic redox imbalance. Treatment with pyridoxine (to activate residual GOT2 activity) and serine fully controlled seizures in two of these patients [94].

11.12 Mitochondrial Citrate Carrier Deficiency

This disorder is responsible for D-2- and L-2-Hydroxyglutaric Aciduria (► Chap. 22).

11.13 Mitochondrial Pyruvate Carrier (MPC) deficiency

MPC1 mutations have been described in 5 cases [95, 96]. Neonatal lactic acidosis in a female baby born to consanguineous parents was associated with generalized hypotonia and facial dysmorphism. Brain MRI revealed cortical atrophy, periventricular leukomalacia and calcifications. Progressive microcephaly, failure to thrive and neurological deterioration led to death at age 19 months. An affected fetus was found in a subsequent pregnancy. Three other patients with a mild progressive encephalopathy have been identified in consanguineous families of North African descent.

The mitochondrial pyruvate carrier (MPC) is a hetero-oligomer consisting in two obligate subunits encoded by the *MPC1* and *MPC2* genes. MPC mediates the proton symport of pyruvate across the inner mito-

chondrial membrane. Consequently, the metabolic derangement should be the same as in pyruvate dehydrogenase deficiency.

High lactate and pyruvate are found with normal L/P ratio. To evidence the transport defect, [2-^{14}C] pyruvate oxidation is measured in digitonin-permeabilised *versus* disrupted fibroblasts. Digitonin induces outer cell membrane permeabilisation, leaving intracellular mitochondrial membranes intact. Oxidation of 2-^{14}C-pyruvate is severely impaired in digitonin-permeabilised fibroblasts but not in disrupted cells. No treatment is known to date.

11.14 NAD(P)HX System Repair Defects

Deficiencies in the enzymes that repair the damages induced by water addition on reduced forms of pyridine dinucleotides have been recently discovered [97]. Hydration of NAD(P)H occurs spontaneously at high temperature or as a side effect of GAPDH, giving rise to hydrates NAD(P)HX which have lost their ability to exchange electrons and may inhibit several dehydrogenases. NAD(P)HX are prone to an irreversible cyclisation but the NAD(P)HX repair system allows the reconversion of both epimers S and R of NAD(P)HX to normal cofactors: a NAD(P)HX epimerase encoded by *NAXE* converts the S-form of NAD(P)HX in the R-form and a NAD(P)HX dehydratase encoded by *NAXD* dehydrates the R-form. Inactivating mutations have been discovered in both *NAXE* and *NAXD* [97, 98]. Most patients with a NAD(P)H system repair defect present with infantile progressive lethal encephalopathy, with skin lesions, elevated lactate in cerebrospinal fluid and episodes of deterioration triggered by fever episodes. Patients with onset in adulthood and a milder course have also been reported. Administration of high doses of vitamin D_3 (nicotinamide) has been suggested as a treatment strategy [97] and has been shown to stabilize milder forms [99].

11.15 Protein-Bound Lipoic Acid Defects and Defects in Cofactors

See ► Chaps. 23 and 29.

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Disorders of Mitochondrial Fatty Acid Oxidation & Riboflavin Metabolism

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Contents

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Mitochondrial Fatty Acid Oxidation

Mitochondrial fatty acid oxidation involves three processes (■ Fig. 12.1).

1. *Entry of fatty acids into mitochondria*. Long-chain fatty acids are activated to coenzyme A (CoA) esters in the cytoplasm but they need to be transferred to carnitine in order to cross the inner mitochondrial membrane; they are transferred back to CoA within the mitochondria. Carnitine palmitoyltransferase I is the main site for the regulation of fatty acid oxidation by cytoplasmic malonyl-CoA. Medium and short-chain fatty acids enter mitochondria independent of carnitine and are activated to CoA esters in the matrix.
2. *β-Oxidation via a spiral pathway*. Each turn of the spiral shortens the acyl-CoA by two carbons and involves four steps. These include two dehydrogenation reactions, linked respectively to flavin adenine dinucleotide (FAD) and nicotinamide adenine dinucleotide (NAD). β-Oxidation is catalysed by enzymes of different chain length specificities. The enzymes for long-chain substrates are membrane-bound; three of the reactions are catalysed by the mitochondrial trifunctional protein (MTP). Medium and short-chain enzymes are located in the matrix.
3. *Electron Transfer.* Electrons are passed to the respiratory chain either directly (from NADH to complex I) or via two transfer proteins (from FADH2 to ubiquinone). Acetyl-CoA released by β-oxidation can either be oxidised in the Krebs cycle or, in the liver, used to synthesise ketone bodies (► Chap. 13).

Riboflavin

Riboflavin (vitamin B_2) is the precursor of flavin mononucleotide (FMN) and FAD. FMN is a cofactor for complex I of the respiratory chain and FAD is a cofactor for complex II and many other dehydrogenation reactions. Three riboflavin transporters have been identified. RFVT1 and RFVT3 are responsible for intestinal riboflavin uptake whereas RFVT2 is involved in transport into tissues, particularly brain. Within cells, riboflavin is phosphorylated to FMN, which can then be converted to FAD by FAD synthase. This enzyme has 2 isoforms, mitochondrial (FADS1) and cytoplasmic (FADS2), generated by alternative splicing. FAD enters mitochondria on the mitochondrial folate/FAD transporter. A mitochondrial riboflavin transporter has been postulated but not proven.

Introduction

Fatty acid oxidation disorders have a high incidence, particularly in populations of European origin. Many countries now have newborn screening programs for these disorders. Before screening was introduced, the commonest clinical presentations were hypoketotic hypoglycaemia and sudden death, usually precipitated by an infection or fasting in the neonatal period or early childhood. Older children or adults may present with exercise-induced rhabdomyolysis. Patients can remain asymptomatic throughout life if they have mild defects and are not exposed to the necessary stress. Treatment should be tailored to the severity of the disorder.

This chapter also considers defects of riboflavin metabolism and transport, including Brown-Vialetto-van Laere syndrome.

12.1 Disorders of Mitochondrial Fatty Acid Oxidation

Fat is an important source of energy and, because of its high energy density, it is the body's principal fuel store. Fatty acids are used by heart muscle in preference to glucose and are the main fuel for skeletal muscle during sustained exercise. During prolonged fasting, most tissues derive energy from fatty acids, allowing glucose to be spared for the brain. As well as releasing energy, hepatic fatty acid oxidation provides acetyl-CoA for ketone body synthesis. By using ketone bodies, even the brain can derive energy indirectly from fatty acids. For non mitochondrial fatty acid metabolism see ► Chap. 42.

12.1.1 Clinical Presentations

Mitochondrial fatty acid oxidation disorders (FAODs) have three characteristic clinical presentations.

- Acute hypoketotic hypoglycaemia and encephalopathy, accompanied by hepatomegaly and liver dysfunction but seldom jaundice. Problems are precipitated by fasting or an infection with vomiting. Some patients die unexpectedly during a minor illness. This is often described as a hepatic presentation or a 'Reye-like illness'.
- Cardiomyopathy (usually hypertrophic), arrhythmias or conduction defects.
- Myopathy, presenting either with weakness or with acute rhabdomyolysis, which may be precipitated by exercise or infection.

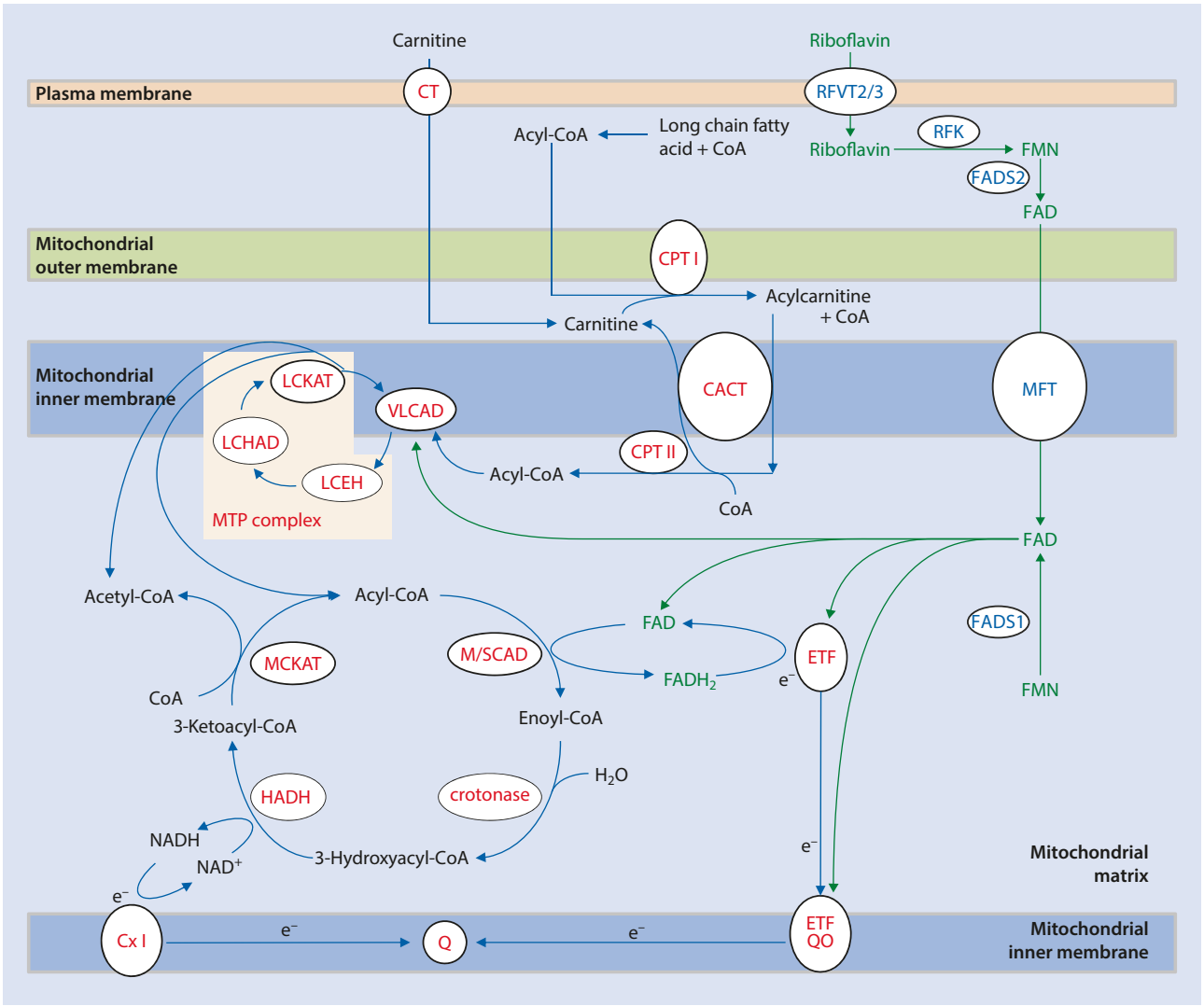

Fig. 12.1 Pathway of mitochondrial fatty acid oxidation. CACT carnitine acylcarnitine translocase, CoA coenzyme A, CPT carnitine palmitoyltranferase, CT carnitine transporter, Cx I complex I of respiratory chain, *e-* electrons, ETF electron transfer flavoprotein, ETFQO ETF ubiquinone oxidoreductase, FAD flavin adenine dinucleotide, FADH2 reduced FAD, FADS1/2 FAD synthase 1/2, FMN flavin mononucleotide, HADH 3-hydroxyacyl-CoA dehydrogenase, LCEH long-chain enoyl-CoA hydratase, LCKAT long-chain ketoacyl-CoA thiolase, LCHAD long-chain 3-hydroxyacyl-CoA dehydrogenase, MCKAT medium-chain ketoacyl-CoA thiolase, MFT mitochondrial folate/FAD transporter, MTP mitochondrial trifunctional protein, M/SCAD medium-chain acyl-CoA dehydrogenase or short-chain acyl-CoA dehydrogenase, NAD^+ nicotinamide adenine dinucleotide, NADH reduced NAD, Q ubiquinone, RFK riboflavin kinase, RFVT2/3 riboflavin transporter 2/3, VLCAD very-long-chain acyl-CoA dehydrogenase. Some substrates and products are omitted for VLCAD and MTP. Extra enzymes are required for the oxidation of unsaturated fatty acids (dodecenoyl-CoA delta isomerase and 2,4-dienoyl-CoA reductase, not shown)

Some disorders are only associated with one of these presentations, whereas others can cause all three problems (Table 12.1), depending on the residual enzyme activity, the age of the patient and exposure to stress. Thus, patients may suffer hypoglycaemia in infancy and rhabdomyolysis as adults. Additional problems occur in specific disorders, as described below. A number of patients never develop symptoms, either because they have a mild defect or because they are not exposed to the necessary environmental stress.

12.1.1.1 Fatty Acid Transport Defects

The mechanisms of fatty acid transport across the plasma membrane are still not completely clear [1]. Impaired uptake has been reported in 2 boys who pre-

Table 12.1 Inherited disorders of mitochondrial fatty acid oxidation

Defect	Gene(s)	Potential clinical manifestations[a]				
		Hypoglycaemia & acute liver dysfunction	Cardio-myopathy	Rhabdo-myolysis	Chronic weakness	Other problems
Carnitine defects						
CT	*SLC22A5*	+	+		+	
CPT I	*CPT1*	+				RTA
CACT	*SLC25A20*	+	+		+	
CPT II	*CPT2*	+	+	+	+	Malformations
β-oxidation defects						
VLCAD	*ACADVL*	+	+	+	+	
MCAD	*ACADM*	+				
SCAD	*ACADS*					
ACAD9	*ACAD9*		+		+	
Crotonase	*ECHS1*		+			Neurodegeneration
LCHAD & MTP	*HADHA, HADHB*	+	+	+	+	Retinopathy, Neuropathy
HADH/SCHAD	*HADHSC*	+				Hyperinsulinism
Dienoyl-CoA reductase[b]	*NADK2*					Neurodegeneration
Mitochondrial enoyl-CoA reductase deficiency	*MECR*					Movement disorder & optic atrophy
Electron transfer defects						
MAD[c]	*ETFA, ETFB, ETFDH*	+	+		+	Malformations

[a]Features depend on the residual enzyme activity and exposure to environmental stress
[b]Some patients with biallelic *NADK2* mutations have secondary dienoyl-CoA reductase deficiency; no patients with primary deficiency have been identified
[c]MAD can also result from defects of riboflavin transport or metabolism ◘ Table 12.3
MAD multiple acyl-CoA dehydrogenase deficiency, *RTA* renal tubular acidosis, other abbreviations in ◘ Fig. 12.1

sented with liver failure [2]. The molecular basis was not identified and the diagnoses remain uncertain.

12.1.1.2 Carnitine Cycle Defects

Carnitine Transporter Deficiency

The organic cation/carnitine transporter OCTN2 (encoded by *SLC22A5*) is responsible for carnitine uptake across the plasma membrane, particularly in heart, muscle and kidney. Defects lead to primary carnitine deficiency with increased renal loss of carnitine, low plasma concentrations and sufficiently low intracellular concentrations to impair fatty acid oxidation [3].

Symptoms may be precipitated by an infection, fasting, pregnancy or antibiotics containing pivalate, which is excreted bound to carnitine further reducing carnitine concentrations [4]. Some patients present in infancy with hypoglycaemia, liver dysfunction and hyperammonaemia, usually before 2.5 years of age. Other children develop heart failure due to cardiomyopathy, with thickened ventricular walls and reduced contractility. This is often accompanied by skeletal muscle weakness. Adults may suffer fatigue or arrhythmias [4] but screening has shown that many subjects with low plasma carnitine remain asymptomatic (for example in the Faroe Islands, where the prevalence is 1:300).

Carnitine Palmitoyltransferase I (CPT I) Deficiency

Different isoforms of CPT I have been found in liver and kidney (CPT Ia), muscle and heart (CPT Ib) and brain (CPT Ic). Only CPT Ia deficiency has been identified in man. Patients usually present by the age of 2 years with hypoketotic hypoglycaemia, induced by fasting

or illness. This is accompanied by hepatomegaly, liver dysfunction and occasionally cholestasis that may take several weeks to resolve. There may also be transient lipaemia and renal tubular acidosis [5]. Paradoxically, a few patients have had cardiac problems or raised plasma creatine kinase (CK) [5].

CPT I deficiency is extremely common in the Inuit population of Canada and Greenland. A few of these patients present with hypoglycaemia as neonates or young children but most remain asymptomatic [6].

▪ Carnitine Acylcarnitine Translocase (CACT) Deficiency

This rare disorder usually presents in the neonatal period, with death by 3 months of age [7]. Problems include severe hypoglycaemia and hyperammonaemia, cardiomyopathy, atrioventricular block and ventricular arrhythmias, which can cause sudden death. A few more mildly affected patients present later with hypoglycaemic encephalopathy, precipitated by fasting or infections.

▪ Carnitine Palmitoyltransferase II (CPT II) Deficiency

The commonest form of this disorder is a partial deficiency that presents with episodes of rhabdomyolysis. Attacks are usually precipitated by prolonged exercise, particularly in the cold or after fasting, and start in adolescents or young adults. In childhood, episodes may be brought on by infections. Muscle pain may begin during or after exercise and can spread from muscles that have been working to those that have not. In moderate or severe episodes there is myoglobinuria, which may lead to acute renal failure and require dialysis for a few days. Plasma CK is markedly raised; it often normalises between episodes but may remain moderately elevated, with chronic weakness.

Severe neonatal onset CPT II deficiency is usually lethal. Patients become comatose within a few days of birth, due to hypoglycaemia and hyperammonaemia. In addition, they may have cardiomyopathy, arrhythmias and congenital malformations, principally renal cysts and neuronal migration defects, such as the Dandy-Walker malformation [8]. There is also an intermediate form of CPT II deficiency that causes episodes of hypoglycaemia and liver dysfunction, sometimes accompanied by cardiomyopathy and arrhythmias.

12.1.1.3 β-Oxidation Defects

▪ Very-Long-Chain Acyl-CoA Dehydrogenase (VLCAD) Deficiency

Mildly affected patients present as adolescents or adults with exercise-induced rhabdomyolysis. Other patients present in childhood with hypoglycaemia but suffer exercise- or illness-induced rhabdomyolysis or chronic weakness at a later age. Severely affected patients present in early infancy with cardiomyopathy, in addition to the problems seen in milder patients. Neonatal screening has shown that VLCAD deficiency is the second commonest fatty acid oxidation disorder in Europe and the USA, with a prevalence between 1:50,000 and 1:100,000 [9]. This is much higher than was detected clinically. Undoubtedly, the diagnosis was missed in some symptomatic patients, particularly those presenting as adults, but it is likely that many patients diagnosed by screening would remain asymptomatic without intervention.

▪ Long-Chain 3-Hydroxyacyl-CoA Dehydrogenase (LCHAD) & Mitochondrial Trifunctional Protein (MTP) Deficiencies

MTP is composed of four α-subunits and four β-subunits; the α-subunit has long-chain enoyl-CoA hydratase (LCEH) and LCHAD activities and the β-subunit has long-chain ketoacyl-CoA thiolase (LCKAT) activity. Patients may have isolated LCHAD deficiency or a generalised deficiency of all three enzyme activities.

Patients with isolated LCHAD deficiency usually present acutely before 6 months of age with hypoglycaemia, accompanied by liver dysfunction and lactic acidosis [10]. Many patients have cardiomyopathy and some have hypoparathyroidism or acute respiratory distress syndrome. Other patients present with chronic symptoms, such as failure to thrive, hypotonia or, occasionally, cholestasis or cirrhosis. Subsequently, episodes of rhabdomyolysis are common. Many patients develop retinopathy, which may start as early as 2 years of age. Granular pigmentation, especially in the macular region, is followed by chorioretinal atrophy with deteriorating central vision [11]. Some patients develop cataracts [11]. Peripheral neuropathy is another common complication with onset at any age from infancy to adulthood. It is axonal, though there may be secondary demyelination; it can be sensorimotor, purely sensory or purely motor with prominent ankle weakness and calf wasting. Occasionally, metabolic decompensation triggers acute worsening of the neuropathy, which is partly reversible [12].

Generalised MTP deficiency is more heterogeneous [13]. Patients with severe deficiency present as neonates with cardiomyopathy, respiratory distress, hypoglycaemia and liver dysfunction; most die within a few months, regardless of treatment. Other patients resemble those with isolated LCHAD deficiency. There is also a milder neuromyopathic phenotype, characterised by episodes of exercise- or illness-induced rhabdomyolysis and/or progressive peripheral neuropathy. Diagnosis is often delayed in patients presenting with isolated neuropathy, particularly as their acylcarnitines are often normal [12].

Mothers who are heterozygous for LCHAD or MTP deficiency have a high risk of illness during pregnancies

when they are carrying an affected fetus [14]. The main problems are HELLP syndrome (Haemolysis, Elevated Liver enzymes and Low Platelets) and acute fatty liver of pregnancy (AFLP).

Medium-Chain Acyl-CoA Dehydrogenase (MCAD) Deficiency

MCAD deficiency is much the commonest fatty acid oxidation disorder in North-West Europe, with an incidence of approximately 1:10,000–20,000 in Europe, the USA and Australia [15]. Before newborn screening, patients usually presented between the ages of 4 months and 4 years with acute hypoglycaemic encephalopathy and liver dysfunction; some deteriorated rapidly and died. Problems are precipitated by prolonged fasting or, more often, by an infection with vomiting. A number of babies still present within 72 hours of birth, before newborn screening results are available, with hypoglycaemia and/or arrhythmias [16]; breast-fed babies are at higher risk, due to the small supply of breast milk at this stage.

Patients with MCAD deficiency only present clinically if exposed to an appropriate environmental stress. Even before newborn screening, many patients, probably 30–50%, remained asymptomatic [15]; with newborn screening and preventative measures, hypoglycaemia is rare. Patients do not develop cardiomyopathy or myopathy and few present as adults [17].

When in good health, MCAD deficient children aged over 1 year can fast for 12–14 hours without problems. If fasting is continued, they deteriorate over a few hours with hypoglycaemia and inappropriately low ketone body concentrations. Shorter periods of fasting may cause problems in infancy, though some patients have fasted regularly for up to 12 hours from 4–6 months of age, prior to diagnosis. Encephalopathy may occur without hypoglycaemia [17], presumably due to the accumulation of free fatty acids and their carnitine and CoA esters.

Short-Chain Acyl-CoA Dehydrogenase (SCAD) Deficiency

Various symptoms have been reported in SCAD deficiency, most frequently developmental delay. However, almost all patients diagnosed by screening or because of an affected relative remain asymptomatic [18]. The pathological significance of SCAD deficiency is, therefore, unclear. It may confer susceptibility to disease or, more probably, it may be a non-disease whose association with symptoms results from ascertainment bias.

3-Hydroxyacyl-CoA Dehydrogenase (HADH) Deficiency

This defect, previously called SCHAD deficiency, causes hyperinsulinaemic hypoglycaemia (▶ Chap. 6).

Short-Chain Enoyl-CoA Hydratase (Crotonase) Deficiency

This defect presents with severe neurological problems, lactic acidosis and sometimes cardiomyopathy (▶ Chap. 18).

Acyl-CoA Dehydrogenase 9 (ACAD9) Deficiency

ACAD9 is an assembly factor for respiratory chain complex I. It is also homologous to VLCAD and has dehydrogenase activity towards long-chain acyl-CoA esters; it is responsible for forming the C14:1 acylcarnitine that accumulates in VLCAD deficiency. Patients with ACAD9 defects usually present in infancy or childhood with hypertrophic cardiomyopathy, myopathy and lactic acidaemia; a few also have neurodevelopmental problems. Most patients show some response to riboflavin, reducing the mortality which is otherwise high in infancy [19].

Dienoyl-CoA Reductase Deficiency

No patients with this primary defect have been identified. The diagnosis was suspected in a few infants with neurodegeneration and raised plasma lactate and C10:2 acylcarnitine levels but these were secondary to mitochondrial NADP(H) deficiency, caused by *NADK2* mutations [20]. The C10:2 acylcarnitine level was normal in a girl with milder NADK2 mutations, who presented with optic atrophy and peripheral neuropathy; like the other patients, she had raised plasma lysine concentrations [21].

Mitochondrial Enoyl-CoA Reductase Deficiency

Biallelic mutations in *MECR* cause *MECR*-related neurologic disorder, also known as Mitochondrial Enoyl-CoA Reductase Protein-Associated Neurodegeneration (MEPAN) as MECR encodes mitochondrial trans-2-enoyl-CoA reductase. The disease is characterised by a progressive dystonia, that may be accompanied by chorea and/or ataxia and by optic atrophy. The movement disorder typically starts in early to mid childhood but the optic atrophy may appear later. Intellect is often but not always, preserved. On MRI there is bilateral hyperintense T_2-weighted signal within the basal ganglia. No consistent biomarkers have been found; diagnosis relies on molecular testing. Only supportive treatment is currently available but all of the 13 known patients were alive when reported and 2 were in their 5th decade [22].

12.1.1.4 Electron Transfer Defects

Multiple Acyl-CoA Dehydrogenase (MAD) Deficiency

Electron transfer flavoprotein (ETF) and ETF ubiquinone oxidoreductase (ETFQO) carry electrons to the respiratory chain from multiple FAD-linked dehydrogenases (◘ Fig. 12.1). These include enzymes of amino acid and choline metabolism in addition to the acyl-CoA dehydrogenases of β-oxidation. Thus, defects

of ETF or ETFQO lead to multiple acyl-CoA dehydrogenase (MAD) deficiency (also known as glutaric aciduria type II). MAD deficiency can also, less often, result from defects of riboflavin transport or metabolism (▶ Sect. 12.2).

ETF and ETFQO deficiencies have a wide range of clinical severity. Severely affected patients present in the first few days of life with hypoglycaemia, hyperammonaemia and acidosis accompanied by hypotonia and hepatomegaly. There is usually an odour of sweaty feet similar to that in isovaleric acidaemia. Some patients have congenital anomalies (including large cystic kidneys, hypospadias and neuronal migration defects that can be detected prenatally by fetal MRI) and facial dysmorphism (low set ears, high forehead and midfacial hypoplasia). The malformations resemble those seen in CPT II deficiency and Zellweger syndrome but the pathogenesis is unknown. Most patients with neonatal presentation die within a week of birth; many of the others develop cardiomyopathy and die within a few months.

Less severe cases can present at any age from infancy to adulthood with hypoglycaemia, liver dysfunction and weakness, usually precipitated by an infection [23]. Cardiomyopathy is common in infants. Rarer problems include stridor and leukodystrophy [24]. Mildly affected children may have recurrent bouts of vomiting. Muscle weakness is the commonest presentation in adolescents and adults. It predominantly affects proximal muscles and may lead to scoliosis, hypoventilation or an inability to lift the chin off the chest. The weakness can worsen rapidly during an infection or pregnancy but myoglobinuria is rare. Many of the milder defects respond to riboflavin (▶ Sect. 12.1.5.3) [23].

12.1.1.5 Other Potential Defects

Medium-chain 3-ketoacyl-CoA thiolase (MCKAT) deficiency has been reported in one patient, who died at 13 days of age with hypoglycaemia, hyperammonaemia, acidosis and myoglobinuria [25].

Long-chain acyl-CoA dehydrogenase (LCAD) appears to be involved in surfactant metabolism and LCAD deficiency has been reported in two cases of sudden infant death [26]. The paucity of cases may be due to the overlapping substrate specificities of LCAD and VLCAD.

12.1.2 Metabolic Derangement

Fasting hypoglycaemia is the classic metabolic disturbance in FAODs and is primarily due to increased peripheral glucose consumption, though hepatic glucose output is also reduced under some conditions [27]. The hypoglycaemia is hypoketotic. Ketone bodies can be synthesised, particularly in medium- or short-chain FAODs [28] or if there is high residual enzyme activity, but the plasma concentrations are lower than expected for the degree of hypoglycaemia or the plasma free fatty acid concentrations. Hyperammonaemia occurs in some severe defects, with normal or low glutamine concentrations; it is thought to result from decreased acetyl-CoA production reducing the synthesis of N-acetylglutamate, which is the physiological activator of carbamoyl phosphate synthetase 1. Hyperuricaemia is common during acute attacks. Lactic acidaemia is seen in long-chain FAODs, particularly LCHAD and MTP deficiencies, and results from the inhibitory effects of metabolites on various steps in pyruvate metabolism [29]. Secondary respiratory chain dysfunction, particularly affecting flavin-dependent complexes, has been demonstrated in MAD deficiency due to *ETFDH* mutations [23] as well as defects of flavin metabolism (▶ Sect. 12.2).

Accumulating long-chain acylcarnitines may be responsible for arrhythmias and may interfere with surfactant metabolism [26]. In LCHAD and MTP deficiencies, long-chain hydroxyacylcarnitine concentrations correlate with the severity of retinopathy [30] and may cause both this and the peripheral neuropathy.

12.1.3 Genetics

All mitochondrial FAODs show an autosomal recessive pattern of inheritance with the exception of transient neonatal MAD deficiency (▶ Sect. 12.2.2). The genes for individual enzymes are listed in ◘ Table 12.1. Heterozygosity seldom causes problems except for mothers heterozygous for LCHAD deficiency, who may develop AFLP or HELLP syndrome when carrying an affected fetus. Rhabdomyolysis has been reported in a few heterozygotes for CPT II deficiency. It has also been suggested that symptoms, such as myopathy, may occur in individuals who are heterozygous for more than one defect of fatty acid oxidation or related pathways [31]; this proposal has been termed ‘synergistic heterozygosity’.

There is molecular heterogeneity in all these disorders but some prevalent mutations have been identified:

- CPT I deficiency. The high frequency of CPT I deficiency in the Inuit is due to a founder effect: in some regions of northern Canada, 70% babies are homozygous for c.1436C>T p.(Pro479Leu) [6]. Though the mutation reduces CPT I activity to about 6% of control values, fatty acid oxidation flux is only modestly decreased. The Inuit traditionally select a high fat diet leading to permanent ketosis; subjects feel unwell if ketogenesis stops abruptly. The mutant

enzyme is less sensitive to inhibition by malonyl-CoA and this may confer a selective advantage by retaining ketosis.
- CPT II deficiency. The c.439C>T (p.Ser113Leu) *CPT2* mutation accounts for approximately 60% mutant alleles in myopathic Caucasian patients [32].
- MCAD deficiency. The c.985A>G p.(Lys304Glu) mutation is common in populations originating from northern Europe: 80% symptomatic patients and 60% patients detected by screening are c.985A>G homozygotes [15].
- SCAD deficiency. There are two polymorphisms, c.625G>A p.(Gly185Ser) and c.511C>T p.(Arg147Trp). In northern Europe, 6% of the general population have one of these variants on both alleles [18]. SCAD deficiency can be associated with these variants or with rare mutations.
- LCHAD & MTP deficiency. Most Caucasian patients are homozygous for the c.1528G>C p.(Glu510Gln) mutation in the LCHAD domain of the α-subunit; this gives rise to isolated LCHAD deficiency. Patients with complete or partial deficiencies of all 3 enzyme activities are said to have generalised MTP deficiency. This can result from mutations affecting either subunit [13] and includes most compound heterozygotes for c.1528G>C and a second α-subunit mutation.

The relationship between genotype and phenotype varies in different FAODs. In CPT II and VLCAD deficiencies, homozygous nonsense mutations are generally associated with severe early onset disease, whereas late onset rhabdomyolysis is associated with conservative missense mutations (such as the c.439C>T) *CPT2* mutation and the c.848T>C p.(Val283Ala) *ACADVL* mutation) [32, 33]. The latter is the commonest mutation in Caucasians with VLCAD deficiency and has only been found in mildly affected or asymptomatic patients. For patients with rare mutations, it is easier to predict the clinical course from the residual enzyme activity or fatty acid oxidation flux.

The genotype correlates less closely with phenotype in MCAD and carnitine transporter deficiencies. MCAD deficient patients with the same genotype may die or remain asymptomatic, depending on their exposure to fasting stress. Some *ACADM* mutations are, however, less likely to cause clinical problems. In particular, the c.199T>C p.(Tyr42His) mutation is associated with significant residual activity and is relatively benign: it accounts for >6% mutant alleles in most screened populations but there have only been a few reports of clinical problems [34].

12.1.4 Diagnostic Tests

The investigation of a suspected FAOD starts by looking for abnormal metabolites, particularly acylcarnitines. If the results suggest a specific diagnosis, this is confirmed by enzyme assays or mutation analysis. If the metabolite results are non-specifically abnormal or if they are normal despite strong clinical suspicion, it may be helpful to measure acylcarnitine production in vitro or flux through the pathway or to sequence a panel of FAOD genes (or the whole exome).

12.1.4.1 Abnormal Metabolites

Acylcarnitines

In most FAODs, acyl-CoA intermediates accumulate proximal to the defect and are transesterified to carnitine. The acylcarnitine abnormalities are best analysed by tandem mass spectrometry (TMS). The usual samples are plasma or dried blood spots on filter paper. Table 12.2 lists the typical abnormalities in different FAODs.

The diagnostic specificity can be increased by measuring the ratios of different acylcarnitines. For example, C8 acylcarnitine is raised in patients with MCAD and MAD deficiencies and in MCAD deficiency carriers at times of stress; the presence of a raised C8/C10 acylcarnitine ratio increases the specificity for MCAD deficiency, which is particularly useful in newborn screening programs. Severe CPT II and CACT deficiencies, however, cause identical acylcarnitine abnormalities, as do LCHAD and MTP deficiencies; distinction requires genetic or enzyme analysis.

The clinical circumstances have a major effect on the acylcarnitine profile. Abnormalities are usually more marked in stressed patients but, if the plasma free carnitine concentration is very low, abnormal acylcarnitines may be hard to detect. Abnormalities may be reduced or masked completely by intravenous glucose or dietary treatment, such as the use of medium-chain triglycerides (MCT) in long-chain FAODs. Interpretation is especially difficult for samples obtained terminally or post-mortem: these often show multiple raised acylcarnitine species, resembling MAD deficiency.

Acylcarnitine analysis can be completely normal in patients with high residual enzyme activity, such as mild VLCAD or MTP deficiencies; abnormalities are sometimes detectable after overnight fasting, exercise or loading with carnitine. Myopathic CPT II deficiency is particularly hard to diagnose; the sum of the C16:0 and C18:1 acylcarnitine concentrations may be raised relative to acetylcarnitine but this is not reliable.

There are no abnormal acylcarnitine species in patients with deficiencies of the carnitine transporter or CPT I but free carnitine concentrations are usually abnormal.

Free and Total Carnitine Concentrations

Plasma free and total carnitine concentrations are best measured by an enzymatic radioisotope tech-

Table 12.2 Abnormal metabolites seen in fatty acid oxidation disorders

Deficiency	Plasma acylcarnitines	Urinary acylglycines	Urinary organic acids[a]
CT	Low free carnitine		±(DCA)
CPT IA	Virtually absent long- & medium-chain acylcarnitines, high free carnitine		(Variable DCA)
CACT and CPT II severe	C18:1, C18:2, C16, C16-DC, C18:2-DC, C18:1-DC		Variable DCA
CPT II mild	↑(C16 + C18:1)/C2[b]		
VLCAD	C16:1, C14:2, **C14:1**, C18:1[b]		Variable DCA
MCAD	C10:1, **C8**, C6	Hexanoyl-, suberyl-, phenylpropionyl-	DCA [suberic > adipic], (KB)
SCAD	C4	Butyryl-	Ethylmalonic, methylsuccinic, KB
LCHAD / MTP	C18:1-OH, C18-OH, C16:1-OH, C16-OH[b]		3-Hydroxydicarboxylic acids, DCA
HADH	±C4-OH		±(3-hydroxybutyric, 3-hydroxyglutaric)
NADK2	±C10:2		±Lactic, ethylmalonic, glutaric, fumaric
MAD: severe	C4, C5, C5-DC, C6, C8, C10, C12, C14:1, C16, C18:1	Isobutyryl-, isovaleryl-, hexanoyl-, suberyl-,	Ethylmalonic, glutaric, 2-hydroxyglutaric, DCA
MAD: mild	C6, C8, C10, C12	Isobutyryl-, isovaleryl-, hexanoyl-, suberyl-	Ethylmalonic, adipic, DCA, KB

[a]These are typical organic acids during acute illness; those in parentheses are mildly elevated. Organic acids are often normal during anabolism. *DCA,* C6-C10 saturated straight-chain dicarboxylic acids; *Variable DCA*, C6-C12 saturated and unsaturated straight-chain dicarboxylic acids

[b]Acylcarnitines can be normal during anabolism (e.g. mild VLCAD and MTP deficiencies) or even during catabolism (e.g. mild CPT II deficiency)

'For VLCAD & MCAD deficiencies, the main abnormal acylcarnitines are in bold'

KB ketone bodies, other abbreviations in Fig. 12.1

nique. Carnitine can be formed from acetyl- and acylcarnitines during derivatisation for TMS. Nevertheless, with careful sample preparation, TMS can provide a reasonable estimate of the plasma free carnitine concentration. Measurement in dried blood spots is less reliable. Plasma free and total carnitine concentrations are usually <5 μmol/l in patients with carnitine transporter deficiency [3], though they may be higher in the newborn period, when they reflect the mother's carnitine status and carnitine transporter deficiency can be missed. Plasma free carnitine concentrations are often reduced in other FAODs, due to the accumulation of acylcarnitines which competitively inhibit the carnitine transporter and increase the renal loss of carnitine.

In CPT I deficiency, the ratio of free carnitine to long-chain acylcarnitines is increased. This abnormality is more reliably detected in blood spots than plasma and allows patients to be detected by newborn screening. Raised free carnitine concentrations are due to increased reabsorption from urine: because the defect prevents the formation of acylcarnitines, there is less competitive inhibition of the carnitine transporter than normal.

Urinary Organic Acids and Acylglycines

Organic acid analysis is often normal in FAODs when the patient is well. Indeed, patients with mild CPT II, MTP or MAD deficiencies can have normal organic acids even during acute crises and patients with SCAD, MCAD and mild MAD deficiencies can have significant ketonuria. Many patients with FAODs, however, have elevated medium-chain (and sometimes long-chain) dicarboxylic acids during fasting or illness, with little or no increase in ketone bodies. Similar patterns can be seen in defects of ketogenesis or the respiratory chain and in patients recovering from ketosis or receiving MCT; a preponderance of sebacic acid helps to distinguish the latter.

Characteristic organic acid patterns are seen in certain FAODs (◘ Table 12.2 and ▶ Chap. 3 ◘ Table 3.2) and abnormal glycine conjugates are found in some FAODs (◘ Table 12.2). Using stable isotope-dilution mass spectrometry, these can be demonstrated even when patients are healthy.

12.1.4.2 In Vitro Studies

- **Enzyme Assays**

Suspected diagnoses need to be confirmed by enzyme assays or mutation analysis. Enzyme assays are generally performed on cultured fibroblasts or lymphocytes [35]. The latter can be prepared from as little as 1–2 ml fresh EDTA blood and results may be available within a few days. For the ß-oxidation spiral, the chain length specificities of different enzymes overlap. This problem can be overcome by finding a substrate that is specific for one enzyme (e.g. MCAD, HADH) or specific under the assay conditions (e.g. VLCAD). Alternatively, the interfering enzyme can be inhibited or immunoprecipitated before performing the assay (e.g. LCHAD, LCKAT). For some enzymes, such as MCAD and VLCAD, the residual activity in vitro correlates with the clinical severity: this may help in managing patients detected by screening. Few laboratories assay ETF or ETFQO and these defects are generally confirmed by mutation analysis.

- **Mutation Analysis**

Molecular studies, such as gene panels and whole exome sequencing, identify FAODs in a few patients with normal plasma acylcarnitines and urine organic acids. This is most likely in patients presenting with rhabdomyolysis or peripheral neuropathy [36]. Molecular studies are also used increasingly often to confirm the diagnosis, as an alternative to enzymology. This is usually satisfactory but the pathogenicity of sequence variants is sometimes hard to assess. Moreover, standard sequencing may miss some mutations, such as large deletions and those in introns that affect splicing. Mutation analysis allows carrier testing and prenatal diagnosis.

- **Whole Cell Techniques**

Quantitative acylcarnitine profiling may indicate the site of a defect if this is not clear from metabolite results. Acylcarnitines are analysed by TMS after incubating fibroblasts or lymphocytes with fatty acids, labelled with stable isotopes [37].

Fatty acid oxidation flux is measured by incubating cells with radio-labelled fatty acids and collecting the oxidation products [38]. This is useful in evaluating the severity of a disorder but acylcarnitine profiling yields more diagnostic information.

12.1.4.3 Fasting Studies

For suspected FAODs, fasting studies have been supplanted by acylcarnitine analysis and in vitro studies. It is, however, still useful to collect blood (and urine) samples if a patient presents with hypoglycaemia: raised plasma free fatty acids with inappropriately low ketone body concentrations suggest an FAOD (▶ Chap. 3).

12.1.4.4 Prenatal Diagnosis

Mutation analysis is the preferred technique, if the molecular defect is known in the index case. All the enzymes of fatty acid oxidation are expressed in chorionic villus biopsies and amniocytes. Prenatal diagnosis is, therefore, also possible using enzyme assays.

12.1.4.5 Newborn Screening

Many countries now screen for FAODs, using acylcarnitine analysis by TMS. The free- and acyl-carnitine abnormalities and value of ratios are discussed in ▶ Sect. 12.1.4.1 and ◘ Table 12.2. The target conditions vary between countries, some only screening for MCAD deficiency. Screening for long-chain FAODs is best performed when patients are catabolic on day 2 or 3 of life; acylcarnitines may be normal subsequently, so an initial abnormal profile should be followed by confirmatory enzyme or genetic testing rather than repeating acylcarnitine analysis. Screening may miss mild deficiencies of CPT II, MAD and MTP. For VLCAD and carnitine transporter deficiencies there is a high false positive rate (in the latter occasionally due to undiagnosed maternal carnitine transporter deficiency or a maternal vegan diet); moreover, many of the cases detected are unlikely to have symptoms during childhood. Patients with VLCAD deficiency can be stratified into high and low risk groups according to the residual enzyme activity in lymphocytes or the fatty acid oxidation flux in fibroblasts. In the future, the metabolomic profile in blood spots may be another tool to distinguish mild and severe cases [39].

12.1.5 Treatment and Prognosis

Most patients with a FAOD need to avoid prolonged fasting and require careful management during acute illnesses to prevent metabolic decompensation. Long-term dietary treatment is needed in patients with severe long-chain FAODs. Carnitine and riboflavin are indicated in specific disorders and various forms of treatment have been proposed for exercise-induced symptoms.

12.1.5.1 Management of Acute Illness

The hormonal changes associated with acute illness lead to lipolysis and increased fatty acid oxidation. In most FAODs, this can lead to metabolic decompensa-

tion. The process can be prevented by providing sufficient glucose to stimulate insulin secretion and suppress lipolysis. Drinks containing an appropriate amount of glucose should be started at the first sign of illness and continued every 2–3 hours until the patient starts to improve; feeds should be reintroduced within 24–48 hours. If the drinks are vomited or the patient deteriorates, hospital admission is needed for intravenous glucose (at least the physiological hepatic glucose production rate, i.e. 3–12 mg/kg/minute, depending on age). Hypoglycaemia is a late event and management should be started without delay, regardless of the blood glucose concentration.

12.1.5.2 Long Term Dietary Management

Prolonged fasting should be avoided in all FAODs to prevent acute metabolic decompensation. Frequent, regular feeds are recommended during the first year of life but subsequently overnight fasting can be tolerated in most disorders (including MCAD and most cases of VLCAD deficiency). In severe FAODs, overnight fasting is avoided until later in childhood, to reduce the risk of cardiomyopathy and long-term complications. These patients may be managed with continuous overnight tube feeding, with extra feeds during the night or, when older, with uncooked cornstarch before bed.

Dietary fat restriction is unnecessary in MCAD deficiency and breast feeding should be allowed. Top-ups of formula should, however, be given for the first 2–3 days, until the supply of breast milk improves. Unfortunately, this can only be implemented if there is a relevant family history, because screening results only arrive after the period of increased risk.

Long-chain fat is restricted in severe long-chain FAODs. Medium-chain fatty acids can enter mitochondria independent of carnitine and also bypass the long-chain ß-oxidation enzymes. Medium-chain triglyceride (MCT) can, therefore, be substituted for long-chain fat in patients with long-chain FAODs, such as VLCAD, MTP, LCHAD, CACT, CPT I and CPT II deficiencies. Dietary MCT has led to the resolution of cardiomyopathy in a number of patients with VLCAD and LCHAD deficiencies; its use has also been associated with the resolution of renal tubular acidosis in CPT I deficiency [5]. Anecdotal evidence suggests that a bolus of MCT before exercise can prevent rhabdomyolysis in patients with myopathic VLCAD deficiency [40].

For symptomatic infants with long-chain FAODs, a formula maximally enriched with MCT is recommended and breast feeding is avoided, at least initially [41, 42]. After weaning, it has been suggested that MCT should provide 20% and long-chain fat only 10% energy intake; this is hard to achieve in older patients, particularly as the long-chain fat has to include adequate essential fatty acids (4% energy intake).

Newborn screening detects a number of mildly affected patients with long-chain FAODs. These do not need a special diet and breast feeding can be continued. Most asymptomatic babies with VLCAD deficiency fall into this category but some authorities recommend dietary modification (as above) if the mutations or enzyme studies predict a severe phenotype [42]. An MCT-enriched formula is recommended for all babies with LCHAD and MTP deficiencies [41] but a limited amount of breast feeding is sometimes allowed.

Triheptanoin has been substituted for MCT in a number of patients with long-chain FAODs. Triheptanoin differs from conventional MCT in containing odd chain fatty acids, which generate propionyl-CoA as well as acetyl-CoA when they are oxidised. In FAODs, it is suggested that tricarboxylic acid cycle intermediates leak out of cells, and that propionyl-CoA can replenish this loss. Substituting triheptanoin for MCT resulted in a statistically significant improvement in cardiac function in a randomised controlled trial [43]. Triheptanoin has been approved by the FDA since 2020 but it is not yet approved by the EMA.

MCT and Triheptanoin are contraindicated in MCAD, ETF and ETFQO deficiencies because medium-chain fatty acids cannot be oxidised in these defects. Instead, patients with severe ETF and ETFQO deficiencies have been treated with sodium 3-hydroxybutyrate (usually a racemic mixture of the D and L isomers). The myopathy, cardiomyopathy, liver disease and leukodystrophy can all improve but it may take weeks or months to see benefit, particularly in myopathy, and large doses are needed (300-2000 mg/kg/d, generally divided into 4 doses) [24, 44]. Modes of action may include the supply of energy and of substrates for cholesterol synthesis, decreases in toxic metabolites (via inhibition of lipolysis) and regulation of gene expression and inflammation. Sodium 3-hydroxybutyrate has also helped to overcome acute decompensation in patients with CACT, CPT II and HMG-CoA lyase deficiencies [45].

Ketone esters can supply 3-hydroxybutyrate without a sodium load. Nausea and abdominal pain can occur with KB salts or esters. When given before exercise, (R)-3-hydroxybutyl (R)-3-hydroxybutyrate lowered abnormal plasma acylcarnitine levels and improved muscle energy balance (assessed by ^{31}P-MRS) in a small trial of adults with VLCAD deficiency, suggesting that it may prevent rhabdomyolysis [46].

12.1.5.3 Drug Treatment

Carnitine treatment is very effective in patients with carnitine transporter deficiency. The usual dose is 100 mg/kg/day, divided into 3 or 4 doses. Plasma concentrations may reach the lower part of the normal range but muscle carnitine concentrations remain less than 5% normal. Nevertheless, treatment prevents hypoglycaemia and arrhythmias; any cardiomyopathy or weakness resolves within a few months.

The value of carnitine therapy in other FAODs is controversial. Plasma free carnitine concentrations are often low, particularly after an acute illness, but tissue concentrations are seldom measured. It has been suggested that carnitine may promote the excretion of metabolites and prevent the sequestration of coenzyme A but this has not been proven. Indeed, carnitine treatment may be harmful in long-chain FAODs, as it increases the concentrations of long-chain acylcarnitines, which are potentially arrhythmogenic.

Patients with mild MAD deficiency due to *ETFDH* mutations often respond to treatment with riboflavin (100 mg/day). Benefit is seen in some children who presented with hypoglycaemia as well as adults who presented with weakness [23]. Symptoms may resolve completely or there may be some residual weakness.

Bezafibrate increases the expression of FAO enzymes by activating peroxisome proliferator-activated receptor (PPAR) α and PPARδ receptors. This allows it to enhance the residual enzyme activity in fibroblasts from patients with partial CPT II or VLCAD deficiencies. Conflicting results have been obtained in clinical trials. In one study, treatment for 6 months increased the enzyme activity in muscle biopsies from adults with CPT II deficiency [47] but a second randomised study found no increase in exercise tolerance or fatty acid oxidation [48].

12.1.5.4 Monitoring

Follow-up is required even for asymptomatic patients but the tests undertaken depend on the defect and its severity. The plasma transaminase levels indicate the recent metabolic status in patients with hepatic involvement, as does the plasma CK if muscle or heart is involved. In unsupplemented patients, the free carnitine concentration is another marker of metabolic status. Essential fatty acids and fat-soluble vitamins should be monitored in patients on fat-modified diets. Hepatic steatosis and cardiomyopathy can be assessed by ultrasound. Ophthalmological and neurophysiological studies are needed in LCHAD and MTP deficiencies.

12.1.5.5 Prognosis

In the past, most FAODs had a significant mortality during the presenting illness but a good prognosis following diagnosis. Newborn screening improves the outcomes but, for many disorders, it identifies a number of patients who would never have developed symptoms. Most data are available for MCAD deficiency. Before screening programmes, approximately 4% patients died in the first 72 hours and a further 5–7% died over the next 6 years [49]. After an episode of encephalopathy, about 7% survivors have neuropsychological deficits. Newborn screening greatly reduces the morbidity and mortality, though it cannot eliminate early neonatal deaths [15]. Following diagnosis, the prognosis is also excellent for patients with carnitine transporter, CPT I and riboflavin-responsive MAD deficiencies.

Recurrent rhabdomyolysis is a long-term problem in mild CPT II deficiency and in some patients with VLCAD deficiency; no current form of treatment completely prevents this. LCHAD deficiency is a more serious condition: about a third of patients died in the presenting illness prior to screening [10]. Moreover, in LCHAD and MTP deficiencies, there is a high long-term risk of retinopathy or peripheral neuropathy and pregnancy is hazardous. The neonatal onset forms of CACT, CPT II, MTP and MAD deficiencies often present before screening results are available; they are usually fatal within a few days or months.

12.2 Defects of Riboflavin Transport & Metabolism

Riboflavin is present in a wide variety of foods; milk and dairy products are major sources in Western diets. Riboflavin deficiency is associated with stomatitis and interferes with iron handling but it is hard to be sure of the true effects as human deficiency is usually accompanied by other dietary deficiencies and animal studies may not be relevant. Riboflavin deficient rats have impaired fatty acid oxidation and excrete dicarboxylic acids and acylglycines similar to those seen in MAD deficiency.

Riboflavin transport and metabolism are summarised in the Overview at the start of the chapter. Defects of riboflavin metabolism cause myopathy or cardiomyopathy whereas defects of the RFVT2 and RFVT3 riboflavin transporters present with neurodegeneration (Brown-Vialetto-van Laere syndrome). The biochemical features often indicate MAD deficiency (◘ Table 12.3).

Table 12.3 Riboflavin transport and metabolism defects

Transporter/ Enzyme	RFVT1	RFVT2	RFVT3	FAD synthase	FAD transporter
Gene	*SLC52A1*	*SLC52A2*	*SLC52A3*	*FLAD1*	*SLC25A32*
Maximum expression	Intestine, placenta	Ubiquitous, esp. brain	Intestine, testis		
Inheritance of defect	Transient problems in child of heterozygous mother	Autosomal recessive		Autosomal recessive	
Clinical features	Neonatal hypoglycaemia & hyperammonaemia	Ponto-bulbar palsy & deafness		Myopathy ± cardiomyopathy	
Biochemical features	Mother: riboflavin deficiency, Child: MADD-like ACs & OAs	± Low riboflavin, ± MADD-like ACs		MADD-like ACs & OAs	

MADD multiple acyl-CoA dehydrogenase deficiency, *ACs* blood acylcarnitines, *OAs* urine organic acids (Table 12.2 for ACs and OAs expected in MADD)

12.2.1 Riboflavin Transporter Deficiencies

Autosomal recessive deficiencies of the RFVT2 or RFVT3 transporters cause a neurodegenerative disorder characterised by ponto-bulbar palsy, neurogenic weakness and deafness. It was previously called Brown-Vialetto-van Laere syndrome; cases without deafness were called Fazio-Londe syndrome but have the same causes. Patients usually present in infancy or early childhood but those with RFVT3 defects occasionally present as adults. In RFVT2 defects the commonest initial symptom is sensory ataxia followed by weakness of the hands, wrists and neck, due to axonal sensorimotor neuropathy; some patients present with nystagmus due to optic atrophy. RFVT3 defects present with more generalised weakness (hypotonia in infancy), including facial weakness, or with bulbar signs, such as stridor or dysphagia; optic atrophy is less common [50]. Deafness (due to auditory neuropathy) is often an early feature in both defects. The initial signs are followed by rapidly progressive bulbar palsy with respiratory failure due to diaphragmatic paralysis; there may be tongue fasciculation, ptosis or ophthalmoplegia.

A pattern of blood acylcarnitines suggesting mild MAD deficiency is seen in approximately 60% patients, regardless of the underlying defect. The organic acids may also suggest mild MAD deficiency and there may be low plasma flavin concentrations. *SLC52A2* and *SLC52A3* sequencing is needed for diagnosis.

Treatment with riboflavin leads to clinical stabilisation or improvement in patients with defects of either transporter. Doses of 10–80 mg/kg/day have been given with minimal side effects [49]. Improvement may occur over a few days or several months but it is less likely if symptoms have been present for long. The biochemical abnormalities always resolve.

12.2.2 RFVT1 Deficiency

This has been implicated in a transient neonatal fatty acid oxidation defect. The baby presented within 24 hours of birth with hypoglycaemia, hyperammonaemia and organic aciduria typical of MAD deficiency. The abnormalities resolved with riboflavin treatment and did not recur when this was withdrawn. The mother had riboflavin deficiency and a heterozygous mutation in *SLC52A1*, which encodes the RFVT1 riboflavin transporter [51]. RFVT1 is expressed in placenta as well as small intestine and haploinsufficiency may have caused severe riboflavin deficiency in the baby at birth. We have seen similar transient clinical and biochemical abnormalities in several neonates whose mothers have not had *SLC52A1* mutations, though some did have riboflavin deficiency.

12.2.3 FAD Synthase and Mitochondrial FAD Transporter Deficiencies

These are rare autosomal recessive neuromuscular disorders. The plasma acylcarnitines and urine organic acids are typical of MAD deficiency. Muscle biopsies show a lipid-storage myopathy and respiratory chain studies show abnormalities particularly affecting the FAD-dependent complex II. Molecular genetic studies reveal biallelic mutations in the gene for FAD synthase (*FLAD1*) or the mitochondrial FAD transporter (*SLC25A32*).

FAD synthase deficiency has been reported in patients from 12 pedigrees. The main problem has been generalised weakness, with onset ranging from the neonatal period to adulthood. The myopathy often leads to dysarthria, dysphagia, scoliosis or respiratory failure; cardiomyopathy and arrhythmias have also been reported [52].

There have been two reports of patients with mitochondrial FAD transporter defects who presented with muscle weakness in childhood [53]; one subsequently developed dysarthria, dysphagia and myoclonus as an adult [54]. Biallelic *SLC25A32* mutations have also been found in an anencephalic fetus and inactivation of the Slc25a32 gene in mice induces neural tube defects [55]. This may reflect another role of the transporter as a folate carrier.

Treatment with riboflavin led to clinical and biochemical improvement in the two patients with FAD transporter defects and in some patients with FAD synthase deficiency but other patients did not respond and several died in infancy.

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Disorders of Ketogenesis and Ketolysis

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Contents

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Ketogenesis and Ketolysis

During fasting, ketone bodies (KB) are an important fuel for many tissues, including cardiac and skeletal muscle. They are particularly important for the brain, which cannot oxidise fatty acids. The principal KB, acetoacetate and 3-hydroxybutyrate, are maintained in equilibrium by 3-hydroxybutyrate dehydrogenase; acetone is formed from acetoacetate non-enzymatically and eliminated in breath. KB are formed in liver mitochondria, predominantly from fatty acids, but also from certain amino acids, such as leucine. KB cross the mitochondrial inner membrane on the mitochondrial pyruvate carrier and cell membranes on monocarboxylate transporters (MCTs) [1]. Export from hepatocytes is facilitated by MCT1 and/or MCT7 (encoded by *SLC16A1* and *SLC16A6*). MCT1 facilitates entry into target cells (such as heart, muscle and neurons) and is needed for KB to cross the blood brain barrier. For use as fuel, KB are converted to acetyl-CoA in the mitochondria of extrahepatic tissues. One of the ketolytic enzymes, mitochondrial acetoacetyl-CoA thiolase (also known as β-ketothiolase or T2), is also involved in the breakdown of isoleucine (Fig. 13.1).

Introduction

Disorders of ketone body metabolism present either in the first few days of life or later in childhood, during an infection or some other metabolic stress.

There are two defects of ketogenesis, 3-hydroxy-3-methylglutaryl (HMG)-CoA lyase deficiency and HMG-CoA synthase deficiency. In these, decompensation leads to encephalopathy, with vomiting and a reduced level of consciousness, often accompanied by hepatomegaly. The biochemical features – hypoketotic hypoglycaemia, with or without hyperammonaemia – resemble those seen in fatty acid oxidation disorders. The organic acids are diagnostic in HMG-CoA lyase deficiency. In HMG-CoA synthase deficiency, the organic acids are characteristic during decompensation but normal at other times.

Ketone body utilisation is catalysed by succinyl-CoA:3-oxoacid CoA transferase (SCOT) and mitochondrial acetoacetyl-CoA thiolase (also known as β-ketothiolase or T2). Deficiencies of SCOT, T2 or the monocarboxylate transporter 1 (MCT1) present with episodes of ketoacidosis. This is often accompanied by dehydration and decreased consciousness. The organic acids usually show characteristic abnormalities in T2 deficiency but there are no specific findings in SCOT or MCT1 deficiencies and diagnosis relies on molecular analysis.

In all these disorders, the primary aim of treatment is to prevent decompensation. Fasting is avoided and a high glucose intake is maintained at times of metabolic stress, such as infections.

This chapter also discusses ketotic hypoglycaemia and the use of ketogenic diets in inherited metabolic disease.

13.1 Ketogenesis Defects

13.1.1 Clinical Presentation

Mitochondrial 3-Hydroxy-3-Methylglutaryl-CoA (HMG-CoA) Synthase Deficiency

HMG-CoA synthase deficiency presents with hypoglycaemia, often accompanied by coma and metabolic acidosis [2]. There is one report of a seizure and coma without hypoglycaemia [3]. Episodes are precipitated by infections with vomiting or poor feeding in early childhood (5 months to 6 years of age). There is usually hepatomegaly, which subsequently resolves. Hyperammonaemia is rare but (surprisingly) ketonuria may be present [4]. Most patients recover promptly with intravenous glucose.

HMG-CoA Lyase Deficiency

HMG-CoA lyase deficiency presents with hypoglycaemia, metabolic acidosis, vomiting and a reduced level of consciousness. In almost 50% patients, the onset is within 5 days of birth after a short symptom-free period [5]. In most others, symptoms are provoked by an infection in the first year. A few patients first present later, occasionally as adults [6].

KB levels are inappropriately low but blood lactate concentrations may be markedly elevated, particularly in neonatal onset cases. Patients often have hyperammonaemia, hepatomegaly and abnormal liver function tests. Cardiomyopathy and pancreatitis are rare complications [7]. Most patients recover but many suffer neurological sequelae, including intellectual handicap, epilepsy, hemiplegia/tetraplegia or cerebral visual loss, particularly after neonatal hypoglycaemia [5, 7].

Neuroimaging shows diffusely abnormal white matter with foci of more severe changes, even in asymptomatic patients [8]. This may be due to impaired myelination because KB are a substrate for the synthesis of myelin cholesterol. Cerebral atrophy and basal ganglia abnormalities are also common, sometimes without an overt movement disorder [5].

13.1.2 Metabolic Derangement

KB are synthesised in hepatic mitochondria, primarily using acetyl-CoA derived from fatty acid oxidation (Fig. 13.1). HMG-CoA synthase catalyses the condensation of acetoacetyl-CoA and acetyl-CoA to form

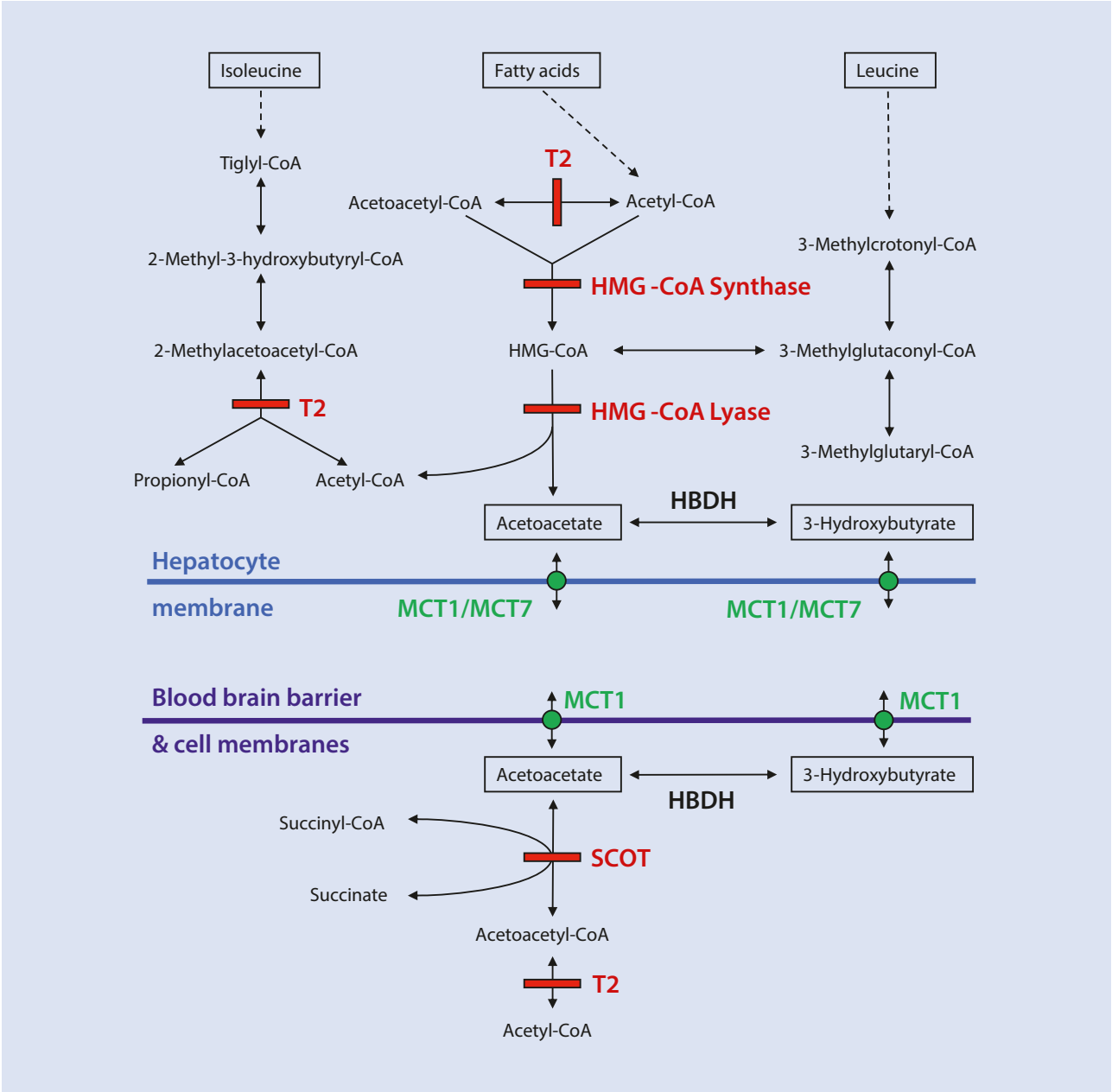

Fig. 13.1 Biochemical pathways involving enzymes of ketogenesis and ketolysis. HMG-CoA 3-hydroxy-3-methylglutaryl coenzyme A, SCOT succinyl-CoA 3-oxoacid CoA transferase, T2 mitochondrial acetoacetyl-CoA thiolase, HBDH 3-hydroxybutyrate dehydrogenase, MCT1 monocarboxylate transporter 1. The thick blue and purple lines represent the cell membranes of hepatocytes and the blood brain barrier / extrahepatic tissues that consume ketone bodies. Mitochondrial membranes are not shown. The enzyme defects discussed in this chapter are depicted by red bars across the arrows

HMG-CoA, which is cleaved by HMG-CoA lyase to release acetyl-CoA and acetoacetate. HMG-CoA can also be derived from the amino acid, leucine. Thus, HMG-CoA synthase and HMG-CoA lyase deficiencies both impair ketogenesis but HMG-CoA lyase deficiency also causes the accumulation of intermediates of the leucine catabolic pathway. During fasting, the lack of KB leads to excessive glucose consumption and hypoglycaemia.

13.1.3 Genetics

HMG-CoA synthase and HMG-CoA lyase deficiencies are inherited as autosomal recessive traits caused by homozygous or compound heterozygous mutations in *HMGCS2* and *HMGCL* respectively. HMG-CoA lyase deficiency is the commonest organic acidaemias in the Iberian peninsula and in Saudi Arabia, where

the prevalent *HMGCL* mutations are c.109G>A p.(Glu37*) and c.122G>A p.(Arg41Gln), respectively [7]. The genotype correlates poorly with the clinical phenotype, which depends on exposure to environmental stress [7].

13.1.4 Diagnostic Tests

A general approach to hypoketotic hypoglycaemia is given in ▶ Chap. 1 (▶ Fig. 1.2 and 1.4).

Samples collected during an episode of hypoglycaemia can be very valuable in disorders of KB metabolism. If the plasma free fatty acid concentration is raised with an inappropriately small rise in total KB (FFA/total KB >2.5) it implies a defect of ketogenesis or fatty acid oxidation [2]. These can be distinguished by analysing metabolites or measuring fatty acid oxidation flux *in vitro*.

▪ HMG-CoA Synthase Deficiency

During decompensation, urine contains saturated, unsaturated and 3-hydroxy-dicarboxylic acids, 5-hydroxyhexanoic acid and other metabolites, of which 4-hydroxy-6-methyl-2-pyrone is the most specific [4]. Blood acylcarnitine analysis is normal when patients are well but acetylcarnitine may be raised during illness. The diagnosis is confirmed by mutation analysis. Enzyme assays require a liver biopsy and are complicated by a cytoplasmic isoenzyme, involved in cholesterol synthesis.

▪ HMG-CoA Lyase Deficiency

Even when healthy, patients excrete increased quantities of 3-hydroxy-3-methylglutaric, 3-hydroxyisovaleric, 3-methylglutaconic and 3-methylglutaric acids (◘ Fig. 13.1); 3-methylcrotonylglycine may also be present. Blood acylcarnitine analysis shows raised 3-hydroxyisovalerylcarnitine concentrations. The diagnosis is confirmed by mutation analysis or measuring HMG-CoA lyase activity in leukocytes or cultured fibroblasts.

HMG-CoA lyase deficiency is included in the newborn screening programs for several countries, including the USA. Cases need to be distinguished from other causes of increased C5-hydroxyacylcarnitines (3-hydroxyisovalerylcarnitine or 2-methyl-3-hydroxybutyrylcarnitine, which have the same mass): 3-methylcrotonyl-CoA carboxylase deficiency (in the infant or mother), T2 deficiency, 2-methyl-3-hydroxybutyryl-CoA dehydrogenase deficiency, multiple carboxylase deficiency (2 disorders) and various disorders associated with 3-methylglutaconic aciduria (▶ Chaps. 10, 18 and 35). Confirmatory tests include urine organic acid analysis (for the infant and mother), plasma acylcarnitine analysis and serum biotinidase assay.

▪ Prenatal Diagnosis

Molecular techniques are used in families where the mutations are known. For HMG-CoA lyase deficiency, enzyme assays can be performed on chorionic villi or cultured amniocytes.

13.1.5 Treatment and Prognosis

Patients should avoid fasting and maintain a high carbohydrate intake during any metabolic stress, such as infections. An intravenous infusion of glucose is required if drinks containing glucose or glucose polymers are refused or vomited. Intravenous sodium bicarbonate may be needed if there is severe acidosis in HMG-CoA lyase deficiency. Sodium DL-3-hydroxybutyrate has been given to an adolescent with HMG-CoA lyase deficiency during acute decompensation and appeared to contribute to his recovery [9].

A moderate protein or leucine restriction is recommended in HMG-CoA lyase deficiency because of its role in leucine catabolism [2, 7]. There is less agreement about the need for a low fat diet [7]. Indeed, some patients have developed normally without any dietary restriction [6]. Carnitine supplements are usually given in HMG-CoA lyase deficiency, though their value is unproven.

HMG-CoA synthase deficiency has a good prognosis after the presenting illness: most patients have no further episodes of encephalopathy. Neurological problems are commoner in HMG-CoA lyase deficiency, particularly in neonatal-onset cases. These patients are also more likely to have recurrent episodes of encephalopathy as older children or adults. Pregnancy carries a high risk in HMG-CoA lyase deficiency: the eight reported pregnancies included one termination, one intrauterine death and one maternal death during decompensation at 9 weeks gestation [10]. Pregnant women with either defect should be given intravenous glucose during labour and during illnesses with vomiting.

13.2 Defects of Ketone Body Utilisation or Transport

Defects of ketone body utilisation or transport include succinyl-CoA:3-oxoacid CoA transferase (SCOT), mitochondrial acetoacetyl-CoA thiolase (T2) and monocarboxylate transporter 1 (MCT1) deficiencies.

13.2.1 Clinical Presentation

Typically, patients present with episodes of severe ketoacidosis in early childhood and are healthy between

episodes, with a normal blood pH. Decompensation is generally triggered by fasting or an infection with poor feeding and vomiting. Tachypnoea, due to acidosis, is accompanied by dehydration, caused by vomiting and an osmotic diuresis; consciousness may be reduced if the acidosis is severe and a few patients have seizures. Blood glucose, lactate and ammonia concentrations are normal in most cases but there may be hypo- or hyperglycaemia or mild hyperammonaemia [11, 12].

Approaching 50% patients with SCOT deficiency become acidotic within a few days of birth, the others presenting within the first two years [2]. In T2 deficiency, neonatal onset is very rare and the initial episode of acidosis is usually between 6 and 24 months of age; a few patients are never acidotic [10]. Only six patients with homozygous MCT1 deficiency have been reported [13–15]. Their acute presentations were indistinguishable from ketolysis defects and occurred by two years of age.

Most patients with SCOT or T2 deficiencies make a full recovery following episodes of acidosis but a few die or are left with mental retardation, ataxia or dystonia [11, 12]. Neuroimaging shows abnormalities in the basal ganglia in a number of patients with T2 deficiency. Some of these patients have presented with hypotonia, dystonia or chorea without any preceding episodes of acidosis [11, 16]. All the patients with homozygous MCT1 deficiency had developmental delay and several had epilepsy; cranial MRI showed abnormal signal involving the subcortical U-fibres and in the basal ganglia and thalami; two siblings had agenesis of the corpus callosum [15].

13.2.2 Metabolic Derangement

KB utilisation occurs in extrahepatic mitochondria, starting with the transfer of coenzyme A from succinyl-CoA to acetoacetate, catalysed by SCOT. This forms acetoacetyl-CoA, which is converted to acetyl-CoA by T2. The second reaction can also be catalysed to some extent by medium-chain 3-ketoacyl-CoA thiolase (T1), which may explain why T2 deficient patients do not have permanent ketosis (unlike those with severe SCOT deficiency). SCOT is not expressed in liver and has no role other than ketolysis. In contrast, T2 is expressed in liver, where it participates in ketogenesis. Patients with T2 deficiency present with ketoacidosis, suggesting that T1 is a more effective substitute in ketogenesis than in ketolysis. T2 also cleaves 2-methylacetoacetyl-CoA in the isoleucine degradation pathway and T2 deficiency causes the accumulation of isoleucine-derived acyl-CoA esters: these may be responsible for the basal ganglia lesions found in some T2 deficient patients [16].

The episodes of ketoacidosis in patients with MCT1 deficiency indicate the need for these transporters to facilitate the rapid entry of KB into target cells at times of stress. MCT1 and other monocarboxylate transporters are also important for lactate transport, including lactate shuttling from glia to neurons. The learning difficulties in MCT1 deficient patients may reflect this rather than the episodes of ketoacidosis.

13.2.3 Genetics

SCOT, T2 and MCT1 deficiencies are inherited as autosomal recessive traits with biallelic mutations in the *OXCT1*, *ACAT1* and *SLC16A1* genes, respectively. Single heterozygous mutations in *OXCT1* or *SLC16A1* have also been found in a number of patients investigated for ketoacidosis, suggesting that carriers have an increased risk of acidosis if exposed to sufficient stress [13, 17, 18]. The episodes of ketoacidosis tend to be less severe in *SLC16A1* heterozygotes than in homozygotes and start later in childhood; cyclical vomiting is initially suspected in some heterozygotes.

Heterozygous *SLC16A1* mutations have also been associated with muscle injury [19] and with exercise-induced hyperinsulinism [20]. The latter is caused by promoter mutations that prevent the normal silencing of MCT1 expression in pancreatic β-cells (► Chap. 6). Apart from this, the genotype shows little correlation with the clinical phenotype in these disorders, though it is related to the severity of the biochemical abnormalities (see below). The frequency of ketoacidosis depends primarily on exposure to environmental stress [2, 11].

13.2.4 Diagnostic Tests

A general approach to ketoacidotic states is presented in ► Chap. 1 (► Fig. 1.3).

■ SCOT & MCT1 Deficiencies

These conditions need to be considered in a number of patients because ketoacidosis is relatively common. A plasma free fatty acid: total KB ratio <0.3 suggests a defect of ketolysis [2]. Urine organic acid analysis reveals high concentrations of KB but no specific abnormalities. Patients with severe SCOT deficiency have persistent ketonuria in the fed state, but patients with a mild mutation do not [2]. The diagnoses are now usually made by mutation analysis, though SCOT enzyme assays can be undertaken on lymphocytes or cultured fibroblasts.

T2 Deficiency

Patients with T2 deficiency typically excrete increased amounts of 2-methylacetoacetate, 2-methyl-3-hydroxybutyric acid and tiglylglycine (Fig. 13.1). However, 2-methylacetoacetate is unstable and patients with mild mutations may only show abnormalities when they are stressed [2, 11], for example by an isoleucine load. 2-Methyl-3-hydroxybutyryl-CoA dehydrogenase deficiency (HSD10 disease) causes a similar pattern of organic acids but 2-methyacetoacetate is not excreted (▶ Chap. 18). The diagnosis must, therefore, be confirmed by mutation analysis or enzyme assay in fibroblasts. Assays are complicated by the presence of other thiolases that act on acetoacetyl-CoA. 2-Methylacetoacetyl-CoA is a specific substrate for T2 but it is difficult to synthesise. One solution is to repeat the assay in the presence and absence of potassium, which enhances the activity of T2 but not the other enzymes.

Blood acylcarnitine analysis in T2 deficiency generally shows raised 2-methyl-3-hydroxybutyrylcarnitine and tiglylcarnitine but the concentrations may be normal in patients with mild mutations [2, 11]. T2 deficiency is included in some expanded newborn screening programs but patients with mild mutations are often missed and cases need to be distinguished from HMG-CoA lyase deficiency and other inborn errors (▶ Sect. 13.1.4).

Prenatal Diagnosis

Prenatal diagnosis is possible using molecular techniques in families where the mutations are known. Alternatively, prenatal diagnosis can be performed by enzyme assays in chorionic villi (T2) or cultured amniocytes (T2 or SCOT).

13.2.5 Treatment and Prognosis

These patients can decompensate rapidly in early childhood. To prevent this, they should have frequent regular feeds during the first year and subsequently they should not fast for longer than 12 hours. A high carbohydrate intake must be maintained during any metabolic stress, such as surgery or infection (▶ Chap. 4). Drinks containing carbohydrate should be started at the first sign of illness; hospital admission is needed if these are vomited or if the patient develops moderate or heavy ketonuria. In hospital, patients require an intravenous infusion of glucose. Dehydration is common and must be corrected. An intravenous infusion of sodium bicarbonate is needed if there is severe ketoacidosis (pH <7.1); it may be given in milder acidosis but electrolytes must be monitored frequently as there is a risk of severe and potentially fatal hypernatraemia.

Mild dietary protein restriction is generally recommended in T2 deficiency as isoleucine metabolites may be responsible for the basal ganglia lesions seen in some patients [16]. Outcomes have not, however, correlated with protein intake and a number of patients have developed normally without dietary modification [16]. Ketogenic diets should be avoided in all 3 disorders but a low fat diet is not needed. Carnitine supplements are often given, particularly if plasma levels are low.

Most patients with T2 deficiency only suffer a single episode of ketoacidosis. Recurrent episodes are more likely in SCOT & MCT1 deficiencies (including heterozygous cases) but ketoacidosis is very rare after mid-childhood in all three conditions [11]. Uncomplicated pregnancies have been reported in patients with SCOT and T2 deficiencies [2] but intravenous glucose should be given during labour and during illnesses with vomiting.

Though the episodes of ketoacidosis can be fatal or lead to brain damage, almost all patients with SCOT deficiency survive without impairment. Approximately 20% of patients with T2 deficiency have some intellectual or neurological problems, including extrapyramidal movement disorders, which may arise without episodes of ketoacidosis. The patients with homozygous MCT1 deficiency all had cognitive impairment and/or abnormal neuroimaging [13–15], probably unrelated to episodes of acidosis (▶ Sect. 13.2.2).

13.3 Cytosolic Acetoacetyl-CoA Thiolase Deficiency

Cytosolic acetoacetyl-CoA thiolase (CAT) is primarily involved in the synthesis of isoprenoid compounds, such as cholesterol (▶ Chap. 37), rather than ketone body metabolism. CAT deficiency has been reported in two patients with mental retardation and persistent ketosis [21] but the diagnosis remains uncertain.

13.4 "Idiopathic" Ketotic Hypoglycaemia

Idiopathic ketotic hypoglycaemia (IKH), sometimes just called 'ketotic hypoglycaemia', is characterised by episodes of hypoglycaemia for which no specific endocrine or metabolic cause can be identified. It is the commonest cause of hypoglycaemia in non-diabetic children aged 6 months to 6 years.

13.4.1 Clinical Presentation

Patients usually present with hypoglycaemia in late infancy or early childhood, precipitated by infections

associated with anorexia and vomiting. Children are seldom subjected to prolonged fasting under other circumstances but a few develop symptomatic hypoglycaemia after an overnight fast of 12–14 hours or less. They have typical adrenergic symptoms (such as pallor, sweating and tachycardia) followed by neuroglycopenic symptoms (confusion, drowsiness or seizures). Patients may smell of acetone and have a mild metabolic acidosis but this is insufficient to cause symptoms.

13.4.2 Metabolic Derangement

During prolonged fasting, blood glucose concentrations gradually fall, despite glycogenolysis and gluconeogenesis and most tissues switching to fatty acid oxidation. In the liver, acetyl-CoA produced by fatty acid oxidation cannot enter the citric acid cycle because gluconeogenesis has depleted the supply of oxaloacetate, and instead it generates KBs; these are a particularly important in the brain as an alternative fuel to glucose. Mild hypoglycaemia with raised KB concentrations – ketotic hypoglycaemia – is, therefore, a normal physiological response to prolonged fasting. The duration of fasting that causes hypoglycaemia varies between children; reasons may include polymorphisms in genes involved in glucose homeostasis or decreased hepatic glycogen following a period of poor nutrition. Stable isotope studies have shown that hypoglycaemia is due to a failure to maintain hepatic glucose production rather than increased glucose oxidation [22, 23]. There may also be impaired KB use, particularly by the brain, if the blood glucose falls rapidly before maximum MCT1 expression has been induced in the blood brain barrier: this explains the neurological symptoms, such as seizures. Though IKH appears to represent the lower tail of the Gaussian distribution of fasting tolerance, it is essential to exclude alternative causes of hypoglycaemia with ketosis; additional causes continue to be identified [24].

In some series, IKH is commoner in children who are underweight or have relative macrocephaly, presumably because of the brain's high glucose consumption. IKH sometimes occurs following intrauterine growth retardation and neonatal hypoglycaemia due to transient hyperinsulinism [25].

13.4.3 Diagnostic Tests

See ▶ Chap. 1 (▶ Sect. 1.5.2).

As there is no specific test for IKH, it is necessary to exclude alternative causes of hypoglycaemia with ketosis. These include drugs (such as β-blockers and alcohol), liver disease (including mitochondrial diseases involving the liver), adrenal insufficiency, hypopituitarism or isolated growth hormone deficiency, glycogen storage diseases (GSDs), glycogen synthase deficiency, Fanconi-Bickel syndrome, defects of gluconeogenesis or ketone body utilisation or transport, organic acidaemias, MSUD and fatty acid oxidation disorders (in many of which KBs can be made). GSD IXa is a relatively common cause of ketotic hypoglycaemia that can easily be missed [26], particularly as the liver is soft and may not be enlarged with some mutations [27] (▶ Chap. 5); this may explain the higher prevalence of IKH reported in boys. It must be stressed that the Glucagon test is potentially dangerous in such fasting situations in which glycogen stores are likely to be depleted. Samples obtained at the time of hypoglycaemia are useful but they cannot exclude all metabolic disorders. Plasma lactate and alanine concentrations are often low acutely with raised branched chain amino acids (in patients who do not have MSUD) reflecting normally activated gluconeogenesis [28].

If acute samples are not available, most potential diagnoses can be excluded without inducing hypoglycaemia by clinical examination, a synacthen test and metabolic/genetic investigations. Nevertheless, a carefully controlled fasting test (▶ Chap. 3) is often useful to establish how long the patient can safely fast when healthy.

13.4.4 Treatment and Prognosis

In most children, problems can be prevented by giving drinks containing maltodextrin every 3 hours, day and night, during illnesses, or an intravenous infusion containing glucose if the drinks are vomited. Children who suffer episodes of hypoglycaemia before breakfast when they are healthy should either have a shorter overnight fasting period or be given uncooked cornstarch during the evening. Home monitoring of blood glucose concentrations can be useful to check that treatment is adequate.

Fortunately, neurological sequelae are rare in these patients, even if hypoglycaemia has been associated with seizures. Most patients stop having hypoglycaemia by 7 years of age and episodes after puberty are extremely rare.

13.5 Ketogenic Diets

Diets that induce ketosis are the optimal treatment for GLUT1 deficiency (▶ Chap. 8); they are usually beneficial in pyruvate dehydrogenase (PDH) deficiency (▶ Chap. 11) and often in patients with drug-resistant

epilepsy. Contraindications include acute intermittent porphyria, pyruvate carboxylase deficiency and defects of fatty acid oxidation, ketogenesis or ketolysis. The mechanism of the anti-epileptic effect remains uncertain [29] but there is a clear rationale for ketogenic diets in GLUT1 and pyruvate dehydrogenase (PDH) deficiencies. GLUT1 deficiency reduces the entry of glucose into the brain but KBs cross the blood brain barrier on MCT1 transporters and can act as an alternative fuel. PDH deficiency interferes with the oxidation of carbohydrates but not of KBs, which can supply acetyl-CoA and energy to the brain.

Diets that are high in fat and low in protein and carbohydrate lead to fatty acid oxidation in the liver and the production of more acetyl-CoA than can enter the citric acid cycle. As in prolonged fasting, this causes ketogenesis. In the classical ketogenic diet, there is a 3:1 or 4:1 ratio by weight of dietary fat to carbohydrate plus protein. Expert dietary supervision and supplements of calcium, trace elements and vitamins are needed; potential side effects include poor growth, constipation, acidosis, hyperlipidaemia and renal stones [30]. Compliance with the classical diet often deteriorates in the long-term, particularly in older patients.

Ketosis can be induced by diets that contain less fat (60–70% energy) if the fat is predominantly medium-chain triglycerides (MCT). After a meal containing MCT, high concentrations of medium chain fatty acids reach the liver because they are transported in the portal vein, rather than lymph vessels. Moreover, medium chain fatty acids are oxidised rapidly within hepatocytes, because they can enter mitochondria independent of carnitine, leading to high acetyl-CoA concentrations and ketogenesis. As yet it is uncertain whether triheptanoin confers additional benefit compared to conventional MCT. Both can cause gastrointestinal side effects.

Adolescents and adults generally find it easier to adhere to the Modified Atkins Diet, in which fat is encouraged and carbohydrate is restricted (usually 10–15 g/day initially) but protein and energy are unrestricted. KB concentrations are generally lower than on the classical or MCT-based diets but it has been used successfully in epilepsy and GLUT1 deficiency [31]. A low glycaemic index diet may lead to mild ketosis but its aim is to stabilise blood glucose concentrations and, although it has been used for epilepsy, it is not recommended for GLUT1 or PDH deficiencies.

13.6 Therapeutic Use of Ketone Bodies and Ketone Esters

Treatment with KBs may help patients with various inborn errors, especially disorders of fatty acid oxidation or ketogenesis. Though they are a particularly important fuel for the brain, KBs are used by many other tissues (such as cardiac and skeletal muscle) in preference to fatty acids [32]. They also decrease fatty acid oxidation by inhibiting lipolysis [33]. Sodium 3-hydroxybutyrate is an established long-term treatment for multiple acyl-CoA dehydrogenase deficiency, with beneficial effects on myopathy, cardiomyopathy, liver disease and leukodystrophy [34]. The latter reflects the role of KBs in the synthesis of myelin cholesterol; KB may also have effects via regulation of gene expression and inflammation. Recently, sodium 3-hydroxybutyrate has been used during acute decompensation in patients with severe defects of fatty acid oxidation and ketogenesis [9]. A racemic mixture of D and L isomers has generally been employed.

Ketone esters may be preferable to sodium 3-hydroxybutyrate as they avoid the sodium load, they only deliver the physiological D isomer and they can achieve higher D-3-hydroxybutyrate concentrations [35]. Gastrointestinal side effects, such as nausea, can occur with the salt or esters. Initial studies in patients with VLCAD deficiency showed that a ketone ester (D-3-hydroxybutyl D-3-hydroxybutyrate) improved muscle energy metabolism when given prior to exercise, suggesting that it may help to prevent rhabdomyolysis [36].

It is possible that ketone esters may enhance or provide an alternative to treatment with a ketogenic diet but, as yet, there are no reports of ketone ester use in GLUT1 or PDH deficiencies or in patients with epilepsy. Treatment with ketone esters has also been proposed for many other diseases, including diabetes mellitus type 2, heart failure, psychiatric and neurodegenerative conditions (such as Parkinson and Alzheimer diseases) [35].

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Small Molecule Disorders

Contents

Disorders of Galactose Metabolism

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Contents

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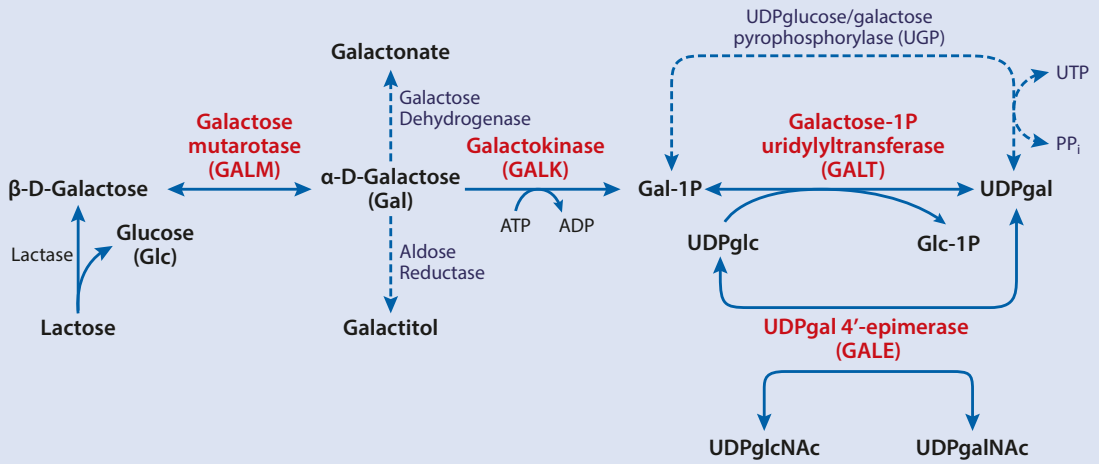
Galactose Metabolism

In nearly all mammals, glucose and uridine diphosphogalactose (UDPgal) are used in mammary tissue to form the disaccharide lactose, which is the principal carbohydrate in milk. Although other foods may contain free or bound galactose, dairy products are, by far, the largest exogenous source of galactose in man. Endogenous galactose production is also significant.

Ingested lactose is hydrolysed by lactase, liberating β-D-galactose and D-glucose (◘ Fig.14.1). Galactose is then absorbed using a sodium/glucose-galactose cotransporter (SGLT1). Although the enzymes involved in galactose metabolism are widespread, the liver is the major organ responsible for galactose metabolism. The integration of galactose within hepatic carbohydrate metabolism is shown in ◘ Fig. 14.2. First, β-D-galactose is converted to α-D-galactose by galactose mutarotase (GALM). Galactose is then phosphorylated to form galactose-1-phosphate (Gal-1P) by galactokinase (GALK). Galactose-1-P-uridylyltransferase (GALT) converts uridine diphosphoglucose (UDPglc) and Gal-1P into UDPgal and glucose-1-phosphate (Glc-1P). Glc-1P is metabolised into glucose-6-P, from which glucose is formed by glucose 6 phosphatase, and pyruvate and lactate by glycolysis (▶ Chap. 5, ▶ Fig. 5.1). Galactose can also be reduced by aldose reductase to form galactitol, or oxidized by galactose dehydrogenase to form galactonate. UDPglc (or UDP-N-acetylglucosamine, UDPglcNAc) can be interconverted with UDPgal (or UDP-N-acetylgalactosamine, UDPgalNAc) by UDP-galactose 4′-epimerase (GALE). The utilisation of UDPgal in the synthesis of glycoconjugates, including glycoproteins, glycolipids and glycosaminoglycans, and their subsequent degradation (◘ Fig. 14.2) may constitute the pathways of de novo and salvage production of endogenous galactose. All four substrates of GALE: UDPgal, UDPglc, UDPgalNAc, and UDPglcNAc, are used for glycoconjugate synthesis (▶ Chap. 43). UDPglc is also the key substrate in all tissues for glycogen production, and as mentioned above, UDPgal is also used in mammary tissue during lactation for lactose synthesis. The UDPglucose pyrophosphorylase (UGP) enzyme (◘ Fig. 14.1) that is primarily responsible for synthesis of UDPglc from Glc-1P and UTP can also catalyse, albeit in a limited way, the synthesis of UDPgal from Gal-1P and UTP, and therefore may also contribute to metabolism of galactose when GALT is deficient.



◘ **Fig. 14.1** Major reactions of galactose metabolism. The four enzymes of the Leloir Pathway (GALM, GALK, GALT, and GALE) are presented in red font. Enzymes that provide alternative or bypass routes of galactose metabolism are presented in blue font. The four UDP sugars listed are all key substrates for glycosylation. Inherited deficiencies of GALM, GALK, GALT, or GALE result in the primary disorders of galactosemia metabolism described here

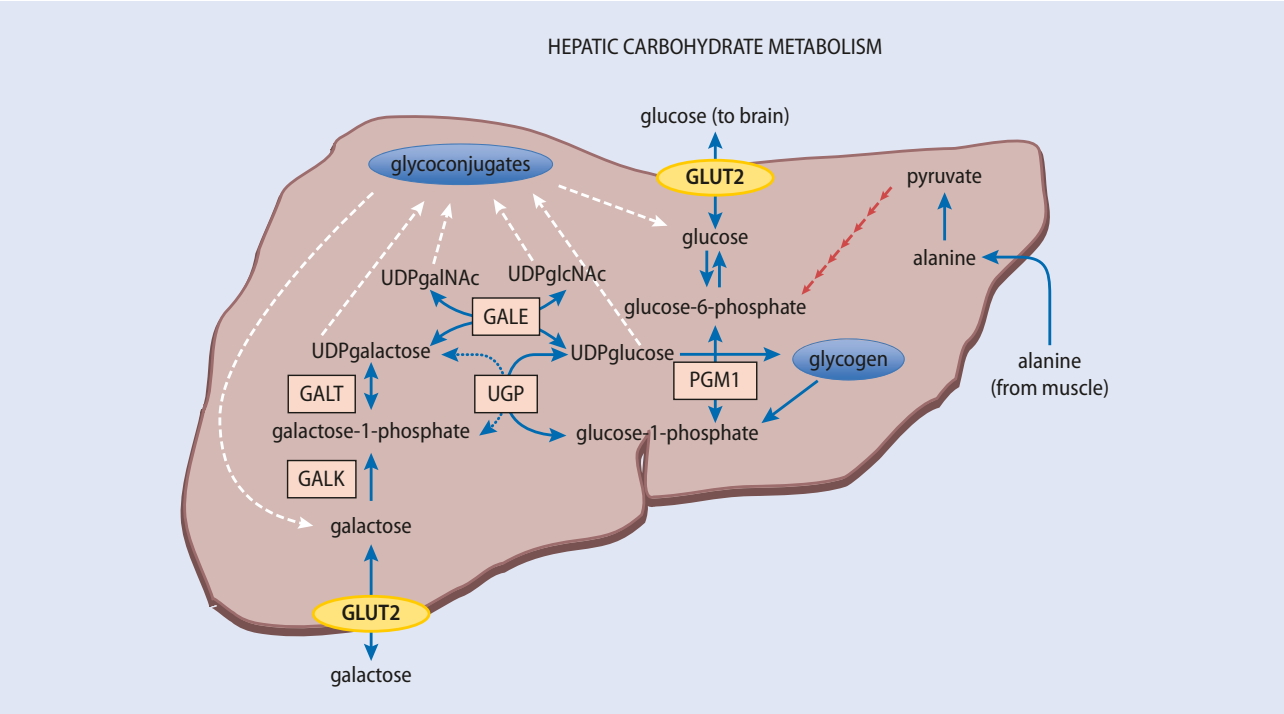


Fig. 14.2 Hepatic carbohydrate metabolism. Red arrows indicate the various reactions in the gluconeogenesis pathway. White dashed lines indicate known or putative steps or reactions in the pathway for glycoconjugate synthesis and catabolism. The fine blue dotted line indicates that galactose-1-phosphate is a poor substrate for the UGP enzyme compared to glucose-1-phosphate

Introduction

Four inborn errors of galactose metabolism are known [1, 2]. The clinically best recognized is classic galactosemia caused by a complete or profound deficiency of galactose-1-phosphate uridylyltransferase (GALT). Classic galactosemia can be life threatening in infancy with multiorgan involvement; long-term developmental and other complications are also common [2]. Partial GALT deficiency ranges from having serious consequences in the newborn period to being generally mild or benign. Uridine diphosphate galactose 4′-epimerase (GALE) deficiency exists as a continuum [3]. The very rare profound, but not complete, GALE deficiency clinically resembles classic galactosemia, at least in the neonatal period. Partial GALE deficiency, which is much more common, at least in some populations, appears to be mild or benign [4], and homozygosity for a novel mutation in *GALE* was linked to thrombocytopenia in one extended family [5]. Galactokinase (GALK) deficiency is extremely rare in many populations, but more common in others, and can lead to the formation of nuclear cataracts and possibly also long-term developmental deficits, often without provoking acute symptoms of intolerance [6–8]. Finally, homozygosity for mutations in galactose mutarotase (GALM) can lead to a variant form of galactosemia [9]. Deficiency of the sodium-dependent monosaccharide carrier SGLT1 causes congenital glucose/galactose malabsorption (▶ Chap. 8). Deficiency of the glucose and galactose transporter, GLUT2, leads to Fanconi-Bickel syndrome (▶ Chap. 8), a congenital disorder characterized by renal tubular dysfunction and glycogen storage that can be misdiagnosed as galactosemia due to hypergalactosaemia in the neonatal period. Other secondary causes of impaired liver handling of galactose in the neonatal period are congenital portosystemic shunting and multiple hepatic arteriovenous malformations [10]. Whenever an infant is suspected of having a disorder of galactose metabolism, it is imperative that all milk feeding is ceased immediately and replaced with soya or elemental formula to minimize the risk of acute disease or death while the diagnosis is pursued. A guideline for the treatment of galactosemia was published in 2017 [11].

14.1 Galactose-1-Phosphate Uridylyltransferase (GALT) Deficiency

14.1.1 Clinical Presentation

A diagnosis of classic galactosemia is most often made following the onset of clinical illness in infancy, or from a positive newborn screening (NBS) result [2]. Infants with classic galactosemia may appear normal at birth,

but within a few days of starting breast or formula milk feeds develop a life-threatening illness with hepatic, renal and cerebral involvement. Early signs include vomiting, diarrhea, poor feeding, jaundice, excessive weight loss, and lethargy. Other findings may include liver enlargement, excessive bruising or bleeding, nuclear cataracts detectable by slit lamp examination, and a full fontanelle. Death from septicaemia, particularly with *E. coli*, may occur. The severity of acute illness may wane when milk feeds are temporarily withdrawn and replaced by intravenous glucose nutrition. Occasionally children may first present with a more chronic illness characterised by failure to thrive and developmental delay.

Blood tests may show liver disease evident by unconjugated or mixed hyperbilirubinaemia, abnormal clotting, raised liver transaminases and an increase in certain amino acids, particularly phenylalanine, tyrosine and methionine (which may result in an abnormal newborn screening test for PKU or homocystinuria). Urine studies may show renal tubular disease manifest by metabolic acidosis, galactosuria, glycosuria and aminoaciduria. The absence of reducing substances or galactose in urine can be misleading, especially if the sample was collected only a few hours after a milk feed. Even if present, they may be masked by the glycosuria and aminoaciduria resulting from renal tubular dysfunction. Hypoglycaemia can occur but is rare. Vitreous hemorrhage has been detected in a minority of affected infants [2]. Partial GALT deficiency or clinical variant galactosemia with up to 10% residual activity may cause acute illness if untreated; individuals with 15% or more GALT activity do not require diet therapy [12–14].

14

14.1.2 Metabolic Derangement

Infants with profound GALT deficiency accumulate galactose, Gal-1P, galactitol and galactonate in blood and tissues, especially following dietary exposure to high levels of lactose or galactose. Disturbances in the glycosylation of glycoproteins also occur prior to dietary galactose restriction, as demonstrated by abnormalities in both N- and O-linked glycans [1]. Cataract formation results from galactitol accumulation in the ocular lens. Metabolites responsible for the hepatic, renal and cerebral pathogenesis remain unknown, but might include Gal-1P and perhaps galactitol.

Although the amounts of galactose and galactose metabolites detected in blood and urine decrease significantly following dietary galactose restriction, they often remain elevated. This may reflect endogenous galactose production and/or cryptic dietary sources. Endogenous galactose production occurs in utero and throughout life [15]. It is age related, with higher levels detected in children than in adults [16, 17]. Studies of adults on restrictive diets showed that endogenous galactose production exceeded the estimated 20–40 mg/day of dietary galactose intake [15].

14.1.3 Genetics

Galactosemia is considered an autosomal recessive disorder with a recurrence risk of 1:4, though one case of de novo mutation has been reported [18]. The birth incidence is 1/50,000 in the United States [19]. In Ireland it is about 1/16,500 [20]. In Asian populations it is extremely rare. Over 350 variants in human *GALT* have been described (▶ https://arup.utah.edu/database/GALT/GALT_display.php) [21]. Q188R (c.563A > G) is the most prevalent mutation in northern European populations; it is particularly common in Ireland and Great Britain where it accounts for over 70% of mutant alleles. S135L (c.404C > T) is common among patients with African ancestry. Though genotype-phenotype matching is complicated by allelic heterogeneity, it appears that *GALT* genotypes associated with even trace residual GALT activity, including S135L and others, are associated with milder clinical outcomes [22, 23].

Many alleles of *GALT* associated with substantial residual activity have been reported. The Duarte variant galactosemia (D2) allele is the best known; it includes an N314D (c.940A > G) polymorphism that exists, in *cis*, with 3 intronic changes and a small deletion in the promoter region of the gene (c.-119_-116delGTCA) that is believed to be causal [24]. Patients who inherit a Duarte allele in *trans* with a 'classic' *GALT* mutation, such as Q188R, have what is called Duarte variant galactosemia (D/G). Of note, the D2 allele, and therefore D/G galactosemia, are found predominantly in individuals of European ancestry [25]. D/G is associated with approximately 25% residual GALT activity and does not require diet therapy [12–14]. D/G is identified by newborn screening in some populations at up to 10 times the prevalence of classic galactosaemia [24].

14.1.4 Diagnostic Tests

Newborn screening (NBS) for galactosaemia is undertaken in many countries by measurement of GALT enzyme activity, with or without blood total galactose measurement, using dried blood spots usually collected within 48 hours after birth [19]. Because the GALT activity assay (Beutler test) is coupled, infants with G6PDH deficiency may show apparently diminished activity. Some programmes quantify Gal-1P in addition to total galactose. Depending on the screening approach and cut-offs used, false positive and even some false negative results can occur [2]. If the NBS turn-around

time is long, infants with classic galactosemia may already be ill before the NBS result is received.

The diagnosis of GALT deficiency galactosaemia is confirmed or refuted by a quantitative assay of GALT activity in freshly drawn erythrocytes. The use of an LC-MS/MS-based erythrocyte GALT enzyme assay may better inform the clinician as to whether the patient has classic galactosemia vs. clinical variant galactosemia [26]. All assays of erythrocyte GALT activity can give a false-negative result if the patient received a blood transfusion within 2–3 months prior to the blood draw. In this situation, more informative tests will include assay of urinary galactitol, erythrocyte Gal-1P, or mutation analysis in *GALT*. Cultured skin fibroblasts can also be used for study. If taken post-mortem, liver or kidney cortex may provide diagnostic enzyme information, but these specimens must be adequately collected and stored.

Infants with D/G or another partial GALT deficiency are detected at high rates by some but not all NBS programs [19]. Follow-up assessment of an infant with suspected partial GALT deficiency involves quantitation of urine galactitol and/or erythrocyte Gal-1P, repeat quantitative investigation of GALT enzyme activity, and *GALT* sequencing, if available. Galactose tolerance tests present a potential risk to the child should they have profound GALT deficiency, or some other significant cause of milk intolerance, and should not be used to evaluate the need for treatment of partial GALT deficiency.

Prenatal Diagnosis

GALT deficiency is inherited as an autosomal recessive trait. If familial *GALT* mutations are known, informative prenatal diagnosis can be performed by genotyping DNA from a chorionic villous (CVS) biopsy or amniotic fluid cells. Full *GALT* sequencing may be informative even in the absence of known familial mutations. Historically, prenatal testing has also been achieved by measuring GALT activity in primary or cultured CVS cells, or in cultured amniotic fluid cells, or by measuring galactitol in amniotic fluid; however, genetic testing of a fetal DNA source may be preferable [2].

14.1.5 Treatment and Prognosis

Prenatal and Newborn Considerations

Due to family history some infants are diagnosed with classic galactosemia prenatally. Though some of these mothers have been advised to restrict their own dietary lactose or galactose intake during pregnancy, this does not seem to prevent the accumulation of Gal-1P and galactitol in the fetus or amniotic fluid, presumably due to endogenous synthesis. Furthermore, the outcomes for infants whose mothers restricted milk intake in pregnancy were no better than for those whose mothers did not [27].

Newborns suspected of classic galactosemia must be treated immediately by exclusion of all lactose from the diet, including both breast milk and milk-based formula. Most newborns suspected of having classic galactosemia are switched to a soya-based formula with no apparent adverse effects [28], although an elemental formula may be used if soya presents a problem [29]. In the presence of significant liver disease, a medium-chain triglyceride containing casein hydrolysate preparation may be preferable. Infants who are seriously ill at diagnosis may require considerable supportive care, including the management of a coagulopathy and septicaemia. When a lactose-free diet is instituted early enough, signs disappear promptly, jaundice resolves within days, cataracts may clear, liver and kidney functions return to normal, and liver cirrhosis may be prevented [2]. A guideline for the treatment of galactosemia was published in 2017 [11].

Infants and Young Children

Dietary restriction of high lactose or galactose foods becomes more complex as the infant with classic galactosemia transitions to solid foods; calcium (+ vitamin D) supplementation may also become advisable. Parents may require assistance in learning to scrutinize food labels to look for hidden sources of galactose or lactose from milk powder or solids, hydrolysed whey (a sweetener), drugs in tablet form, toothpaste, baking additives, fillers, sausages, etc. Further, not all dairy products are problematic; for example, some hard cheeses contain very little, if any, galactose because milk sugars were cleared by the fermenting microorganisms [30].

Galactose is present at high levels in dairy milk but also at low levels in a great number of vegetables, fruits, legumes (beans, peas, lentils etc.) and other foods [30]. When compared with endogenous production, however, it is unlikely that absorption of galactose from these cryptic dietary sources has a significant impact on the expanded whole-body pool. Further, an observational study of 231 adults and children with classic galactosemia, some of whom consumed diets that restricted both dairy and many non-dairy sources of galactose, and others whose diets restricted only dairy, showed no significant association between markers of long-term outcome and rigor of non-dairy galactose restriction in childhood or later life [31].

Older Children and Adults

Current recommendations are that patients with classic galactosemia should continue dietary restriction of galactose for life; however, it remains unclear how rigorous that restriction should be for older children and adults. Anecdotal reports suggest that children and/or adults may experience elevated red blood cell Gal-1P and develop cataracts after ingesting high levels of

lactose ([32], G.T. Berry, unpublished observations). However, at least two cases of classic galactosaemia have been reported in which stopping dietary restriction of galactose after early childhood resulted in outcomes that were no worse than those of patients who continued treatment [2]. Several small studies have also demonstrated that defined quantities of galactose appear to be well tolerated by children and adults with classic galactosaemia [33, 34], although individual differences among patients may render some more sensitive to exposure than others. Finally, the failure to understand long-term disease mechanisms, the lack of true disease-related biomarkers, and the marked variability in clinical outcomes among patients has led to a continued diversity in degrees of dietary galactose restriction, especially among older children and adults [31, 35].

Biochemical Monitoring

Erythrocyte Gal-1P concentration is the most common biochemical marker used to monitor treatment. The level can be very high at diagnosis if the infant is drinking dairy milk, and then falls gradually over weeks to months after the initiation of dietary galactose restriction. Even with good dietary compliance, the Gal-1P concentration may plateau above normal. The usefulness of monitoring Gal-1P is open to question, however, as most, but not all, studies addressing the question have shown no correlation between erythrocyte Gal-1P and long-term outcome in patients with classic galactosemia [1, 2]. Other metabolites, including erythrocyte or plasma galactose, erythrocyte, plasma, or urine galactitol, and erythrocyte or urine galactonate, are also consistently increased in patients and have been suggested as alternative or additional markers [2]. Untargeted metabolomic studies have also revealed numerous other perturbed pathways and markers in treated patients as compared with controls [36]. Abnormal glycans may also be informative, at least in infants [2]. However, there are currently no data available to demonstrate the superiority of any of these other markers over Gal-1-P for biochemical monitoring.

Long-Term Outcome and Complications

Despite the rapid clinical response to lactose exclusion in newborns with classic galactosaemia, long-term complications are common, and appear to be largely independent of the severity of initial illness or the strictness of dietary compliance [1, 2, 20, 27, 31]. Decreased bone density is common and early identification by DXA scan may allow intervention to help reduce the risk of osteoporosis later in life [2, 37]. Mild growth retardation, delayed speech development, verbal dyspraxia, difficulties in spatial orientation and visual perception, and intellectual deficits have been variably described as complications of treated galactosaemia [1, 2, 38]. Reduced leptin levels [39] tremor, ataxia, dystonia and choreic movements [20, 40, 41], increased frequency of gastrointestinal problems [42], and introverted personality and/or anxiety/depression [20, 41] have also been reported. The quality of life in treated patients has been unfavourable compared with that of patients with PKU [43]. Patients with classic galactosemia may require intense professional help and/or oversight in many spheres [44, 45].

By mid-childhood or later, patients vary markedly in terms of the number of long-term sequelae present and the severity of those sequelae. Contrary to some early reports, IQ is no longer thought to decrease with age [1, 2]. A minority of patients also develop severe neurological disease with cerebellar dysfunction, and brain MRI and FDG-PET scans may reveal abnormalities, though results have been highly variable [20, 46].

Fertility and Pregnancy

Hypergonadotropic hypogonadism or primary ovarian insufficiency (POI) occurs in almost all women with classic galactosaemia [22, 47], but not in Duarte variant galactosemia [48] nor has it been reported in females homozygous for the S135L mutation [1, 2]. Galactosemia-associated POI presents clinically with delayed puberty and menarche, primary or secondary amenorrhoea, or oligomenorrhoea [2, 22]. About 2/3 of girls with classic galactosemia achieve spontaneous menarche, although most of those who do, cease having regular spontaneous menses within 5 years [22]. Male gonadal function appears largely unaffected [2, 49].The cause of ovarian dysfunction in classic galactosemia remains unclear, but is often signalled early in infancy or childhood by hypergonadotropism with a perturbation in granulosa cell function, as evidenced by reduced circulating levels of anti-Müllerian hormone (AMH) [2, 22, 47]. Indeed, by AMH levels, a vast majority of girls with classic galactosemia show evidence of severely diminished numbers of normally-developing ovarian follicles by 2–6 months of age [47], suggesting the initial damage may have occurred very early in development, perhaps in utero. Of note, histological studies of ovarian tissue from a small number of girls below the age of 5 with classic galactosemia suggest that despite low levels of detected circulating AMH, a substantial pool of primordial follicles may nonetheless exist [50].

The hormonal intervention required to help some girls achieve or complete puberty, and women avoid the negative general health consequences of early menopause, can be complicated by the fact that seemingly all oral hormone drug tablets contain lactose as an »inactive« filler. However, some women with classic galacto-

semia have received these pills for many years with no obvious negative side effects (G.T. Berry, personal observation). It is important to stress that the hormonal treatments that enable girls and women with classic galactosemia to achieve or complete puberty and maintain good bone health, etc., are not known to have any impact on fertility [22, 50]. A minority of women with classic galactosaemia, including those with no detectable residual GALT activity in blood, have experienced one or more successful pregnancies and deliveries. Some of these women subsequently developed secondary amenorrhoea. In a recent study, over 40% of a small number of women with galactosemia who actually tried to become pregnant were successful [51]. Whether these women are representative of the larger population remains unclear. Galactose metabolite concentrations in blood do not appear to increase significantly during pregnancy, or following 1 week postpartum even in those who choose to breast feed [52]. Infants born to mothers with classic galactosaemia appear normal and healthy.

- **Management of Partial GALT Deficiency Attributable to Duarte Galactosemia (D/G)**

Duarte (D2) galactosaemia (D/G) does not require diet therapy. Yet, at present there is still no uniform standard of care for infants with Duarte (D2) galactosaemia (D/G), though this is expected to change (McCandless, Pediatrics) [12, 14, 48]. Historically, some centres advised lactose restriction in infancy or until erythrocyte Gal-1P levels normalised and remained normal following a lactose challenge or a normal lactose-containing diet, often tested at 1 year of age. Other centres advised no follow-up testing or intervention. However, the recent publications of a well-powered study demonstrating no detectable difference in developmental outcomes in subjects 6-12 years of age with D/G, whether or not they drank milk in infancy [12], and another showing no increased risk for acute complications or adverse effects on early childhood developmental in those younger than 6 years of age [13], has encouraged many healthcare providers who used to recommend dietary intervention for infants with D/G to reconsider their approach [14].

- **Heterozygotes for Classic Galactosaemia**

Heterozygotes for GALT deficiency are predicted to occur at a frequency of about 1/112 individuals in populations where classic galactosemia impacts about 1/50,000 birth. Heterozygotes, or carriers, have not been shown to be at increased risk of premature menopause, presenile cataracts, or other disease manifestations associated with classic galactosemia [53].

14.2 Uridine Diphosphate Galactose 4′-Epimerase (GALE) Deficiency

14.2.1 Clinical Presentation

GALE deficiency ranges from an apparently benign 'peripheral' condition associated with GALE deficiency restricted to circulating red and white blood cells to a severe 'generalized' disorder resulting from widespread GALE impairment that presents with life-threatening illness in the newborn period [3]. Of note, unlike GALT deficiency, even the most severely affected patients with GALE deficiency exhibit some residual GALE activity, at least in some tissues. The lack of infants detected with complete absence of GALE deficiency, although true null alleles of *GALE* have been identified in the compound heterozygous state, is presumed to reflect ascertainment bias; namely, that complete loss of GALE may be incompatible with survival of a fetus to term [3]. The severe or generalized form of GALE deficiency is extremely rare, with a total of 6 patients from three families reported. However, a new enigmatic phenotype with features of a congenital defect of glycosylation has recently surfaced associated with the presence of homozygosity for the severe mutation [4].

The original affected infants exposed to milk showed clinical presentation similar to classic galactosemia; they were rapidly switched to low-galactose formula. One child died from unexplained liver failure at 4 months of age. Despite continued dietary restriction, some, but not all, affected children showed learning difficulties, sensorineural hearing loss, and other long-term complications; however, POI has not been reported. Patients with an intermediate form of GALE deficiency have also been described, with clinical findings ranging from transient illness with seizures, to vomiting and hypoglycaemia that resolved upon dietary restriction of galactose, to juvenile cataracts and developmental delay [3]. Six members of an extended family homozygous for a novel missense variant of GALE (p.R51W) [5] have recently been reported with thrombocytopenia and a further patient homozygous for a different variant, (p.Thr150Met), reported with chronic thrombocytopenia, dysmegakaryopoiesis, macrocytosis, and lymphopenia [54].

14.2.2 Metabolic Derangement

Patients with GALE deficiency exposed to milk accumulate galactose, galactitol, Gal-1P, and UDPgal in blood (► Fig. 6.1). As in GALT deficiency, patients with severe GALE deficiency exposed to high levels of dietary lactose may also show abnormal glycosylation of

proteins in blood]; this may be a secondary biochemical complication not primarily related to the genetic defect. Of note, erythrocyte GALE activity does not correlate well with that seen in other tissues, such as lymphoblasts, and is poor at differentiating between peripheral and generalised forms of the disease.

14.2.3 Genetics

GALE deficiency is inherited as an autosomal recessive trait; the recurrence risk for couples with an affected child is therefore 1:4. Due to incomplete ascertainment, the true population incidence is not known, and may vary significantly between groups [3]. A number of mutations have been identified and characterised. The c.280G > A (V94M) mutation has been found in the homozygous state in the majority of patients with the severe phenotype, whereas other mutations are associated with the intermediate or asymptomatic phenotypes. Of note, some mutations identified in the compound heterozygous state in mildly affected patients appear to be profoundly impaired when expressed in model systems, suggesting that these naturally-occurring mutations could also result in severe disease if inherited together with another severe allele.

14.2.4 Diagnostic Tests

Infants with GALE deficiency may be detected by NBS on the basis of elevated total galactose or Gal-1P but normal GALT [3]. However, many newborn screening programs only measure total blood galactose in follow-up to an abnormal GALT activity result; by definition, these programs will not detect cases of GALE deficiency [19]. Diagnosis of GALE deficiency is confirmed by quantitative assay of GALE in freshly drawn erythrocytes or other cells. Further studies of GALE activity in transformed lymphoblasts, and erythrocyte Gal-1P or urinary galactitol measured while on and off dietary galactose, may help characterise the disorder further. If the familial *GALE* mutations are known, DNA analysis may be the fastest method of determining whether or not an infant is affected.

14.2.5 Treatment and Prognosis

Newborns at risk for generalized GALE deficiency should be maintained on low galactose formula until the diagnosis can be confirmed or excluded. Once confirmed, patients with generalized GALE deficiency should be treated and followed much like patients with classic galactosemia, though less stringent dietary galactose restriction may be advisable to ensure sufficient exogenous galactose for synthesis of galactoproteins and galactolipids. As with GALT deficiency, erythrocyte Gal-1P levels tend to remain slightly elevated in treated patients. The oldest patients reported with generalized GALE deficiency are now in their third decade; they have not shown evidence of progressive disease (JH Walter, personal communication).

True peripheral GALE deficiency does not require galactose restriction. However, since intermediate forms are now recognised, measurement of erythrocyte Gal-1P and urine galactitol while the patient is on a normal galactose intake, and monitoring of psychomotor progress, may be advisable.

14.3 Galactokinase (GALK) Deficiency

14.3.1 Clinical Presentation

Historically, untreated galactokinase deficiency has been considered largely benign except for diet-dependent cataracts and in rare cases pseudotumour cerebri [7]. However, a recent report detailing the outcomes of 18 patients with profound galactokinase deficiency identified by newborn screening in Germany [8] raised concern as many experienced long-term complications. The large majority of these patients were homozygous for the Romani founder mutation c.82C > A (p P28T). Most were clinically well as infants regardless of diet; however, complications were reported in close to 30%. These included hypoglycaemia, failure to thrive, microcephaly, intellectual disability, and hypercholesterolemia, with most symptoms seemingly more prevalent in those with poor dietary compliance. Complications did not appear to correlate with known consanguinity. It remains unclear whether GALK deficiency was causal.

14.3.2 Metabolic Derangement

Patients with profound GALK deficiency lack the ability to phosphorylate galactose (Fig. 14.1) and consequently accumulate galactose and galactitol, but not Gal-1P. As in classic galactosemia, these patients accumulate galactitol in the lens when consuming a high galactose diet, causing osmotic swelling, denaturation of proteins, and cataracts.

14.3.3 Genetics

GALK deficiency is inherited as an autosomal recessive disorder. Historically, GALK deficiency was considered extremely rare (<1/150,000) in most parts of Europe, the

USA, and Japan, but higher in the Balkan countries [8], the former Yugoslavia, Romania and Bulgaria. Among Roma people, the birth incidence is close to 1/2500. Following the civil war in Yugoslavia, which caused a mass relocation of many Eastern Europeans, the birth incidence of GALK deficiency in Western Europe rose. Between 1991–2010 GALK deficiency was detected at 1/40,000 screened births in Germany, making it comparable in prevalence to classic galactosemia in the same population.

Two genes have been reported to encode human galactokinase [1]. However, all mutations associated with clinical GALK deficiency map to *GK1*. The *GK2* gene product appears to function primarily as an *N*-acetylgalactosamine kinase. Of the many *GK1* mutations described in patients with GALK deficiency, P28T (c.82C > A) was identified as the founder mutation in most Roma patients and in German patients who immigrated from Bosnia [6].

14.3.4 Diagnostic Tests

Newborns with profound GALK deficiency may be discovered by NBS due to elevated total blood galactose following exposure to high levels of dietary galactose. These infants will be missed if they have not been exposed to milk, or if the NBS protocol does not test total blood galactose, or only tests samples secondary to low GALT activity [19]. Older children and adults with nuclear cataracts should be tested for possible GALK deficiency by enzyme assay of freshly drawn erythrocyte or another cell type. Elevated galactose and galactitol may also be detected in urine if the patient is on a high galactose diet.

14.3.5 Treatment and Prognosis

Initial treatment of GALK deficiency involves elimination of milk and other high galactose foods from the diet. Cryptic sources of dietary galactose, such as fruits and vegetables, are generally allowed. Once a patient is on a galactose-restricted diet urinary levels of galactitol should normalize. When diagnosis and intervention occur within the first few weeks of life, cataracts may be prevented or may resolve over time. However, when treatment is late and cataracts are already dense, they may require surgical removal. Patients who have had their lenses surgically removed remain at risk for recurrent cataracts originating from remnants of the posterior lens capsule. Recurrence can be avoided by continuing the galactose-restricted diet.

As for carriers of *GALT* mutations, the speculation that heterozygosity for GALK deficiency predisposes to the formation of presenile cataracts remains unproven [55, 56].

14.4 Galactose Mutarotase (GALM) Deficiency

Eight infants from a Japanese cohort who presented with elevated total galactose while consuming milk, but who proved negative for all other anticipated possible causes of galactosemia, were determined to be homozygous for pathogenic mutations in *GALM* encoding galactose mutarotase (GALM). Two of the patients were reported to have congenital cataracts and two others transient cholestasis but were otherwise well [9]. GALM catalyzes the epimerization of β-D-galactose, which is released by cleavage of lactose, to α-D-galactose, a substrate for GALK. While this epimerization can occur spontaneously in aqueous solution, GALM speeds the process in vivo, enabling efficient metabolism of large quantities of dietary galactose. Patients with GALM deficiency should be treated with dietary galactose restriction to prevent the buildup of galactose metabolites and the occurrence of cataracts, and perhaps other outcomes. Functional and prevalence studies of GALM variants suggest that GALM deficiency may be very rare worldwide but present in about 1/10,000 individuals of African ancestry, and about 1/80,000 individuals of Japanese ancestry [57].

14.5 Fanconi-Bickel Syndrome

Fanconi-Bickel Syndrome is a rare, recessively inherited disorder of glucose and galactose transport resulting from deficiency of glucose transporter 2 (GLUT2). A few cases have been discovered during newborn screening for galactose in blood. The clinical features of the disorder are those of glycogen storage disease and renal tubular dysfunction. The diagnosis is confirmed by mutation analysis. For further details, see ► Chap. 8.

14.6 Portosystemic Venous Shunting and Hepatic Arteriovenous Malformations

Portosystemic bypass of splanchnic blood via ductus venosus or intrahepatic shunts causes alimentary hypergalactosaemia, which may be discovered during metabolic NBS [10].

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Disorders of Fructose Metabolism

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Contents

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Fructose Metabolism

Fructose is one of the main sweetening agents in the human diet. It is found in its free form in honey, fruits and many vegetables, and is associated with glucose in the disaccharide sucrose in numerous foods and beverages. Sorbitol, also widely distributed in fruits and vegetables, is converted into fructose in the liver by sorbitol dehydrogenase (Fig.15.1). In recent years, increased consumption of fructose, particularly from sweetened beverages using industrially produced high-fructose corn syrup (HFCS), has been associated with an increased prevalence of obesity, metabolic syndrome, non-alcoholic fatty liver disease, type-2 diabetes, and gout [1] which underscores the importance of understanding the metabolic consequences of fructose consumption.

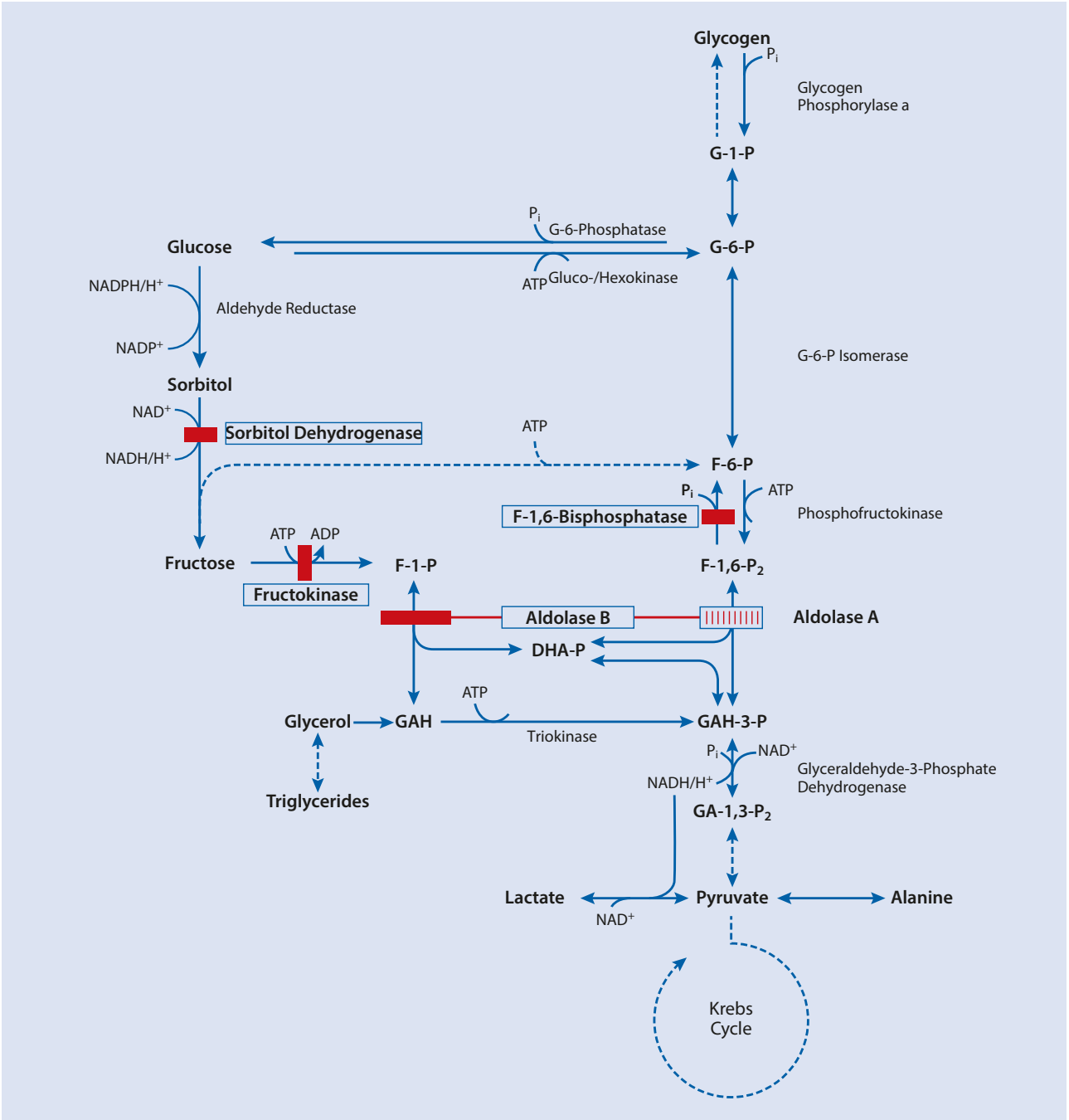

Fig. 15.1 Fructose metabolism. The four enzyme defects related to fructose metabolism are boxed and depicted by solid red bars across the arrows; the diminished activity of aldolase B toward fructose-1,6-bisphosphate is depicted by a broken bar. DHA-P dihydroxyacetone phosphate, F fructose, G glucose, GA glycerate, GAH glyceraldehyde, P phosphate, Pi inorganic phosphate

Fructose is transported by the facilitative glucose transporter-5 (GLUT5) across the intestinal apical membrane into the cytosol and may enter portal circulation by basolateral GLUT2 [2]. Fructose can then be taken up by GLUT2 in the liver and the renal cortex and is metabolised in a pathway composed of fructokinase, aldolase B and triokinase. While for years the liver has been considered the most important organ in fructose metabolism, this notion has recently been challenged by isotope tracing studies in rodents demonstrating that low doses of fructose are almost completely converted to glucose by the intestine while higher doses are only partially metabolised, and fructose and organic acids produced by colonic bacteria from unabsorbed sugars, appear in the portal vein [3].

Introduction

Four inborn errors related to the pathway of fructose metabolism are depicted in Fig. 15.1. Essential fructosuria is a harmless anomaly characterised by the appearance of fructose in the urine after the intake of fructose-containing food. In hereditary fructose intolerance (HFI), fructose may provoke prompt gastrointestinal discomfort and hypoglycaemia upon ingestion, symptoms that can vary from patient to patient and depend on the ingested dose. Fructose may cause liver and kidney failure when taken persistently and its intake becomes life-threatening when given intravenously. Sorbitol dehydrogenase deficiency has recently been reported to cause a slowly progressive neuropathy. The pathomechanism is unclear but may be related to accumulation of intracellular sorbitol. Fructose-1,6-bisphosphatase (FBPase) deficiency is also usually considered an inborn error of fructose metabolism although, strictly speaking, it is a defect of gluconeogenesis. The disorder is manifested by the appearance of hypoglycaemia and lactic acidosis (neonatally, or later during prolonged fasting or induced by fructose) and may be life-threatening.

15.1 Essential Fructosuria

15.1.1 Clinical Presentation

Essential fructosuria is a rare non-disease; it is detected by routine screening of urine for reducing sugars. It is caused by a deficiency of fructokinase, also known as ketohexokinase (KHK), the first enzyme of the main fructose pathway (Fig.15.1).

15.1.2 Metabolic Derangement

In essential fructosuria, ingested fructose is partly (10–20%) excreted as such in the urine, the rest is slowly metabolised by an alternative pathway, namely conversion into fructose-6-phosphate by hexokinase in adipose tissue and muscle (Fig.15.1).

15.1.3 Genetics

The mode of inheritance is autosomal recessive and the frequency has been estimated at 1:130,000. However, since the condition is asymptomatic and harmless and since laboratories are abandoning tests for reducing substances in urine in favour of specific glucose tests, it may be more prevalent than reported.

Tissue-specific alternative splicing of *KHK* results in two isoforms, ketohexokinase A, widely distributed in most foetal and adult organs but with no clear physiological role, and ketohexokinase C, expressed in adult liver, kidney and small intestine, which is affected in essential fructosuria [4]. Numerous variants have been reported to databases from exome or genome studies but only two pathogenic variants of *KHK*, p.G40R and p.A43T have been detected in affected individuals, i.e., a family with three compound heterozygotes [5]. The effect of these variants on protein function has been characterised based on the crystallographic structure [6].

15.1.4 Diagnosis

Fructose gives a positive test for reducing sugars and a negative reaction with glucose oxidase. It can be identified by various techniques, such as thin-layer chromatography, and quantified enzymatically. Fructosuria depends on the time and amount of fructose, sorbitol and sucrose intake and, thus, is inconstant. Fructose-tolerance tests neither provoke an increase in blood glucose as in normal subjects, hypoglycaemia or other changes as occur in HFI and FBPase deficiency, nor are metabolic changes in liver detectable by ^{31}P-magnetic resonance spectroscopy (MRS) [7].

15.1.5 Differential Diagnosis

Acute liver diseases such as e.g. tyrosinaemia or galactosaemia may lead to fructosuria.

15.1.6 Treatment and Prognosis

Dietary treatment is not indicated, and the prognosis is excellent. Judged from animal studies this condition may be protective against the metabolic syndrome [8].

15.2 Hereditary Fructose Intolerance

15.2.1 Clinical Presentation

Individuals with hereditary fructose intolerance (HFI) are perfectly healthy as long as they do not ingest food containing fructose, sucrose and/or sorbitol. Consequently, no metabolic derangement occurs during breast-feeding. The younger the child and the higher the dietary fructose load, the more severe the reaction. In the acute presentation of HFI, an affected newborn infant who is not breast-fed but receives a cow's milk formula sweetened and enriched with fructose or sucrose – formulas which should be obsolete today – is in danger of severe liver and kidney failure and death.

At weaning from breast-feeding or from a fructose/sucrose-free infant formula, the first symptoms appear with the intake of fruits and vegetables [9, 10]. They are generally those of gastrointestinal discomfort, nausea, vomiting, restlessness, pallor, sweating, trembling, lethargy and, eventually, apathy, coma, jerks and convulsions. At this stage, laboratory signs may be those of acute liver failure and generalised dysfunction of the renal proximal tubules. If the condition is unrecognised and fructose not excluded from the diet, the disease may take a chronic, fluctuating course with failure to thrive, liver disease manifested by hepatomegaly, jaundice, bleeding tendency, oedema, ascites, and signs of proximal renal tubular dysfunction. Laboratory findings are those of liver failure, proximal renal tubular dysfunction and derangements of intermediary metabolism. Note that hypoglycaemia after fructose ingestion is short-lived and can be easily missed or masked by concomitant glucose intake.

HFI can be suspected in an asymptomatic infant, if the parents have excluded certain foods from the diet, having become aware that they are not tolerated. In older children, a distinct aversion towards foods containing fructose may develop; these feeding habits protect them but are sometimes considered as neurotic behaviour. At school age, HFI is occasionally recognised when hepatomegaly or growth delay is found [11]. Some adults were diagnosed after developing life-threatening reactions with infusions containing fructose, sorbitol or invert sugar (a mixture of glucose and fructose obtained by hydrolysis of sucrose) when these IV solutions were still in use [12]. Because approximately half of all adults with HFI are free of caries, the diagnosis has also been suspected by dentists. Although several hundred patients with HFI have been identified since its recognition as an inborn error of metabolism in the 1950s [9, 10], these observations indicate that affected subjects may remain undiagnosed and still have a normal life span.

15.2.2 Metabolic Derangement

HFI is caused by deficiency of the second enzyme of the fructose pathway, aldolase B (◘ Fig. 15.1), which splits fructose-1-phosphate (F-1-P) into dihydroxyacetone phosphate and glyceraldehyde. As a consequence of the high activity of fructokinase, intake of fructose results in accumulation of F-1-P and trapping of phosphate. This has two major effects [13]: *(i)* inhibition of glucose production by blockage of gluconeogenesis (by inhibition of aldolase A, ◘ Fig. 15.1) and of glycogenolysis (by inhibition of glycogen phosphorylase a) which induces a rapid drop in blood glucose, and *(ii)* overutilisation and diminished regeneration of ATP; this depletion of ATP results in an increased production of uric acid, a release of magnesium, and a series of other disturbances, including impaired protein synthesis and ultrastructural lesions which are responsible for hepatic and renal dysfunction. The accumulation of F-1-P has also been shown to result in deficient glycosylation of proteins, e.g., serum transferrin, by inhibiting phosphomannose isomerase [14] (▶ Chap. 43).

Residual activity measurable with fructose-1,6-bisphosphate as substrate (see below) is mainly due to the isozyme aldolase A. Thus, glycolysis and gluconeogenesis are not impaired in the fasted state in HFI patients and thus they tolerate long fasting periods.

It should be noted that the IV administration of fructose to normal subjects also induces the metabolic derangements described above (including the drop in ATP and Pi, and rise in urate and Mg^{++}) to an equivalent extent, although they are more transient than in patients with HFI, as demonstrated by ^{31}P-MRS [7]. In normal subjects, IV fructose results in increased glycaemia because of its rapid conversion into glucose. However, the equally rapid conversion of fructose into lactate may provoke metabolic acidosis. For these reasons, the use of fructose, sorbitol and invert sugar has been strongly discouraged for parenteral nutrition in general [15].

15.2.3 Genetics

HFI is an autosomal-recessive disorder. Three different genes coding for aldolases have been identified. While

isozymes A and C are mainly expressed in muscle and brain, respectively, aldolase B is the major fructaldolase of liver, renal cortex, and small intestine. At present, according to different databases approximately 70 causative variants of the aldolase B gene (*ALDOB*) have been reported. Among them, three amino acid substitutions, p.A150P,[1] p.A175D, and p.N335K are relatively common among patients of European descent [16].

Since the three most common pathogenic variants are responsible for more than 90% of HFI cases in some European regions and still more than 50% of cases from the more heterogeneous population in North America, a non-invasive diagnostic approach using molecular genetic methods has been advocated [17, 18]. Among these methods, multiplex ligation-dependent probe amplification (MLPA) assays can also detect copy number variations present on approximately 6% of mutant alleles not detectable by standard sequencing techniques [Santer, unpublished].

From molecular genetic neonatal screening studies in England and Germany, the prevalence of HFI has been calculated as 1:18,000 [1] and 1:29,600, respectively [17].

15.2.4 Diagnosis

Whenever HFI is suspected, fructose should be eliminated from the diet immediately. The beneficial clinical and chemical effects of withdrawal, usually seen within days, provide a first diagnostic clue. Laboratory findings will subsequently show a fall in the elevated serum transaminases and bilirubin, improved levels of blood clotting factors, and amelioration of proximal tubular dysfunction (proteinuria, glucosuria, generalised hyperaminoaciduria, hyperphosphaturia, hypophosphataemia, hyperuricuria, metabolic acidosis).

A cornerstone in the diagnosis of HFI is a careful nutritional history, with special emphasis on the time of weaning when fruits and vegetables were introduced [1, 19]. If the nutritional history is suggestive or other aspects are indicative of HFI (e.g., a positive family history), the disorder should be confirmed by molecular diagnosis (above) on DNA from peripheral leukocytes. This is a non-invasive approach and has the advantage over enzymatic measurement in liver tissue in that it eliminates the complication of secondarily lowered aldolase B activity in a damaged liver.

If no pathogenic variants can be found despite a strong clinical and nutritional history suggestive of HFI, an enzymatic determination or a functional test should be undertaken after a few weeks of fructose exclusion. In liver biopsies from HFI patients, the capacity of aldolase to split F-1-P is reduced, usually to a few percent of normal (mean 5%, range 0–15%) [19], although residual activities as high as 30% of normal have been reported [12]. There is also a distinct (but less marked) reduction in the activity of aldolase B toward fructose-1,6-bisphosphate (mean 17%, range 5–30%). As a consequence, the ratio of V_{max} towards fructose-1,6-bisphosphate versus the V_{max} towards F-1-P, which is approximately 1 in control liver, is increased to 2 to ∞ in HFI patients [19]. Aldolase activity is normal in blood cells, muscle, and skin fibroblasts, which contain a different isozyme, aldolase A. The enzymatic determination of aldolase B in small intestinal mucosa is not recommended because of inconsistent results. For post-mortem diagnosis, molecular studies and measurements of enzyme activity in liver and kidney cortex should be done.

It should be noted that the level of residual activity has never been shown to correlate with the degree of tolerance to fructose.

However, if the clinical history is suggestive for HFI in the absence of pathogenic variants within *ALDOB,* an IV fructose tolerance test is recommended, in which fructose (200 mg/kg b.w.) is injected as a 20% solution intravenously within 2 minutes. Blood samples are taken at 0 (2), 5, 10, 15, 30, 45, 60 and 90 minutes for determination of glucose and phosphate. In normal subjects, blood glucose increases by 0–40%, with no or minimal changes in phosphate [19]. In HFI patients, glucose and phosphate decrease within 10–20 minutes. As a rule, the decrease of phosphate precedes and occurs more rapidly than that of glucose. The test should only be undertaken in an experienced metabolic centre, with careful monitoring of glucose and an indwelling catheter for the (exceptional) case of symptomatic hypoglycaemia and its treatment by IV glucose administration. Oral fructose tolerance tests are not recommended, because they provoke more ill effects and are less reliable [19].

15.2.5 Differential Diagnosis

A high degree of diagnostic awareness is often needed in HFI because the spectrum of symptoms and signs is wide and nonspecific; HFI has been misdiagnosed as pyloric stenosis, gastro-oesophageal reflux, galactosaemia, tyrosinaemia, non-IgE-mediated gastrointestinal food hypersensitivity, intrauterine infection, glycogen and other storage disorders, ornithine transcarbamylase deficiency, and later in life as Wilson disease, leukaemia, and growth retardation. Fructosuria may be secondary to liver damage, e.g., in tyrosinaemia.

1 *Note* that the initiation codon ATG for methionine in the *ALDOB* cDNA was ignored in previous designations and that, e.g., 'p.A150P' was originally named 'p.A149P'

HFI is frequently confused with fructose malabsorption [20], a condition caused by defective fructose transport in the small intestine whose metabolic basis, however, is not well understood. The ingestion of fructose, and to a considerably lesser extent of sucrose, leads to abdominal pain and diarrhoea. Since this condition is diagnosed by breath hydrogen analysis after an oral load of fructose, HFI has to be excluded before such a tolerance test is performed, otherwise deleterious effects may occur [21]. In sucrase-isomaltase deficiency, the ingestion of sucrose results in bloating, abdominal cramps and fermentative osmotic diarrhoea; free fructose, however, is well tolerated.

15.2.6 Treatment and Prognosis

In acute intoxication, intensive care may be required and supportive measures such as fresh frozen plasma may be needed. The main therapeutic step in HFI, however, is the immediate elimination of all sources of fructose from the diet. This involves the avoidance of all types of food in which fructose, sucrose and/or sorbitol occur naturally or have been added during processing. Fructose and sorbitol may be present in medications (e.g., syrups, immunoglobulin solutions, rinsing fluids, enema solutions, amiodarone infusion containing polysorbate 80 [22]) and infant formulas (without adequate declaration of the carbohydrate composition). In this respect, it is deplorable that European Union regulations allow infant formulae to contain up to 20% of their total carbohydrate content as sucrose [23].

Sucrose should be replaced by glucose, maltose and/or starch to prevent the fructose-free diet from containing too much fat. Despite the availability of books and online information on food composition, a dietician should be consulted and practical aspects of the diet (e.g., the considerable variability of the fructose content of different food types, and the influence of storage temperature or method of preparation and manner of cooking on bioavailability) be discussed. Substitution of vitamins, especially ascorbic acid and folates, in the form of a multivitamin preparation should be prescribed to make up for their diminished intake from fruits and vegetables.

After institution of a fructose-free diet most abnormalities disappear rapidly, except for hepatomegaly, which may persist for months or even years [24]. It has recently been shown by H^+-MRS studies that patients with HFI have an increased intrahepatic triglyceride content [25], a fact which has been explained in animals by the endogenous synthesis of fructose by the polyol pathway [26] (▶ Chap. 7). In AldoB knock-out mice F-1-P leads to increased concentration of intrahepatic triglycerides, but by blocking fructokinase with osthole, the triglyceride accumulation could be prevented [27]. Hence, osthole, a coumarinic derivative obtained from plants may be a future therapeutic approach in HFI patients.

Different thresholds of fructose intake for the development of certain symptoms have appeared in the literature, ranging from 40–250 mg/kg b.w./day as compared with an average intake of 1–2 g/kg/day in Western societies [1]. Insufficient restriction of fructose has been reported to cause isolated growth retardation, as evidenced by catch-up growth on a stricter diet [11]. Recommendations for maximum doses have not been validated in different genotypes and sensitivity is known to be different in individual patients with identical *ALDOB* variants.

Thus, it should be suggested to parents that they keep fructose intake as low as possible and that, at least in childhood, it should not be determined by subjective tolerance. For dietary control, the regular taking of the nutritional history is still best, as there are no good sensitive chemical parameters except, perhaps, transaminases. Quantification of carbohydrate-deficient proteins, e.g., transferrin, has been suggested for dietary monitoring [14]; however, the sensitivity of this procedure has not been validated. Patients (and their parents) must be made aware that infusions containing fructose, sorbitol or invert sugar are life-threatening. There are numerous reports in the literature of fructose ingestion by mistake and that is why HFI, if present, should be reported on any hospital admission by an emergency card.

The prognosis of HFI on diet is excellent with normal growth, intelligence and life span.

15.3 Fructose-1,6-Bisphosphatase Deficiency

15.3.1 Clinical Presentation

In about half of all cases, fructose-1,6-bisphosphatase (FBPase) deficiency presents in the first 1 to 4 days of life with severe hyperventilation caused by profound lactic acidosis and marked hypoglycaemia. Later on, episodes of irritability, somnolescence or coma, apnoeic spells, dyspnea and tachycardia, muscular hypotonia, and moderate hepatomegaly may occur. Most affected children experience a number of acute attacks before the diagnosis is made. As reported in the first patient described [28], episodes are typically triggered by a febrile episode accompanied by refusal to feed and vomiting. Attacks may also follow ingestion of large amounts

of fructose (~1 g/kg b.w. in one dose) especially after a period of fasting. FBPase deficiency may be life-threatening and, as in HFI, administration of IV fructose is contraindicated and may lead to death. In between attacks, patients are usually well, although mild, intermittent or chronic acidosis may exist. The frequency of the attacks decreases with age, and the majority of survivors display normal somatic and psychomotor development [29].

In contrast to HFI, chronic ingestion of fructose does not lead to gastrointestinal symptoms – hence there is no aversion to sweet foods – or failure to thrive, and only exceptionally is there disturbed liver function. Analysis of plasma during acute episodes reveals lactate accumulation (up to 15–25 mM) accompanied by a decreased pH and an increased lactate/pyruvate ratio (up to 40), hyperalaninaemia, an increase in glycerol which may mimic hypertriglyceridaemia [30], and glucagon-resistant hypoglycaemia. Hyperketonaemia may be found, but in several patients ketosis has been reported to be moderate or absent (below and [31]). Increased levels of free fatty acids and uric acid may also be found. Urinary analysis reveals increased lactate, alanine, glycerol, and, in most cases, ketones and glycerol-3-phosphate. Clinicians should be aware that detection of the strongly hydrophilic compounds glycerol and glycerol-3-phosphate is best when the urease pretreatment non-extraction method [32], hydrophilic interaction chromatography (HILIC) or ion-pairing reverse phase chromatography is used.

15.3.2 Metabolic Derangement

Deficiency of hepatic FBPase, a key enzyme in gluconeogenesis, impairs the formation of glucose from all gluconeogenic precursors, including dietary fructose (Fig. 15.1). Consequently, maintenance of normoglycaemia in patients with the defect is exclusively dependent on glucose (and galactose) intake and degradation of hepatic glycogen and, to a minor degree, on glucose production by the muscle [33]. Thus, hypoglycaemia is likely to occur when glycogen reserves are limited (as in newborns) or exhausted (as when fasting). The defect provokes accumulation of the gluconeogenic substrates lactate, pyruvate, alanine, and glycerol. The lactate/pyruvate ratio is usually increased which is explained by secondary impairment of conversion of 1,3-bisphosphoglycerate to glyceraldehyde-3-phosphate; this results in accumulation of $NADH/H^+$ which shifts the equilibrium of pyruvate and lactate (Fig. 15.1). Hyperketonaemia and ketonuria, which usually accompany hypoglycaemia, may be absent in some patients with FBPase deficiency [31]. This may be explained by pyruvate accumulation resulting in an increase of oxaloacetate and, hence, in the diversion of acetyl-CoA away from ketone-body formation into citrate synthesis. This, in turn, results in increased synthesis of malonyl-CoA in the cytosol. Elevated malonyl-CoA, by inhibiting carnitine-palmitoyl transferase I, prevents the entry of long-chain acyl-CoA into the mitochondria and, thereby, further reduces ketogenesis. It also promotes accumulation of fatty acids in liver and plasma, as documented in some patients.

Children with FBPase deficiency generally tolerate sweet foods, up to 2 g fructose/kg b.w. per day, when given regularly distributed over the day and, in contrast to subjects with HFI, they thrive on such a diet [34]. Nevertheless, loading tests with IV fructose do induce hypoglycaemia, as in HFI. This is caused by the inhibitory effect of the rapidly formed but slowly metabolised F-1-P on liver glycogen phosphorylase a. That higher doses of fructose are required for hypoglycaemia to occur is explained by the fact that, in contrast to the aldolase B defect in HFI, FBPase deficiency still allows F-1-P to be converted into lactate. ^{31}P-MRS of the liver following IV administration of fructose (200 mg/kg b.w.) has documented a slower decrease in the fructose-induced accumulation of F-1-P and a delayed recovery of the ensuing depletion of Pi and ATP (both of which are signs of fructose toxicity) in patients with FBPase deficiency as compared with healthy controls [7].

15.3.3 Genetics

FBPase deficiency is a rare autosomal-recessive disorder. In addition to European and North American patients, many cases have been diagnosed in Japan. The high proportion of Turkish patients in our own series might simply be the result of the high rate of parental consanguinity.

There is evidence for the existence of more than one isozyme with FBPase activity in humans. The muscle isoform has different kinetic characteristics to the liver isoform and is not affected in FBPase deficiency. Only the liver-type isoform gene (*FBP1*) has been cloned and characterised. To date, more than 50 different mutations in all regions of the gene have been published. Among them, a gross deletion including the entire exon 2 (c.-24-26_170+5192del) is common in patients of Turkish and Armenian descent [35]. The c.959dupG mutation has been reported to be responsible for 46% of mutated alleles in Japan [36] but only 14% in Central Europe [38]; c.841G>A has repeatedly been detected in Pakistani patients [30].

There are several patients in whom no mutation of the coding region of *FBP1* could be found. Therefore, we have supposed that these patients carry mutations within the promoter region of *FBP1* or, more hypothetically, in the gene for the bifunctional enzyme which controls the concentration of fructose-2,6-bisphosphate, the main physiological regulator of FBPase [37].

15.3.4 Diagnosis

Whenever possible, the diagnosis of FBPase deficiency should be made by molecular analysis on DNA from peripheral leukocytes. If no mutation is found despite highly suggestive clinical and laboratory findings, the determination of enzymatic activity in a liver biopsy should be undertaken; the residual activity may vary from zero to 30% of normal, indicating genetic heterogeneity of the disorder. Obligate heterozygotes have intermediate activity. Diagnosis is not possible in mixed leukocytes but seems to be reliable in isolated and stimulated monocytes [29]; however, cultured skin fibroblasts, amniotic fluid cells and chorionic villi do not express FBPase.

Loading tests with fructose (or with glycerol or alanine) or fasting tests should not be part of the initial investigations as they provide only a tentative diagnosis. However, such functional tests may be useful, and may point to a disturbance in the regulation of the fructose-6-phosphate – fructose-1,6-bisphosphate substrate cycle if mutation analysis and enzyme activity are normal despite a strong clinical and chemical suspicion of FBPase deficiency.

15.3.5 Differential Diagnosis

Since FBP has only recently been shown to be inhibited by metformin [38], it is not surprising that diminished glucose production and a propensity to lactic acidosis is seen under this medication. Congenital disorders with primary or secondary affection of gluconeogenesis and pyruvate metabolism have to be considered, e.g., *(i)* glycogenosis type Ia and Ib presenting with the same metabolic profile (fasting hypoglycaemia and lactic acidosis) and hepatonephromegaly, hyperlipidaemia, and hyperuricaemia; *(ii)* pyruvate carboxylase deficiency when in context with neurological symptoms; *(iii)* fatty acid oxidation defects; and *(iv)* some rare liver-specific presentations of respiratory chain disorders with fasting hypoketotic hypoglycaemia and hyperlactataemia due to deficient energy production required for gluconeogenesis in the liver may also mimick FBP (► Chap. 1).

15.3.6 Treatment and Prognosis

Whenever FBPase deficiency is suspected, adequate amounts of IV or oral glucose should be given. The acute, life-threatening episodes should be treated with an IV bolus (1 ml/kg b.w. of 20% glucose as a rule of thumb) followed by a continuous infusion of glucose at high rates (e.g., 10–12 mg/kg b.w./ min for newborns) and bicarbonate to control hypoglycaemia and acidosis. If correction of acidosis is not really needed, recovery from it in response to glucose is a good (positive) indicator for the diagnosis of FBPase deficiency. Furthermore, the infusion of glycerol (that may even contain additional fructose), as frequently practiced in patients with brain oedema and hypoglycaemia in Japan, is extremely dangerous unless FBPase deficiency is excluded [34, 39].

Maintenance therapy should be aimed at avoiding fasting, particularly during febrile episodes. This involves frequent feeding, the use of slowly absorbed carbohydrates (such as uncooked starch), and a gastric drip, if necessary. In small children, restriction of fructose, sucrose and sorbitol is also recommended, as are restrictions of fat (to 20–25%) and protein (to 10% of energy requirements). In the absence of any triggering effects leading to metabolic decompensation, individuals with FBPase deficiency are healthy and no carbohydrate supplements are needed.

Once FBPase deficiency has been diagnosed and adequate management introduced, its course is usually benign. Growth, psychomotor and intellectual development are unimpaired, and tolerance to fasting improves with age with the effect that the disorder in general does not present a problem in later life [30]. Pregnancies were reported to be uncomplicated [29, 40]. Many patients, however, become obese because their concerned parents overfeed them and they continue these eating habits when older. Under carefully observed conditions, a hypocaloric fructose-free diet (800–900 kcal/m^2/day) can lead to a considerable weight loss in obese patients without the development of lactic acidosis and hypoglycaemia [Steinmann, personal observation].

Note added in proof: A large retrospective series of 18 patients with FBPase deficiency has been recently published; most displayed a consistent metabolic phenotype with fasting hypoglycaemia and lactic acidosis, liver abnormalities and developed adult steatosis. [M Gorce et al. JIMD 2022, 45:215–222].

15.4 Sorbitol Dehydrogenase Deficiency

For years, the sorbitol dehydrogenase pathway was a matter of interest to explain complications of diabetes mellitus. In diabetes large amounts of glucose may enter

cells with low activity of sorbitol dehydrogenase, such as the retina, lens or nerve cells. Glucose is there converted by aldehyde reductase, the first step of the polyol pathway, to sorbitol (◘ Fig. 15.1) which accumulates and is supposed to cause osmotic effects and shortage of NADPH with the result of retinopathy, cataract formation, or peripheral neuropathy.

15.4.1 Clinical Presentation

Only recently, a congenital defect of sorbitol dehydrogenase (SORD) has been described in patients who presented with a slowly progressive neuropathy clinically classified as the axonal type of Charcot-Marie-Tooth disease (CMT2) or as distal hereditary motor neuropathy (dHMN) [41]. Hallmark of the disorder is a mild to moderate limb weakness, typically affecting the distal muscle groups of the lower extremities and often accompanied by foot deformities. Cataracts have not been observed. Age of onset typically is late childhood or early adulthood. Delayed milestones are uncommon but onset as early as 2 years has been reported.

15.4.2 Metabolic Derangement

Reported patients showed a complete loss of the SORD protein and a diminished enzymatic activity in fibroblasts, with the effect of an increased intracellular sorbitol concentration. Serum fasting sorbitol concentration was markedly increased. Although the exact pathomechanisms for progressive synaptic degeneration and motor impairment are not known, similar mechanisms as in diabetic complications are suggested.

15.4.3 Genetics

SORD deficiency is an autosomal-recessive disorder, nonetheless almost 70% of the cases are sporadic and a history of consanguinity is uncommon. This is due to a common variant, c.757delG in exon 7 of *SORD*, that most probably occurs because of recurrent gene conversion events from a highly homologous non-functioning pseudogene most likely arisen from gene duplication on chromosome 15. *SORD* has long been unrecognised even in the era of whole exome sequencing probably because of the existence of this pseudogene and despite its relatively high frequency. Homozygosity for the common variation alone causes a frequency of 1:100,000 which means that sorbitol dehydrogenase deficiency is a frequent form of hereditary neuropathies.

15.4.4 Diagnosis

Since detailed nerve conduction studies in SORD-deficient patients invariably showed a motor axonal neuropathy, this patient group should systematically be screened for elevated serum sorbitol concentration (► Chap. 1). An approximately 10% detection rate in undiagnosed CMT2 and dHMN has been reported. Targeted genetic diagnosis is possible using Sanger based sequencing techniques or neuropathy panels if attention is payed to the existence of the pseudogene.

15.4.5 Treatment and Prognosis

SORD deficiency is a potentially treatable disorder. Substrate reduction therapy by aldose reductase inhibitors has been shown to normalise intracellular sorbitol concentration in patient-derived fibroblasts, and also significantly ameliorated the motor phenotype in an animal model [41]. Likewise, clinical trials with aldose reductase inhibitors in diabetic patients show a good safety profile and an improvement of nerve conduction [42]. Hence these substances may be a therapeutic option in the future.

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Hyperphenylalaninaemia

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Contents

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Phenylalanine Metabolism

Phenylalanine (PHE), an essential aromatic amino acid, is mainly metabolised in the liver by the PHE hydroxylase (PAH) system (Fig. 16.1). The first step in the irreversible catabolism of PHE is hydroxylation to tyrosine (TYR) by PAH. This enzyme requires the active pterin, tetrahydrobiopterin (BH_4), which is formed in three steps from guanosine triphosphate (GTP), and DNAJC12 which functions as a co-chaperone with HSP70 for correct folding and stability of the aromatic amino acid hydroxylases. During the hydroxylation reaction BH_4 is converted to the inactive pterin-4a-carbinolamine. Two enzymes regenerate BH_4 via quinoid-dihydrobiopterin (qBH_2). BH_4 is also an obligate co-factor for tyrosine hydroxylase and tryptophan hydroxylases 1 & 2, (and thus necessary for the production of dopamine, catecholamines, melanin and serotonin), and for alkylglycerol monooxygenase (AGMO) and 3 isoforms of nitric oxide synthase [1]. The physiological role of AGMO, which is involved in ether lipid metabolism, is not yet fully characterised.

Defects in either PAH, the production or recycling of BH_4 or DNAJC12 may result in hyperphenylalaninaemia (HPA), as well as in deficiency of TYR, L-dopa, dopamine, melanin, catecholamines and 5-hydroxytryptophan (5HT). When hydroxylation to TYR is impeded, PHE may be transaminated to phenylpyruvic acid (a ketone excreted in increased amounts in the urine, whence the term phenylketonuria or PKU), and further reduced and decarboxylated.

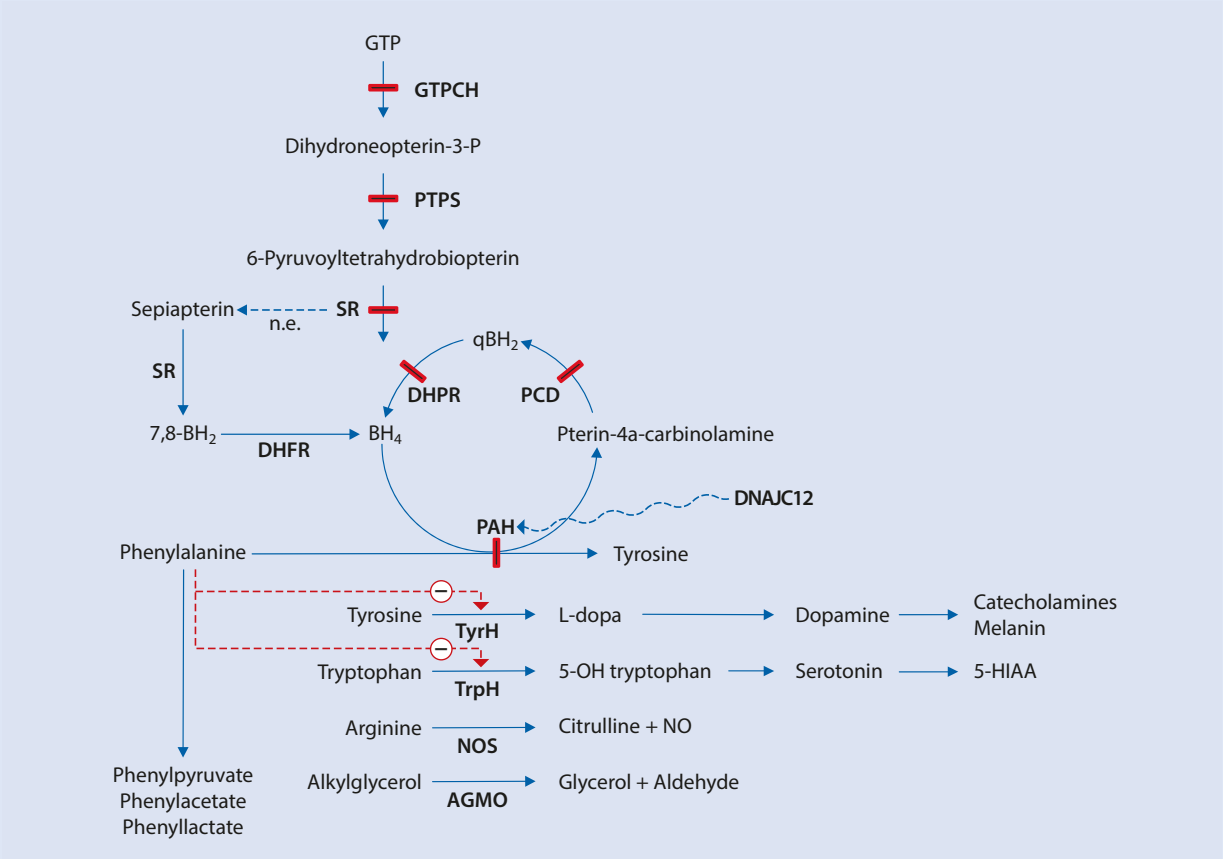

Fig. 16.1 The phenylalanine hydroxylation system, including the synthesis and regeneration of pterins and other pterin-requiring enzymes. AGMO, alkylglycerol monooxygenase; BH_2, dihydrobiopterin (quinone); BH_4, tetrahydrobiopterin; DHFR, dihydrofolate reductase; DHPR, dihydropteridine reductase; DNAJC12, heat shock protein of the HSP40 family; GTP, guanosine triphosphate; GTPCH, guanosine triphosphate cyclohydrolase; 5HIAA, 5-hydroxyindoleacetic acid; NO, nitric oxide; n.e., non-enzymatic; NOS, nitric oxide synthase; P, phosphate; PAH, PHE hydroxylase; PCD, pterin-4a-carbinolamine dehydratase; PTPS, pyruvoyl-tetrahydrobiopterin synthase; SR, sepiapterin reductase; TrpH, tryptophan hydroxylase; TyrH, tyrosine hydroxylase. Encircled minus sign indicates inhibition. The enzyme defects are depicted by *bars* across the *arrows*

Introduction

Mutations within the gene for the hepatic enzyme phenylalanine hydroxylase (PAH) and those involving production or recycling of tetrahydrobiopterin metabolism or DNAJC12 are associated with hyperphenylalaninaemia (HPA). Severe PAH deficiency, which results in a blood phenylalanine (PHE) greater than 1200 μmol/L when individuals are on a normal protein intake, is referred to as classic phenylketonuria or just PKU. Milder defects associated with concentrations between 600 μmol/L and 1200 μmol/L are termed HPA, and those with concentrations less than 600 μmol/L but above 120 μmol/L, mild HPA (MHP). Disorders of biopterin metabolism have in the past been called malignant PKU or malignant HPA. However, such disorders are now best named according to the underlying enzyme deficiency. A comprehensive nomenclature is provided in [2]. Deficiency of DNAJC12, a heat-shock protein (HSP40) which functions as a co-chaperone with HSP70, necessary for correct folding and stability of the PAH protein, also leads to HPA in individuals without mutant PAH genes [3]. PKU if left untreated leads to permanent central nervous system damage. Dietary restriction of PHE along with amino acid, vitamin and mineral supplements, started in the first weeks of life and continued through childhood, is an effective treatment and allows for normal cognitive development. Pharmacologic treatment with BH_4 can reduce blood PHE concentrations in individuals with residual PAH activity. Therapy with PEGylated recombinant *Anabaena variabilis* phenylalanine ammonia lyase (PAL), a non-human enzyme, can also reduce blood PHE concentration. Lifelong treatment is now generally recommended for all patients with PKU, although, as yet, there is insufficient data to know how necessary this is. Less severe forms of PAH deficiency may or may not require treatment, depending on the degree of HPA; however, there is no evidence-based concentration for a raised blood PHE below which treatment is not required. PHE is a potent teratogen and high blood concentrations during pregnancy lead to the maternal PKU syndrome [4]. This can be prevented by strict dietary control of maternal blood PHE throughout pregnancy. Disorders of pterin metabolism lead to both HPA and disturbances in central nervous system amines. Generally, they require treatment with oral BH_4 and neurotransmitters.

16.1 Phenylalanine Hydroxylase Deficiency

16.1.1 Clinical Presentation

The natural history of untreated PKU is progressive, irreversible neurological impairment during infancy with the subsequent development of mental, behavioural, neurological and physical impairments. The most common outcome is moderate to profound intellectual developmental disorder (IQ ≤ 50), often associated with a mousey odour (resulting from the excretion of phenylacetic acid), eczema (20–40%), reduced hair, skin and iris pigmentation (a consequence of impaired melanin synthesis), reduced growth and microcephaly, and neurological impairments (25% epilepsy, 30% tremor, 5% spasticity of the limbs, 80% EEG abnormalities). The brains of patients with PKU untreated in childhood have reduced arborisation of dendrites, impaired synaptogenesis and disturbed myelination. Other neurological features include pyramidal signs with increased muscle tone, hyperreflexia, Parkinsonian signs and abnormalities of gait and tics. Almost all untreated patients show behavioural problems, which include autistic spectrum disorders, hyperactivity, stereotypy, aggressiveness, anxiety and social withdrawal. The clinical phenotype correlates with PHE blood concentrations, reflecting the degree of PAH deficiency.

16.1.2 Metabolic Derangement

Although the pathogenesis of brain damage in PKU is not fully understood, it is causally related to the increased concentrations of blood PHE. Tyrosine (TYR) becomes a semi-essential amino acid, with reduced blood concentrations leading to impaired synthesis of other biogenic amines, including melanin, dopamine and norepinephrine. Increased blood PHE concentrations cause an imbalance of other large neutral amino acids (LNAA) within the brain, resulting in decreased brain concentrations of methionine, TYR and serotonin. The ratio of PHE concentrations in blood/brain is about 4:1 [5]. In addition to the effects on amino acid transport into the brain, elevated PHE inhibits TYR hydroxylation to dopamine and tryptophan decarboxylation to serotonin. The phenylketones phenylpyruvate, phenylacetate, phenylacetylglutamine and phenyllactate are not abnormal metabolites, but appear in increased concentration and are excreted in the urine.

16.1.3 Genetics

PAH deficiency is autosomal-recessively transmitted. At the time of writing >1200 different *PAH* mutations have been described (► http://www.biopku.org/home/pah.asp). Most patients are compound heterozygous. Although there is no single prevalent mutation, certain ones are more common in different ethnic populations: the R408W mutation accounts for approximately 30% of alleles in Europeans with PKU; in East Asians and

South East Asians the R243Q mutation is the most prevalent (13% of alleles). The prevalence of PAH deficiency varies between different populations (e.g. 1 in 1,000,000 in Finland and 1 in 4,200 in Turkey). Overall global prevalence in screened populations is approximately 1 in 12,000, giving an estimated carrier frequency of 1 in 55.

Genotypes correlate well with biochemical phenotypes, pre-treatment PHE concentrations and PHE tolerance [6], which are determined by the milder mutation in compound heterozygotes. However, owing to the many other factors that affect clinical phenotype, correlations between mutations and neurological, intellectual and behavioural outcome are weak. Genetic analysis is of limited practical use in clinical management, but may be of value in determining genotypes associated with BH_4 responsiveness (► http://www.biopku.org/BioPKU_DatabasesBIOPKU.asp) [6] and is essential to diagnose DNAJC12 deficiency [3].

16.1.4 Diagnostic Tests

Blood PHE is normal at birth but rises rapidly within the first days of life. In most Western nations PKU is detected by newborn population screening (NBS). There is variation between different countries and centres in the age at which screening is undertaken (day 1 to day 5), in the methodology used (Guthrie microbiological inhibition test, enzymatic techniques, HPLC, or tandem mass spectrometry) and the concentration of blood PHE that is taken as a positive result requiring further investigation (120–240 µmol/L, but with some laboratories also using a PHE/TYR ratio >3).

Co-factor defects must be excluded by investigation of pterins in blood or urine and dihydropteridine reductase (DHPR) in blood and DNAJC12 deficiency by mutation analysis (► Sect. 16.2). HPA may be found in preterm and sick babies, particularly after parenteral feeding with amino acids and in those with liver disease (where blood concentrations of methionine, TYR, leucine/isoleucine and PHE are usually also raised), and in treatment with chemotherapeutic drugs or trimethoprim.

PAH deficiency may be classified according to the blood PHE concentration when patients are on a normal protein-containing diet, after a standardised protein challenge, or after standardised loading with BH_4 [2].

- Classic PKU (PHE ≥1200 µmol/L; less than 1% residual PAH activity),
- Hyperphenylalaninaemia (HPA) or mild PKU (PHE >600 µmol/L and <1200 µmol/L; 1–5% residual PAH activity), and
- Non-PKU-HPA or mild hyperphenylalaninaemia (MHP) (PHE ≤ 600 µmol/L; >5% residual PAH activity),
- BH_4-Responsive PKU/HPA (blood PHE concentrations decrease substantially after oral administration of BH_4, thus increasing dietary PHE tolerance.

Although the spectrum of severity is continuous, such a classification has some use in terms of indicating the necessity for and type of treatment.

Prenatal diagnosis, rarely requested, is possible by means of *PAH* analysis on chorion villus biopsy (CVB) or amniocentesis where the index case has mutations identified previously.

16.1.5 Treatment and Prognosis

16.1.5.1 Principles of Treatment

Dietary Treatment

Dietary treatment for PKU has proved highly successful and has provided a model for the dietary management of other aminoacidopathies, such as MSUD and classical homocystinuria. The principle of treatment in PAH deficiency is to reduce the blood PHE concentration sufficiently to prevent the neuropathological effects but also to fulfil age-dependent requirements for protein synthesis. Blood PHE is primarily a function of residual PAH activity and PHE intake. For the majority of patients with PKU the former cannot be altered, so that blood PHE must be reduced by restricting dietary PHE intake. The blood PHE concentration while on a normal protein-containing diet, defines whether treatment is indicated. There are some differences in the recommended cut-off above which PHE restriction is required: Germany >600 µmol/L [7], France, USA, Australasia >360 µmol/L [8–10], and 2016 European Society for PKU (ESPKU) guidelines >360 µmol/L [11]. To stay below these, patients with classic PKU have to reduce nutritional PHE intake to 200–400 mg/day or 4–8 g natural protein per day. In all but the USA recommendations, treatment target blood PHE concentrations are age related but show substantial variation. ◘ Table 16.1 shows recommendations for Germany, the USA, France, the Netherlands, Switzerland, Australasia, and the 2016 ESPKU guidelines. With the exception of blood PHE concentrations for the first decade of life and during pregnancy, reported evidence levels are most often low (quasi-experimental designs, non-analytic studies or expert opinion) or not specified and most recommendations are classified as weak. Blood PHE target ranges differ particularly for age groups older than ten years, without clinical evidence that these differences matter. French guidelines accept 900 µmol/L for adults without

Table 16.1 Daily phenylalanine (PHE) tolerances and target blood ranges, showing different targets aimed for in various countries

		Germany [7]	Netherlands [26]	Switzerland [100]	USA [10]	Australasia [7, 71]	Europe [11]	France [8]
Blood PHE concentration indicating treatment (μmol/l)		>600	Not specified	>400	>360	>360	>360	>360
Patient age (years)	PHE tolerance mg/day	Target blood PHE range (lower- upper boundary; μmol/L)						
0	130–400	40–240	120–240	100–300	60/120–360	120–360	120–360	120–360
1	200–400		120–360					
2	200–400			100–400				
3–4	200–400							
5–9	200–400		120–480					
10–11	350–800	40–900		100–600				
12–14	350–800					120–360 (>360[b])	120–600	120–600
15	350–800		120–600					
16–17	450–1000	40–1200						
>17	450–1000							120–600 (900[c])
Pregnancy	120–400[a]	120-360	Not specified	100–300	120–360	70–250	120–360	120–360

[a]tolerance will usually increase in later stages of pregnancy
[b]acceptable after informed decision
[c]acceptable in individuals without clinical signs

clinical signs and Australasian guidelines recommend accepting patients' informed decisions for concentration above 360 μmol/L after childhood.

Since PHE is an essential amino acid, excessive restriction is also harmful and, particularly in infancy, will result in impaired growth and cognitive development. In order to prevent PHE deficiency a lower limit for blood PHE is also defined. The lower limit is formulated ambiguously in the US guideline recommending 120 μmol/L but stating that concentrations 60–120 μmol/L should not be regarded as too low.

The degree of protein restriction required means that in order to provide a nutritionally adequate supply a semi-synthetic diet is necessary. This is composed of the following:

- Unrestricted natural foods with a very low PHE content (<30 mg/100 g; e.g. carbohydrate; fat, some fruit and vegetables).
- Calculated amounts of restricted natural and manufactured foods with medium PHE content (30–100 mg/100 g; e.g. potato, spinach, broccoli; special bread and special pasta). In the United Kingdom a system of 'protein exchanges' is used, with each 1 g of natural protein representing a PHE content of approximately 50 mg.
- Calculated amounts of PHE-free amino acid mixtures (AAMs) supplemented with vitamins, minerals and trace elements. The biological value of AAMs is lower than that of natural protein; the equivalent daily protein from this source needs to be 20% higher than the age-related reference values for natural protein.

Intake of these three components – including the PHE-free amino acid mixture – should be distributed as evenly as possible with meals during the day.

Foods with a higher concentration of PHE (e.g. meat, fish, cheese, egg, milk, yoghurt, cream, rice, corn) are not allowed. Aspartame (L-aspartyl L-phenylalanine methyl ester), a sweetener for foods (e.g. in soft drinks) contains 50% PHE and is therefore inappropriate in the PKU diet.

PHE-free amino acid infant formulas that also contain adequate essential fatty acids, minerals and vita-

mins are available. Human breast milk has relatively low PHE content; in breast-fed infants, PHE-free formulas are given in measured amounts followed by breast feeding to appetite. In the absence of breast feeding a calculated quantity of a normal formula is given to provide the essential daily requirement of PHE. In older patients Glycomacropetide, a 64-amino acid glycophosphopeptide containing 2.0–5.0 mg PHE per gram [12] may partly substitute AAMs thereby improving bioavailability and palatability but there is insufficient evidence to advocate its use as an alternative to traditional treatment [13].

With intercurrent illness, individuals may be unable to take their prescribed diet. During this period high-energy fluids may be given to counteract catabolism of body protein.

Treatment with BH_4

Pharmacological doses of BH_4 can reduce blood phenylalanine concentrations in some patients with PKU [14]; sapropterin dihydrochloride (Kuvan®), a synthetic formulation of the active 6R-isomer of BH_4 is approved in Europe and the USA for the treatment of patients with HPA and PKU, of all ages, who have been shown to be responsive to such treatment. Most frequently BH_4 responsiveness is defined by a reduction of ≥30% in blood PHE concentration after a single dose of 20 mg BH_4/kg body weight, but there are alternative criteria [15]. It has been suggested that a more clinically relevant assessment is to initially determine BH_4-responsiveness with a screening test, measuring the decrease of blood PHE after a single BH_4 dose of 20 mg/kg, followed, if there has been a decrease ≥30%, by a further period of BH_4 treatment to assess the increase in natural protein tolerance setting a goal (e.g. an increase of at least 100%) to define responsiveness in clinical practice [2, 11].

Studies on the PKU Pah^{enu1} mouse, a model of the mild hyperphenylalaninaemia phenotype, and expression studies of mutations found in BH_4-responsive patients have shown that reduced function of PAH can result from misfolding, aggregation and accelerated degradation of the enzyme. BH_4 may act as a chaperone, providing conformational stabilisation and augmenting the effective PAH concentration [16], with different genotypes showing optimal responses at different PHE concentrations [17]. Treatment with BH_4 consists of single daily doses of 5–20 mg/kg body weight, with the aim of decreasing blood PHE concentrations or increasing dietary PHE tolerance. Both effects have been demonstrated in placebo controlled trials [18].

BH_4 responsiveness is most often found in those with mild PKU, who have a higher residual PAH activity. Except for where there are two null-mutations, the association between genotype and BH_4-responsiveness is probabilistic, and BH_4 responsiveness should always be tested clinically. The manufacturer's prescribing information [19] and a US FDA drug review recommend that Kuvan be used in combination with a PHE-reduced diet, leaving open the question of BH_4 monotherapy for those patients who would with treatment have PHE concentrations sufficiently low not to require diet. There are no serious side effects in the short and mid term [20]. Given the different protocols and the limitations of the 30% criterion in determining BH_4 responsiveness, it is impossible to predict the proportion of patients who might benefit significantly from long-term treatment [18]. Limited data suggest that the use of BH_4 in pregnancy is effective and safe in controlling PHE concentrations in responsive patients [20, 21] but diet remains the first-line treatment for pregnant women. Despite increased cost and regimen complexity, treatment with BH_4 can result in improvement in the quality of life in a subgroup of patients with PKU [22].

Sepiapterin, a natural precursor of BH_4 in the salvage pathway of pterins is more stable and crosses cell membranes more efficiently than BH_4. Exploratory studies in adult healthy volunteers showed that after oral doses of CNSA-001, a pharmaceutical preparation of sepiapterin, increases of BH_4 in CSF [23], and plasma BH_4 concentrations were larger than after equivalent doses of sapropterin dihydrochloride [24]. However, trials are required to evaluate possible clinical effects.

Treatment with Pegvaliase

Enzyme substitution therapy with pegvaliase (PEGylated recombinant *Anabaena variabilis* phenylalanine ammonia lyase (PAL) (Palynziq®) is approved for individuals >16 years. The enzyme converts PHE independently from PAH and BH_4 to a harmless compound, transcinnamic acid, and ammonia metabolised in the liver to urea. Covalent attachment of polyethylene glycol polymer chains (PEGylation) 'mask' the agent from the host's immune system, reducing immunogenicity and antigenicity. However, during early treatment (≤6 months) but also later (at year 1) all patients develop antibodies against PEG and PAL and more than 90% experience adverse events like hypersensitivity, arthralgia/arthritis, injection site/generalized skin reactions or lymphadenopathy, and about 9% anaphylaxis episodes [25]. Treatment is initiated by a titration phase when subcutaneous injections must be accompanied by a trained observer (able to recognise signs of acute systemic hypersensitivity/anaphylaxis, to administer an epinephrine autoinjector, and call emergency services if necessary) for at least one hour following each injection. Patients must always carry the epinephrine autoinjector and be able to master its application. Daily subcutaneous injection of 20–60 mg of the enzyme per maintenance dose is effective in reducing PHE concentrations below 120 μmol/L and often allows a normal diet. In clinical trials up to 40% of

patients showed episodes of hypophenylalaninaemia (<30 µmol/L). As the treatment does not increase TYR concentration, blood TYR should also be monitored and if necessary supplemented. Pegvaliase is not recommended for use in women who are currently planning to become pregnant [25].

16.1.5.2 Monitoring of Treatment

A low-protein diet brings the risk of nutritional deficiency. Therefore, treatment is monitored by regular assessment of dietary intake and blood PHE concentrations, as well as neurological, physical, intellectual and behavioural development. Timetables and procedures vary between recommendations [7–10, 26, 27]. Blood PHE should be measured weekly during the first year of life, fortnightly during childhood, and monthly afterwards. Samples ideally should be taken early morning when concentrations are likely to be at a peak or at least 4 hours post-prandially.

16.1.5.3 Alternative Therapies/Experimental Trials

Although dietary treatment is highly successful, it is difficult and compliance is often poor, particularly as individuals reach adolescence. Hence there is a need to develop more acceptable therapies.

- The **large neutral amino acids (LNAA;** phenylalanine, tyrosine, tryptophan, leucine, isoleucine and valine) compete for the same transport mechanism (the L-type amino acid carrier) to cross the blood-brain barrier as well as for the absorption by the intestinal mucosa [28]. Studies in the $PAH^{enu2/2}$ mouse and in patients have shown a reduction in brain PHE concentrations and some positive effect on neuropsychological functions when LNAAs (apart from PHE) have been given enterally [29]. The greatest benefit may be to patients who are unable to comply with conventional dietary management, but it is likely to be of limited efficacy.
- **Gene therapy**. A number of different PAH gene transfer vehicles have been tried in the $PAH^{enu2/2}$ mouse. Vectors based on recombinant adeno-associated viruses (rAAVs) expressed in either liver or muscle are currently the favoured vector system. An rAAV vector with genes for PAH and BH_4 synthesis injected into skeletal muscle or infused into the intraportal vein or naked DNA injected in the tail vein of $PAH^{enu2/2}$ mice, showing a classical PKU phenotype, resulted in correction of PHE for more than 1 year [30]. A phase 1/2 trial of liver delivery with an AAV vector is now underway in adults with PKU (► ClinicalTrials.gov Identifier: NCT04480567).
- **Liver transplantation** fully corrects PAH deficiency [31], but the risks of transplantation surgery and post-transplantation immune suppressive medication are too high for it to be a realistic alternative to dietary treatment. The same is true for liver repopulation with PAH-expressing cells following hepatocyte or haematopoietic stem cell transplantation [32].

16.1.5.4 Compliance with Treatment

Compliance with treatment is best in infancy and childhood. The diet severely interferes with culturally normal eating habits, particularly in older children and adolescents, and this often results in problems with keeping to treatment recommendations. Up to the age of 10 years only 40% of the German Collaborative Study of PKU patients could keep their PHE concentrations in the recommended range [33]. After the age of 10 years 50–80% of blood PHE concentrations measured in a British and Australian sample were above recommendation [34]. In the US, patient, social, and economic factors prevent >70% of adult patients from accessing treatment [35] and two thirds have PHE concentrations above the recommended range [36]. Dietary treatment of PKU is almost impossible without the support of a specialised team, which should include a dietitian, a metabolic paediatrician or physician for adult patients, a biochemist running a metabolic laboratory and a psychologist skilled in the behavioural management of a life-long diet. All professionals, and the families themselves, must fully understand the principles and practice of the diet. The therapeutic team should be trained to work in an interdisciplinary way in a treatment centre, training camps can improve long-term knowledge about the condition and its treatment [2, 11, 37].

16.1.5.5 Outcome

The outcome for PKU mainly depends upon the age at start of treatment, blood PHE concentrations in different age periods, duration of periods of blood PHE deficiency and the individual gradient for PHE transport across the blood-brain barrier. The most important single factor is the blood PHE concentration in infancy and childhood. Dietary treatment started within the first 3 weeks of life with average blood PHE concentrations ≤ 400 µmol/L in infancy and early childhood result in near-normal intellectual development. However, for each 300 µmol/L increase in blood PHE during the first 6 years of life, IQ is reduced by 0.5 of a standard deviation (SD), and during age 5–10 years the reduction is 0.25 SD. Furthermore, IQ at the age of 4 years is reduced by 0.25 SD for each 4 weeks of delay in the start of treatment and for each 5 months of insufficient PHE intake. After the age of 10 years all large studies show stable IQ performance, at least until mid-adulthood irrespective of PHE concentrations [38–41], and a normal school career if compliance during the first 10 years has been accord-

ing to treatment recommendations [42–44]. A Bayesian meta-analysis covering the age range from 2 to 35 years distinguished long-term and concurrent blood PHE concentrations in a critical (<6 years) and non-critical period (≥6 years) as predictors for an IQ <85 [45]. Effects of long-term PHE were larger than for concurrent values and those for the critical period were stronger than for the later period. The associations of PHE with IQ were negative, with PHE measurements <400 µmol/L predicting probabilities of IQ >85, close to the general population. However, correlations between concurrent and long-term PHE values, between the critical and noncritical period as well as age at start of treatment, degree of PAH activity, different IQ tests and socioeconomic status were not controlled. Compared with a matched control group over a 5 year period IQ of early-treated patients with classical PKU aged 10–41 years with blood PHE concentrations between 600 and 900 µmol/L remained stable. Older adults performed worse than younger ones, explained by higher PHE concentrations during childhood or adolescence. IQs of early and well-treated adults with PKU are similar to those of their unaffected family members [44] however, longitudinal studies covering adulthood are still rare [38, 39]. Quality of life (QoL) has become an important outcome. Despite the burden of strict dietary control, early treated patients can have a normal QoL [46, 47]. QoL issues also apply to parents of patients with PKU [48].

16.1.5.6 Complications in Adulthood

The majority of early-treated patients are now adults. Dietary treatment has transformed their prospects: they can expect normal development, professional careers, to start families and to live independently [47, 49]. Nonetheless, when studied in detail, subtle neuropsychological deficits have been found. Neuroimaging abnormalities are common but frank neurological disease has been reported in only in a few. This is concerning and it is important to define the precise phenotype of adults with early-treated PKU and determine which features relate to raised PHE concentrations, either historical or concurrent.

Neurological Abnormalities

Frank neurological disease is rare and may not be related to PHE concentrations [49]. Subacute combined degeneration of the spinal cord has been reported in adults after dietary relaxation. These patients had all developed profound vitamin B_{12} deficiency because they had stopped taking their amino acid supplements, but continued to follow diets low in high quality natural protein [50]. Early and well treated patients as well as those on relaxed diet can show tremor and brisk reflexes [51] the aetiology and clinical significance of which are unclear. Other complications such as cortical visual loss [52, 53] are very infrequent and possibly associated with poor control in childhood or adolescence [54]. Reinstitution of dietary treatment and amino acid supplements can lead to improvement.

Neuropsychological Abnormalities

Subtle changes in executive function, vigilance, working memory, and motor skills have been described in children, adolescents and adults with early-treated PKU, however, findings are inconclusive regarding the nature of executive impairments as well as their specificity, impact on everyday life, persistence over time, association with PHE concentrations, and aetiology [47, 55, 56]. Meta-analytic results suggest that for the prevention of any impairment there is an upper threshold PHE concentrations of 320 µmol/L for children (7–12 years) and 570 µmol/L for adolescents (13–18 years). In adults the negative effect remained stable between PHE concentrations of 750–1500 µmol/L [57]. Information processing was also stable in a 5 year longitudinal controlled study of adult patients with classical PKU [58]. In one study, the latency of visual saccades [59] in adults with PKU with concurrent PHE concentrations greater than 1200 µmol/L was significantly longer than in control patients, whilst no difference was detected with PHE concentrations below 800 µmol/L. Saccadic latencies normalised with improved metabolic control. Others however have failed to find a relationship between saccadic latency and PHE concentrations [60].

Neuroimaging Abnormalities

White matter abnormalities on brain MRI appear after longer periods of increased PHE concentrations, but are reversible after 3–6 months of strict dietary treatment [61]. In all but one study MRI did not correlate with intellectual or neurological abnormalities. In one study, 67% of patients under 10 years old had normal-appearing white matter. This decreased to only 4% in those over 20 [62] although there was no evidence of any clinical neurological deterioration over this period. Alterations were associated with long-term PHE concentrations. Diffusion tensor imaging demonstrated reduced diffusivity of water molecules with intact fractional anisotropy [63, 64], suggesting that the axons in PKU white matter are structurally intact, but that water molecules move more slowly along them. The clinical relevance of any of these imaging findings needs to be demonstrated [49].

Neuropsychiatric Abnormalities

Although no association of early treated PKU with psychiatric disease has been demonstrated [47, 49], increased emotional and behavioural symptoms have been described [65]. Patients with poor dietary control during infancy show hyperactivity, temper tantrums, increased

anxiety and social withdrawal, frequently associated with intellectual deficits. Well-treated subjects show increased risks of depressive symptoms and low self-esteem. However, without correlation to PHE concentrations, causality remains obscure, and such problems are also common in other chronic disease populations [66, 67].

Dietary Deficiencies

Vitamin B_{12} deficiency is well recognised in patients who have stopped their vitamin supplements but continue to restrict their natural protein intake. For patients on a strict diet there have been concerns regarding deficiencies in vitamins and minerals, including selenium, zinc, iron, retinol and long-chain omega-3 polyunsaturated fatty acids (LC-PUFA). Such deficiencies are sometimes found but it is unclear whether they are of any clinical significance. Low calcium, osteopenia and an increased risk of fractures have also been reported, however despite individual studies reporting reduced bone mineral density, pooled data suggest that these reductions are not clinically important [68]. LC-PUFAs, already added in PHE-free infant formulas, have been shown to be low in children aged >4 years. Experimental supplementation has been tolerated well and resulted in increased visual evoked potentials and motor performance, but the optimal type and dose of supply still needs to be determined [69]. In a double-blind randomized six month supplementation study DHA uptakes up to 7 mg/kg and day did not improve neurological functions in children 5–13 years old [70].

Diet for Life

Adults with PKU face different challenges from children, and it is much more difficult to be proscriptive about their dietary management. Although biochemically attractive, the concept of diet for life does pose substantial obstacles. For adolescents with PKU, the low-protein diet is restrictive, imposes a stark differentiation between them and their peers and is enforced by their parents: it is not surprising that many rebel against it. Those who wish to stay on diet when they leave the parental home may lack the skills, financial resources and time required. With suitable support these problems can be overcome, but the majority of adults are poorly compliant with dietetic advice with less than 30% of PHE concentrations falling within target ranges [34, 71]. This is likely to be because most adults who have had a period when their diet has lapsed have noticed no ill effects. A recent large study of adults with PKU shows that patients who were diagnosed on NBS and started on dietary treatment within the first year of life have excellent educational, occupational, and social outcomes regardless of whether they maintain strict dietary control in adulthood [47]. Although there was evidence of some subtle neurocognitive defects, these were mostly related to metabolic control in childhood and did not seem to affect the ability of people with PKU to function in society.

Despite the lack of consistent evidence for any irreversible effects of PHE on the adult brain, recent guidelines are recommending much more stringent PHE control in adults than has historically been the case [10, 72]. To date, no evidence has been published to show that these new targets have had any effect on the number of adult patients following dietary treatment or on the PHE concentrations they obtain. Although experience with maternal PKU (see below) suggests that women can obtain PHE concentrations below 360 μmol/L if they need to, most don't choose to maintain such a strict diet once their children have been born [73], suggesting that for many the strictures involved in maintaining such a diet do not justify any perceived clinical benefits [74].

It has always been difficult to persuade the many patients who are leading a normal life and eating a normal diet of the need to return to the restrictions of their childhood. It is important, however, that these individuals remain under expert care by metabolic physicians and dietitians with a training in behavioural and adult medicine, to ensure that they are following a nutritionally adequate diet, to monitor their long-term outcome, and to keep them informed about new evidence and treatments. A pragmatic approach is likely to be most productive, recommending broader corridors for PHE target concentrations, and giving adults with PKU the support, training and resources they need to follow their own choices [75]. The policy of personalised treatment is supported by a growing evidence of individual vulnerability to the neurotoxic effect of PHE [76].

16.1.5.7 Management of Late-Diagnosed PKU

Children with classic PKU who have not been screened in the newborn period and who are diagnosed later in infancy or childhood will have suffered permanent brain injury. However, the initiation of dietary treatment to control blood PHE concentrations will often lead to improvement in their neurodevelopmental state, albeit limited, and by preventing further damage allow for some developmental progress. The outcome for such children will depend on a number of factors but most importantly on the age at diagnosis and start of treatment.

Caring for adults with late-diagnosed PKU poses a unique set of problems. Although there is a wide spectrum, most of these patients will not be able to live independently. These older patients were either never treated, or treated late, when brain damage was already established, and often came off a low-protein diet at a young age. Although returning to diet does not affect established neurological disease, it can improve difficult

behaviour. In a randomised double-blind cross-over trial of the reintroduction of diet in patients with late-diagnosed PKU, carers rated behaviour as significantly better when subjects were on a low-PHE diet [77]. A 6-month trial of dietary treatment is warranted in late-diagnosed patients with challenging behaviour.

For late-diagnosed patients who remain at home into adulthood, the major challenge is planning for when their parents are no longer able to care for them. Eventually, these individuals will need alternative arrangements for their long-term care. This is best done in good time and with parental participation. If arrangements have to be made in an emergency, because of ill health or death of a carer, the results can be disastrous.

16.2 DNAJC12 Deficiency

Over 45 patients have now been described with HPA due to deficiency of DNAJC12, a 40 kDa heat-shock protein (HSP40) which functions as a co-chaperone with HSP70 required for the correct protein folding of the aromatic amino acid hydroxylases, PAH, tyrosine hydroxylase and tryptophan hydroxylases 1 and 2. For review see [3].

16.2.1 Clinical Presentation

Blood PHE concentrations at diagnosis have been <600 μmol/L. The clinical phenotype is heterogeneous, ranging from severe intellectual disability, dystonia and early-onset dopa–responsive parkinsonism to mild autistic features or hyperactivity and to individuals who are asymptomatic.

16.2.2 Metabolic Derangement

Deficiency of DNAJC12 is associated with a reduction in the activity of the aromatic amino acid hydroxylases leading to HPA and a reduction in biogenic CSF amines. CSF 5-hydroxyindoleacetic acid (5-HIAA) and homovanillic acid (HVA) concentrations are markedly decreased and the HVA/HIAA ratio is elevated. Neopterin and biopterin in CSF, urine and blood are normal. Additionally, DNAJ co-chaperones are thought to be involved in protein folding and degradation, and there is an association of DNAJC-family members with Parkinson's disease, parkinsonism and neurodegenerative diseases. In particular, they are involved in endocytosis of the synaptic vesicle (► Chap. 30).

16.2.3 Genetics

DNAJC12 deficiency is an autosomal recessive disorder caused by biallelic mutations in *DNAJC12*.

16.2.4 Diagnostic and Confirmatory Tests

The diagnosis should be suspected in any patient with HPA in whom PAH deficiency and biopterin synthesis disorders have been excluded. A BH_4 challenge results in a reduction in blood PHE. DHPR activity and urinary and blood pterin levels are normal. The diagnosis is confirmed by finding pathogenic mutations in *DNAJC12* [3].

16.2.5 Treatment and Prognosis

Treatment consists of BH_4 and/or L-dopa/carbidopa and 5-hydroxytryptophan. Normal development should follow early diagnosis and treatment. Late diagnosis can be associated with permanent neurological disease, however, treatment started in older symptomatic individuals may still be of benefit [78].

16.3 Maternal PKU

16.3.1 Clinical Presentation

Before the introduction of NBS and early treatment, it was unusual for women with PKU to have children of their own: as with other women with learning difficulties, positive steps were often taken to control their fertility. Initial observations that some children of mothers with PKU also had learning difficulties and behavioural problems were interpreted as genetic transmission rather than an environmental problem but the first description of maternal PKU syndrome (MPKUS) recognised the teratogenic effects of high maternal PHE concentrations [79]. Offspring of women with untreated classical PKU suffer developmental delay (92%), microcephaly (73%), cardiac defects (12%), low birth weight (40%) and dysmorphic features [4]. Blood PHE should be measured in a mother who delivers a baby with such symptoms, particularly in areas where NBS for PKU is not practiced or has been introduced relatively recently.

Although the pathogenesis of this condition is still poorly understood, much progress has been made and the MPKUS is now a preventable disease.

16.3.2 Metabolic Derangement

Teratogenic Effects of Phenylalanine

The Maternal PKU Collaborative Study (MPKUCS) showed that for maternal PHE concentrations below about 360 μmol/L there was no evidence of any deleterious effect on the foetus [80]. Above 360 μmol/L, developmental indices decreased by about three points for every 60 μmol/L rise in average concentration. Congenital heart disease (CHD) was only seen with much higher PHE concentrations (≥900 μmol/L) [81]. The risk of CHD increased with increasing PHE exposure; 50% of mothers who had children with CHD had average PHE concentrations ≥1500 μmol/L.

16.3.3 Treatment and Prognosis

16.3.3.1 Prevention of the Maternal PKU Syndrome

Strict dietary control for pregnant women with PKU and, preferably, those who are planning pregnancy, is necessary: the institution of strict metabolic control before conception and throughout pregnancy is associated with normal outcomes [80].

The plasma PHE targets used in maternal PKU have changed over time. Initially target concentrations of below 600 μmol/L led to improved outcomes and reduced incidence of CHD to background frequency. The policy to aim for even lower concentrations was based on the fact that, in infancy, PHE concentrations below 360 μmol/L minimised the risk of brain damage and that active placental transport led to an enrichment of PHE in the foetal circulation [82].

16.3.3.2 Current Practice

Prevention of the MPKUS requires time and resources, and the best outcomes are obtained by the centres with the most experience [83]. In 109 pregnancies cared for in a single centre over a 30-year period, preconception diet was established in 69.5% [84]. This centre looks after 15–20 PKU pregnancies annually. Prospective mothers are offered dietary education with partners or families. PHE is monitored twice a week preconceptionally and three times a week in pregnancy, with next day reporting. This level of service requires clinicians trained in metabolic medicine, specialist dietitians, specialised laboratory services, foetal medicine services, and close cooperation with child neuropsychologists to monitor outcomes. These resources are only available in units caring for adults with PKU, and any woman with PKU already pregnant or considering pregnancy should be referred to the nearest such centre.

16.3.3.3 Outcome

All women who plan their pregnancies and start diet before conception can maintain excellent metabolic control throughout pregnancy irrespective of their baseline PHE concentrations [84]. Concentrations may rise transiently during morning sickness or intercurrent illness, but these episodes can be controlled by reducing natural protein intake and increasing amino acid supplements. With morning sickness it is important to maintain calorie and supplement intake, in order to prevent catabolism and early use of anti-emetics is recommended.

After the first trimester protein tolerance increases markedly as the baby grows. For women who remain on a low-protein diet after delivery, greater protein restriction is often required postpartum.

Although PHE concentrations can usually be quickly brought under control, women who start diet after conception have significantly higher PHE concentrations throughout pregnancy [84]. A small subgroup of women, unable to fully comply with a low-protein diet, never obtain satisfactory metabolic control. Admission for full supervision of their diet will bring PHE concentrations down, but prolonged in-patient stays are neither practicable nor acceptable to the patients. Outcomes of such pregnancies remain poor. Often successive pregnancies are affected in the same way. In such pregnancies monitoring of PHE concentrations is infrequent, but often the absolute concentrations remain below 1000 μmol/l. For these women, new interventions are desperately required. BH_4, which is licensed for use in pregnancy, may have a role to play in responsive patients [21, 85]; any significant improvements in IQ for the offspring would justify the cost.

Any effects of the low protein diet on the foetus are much less severe than those of PHE but may still be significant. Maternal PHE below 120 μmol/L is associated with intrauterine growth retardation [86]. Low essential fatty acid intakes have led to the use of amino acid supplements fortified with DHA. Some centres use tyrosine supplements to maintain maternal TYR within the normal range.

The key to preventing MPKUS is planning, with dietary treatment being established prior to conception. This requires all women with HPA to be educated from an early age with the information repeated regularly thereafter.

16.4 HPA and Disorders of Biopterin Metabolism

Disorders of tetrahydrobiopterin (BH_4) associated with HPA and biogenic amine deficiency include deficiencies of GTP cyclohydrolase I (GTPCH), 6-pyruvoyl-tetrahydropterin synthase (PTPS), dihydropteridine reductase (DHPR) and pterin-4a-carbinolamine dehydratase (PCD) (primapterinuria). Dopa-responsive dystonia (DRD), which is due to a dominant form of GTPCH deficiency, and sepiapterin reductase (SR) deficiency, also lead to CNS amine deficiency but are associated with normal blood PHE (although HPA may occur in DRD after a PHE load); these conditions are not considered further here (► Chap. 30). Consensus guidelines for the diagnosis and treatment of BH_4 deficiencies have recently been published [87].

16.4.1 Clinical Presentation

These conditions can present in one of three ways:

- Asymptomatic, but with raised PHE found following NBS; as part of the standard screening protocol the infant is then investigated further for biopterin defects.
- Symptomatic, with neurological deterioration in infancy despite a low-PHE diet. This will occur where no further investigations are routinely undertaken after a finding of HPA in NBS which is wrongly assumed to be PAH deficiency.
- Symptomatic, with neurological deterioration in infancy on a normal diet. This will occur either where there has been no NBS for HPA or if the PHE concentration is not sufficiently raised to have resulted in a positive screen or to require dietary treatment.

Symptoms may be subtle in the newborn period and not readily apparent until several months of age. Birth weight and birth head circumference may be low in some infants, suggesting intrauterine involvement. All conditions apart from PCD deficiency are associated with abnormal and variable tone, abnormal movements, irritability and lethargy, seizures, poor temperature control, progressive developmental delay and microcephaly. An abnormal EEG and cerebral atrophy can occur in PTPS and in DHPR deficiency and basal ganglia calcification is reported in the latter [88]. There is a mild (peripheral) form of PTPS associated with HPA but without neurotransmitter deficiency, where there are usually no neurological symptoms [89]. In PCD deficiency symptoms are mild and transient.

16.4.2 Metabolic Derangement

Disorders of pterin synthesis or recycling are associated with decreased activity of PAH, tyrosine hydroxylase, tryptophan hydroxylase and nitric oxide synthase (◘ Fig. 16.1). The degree of HPA is highly variable, with blood PHE concentrations ranging from normal to >2000 µmol/L. Central nervous system (CNS) amine deficiency is most often profound and responsible for the clinical abnormalities. Decreased concentration of HVA in cerebrospinal fluid (CSF) is a measure of reduced dopamine turnover, and similarly 5-HIAA deficiency is a measure of reduced serotonin metabolism (see also ► Chap. 30).

16.4.3 Genetics

All disorders are autosomal recessive. Descriptions of the relevant genes and a database of mutations are available at ► http://www.biopku.org/pnddb/home.asp. In most series biopterin disorders account for 1–3% of infants found to have a raised PHE on newborn screening; PTPS deficiency is the most common disorder, followed by DHPR deficiency [88]. PTPS deficiency has a higher frequency in Chinese populations, and a genotype phenotype correlation has been reported [90].

16.4.4 Diagnostic and Confirmatory Tests

Diagnostic protocols and interpretation of results are as follows.

16.4.4.1 Urine or Blood Pterin Analysis and Blood DHPR Assay

All infants found to have HPA on NBS should have blood DHPR and urine or blood pterin analysis. The interpretation of results is shown in ◘ Table 16.2.

16.4.4.2 BH_4 Loading Test

If dietary PHE restriction is in place this is stopped 2–3 days before the test. Blood PHE concentrations should be at least 400 µmol/L at the start. An oral dose of 20 mg BH_4/kg is given approximately 30 min before a feed. Blood samples are collected for PHE and TYR at 0, 4, 8 and 24 h. The test is positive if plasma PHE falls to normal (usually by 8 h) with a concomitant increase in TYR. The rate of fall of PHE may be slower in DHPR deficiency. Blood for pterin analysis at 4 h will confirm that the BH_4 has been taken and absorbed.

A combined PHE (100 mg/kg) and BH_4 (20 mg/kg) loading test may be used as an alternative. This combined

Table 16.2 Interpretation of results of investigations in disorders of biopterin metabolism

Deficiency	Blood PHE µmol/L	Blood or urine biopterin	Blood or urine neopterin	Blood or urine primapterin	CSF 5-HIAA and HVA	Blood DHPR activity	Gene
PAH	>120	↑	↑	–	↓[a]	N	*PAH*
GTPCH	50–1200	↓↓	↓↓	–	↓	N	*GTCH1*
PTPS	240–2500	↓↓	↑↑	–	↓	N	*PTS*
DHPR	180–2500	↓↓	N or ↑	–	↓	↓	*QDPR*
PCD	180–1200	↓	↑	↑↑	N	N	*PCBD1*
DNAJC12	>120	**N**	**N**	–	↓	N	*DNAJC12*

CSF, cerebrospinal fluid; DHPR, dihydropterin reductase; GTPCH, guanosine triphosphate cyclohydrolase I; 5-HIAA, 5-hydroxyindole acetic acids; HVA, homovanillic acid; N, normal; PAH, phenylalanine hydroxylase; PCD, pterin-4a-carbinolamine dehydratase; PHE, phenylalanine; PTPS, 6-pyruvoyl-tetrahydropterin synthase

[a]In PAH deficiency, as long as PHE concentrations remain elevated, there is a secondary inhibition of tyrosine and tryptophan hydroxylases causing depletion in CSF amines

loading test is reported to identify BH_4-responsive PAH deficiency and discriminate between co-factor synthesis or regeneration defects and is useful if pterin analysis is not available [91, 92].

16.4.4.3 CSF Neurotransmitters

The measurement of HVA and 5-HIAA is an essential part of the diagnostic investigation and is also subsequently required to monitor amine replacement therapy with L-dopa and 5HT. CSF must be frozen in liquid nitrogen immediately after collection and stored at –70 °C prior to analysis. If blood stained, the sample should be centrifuged immediately and the supernatant then frozen. The reference ranges for HVA and 5-HIAA are age related [93] (see also ► Chap. 30).

16.4.4.4 Confirmatory Tests

Apart from DHPR measurement in erythrocytes, measurement of enzyme activity is not necessary for the initial diagnosis. Molecular analysis is available for all conditions and is now likely to be the method of choice for confirmation of the diagnosis. Where results can be obtained in an acceptable time frame gene panels or next generation sequencing may be used as an alternative to pterin analysis as a first line investigation in infants with HPA on NBS. Where necessary, for further confirmation DHPR activity can be measured in fibroblasts, PTPS activity in erythrocytes and fibroblasts and GTPCH activity in liver, cytokine-stimulated fibroblasts and stimulated lymphocytes. If PAH deficiency and disorders of biopterin metabolism cannot be confirmed as a cause of HPA, molecular analysis should be undertaken for *DNAJC12* mutations [3].

16.4.4.5 Prenatal Diagnosis

If the mutation of the index case is already known prenatal diagnosis can be undertaken in the first trimester by mutation analysis following chorionic villus biopsy. Analysis of amniotic fluid neopterin and biopterin in the second trimester is available for all conditions. Enzyme analysis can be undertaken in foetal erythrocytes or in amniocytes in both DHPR deficiency and PTPS deficiency. GTPCH is only expressed in foetal liver tissue.

16.4.5 Treatment and Prognosis

For GTPCH deficiency, PTPS deficiency and DHPR deficiency the aim of treatment is to control the HPA and to correct CNS amine deficiency. In DHPR deficiency treatment with folinic acid is necessary to prevent CNS folate deficiency [58], and it may also be required in GTPCH and PTPS deficiency, where a reduction in CSF folate can be a consequence of long-term treatment with L-dopa. PCD deficiency does not usually require treatment, although BH_4 may be used initially if the child is symptomatic.

In PTPS and GPCH deficiency, blood PHE responds to treatment with oral BH_4 (available as sapropterin dihydrochloride). In DHPR deficiency, BH_4 may also be effective in reducing blood PHE, but higher doses may be required than in GTPCH and PTPS deficiency. This, in theory, might lead to an accumulation of BH_2 and inhibition of BH_4 dependent enzymes [94]. Consequently, it has been recommended that in DHPR deficiency HPA should be corrected by dietary means and BH_4 should

not be given. However, a number of patients with DHPR deficiency have been successfully treated with BH_4 and in a single case report, BH_4 up to a dose of 40 mg/kg/day did not cause a further increase in CSF BH_2 [95].

CNS amine replacement therapy is given as oral L-dopa with carbidopa (usually in 1:10 ratio, but also available in 1:4 ratio) and 5HT. Carbidopa is a dopa-decarboxylase inhibitor that reduces the peripheral conversion of L-dopa to dopamine, thus limiting side effects and allowing a reduced dose of L-dopa to be effective. Side effects (nausea, vomiting, diarrhoea, irritability) may also be seen at the start of treatment. For this reason L-dopa and 5HT should initially each be started in a low dose (■ Table 16.3), which is increased gradually to the recommended maintenance dose. Further dose adjustment depends on the results of CSF HVA and 5-HIAA concentrations.

Additional medications, developed primarily for treatment of Parkinson's disease, have been used as an adjunct to therapy, with the aim of reducing the dose and frequency of amine replacement medication and improving residual symptoms and preventing diurnal variation. These include selegiline (L-deprenyl), a monoamine oxidase-B inhibitor [96], entacapone, a catechol-O-methyltransferase (COMT) inhibitor and pramipexole, a dopamine agonist. Pramipexole, in higher doses, has been reported to cause adverse psychiatric effects [97]. For further discussion on the use of medication see [87].

16.4.5.1 Monitoring of Treatment

CSF amine concentrations should be monitored 3-monthly in the 1st year, 6-monthly in early childhood and yearly thereafter. Where possible, CSF should be collected before a dose of medication is given. CSF folate should also be measured.

Hyperprolactinaemia occurs as a consequence of dopamine deficiency and measurement of serum prolactin can be used as a method to monitor treatment, with normal values indicating adequate L-dopa replacement. It has been suggested that 3 blood prolactin measurements over a 6 hour period may be a more sensitive and less invasive marker than the CSF HVA concentration in deciding on dose adjustment [98].

Blood PHE must also be monitored, but this only needs to be undertaken frequently in DHPR deficiency where a low-PHE diet is used.

■ **Table 16.3** Medication used in the treatment of disorders of biopterin metabolism

Drug	Dose (oral)	Frequency	GTPCH	PTPS	PCD	DHPR
BH_4	2–5 mg/kg/day, increasing to 5–10 mg/day according to blood PHE response	Once daily	+	+	±	±[a]
5HT	1–2 mg/kg/day, increasing by 1–2 mg/kg/day every 4–5 days up to maintenance dose of 8–10 mg/kg/day	Give in four divided doses; final maintenance dose dependent on results of CNS neurotransmitters	+	+	–	+
L-Dopa (as combined preparation with carbidopa)	1–2 mg/kg/day, increasing by 1–2 mg/kg/day every 4–5 days up to maintenance dose of 10–12 mg/kg/day	Give in four divided doses; final maintenance dose dependent on results of CNS neurotransmitters	+	+	–	+
Selegiline (L-deprenyl)	0.1–0.25 mg/day	In three or four divided doses (as adjunct to 5HT and L-dopa; see text)	±	±	–	±
Entacapone	15 mg/kg/day	In two or three divided doses	±	±	–	±
Pramipexole	0.006 mg/kg/day increasing to 0.010 mg/kg/day[b]	In two to three divided doses	±	±	–	±
Calcium folinate (folinic acid)	15 mg/day	Once daily	±	±	–	+

BH_4, tetrahydrobiopterin; CNS, central nervous system; DHPR, dihydropterin reductase; GTPCH, guanosine triphosphate cyclohydrolase I; 5HT, 5-hydroxytrytophan; PCD, pterin-4a-carbinolamine dehydratase; PTPS, 6-pyruvoyl-tetrahydropterin synthase

[a]See text

[b]Higher doses (0.030–0.033 mg/kg/day) have been used but may cause psychiatric adverse effects [97]

16.4.5.2 Outcome

Without treatment the natural history of GTPCH, PTPS and DHPR deficiency is poor, with progressive neurological disease and early death. The outcome with treatment depends upon the age at diagnosis and initiation of therapy and the phenotypic severity [88]. Most children with GTPCH deficiency have some degree of learning difficulties despite adequate control. Patients with PTPS deficiency may have a satisfactory cognitive outcome if detected early. Those with DHPR deficiency, if started on diet, amine replacement therapy and folinic acid within the first months of life, can show normal development and growth. Late diagnosis in all these conditions is associated with a much poorer outcome. The outcome of pregnancies in women with biopterin synthesis disorders on treatment appears to be good without worsening of symptoms or other disease specific complications. Foetal outcome was also satisfactory [99].

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Disorders of Tyrosine Metabolism

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Contents

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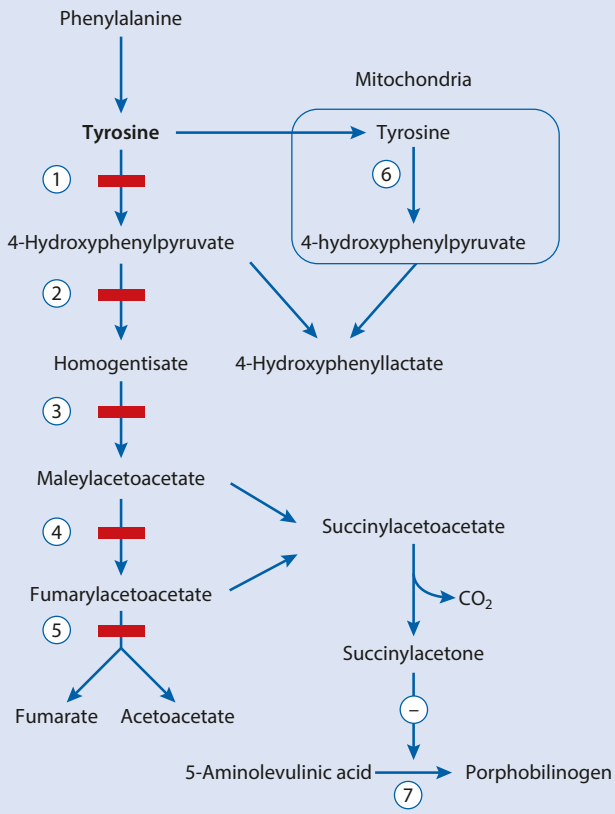
Tyrosine Metabolism

Tyrosine is a non-essential amino acid that is derived from two sources, diet and hydroxylation of phenylalanine (■ Fig. 17.1). Besides forming an integral part of proteins, it is a precursor of DOPA, thyroxine and melanin. Post- translational modifications of tyrosine residues in proteins by phosphorylation and sulfation have important roles in signal transduction and modulation of interaction between proteins. Tyrosine is both glucogenic and ketogenic, since its catabolism, which proceeds predominantly in the liver cytosol, results in the formation of fumarate and acetoacetate. The first step of tyrosine catabolism is conversion into 4-hydroxyphenylpyruvate by cytosolic tyrosine aminotransferase. Transamination of tyrosine can also be accomplished in the liver and in other tissues by mitochondrial aspartate aminotransferase, but this enzyme plays only a minor role under normal conditions. The penultimate intermediates of tyrosine catabolism, maleylacetoacetate and fumarylacetoacetate, are reduced to succinylacetoacetate, followed by decarboxylation to succinylacetone. The latter is the most potent known inhibitor of the heme biosynthetic enzyme, 5-aminolevulinic acid dehydratase (porphobilinogen synthase; ► Fig. 39.1).



■ **Fig. 17.1** The tyrosine catabolic pathway. 1, Tyrosine aminotransferase (deficient in tyrosinaemia type II); 2, 4-hydroxyphenylpyruvate dioxygenase (deficient in tyrosinaemia type III, hawkinsinuria, site of inhibition by NTBC); 3, homogentisate dioxygenase (deficient in alkaptonuria); 4, Maleylacetoacetate isomerase (deficient in maleylacetoacetate isomerase deficiency; 5, fumarylacetoacetase (deficient in tyrosinaemia type I); 6, aspartate aminotransferase; 7, 5-aminolevulinic acid dehydratase (porphobilinogen synthase). Enzyme defects are depicted by solid red bars across the arrows

■■ Introduction

Six inherited disorders of tyrosine metabolism are known (► Tyrosine Metabolism). Hereditary tyrosinaemia type I is characterised by progressive liver disease and renal tubular dysfunction with rickets. Hereditary tyrosinaemia type II (Richner-Hanhart syndrome) presents with keratitis and blistering lesions of the palms and soles and neurological complications. Tyrosinaemia type III may be asymptomatic or associated with mental retardation. Hawkinsinuria may be asymptomatic or present with failure to thrive and metabolic acidosis in infancy. In alkaptonuria, symptoms of osteoarthritis usually appear in adulthood. Maleylacetoacetate isomerase deficiency is associated with asymptomatic mild hypersuccinylacetonaemia. Other inborn errors of tyrosine metabolism include oculocutaneous albinism caused by a deficiency of melanocyte-specific tyrosinase, converting tyrosine into DOPA-quinone; deficiency of tyrosine hydroxylase, the first enzyme in the synthesis of dopamine from tyrosine; and deficiency of aromatic L-amino acid decarboxylase, which also affects tryptophan metabolism. The latter two disorders are covered in ► Chap. 30.

17.1 Hereditary Tyrosinaemia Type I (Hepatorenal Tyrosinaemia): Fumarylacetoacetate Hydrolase Deficiency

17.1.1 Clinical Presentation

The clinical manifestations of tyrosinaemia type I are very variable, and an affected individual can present at any time from the neonatal period to adulthood. There is considerable variability of presentation even between members of the same family.

Clinically, tyrosinaemia type I may be classified based on the age at onset of symptoms, which broadly

correlates with disease severity: an acute form that manifests before 6 months of age (but rarely in the first 2 weeks of life) with acute liver failure; a subacute form presenting between 6 months and 1 year of age with liver disease, failure to thrive, coagulopathy, hepatosplenomegaly, rickets and hypotonia; and a more chronic form that presents after the first year with chronic liver disease, renal disease, rickets, cardiomyopathy and/or a porphyria-like syndrome. Treatment of tyrosinaemia type I with nitisinone in the last 25 years (► Sect. 17.1.5) has dramatically altered its natural history.

Hepatic Disease

The liver is the major organ affected in tyrosinaemia type I, and its involvement is a major cause of morbidity and mortality. Liver disease can manifest as acute hepatic failure, cirrhosis or hepatocellular carcinoma; all three conditions may occur in the same patient. The more severe forms of tyrosinaemia type I present in infancy with vomiting, diarrhoea, bleeding diathesis, hepatomegaly, mild jaundice, hypoglycaemia, oedema and ascites. Typically, liver synthetic function is most affected and, in particular, coagulation is markedly abnormal compared with other tests of liver function. Sepsis is common, and early hypophosphataemic bone disease may be present secondary to renal tubular dysfunction. Acute liver failure may be the presenting feature or may occur subsequently, precipitated by intercurrent illnesses, as hepatic crises which are associated with hepatomegaly and coagulopathy. Mortality is high in untreated patients [1].

Chronic liver disease leading to cirrhosis eventually occurs in most individuals with tyrosinaemia type I – both as a late complication in survivors of early-onset disease and as a presenting feature of the later-onset forms. The cirrhosis is usually a mixed micro and macronodular type with a variable degree of steatosis. Hepatocyte dysplasia is common, with a high risk of malignant transformation [1, 2]. Unfortunately, the heterogeneity of the nodules make it difficult to detect malignant changes at an early stage (► Sect. 17.1.5).

Renal Disease

A variable degree of renal dysfunction is detectable in most patients at presentation, ranging from mild tubular dysfunction to renal failure. Proximal tubular disease is very common and may deteriorate during hepatic crises. Hypophosphataemic rickets is the most common manifestation of proximal tubulopathy, but generalised aminoaciduria, renal tubular acidosis and glycosuria may also be present [3]. Prior to the nitisinone era 40% developed nephrocalcinosis [4]. Rare renal manifestations include distal renal tubular disease and renal impairment.

Neurological Crises

Acute neurological porphyria like crises can occur at any age. Typically, the crises follow a minor infection associated with anorexia and vomiting, and occur in two phases: an active period lasting 1–7 days characterised by progressive ascending polyneuropathy, painful paresthesias and autonomic signs that may progress to paralysis, followed by a recovery phase over several days to months [5]. Complications include seizures, extreme hyperextension, self-mutilation, respiratory paralysis and death. These neurological crises are generally seen after discontinuation of Nitisinone treatment.

Neurodevelopmental

It has recently been recognized that many patients with tyrosinaemia type I have a spectrum of significant learning difficulties; these include lowered IQ and school performance, limited attention span and impaired executive function [6, 7]. The aetiology of these cognitive deficits is uncertain; whether they are related to nitisinone treatment, high tyrosine levels, low phenylalanine levels, a complication of liver failure, or are an intrinsic feature of tyrosinaemia type I per se, is currently unknown.

Other Manifestations

Cardiomyopathy is an occasional incidental finding but may be clinically significant [8]. Pancreatic cell hypertrophy may result in clinically significant hyperinsulinism [9].

17.1.2 Metabolic Derangement

Tyrosinaemia type 1 is caused by a deficiency of the enzyme fumarylacetoacetate hydrolase (FAH), (◘ Fig. 17.1, enzyme 5) which is mainly expressed in the liver and kidney. The compounds immediately upstream from the FAH reaction, maleylacetoacetate (MAA) and fumarylacetoacetate (FAA), and their derivatives, succinylacetone (SA) and succinylacetoacetate (SAA) accumulate and have important pathogenic effects. The effects of FAA and MAA occur only in the cells of the organs in which they are produced; these compounds are not found in body fluids of patients. On the other hand, their derivatives, SA and SAA are readily detectable in plasma and urine and have widespread effects.

FAA, MAA and SA disrupt sulfhydryl metabolism by forming glutathione adducts, thereby rendering cells susceptible to free radical damage [10]. Disruption of sulfhydryl metabolism is also believed to cause secondary deficiency of two other hepatic enzymes, 4-hydroxyphenylpyruvate dioxygenase and methionine adenosyltransferase, resulting in hypertyrosinemia and hypermethioninemia. Additionally, FAA and MAA are

alkylating agents and can disrupt the metabolism of thiols, amines, DNA and other important intracellular molecules including inhibition of base excision repair by FAA, suggesting a mechanism for carcinogenesis in tyrosinaema type I [11]. As a result of these widespread effects on intracellular metabolism, hepatic and renal cells exposed to high levels of these compounds undergo either apoptotic cell death or a significant alteration of gene expression [12, 13]. In patients who have developed cirrhosis, self-induced correction of the genetic defect and the enzyme abnormality occurs within some nodules. The clinical expression of hepatic disease may correlate inversely with the extent of mutation reversion in regenerating nodules [14].

SA is a potent inhibitor of the enzyme 5-aminolevulinic acid (5-ALA) dehydratase (block 7, ◘ Fig. 17.1). 5-ALA, a neurotoxic compound, accumulates and is excreted at high levels in patients with tyrosinemia type I and is believed to cause the acute neurological crises seen during decompensation [5]. SA is also known to disrupt renal tubular function, heme synthesis and immune function [15–17].

▪ Newborn Screening

There is strong clinical evidence to support newborn screening for tyrosinemia type I, as the detection and treatment of patients in early life results in a dramatically better outcome than when treatment is initiated late [18, 19]. Screening using tyrosine levels alone has been used in the past and has resulted in very high false-positive and false-negative rates [20]. SA is a highly sensitive and specific marker for tyrosinaemia type I, and assays based on the inhibitory effects of SA on 5-ALA dehydratase, either alone or in combination with tyrosine levels, have greatly improved diagnostic accuracy [20]. Screening methods based on the direct measurement of SA in dried blood spots by tandem mass spectrometry have also demonstrated excellent test accuracy; several laboratory-based methods have been described and commercial kit-based assays are available, facilitating the routine inclusion of tyrosinaemia type I in many newborn screening programmes [21]. Maleylacetoacetate isomerase deficiency (▶ Sect. 17.2) can cause mild hypersuccinylacetonaemia and may be detected on newborn screening using SA as the primary biomarker, depending on the limits of detection used in individual screening laboratories. This condition is believed to be asymptomatic and SA levels are orders of magnitude lower than those seen in Tyrosinemia type 1.

▪ Prenatal Diagnosis

The description of the geographical and ethnic distribution of causative mutations in many populations worldwide has enabled improved carrier detection, prenatal diagnosis and pre-implantation diagnosis [22]. Antenatal diagnosis is best performed by mutation analysis on chorionic villus sampling (CVS) or amniocytes. Alternative methods include FAH assay on CVS or amniocytes and determination of SA levels in amniotic fluid. However, FAH is expressed at low levels in chorionic tissue and interpretation of results may be difficult. Assay for elevated SA levels in amniotic fluid is very reliable and can be performed as early as 12 weeks; however, in occasional affected pregnancies normal SA amniotic fluid levels have been reported [23]. When mutation analysis is not available for prenatal diagnosis, we recommend a strategy combining initial screening for the common pseudodeficiency mutation and FAH assay on CVS at 10 weeks; in the case of low FAH activity revealed by CVS, amniocentesis for amniotic fluid SA levels is subsequently performed at 11–12 weeks for confirmation.

17.1.3 Genetics

Tyrosinaemia type I is inherited as an autosomal recessive trait. Almost 100 mutations have been reported in *FAH* [24]. The most common mutation, I c.1062 + 5G > A, is found in about 25% of the alleles worldwide and is the predominant mutation in the French-Canadian population, in which it accounts for >90% of alleles. Another mutation, c.554-1G > T, is found in around 60% of alleles in patients from the Mediterranean area. Other *FAH* mutations are common within certain ethnic groups: W262X in Finns, D233V in Turks, and Q64H in Pakistanis. There is no clear genotype-phenotype correlation; spontaneous correction of the mutation within regenerative nodules may influence the clinical phenotype [14]. A novel mutation c.103G > A was found in a patient with a mild phenotype who did not excrete succinylacetone and was successfully treated with diet alone [25]. A pseudodeficiency mutation, R341W, has been reported in healthy individuals who have in vitro FAH activity indistinguishable from that in patients with tyrosinaemia type I [26]. The frequency of this mutation in various populations is unknown, but it has been found in many different ethnic groups.

17.1.4 Diagnostic Tests

In symptomatic patients, biochemical tests of liver function are usually abnormal. In particular, liver synthetic function is severely affected – coagulopathy and/or hypoalbuminaemia are often present even if other tests of liver function are normal. In most acutely ill patients, α-fetoprotein levels are greatly elevated. A Fanconi-type

tubulopathy is often present with aminoaciduria, phosphaturia and glycosuria, and radiological evidence of rickets may be present.

Significantly elevated levels of succinylacetone in dried blood spots, plasma or urine are pathognomonic of tyrosinaemia type I. Mildly elevated levels (<1 μmol/l) may be seen in maleylacetoacetate isomerase deficiency or where a pathogenic mutation in FAH is combined with the R341W pseudodeficiency variant. These levels are at least ten fold lower than seen in tyrosinaemia type 1. In both scenarios there is no evidence of liver dysfunction or coagulopathy and no intervention is needed [27]. Also, very rarely, succinylacetone elevation may be undetectable in mild cases [25]. Other metabolite abnormalities that are suggestive of the diagnosis include elevated plasma levels of tyrosine, phenylalanine and methionine, reduced erythrocyte 5-ALA dehydratase activity and increased urinary 5-ALA excretion.

Confirmation of the diagnosis is usually by mutation analysis. Failing this, FAH assays may be performed on liver biopsy, fibroblasts, lymphocytes or dried blood spots. Falsely elevated enzyme results may be obtained on liver biopsy if a reverted nodule is inadvertently assayed. Enzyme assay results should therefore be interpreted in the context of the patient's clinical and biochemical findings.

17.1.5 Treatment and Prognosis

Nitisinone, also known as NTBC (2-[2-nitro-4-(trifluoromethyl)benzoyl]cyclohexane-1,3-dione), has revolutionised the treatment of type I tyrosinaemia and is now the recommended therapy, in combination with a tyrosine- and phenylalanine-restricted diet [19, 28, 29].

▪ Nitisinone and Dietary Treatment

The rationale for the use of nitisinone is to block tyrosine degradation at an early step so as to prevent the production of toxic downstream metabolites such as FAA, MAA and SA; the levels of tyrosine, 4-hydroxyphenylpyruvate and 4-hydroxyphenylpyruvate concomitantly increase (◘ Fig. 17.1). Nitisinone acts within hours of administration and has a long half-life of about 54 hours [30]. In patients presenting acutely with hepatic decompensation, rapid clinical improvement occurs in over 90%, with improvement of prothrombin time within days of starting treatment. Other biochemical parameters of liver function may take longer to normalise: α-fetoprotein concentrations should show a logarithmic fall but may not normalise for up to several months after the start of treatment. Nitisinone is recommended in an initial dose of 2 mg/kg body weight per day in liver failure or 1 mg/kg/day otherwise [28]. Individual dose adjustment is subsequently based on the biochemical response and the aim is to maintain a plasma nitisinone concentration of >50 μmol/l or a whole blood concentration of 20–40 μmol/l.

Dietary restriction of phenylalanine and tyrosine is necessary to prevent the known adverse effects of hypertyrosinaemia (▶ Sect. 17.2). We currently aim to maintain tyrosine levels between 200 and 400 μmol/l with a phenylalanine level of >30 μmol/l using a combination of a protein-restricted diet and phenylalanine- and tyrosine free amino acid mixtures. Occasionally specific phenylalanine supplementation is necessary.

A small proportion of acutely presenting patients (<10%) do not respond to nitisinone treatment; in these patients, coagulopathy and jaundice progress and mortality is very high without urgent liver transplantation. If encephalopathy or significant jaundice develops or if prothrombin time does not improve within 1 week, urgent liver transplantation should be considered.

Adverse events of nitisinone therapy have been few. Transient thrombocytopenia and neutropenia and transient eye symptoms (burning/photophobia/corneal erosion/corneal clouding) have been reported in a small proportion of patients [28]. The short to medium term prognosis in responders appears to be excellent. Hepatic and neurological decompensations are not known to occur on nitisinone treatment, and progression of chronic liver disease is rare. Renal tubular dysfunction responds quickly, and tubular function usually normalises within the first year of treatment, unless nephrocalcinosis is already established. Neurological crises have never been reported in patients compliant with nitisinone.

The risk of hepatocellular carcinoma (HCC) appears to be related to the age nitisinone is commenced. HCC has not been reported in those treated in the first month of life, with the relative risk increasing with age at treatment [18, 19]. Where nitisinone is introduced after 2 years of age, the risk is similar to that in historical controls (◘ Table 17.1) [29]. Long-term vigilance is however necessary in all patients as the lifelong risk of HCC is still unknown.

Monitoring of patients on nitisinone treatment should include regular blood tests for liver and renal function, blood counts, clotting until these normalize, when biannually will suffice. Metabolic monitoring can be limited to nitisinone levels and plasma SA which can be measured in bloodspots. Blood levels of phenylalanine and tyrosine should be frequently monitored and the diet supervised closely. While tyrosine levels are stable there appears to be considerable diurnal variation in phenylalanine levels and sampling should be done at a consistent time of the day. Monitoring for HCC consists of α-fetoprotein checked every 3 months, in combination with hepatic imaging by ultrasound every 6 months and by MRI annually.

Table 17.1 Risk of hepatocellular carcinoma (HCC) in tyrosinaemia type I

	Reference	Age at start of treatment with NTBC	Number of patients	Patient age (in years) at assessment	Patients developing HCC (%)
Pre-NTBC	[2]	n/a	43	>2	16 (37%)
	[1]	n/a	55	2–12	10 (18%)
Post-NTBC	[29]	<6 months	180	2–13	1 (0.6%)
		6–12 months	61	2–12	1 (1.6%)
		1–2 years	44	2–12	3 (7%)
		2–7 years	65	2–19	14 (21%)
		>7 years	26	7–31	9 (35%)

HCC hepatocellular carcinoma, *n/a* not applicable, *NTBC* 2-[2-nitro-4-(trifluoromethyl)benzoyl]cyclohexane-1,3-dione, or nitisinone

Liver Transplantation

Liver transplantation provides a functional cure of tyrosinaemia type I and allows a normal unrestricted diet [31]. However, even in optimal circumstances, it is associated with approximately 5-10% mortality and necessitates lifelong immunosuppressive therapy. Therefore, at present liver transplantation in tyrosinaemia type I is restricted to patients with acute liver failure who fail to respond to nitisinone therapy, patients with proven or suspected HCC or where nitisinone is unavailable.

The long-term impact of liver transplantation on renal disease in patients with tyrosinaemia type I relates to the era in which they were treated. Prior to nitisinone, all patients had tubular dysfunction and some had glomerular dysfunction before receiving transplants. In this group tubular function improved in most patients but they had higher rates of glomerular dysfunction owing to nephrotoxic immunotherapy [31, 32]. Patients pretreated with nitisinone usually have normal renal function at transplant, and this combined with the modern immunosuppression regimens ensures they have a much improved renal prognosis [33]. After transplantation, when nitisinone is discontinued, renal production results in significantly elevated plasma and urinary SA levels. The functional significance of these findings is unclear, but does not seem to be associated with renal dysfunction or malignancy. At present nitisinone treatment, which would probably necessitate reintroduction of dietary restriction, is not indicated.

Supportive Treatment

In the acutely ill patient supportive treatment is essential. Clotting factors, albumin, electrolytes and acid/base balance should be closely monitored and corrected as necessary. Tyrosine and phenylalanine intake should be kept to a minimum during acute decompensation. Vitamin D is necessary to treat rickets. Infections should be treated aggressively.

Pregnancy

A few pregnancies in patients on nitisinone treatment have been reported with encouraging outcomes and where pregnancy occurs Nitisinone should be continued [34, 35]. Pregnancy is a realistic expectation for the majority of women who have had liver transplantation for any indication. Although close monitoring is required the outcome is excellent for both mother and infant. In our experience, a number of women have had successful pregnancies after liver transplant for tyrosinaemia type I.

17.2 Maleylacetoacetate Isomerase Deficiency (Mild Hypersuccinylacetonaemia, MHSA)

17.2.1 Clinical Presentation

All six cases of MHSA described to date [27] have been asymptomatic up to 13 years of age. All were detected incidentally during the course of newborn screening for Tyrosinemia type 1 in Quebec using succinylacetone as the primary biomarker. No abnormalities in coagulation, liver function, AFP, and plasma amino acids were detected on follow up.

17.2.2 Metabolic Derangement and Genetics

Mildly elevated succinylacetone levels are caused by deficiency of the enzyme maleylacetoacetate isomerase (Fig. 17.1, enzyme 4). Five of the six reported patients

have been found to have pathogenic biallelic mutations in *GSZT1* which encodes for the enzyme. One patient, with the lowest SA levels at diagnosis, was found to have only one sequence variant, and it is speculated that certain single deleterious mutations may also cause MHSA; alternatively, the biochemical phenotype may be determined by alternative pathways of maleylacetoacetate metabolism [27].

17.2.3 Diagnostic Tests

In the Quebec MHSA cohort diagnosed on newborn screening, plasma SA levels were elevated to around tenfold the upper limit of normal. Coagulation studies (INR and Prothrombin time), AFP, and plasma tyrosine levels were normal. In contrast, all cases of Tyrosinemia type 1 were associated with SA levels 1000-fold the upper limit of normal, along with abnormal coagulation studies, AFP, and plasma tyrosine levels at presentation. SA levels in maleylacetoacetate isomerase deficiency were found to decrease over time, but remained above the reference range. The enzyme deficiency has been demonstrated in bacterial mutation expression assays, and direct liver enzyme assay has not yet been reported.

17.2.4 Treatment and Prognosis

Theoretically, treatment with Nitisinone plus dietary restriction would be expected to be effective. The parents of the six reported patients were offered this option, but all instead chose non-treatment alongside a surveillance programme with regular monitoring of liver and kidney function through clinical, biochemical, and imaging techniques [27]. All parameters remained normal without any treatment for up to 13 years, with a progressive decline in plasma and urine SA levels to just above the normal range.

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17.3 Hereditary Tyrosinaemia Type II (Oculocutaneous Tyrosinaemia, Richner-Hanhart Syndrome): Hepatic Cytosolic Tyrosine Aminotransferase Deficiency

17.3.1 Clinical Presentation

The disorder is characterised by ocular lesions (about 75% of the cases), skin lesions (80%), or neurological complications (60%), or by any combination of these [36]. The disorder usually presents in infancy but can become manifest at any age.

Eye symptoms are often the presenting problem and may start in the first months of life with photophobia, lacrimation and intense burning pain. The conjunctivae are inflamed and on slit-lamp examination herpetic-like corneal ulcerations are found. The lesions stain poorly with fluorescein. In contrast with herpetic ulcers, which are usually unilateral, the lesions in tyrosinaemia type II are bilateral. Neovascularisation may be prominent. Untreated, serious damage may occur with corneal scarring, visual impairment, nystagmus and glaucoma.

Skin lesions specifically affect pressure areas and most commonly occur on the palms and soles. They begin as blisters or erosions with crusts and progress to painful, nonpruritic hyperkeratotic plaques with an erythematous rim, typically ranging in diameter from 2 mm to 3 cm. Clinically, tyrosinemia II has to be differentiated from other severe forms of palmoplantar keratoderma such as Olmsted syndrome [37].

Neurological complications are highly variable: some patients are developmentally normal, whilst others have variable degrees of developmental retardation. More severe neurological problems, including microcephaly, seizures, self-mutilation and behavioural difficulties, have also been described [38].

It should be noted that the diagnosis of tyrosinaemia type II has only been confirmed by enzymatic and/or molecular genetic analysis in a minority of the early described cases and it is possible that some of these patients have actually had tyrosinaemia type III.

17.3.2 Metabolic Derangement

Tyrosinaemia type II is due to a defect of hepatic cytosolic tyrosine aminotransferase (◘ Fig. 17.1, enzyme 1). As a result of the metabolic block, tyrosine concentrations in serum and cerebrospinal fluid are markedly elevated. The accompanying increased production of the phenolic acids 4-hydroxyphenylpyruvate, 4-hydroxyphenyllactate and 4-hydroxyphenylacetate (not shown in ◘ Fig. 17.1) may be a consequence of direct deamination of tyrosine in the kidneys, or of tyrosine catabolism by mitochondrial aminotransferase (◘ Fig. 17.1). Corneal damage is thought to be related to crystallisation of tyrosine in the corneal epithelial cells, which results in disruption of cell function and induces an inflammatory response. Tyrosine crystals have not been observed in the skin lesions. It has been suggested that excessive intracellular tyrosine enhances cross-links between aggregated tonofilaments and modulates the number and stability of microtubules [39]. As the skin lesions occur on pressure areas, it is likely that mechanical factors also play a role. Studies on a

rat model of Tyrosinemia type II have suggested that hypertyrosinemia-induced disruption of energy metabolism and oxidative stress may underlie the neurological complications [40].

17.3.3 Genetics

Tyrosinaemia type II is inherited as an autosomal recessive trait due to mutations in *TAT*. Several different mutations have so far been reported [24]. Prenatal diagnosis using mutation analysis on chorionic villus sampling has been reported.

17.3.4 Diagnostic Tests

Plasma tyrosine concentrations are usually above 1200 µmol/l. When the tyrosinaemia is less pronounced a diagnosis of tyrosinaemia type III should be considered (▶ Sect. 17.4). Urinary excretion of the phenolic acids 4-hydroxyphenylpyruvate, 4-hydroxyphenyllactate and 4-hydroxyphenylacetate is highly elevated, and *N*-acetyltyrosine and 4-tyramine are also increased. The diagnosis can be confirmed by mutation analysis. Patients diagnosed using tyrosine levels as part of expanded neonatal screening programmes have been reported. In a neonatally diagnosed patient early detection by screening facilitated presymptomatic treatment and identification of an affected 8-year old sibling who suffered with plantar hyperkeratosis [41].

17.3.5 Treatment and Prognosis

Treatment consists in a phenylalanine- and tyrosine-restricted diet, and the skin and eye symptoms resolve within weeks of treatment [42]. Generally, skin and eye symptoms do not occur at tyrosine levels <800 µmol/l; however, as hypertyrosinaemia may be involved in the pathogenesis of the neurodevelopmental symptoms, it may be beneficial to maintain much lower levels [41]. We currently aim to maintain plasma tyrosine levels of 200–500 µmol/l using a combination of a protein-restricted diet and a phenylalanine- and tyrosine-free amino acid mixture. Growth and nutritional status should be regularly monitored.

■ Pregnancy

There have been several reports of pregnancies in patients with tyrosinaemia type II: some have suggested that untreated hypertyrosinaemia may result in fetal neurological abnormalities, such as microcephaly, seizures and mental retardation; however, other untreated pregnancies have been followed by normal fetal outcome [42, 43], although these have only been associated with mild hypertyrosinaemia. In view of the uncertainty regarding possible fetal effects of maternal hypertyrosinaemia, dietary control of maternal tyrosine levels during pregnancy is recommended. In one pregnancy [44] treated with a low-protein diet to maintain plasma tyrosine levels of 100–200 µmol/l and phenylalanine levels of 200–400 µmol/l, a normal fetal and maternal outcome was reported.

17.4 Hereditary Tyrosinaemia Type III: 4-hydroxyphenylpyruvate Dioxygenase Deficiency

17.4.1 Clinical Presentation

Only a few cases of tyrosinaemia type III have been described, and the full clinical spectrum of this disorder is not completely known [45]. Many of the patients have presented with neurological symptoms, including intellectual impairment, ataxia, increased tendon reflexes, tremors, microcephaly and seizures; some have been detected by the finding of a high tyrosine concentration on neonatal screening. The most common long-term complication has been intellectual impairment, found in 75% of the reported cases. None have developed signs of liver disease. Eye and skin lesions have not been reported so far, but as oculocutaneous symptoms are known to occur in association with hypertyrosinaemia it is reasonable to be aware of this possibility.

17.4.2 Metabolic Derangement

Tyrosinaemia type III is due to deficiency of 4-hydroxyphenylpyruvate dioxygenase (HPD) (◘ Fig. 17.1, enzyme 2), which is expressed in liver and kidney. As a result of the enzyme block there is an increased plasma tyrosine concentration and increased excretion in urine of 4-hydroxyphenylpyruvate and its derivatives 4-hydroxyphenyllactate and 4-hydroxyphenylacetate. The aetiology of the neurological symptoms is not known, but they may be related to hypertyrosinaemia, as in tyrosinaemia types I and II.

17.4.3 Genetics

Tyrosinaemia type III, which follows an autosomal recessive inheritance, is due to mutations in *HPD*; several mutations associated with tyrosinaemia III have

been described [24]. There is no apparent genotype-phenotype correlation [44].

17.4.4 Diagnostic Tests

Elevated plasma tyrosine levels of 300–1300 μmol/l have been found at diagnosis. Elevated urinary excretion of 4-hydroxyphenylpyruvate, 4-hydroxyphenyllactate and 4-hydroxyphenylacetate usually accompanies the increased plasma tyrosine concentration. Diagnosis can be confirmed by enzyme assay in liver or kidney biopsy specimens or by mutation analysis.

17.4.5 Treatment and Prognosis

At present, tyrosinaemia type III appears to be associated with intellectual impairment in some cases, but not in others. It is unknown whether lowering plasma tyrosine levels will alter the natural history. Amongst the patients described, the cases detected by neonatal screening and treated early appear to have fewer neurological abnormalities than those diagnosed on the basis of neurological symptoms [45]; Until there is a greater understanding of the aetiology of the neurological complications, it is reasonable to treat patients with a diet that is low in phenylalanine and tyrosine, at least in early childhood. We currently recommend maintaining plasma tyrosine levels between 200 and 500 μmol/l. After infancy, many patients appear to be able to maintain these levels without dietary restriction or supplementation. No pregnancy data is available to date.

17.5 Transient Tyrosinaemia

Transient tyrosinaemia is one of the most common amino acid disorders, and is believed to be caused by late fetal maturation of 4-hydroxyphenylpyruvate dioxygenase (◘ Fig. 17.1, enzyme 2). It is more common in premature infants than in full-term newborns. The level of protein intake is an important aetiological factor: the incidence of transient tyrosinaemia has fallen dramatically in the last 4 decades, with a concomitant reduction in the protein content of newborn formula milks. Transient tyrosinaemia is clinically asymptomatic. Tyrosine levels are extremely variable and can exceed 2000 μmol/l. Hypertyrosinaemia usually resolves spontaneously by 4–6 weeks; protein restriction to less than 2 g/kg/day, with or without vitamin C supplementation, results in more rapid resolution in most cases. Although the disorder is generally considered benign, some reports have suggested that it may be associated with mild intellectual deficits in the long term [46, 47]. However, large systematic studies have not been performed.

The liver plays a central role in the metabolism of many amino acids, especially tyrosine, phenylalanine and methionine, and plasma levels of these and other amino acids are nonspecifically elevated in liver disease. In the context of newborn screening, elevated plasma tyrosine levels can occur secondary to neonatal liver disease; phenylalanine and methionine levels may also be elevated. Urgent investigations to evaluate liver function and to exclude treatable metabolic disorders such as galactosaemia and tyrosinaemia type I may be indicated in this situation.

17.6 Alkaptonuria: Homogentisate Dioxygenase Deficiency

17.6.1 Clinical Presentation

Some cases of alkaptonuria are diagnosed in infancy due to darkening of urine when exposed to air. However, clinical symptoms first appear in adulthood. The most prominent symptoms relate to joint and connective tissue involvement; significant cardiac disease and urolithiasis may be detected in the later years [48].

The pattern of joint involvement resembles that of osteoarthritis. In general, joint disease tends to be worse in males than in females. The presenting symptom is usually either limitation of movement of a large joint or low back pain starting in the third or fourth decade. Spinal involvement is progressive and may result in kyphosis, limited spine movements and height reduction. On X-ray examination, narrowing of the disc spaces, calcification and vertebral fusion may be evident. In addition to the spine, the large weight-bearing joints such as the hips, knees and ankles are usually involved. Radiological abnormalities may range from mild narrowing of the joint space to destruction and calcification. Synovitis, ligament tears and joint effusions have also been described. The small joints of the hands and feet tend to be spared. Muscle and tendon involvement is common: thickened Achilles tendons may be palpable, and tendons and muscles may be susceptible to rupture with trivial trauma. The clinical course is characterised by episodes of acute exacerbation and progressive joint disability; joint replacement for chronic pain may be required. Alkaptonuria is also associated with osteopenia, osteoporosis, and a risk of pathological fractures [49]. Physical disability increases with age and may become very severe by the sixth decade.

A greyish discolouration (ochre on microscopic examination, thus the name ochronosis) of the sclera

and the ear cartilages usually appears after 30 years of age. Subsequently, dark colouration of the skin, particularly over the nose and cheeks and in the axillary and pubic areas, may become evident. Cardiac involvement probably occurs in most patients eventually; aortic or mitral valve calcification or regurgitation and coronary artery calcification is evident on CT scan and echocardiography in about 50% of patients by the sixth decade. A high frequency of renal and prostatic stones has also been reported. Alkaptonuria is associated with secondary amyloidosis in many tissues [48].

17.6.2 Metabolic Derangement

Alkaptonuria was the first disease to be interpreted as an inborn error of metabolism in 1902 by Garrod [48]. It is caused by a defect of the enzyme homogentisate dioxygenase (◘ Fig. 17.1, enzyme 3), which is expressed mainly in the liver and the kidneys. There is accumulation of homogentisate and its oxidised derivative benzoquinone acetic acid, the precursor to the dark pigment, which is deposited in various tissues. Damage to articular cartilages and connective tissues is believed to be caused by the direct and indirect toxic effects of the binding of a homogentisate-derived melanin-like polymer, which results in protein oxidation and oxidative stress [50, 51]. Additionally, increased osteoclastic activity may also contribute to osteoarthropathy [52].

17.6.3 Genetics

Alkaptonuria is an autosomal recessive disorder. Over 200 mutations have been identified in the gene for homogentisate dioxygenase (*HGD*) most of them private missense mutations [50]. There is no apparent correlation between the genotype, biochemical findings, and clinical manifestations [24, 50]. The estimated incidence is between 1:250,000 and 1:1,000,000 live births.

17.6.4 Diagnostic Tests

Alkalinisation of the urine from alkaptonuric patients results in immediate dark brown colouration of the urine. Excessive urinary homogentisate also results in a positive test for reducing substances. Gas chromatography-mass spectrometry (GC-MS)-based organic acid screening methods can specifically identify and quantify homogentisic acid. Homogentisate may also be quantified by HPLC and by specific enzymatic methods. Genetic testing is confirmatory and widely available.

17.6.5 Treatment and Prognosis

There is no curative treatment for the musculoskeletal and cardiovascular problems associated with Alkaptonuria, and supportive care is the mainstay of management. Nitisinone is an inhibitor of 4-hydroxyphenylpyruvate dioxygenase, the enzyme that catalyses the immediate upstream reaction and has been shown to dramatically reduce plasma and urine homogentisic acid levels in adult patients, but without significant clinical benefit over a 3-year period [53]. However, a more recent clinical follow up study of adults given a daily dose of 2 mg nitisinone has suggested slowing of the progression of the disease over 3 years [54]. The main side effect of nitisinone treatment is hypertyrosinemia, which may lead to keratopathy, skin lesions, and adverse neuropsychological effects, and a phenylalanine and tyrosine restricted diet is recommended to prevent these complications [54, 55]. Very low dose nitisinone (0.2 mg/day) with mild protein restriction is reported to be equally effective in reducing homogentisic acid levels but without increasing tyrosine levels to >500 micromol/L, but the long term clinical efficacy of this approach is not yet known [56].

One patient has been treated with low dose nitisinone (0.2–0.5 mg/day) in conjunction with a low protein diet through pregnancy, without any adverse fetal effects [56].

17.7 Hawkinsinuria

17.7.1 Clinical Presentation

This rare condition, which has only been described in a few families [57], is characterised by failure to thrive and metabolic acidosis in infancy. After the first year of life the condition appears to be asymptomatic. Early weaning from breastfeeding seems to precipitate the disease; the condition may be asymptomatic in breastfed infants.

17.7.2 Metabolic Derangement

The abnormal metabolites produced in hawkinsinuria (hawkinsin (2-cysteinyl-1,4-dihydroxycyclohexenylacetate) and 4-hydroxycycloxylacetate) are thought to derive from incomplete conversion of 4-hydroxyphenylpyruvate to homogentisate caused by a defect in 4-hydroxyphenylpyruvate dioxygenase (HPD; ◘ Fig. 17.1, enzyme 2). Hawkinsin is thought to be the product of a reaction of an epoxide intermediate with glutathione, which may be depleted. The metabolic

acidosis is believed to be due to pyroglutamic acid accumulation secondary to glutathione depletion.

17.7.3 Genetics

Hawkinsinuria is a condition allelic to tyrosinaemia type III, and a single dominant mutation *HPD*, c.772A > G (Asn241Ser) has been reported in affected patients [57]. Using bioinformatic analysis of protein structure the authors concluded that hawkinsinuria is caused by mutations associated with the retention of partial HPD function and which leads to the production of hawkinsin and 4-hydroxycyclohexylacetate.

17.7.4 Diagnostic Tests

Identification of urinary hawkinsin or 4-hydroxycyclohexylacetate by GC-MS is diagnostic [57]. Hawkinsin is a ninhydrin-positive compound, which appears between urea and threonine in ion-exchange chromatography of urine amino acids. Increased excretion of 4-hydroxycyclohexylacetate is detected on urine organic acids analysis. In addition to hawkinsinuria there may be moderate tyrosinaemia, increased urinary 4-hydroxyphenylpyruvate and 4-hydroxyphenyllactate, metabolic acidosis and 5-oxoprolinuria during infancy. Mutation analysis of *HPD* is confirmatory.

17.7.5 Treatment and Prognosis

Symptoms in infancy respond to a return to breastfeeding or a diet restricted in tyrosine and phenylalanine along with vitamin C supplementation. N-Acetylcysteine has also been reported to be effective [58]. The condition is asymptomatic after the first year of life, and affected infants are reported to have developed normally.

17

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Branched-Chain Organic Acidurias/Acidaemias

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Contents

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Catabolism of Branched-Chain Amino Acids

The three essential branched-chain amino acids (BCAAs), leucine, isoleucine and valine, are initially catabolised by a common pathway (Fig. 18.1). The first reaction, which occurs primarily in muscle, involves reversible transamination to 2-oxo- (or keto) acids and is followed by oxidative decarboxylation to coenzyme A (CoA) derivatives by branched-chain oxo- (or keto) acid dehydrogenase (BCKD). The latter enzyme is similar in structure to pyruvate dehydrogenase (▶ Chap. 11, ▶ Fig. 11.2). Subsequently, the degradative pathways of BCAAs diverge. Leucine is catabolised to acetoacetate and acetyl-CoA, which enters the Krebs cycle. The final step in the catabolism of isoleucine involves cleavage into acetyl-CoA and propionyl-CoA, which also enters the Krebs cycle via conversion into succinyl-CoA. Valine is also ultimately metabolised to propionyl-CoA. Methionine, threonine, fatty acids with an odd number of carbons, the side chain of cholesterol, and bacterial gut activity also contribute to the formation of propionyl-CoA.

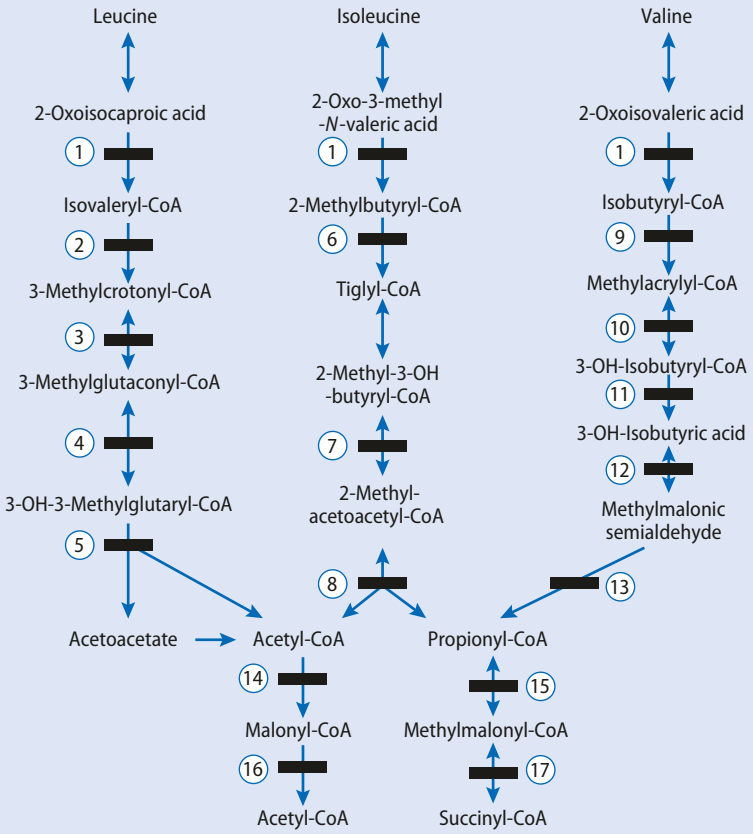

Fig. 18.1 Pathways of branched-chain amino acid catabolism. *1*, Branched-chain 2-ketoacid dehydrogenase complex; *2*, isovaleryl-coenzyme A (CoA) dehydrogenase; *3*, 3-methylcrotonyl-CoA carboxylase; *4*, 3-methylglutaconyl-CoA hydratase; *5*, 3-hydroxy-3-methylglutaryl-CoA lyase; *6*, short/branched chain acyl-CoA dehydrogenase deficiency; *7*, 2-methyl-3-hydroxybutyryl-CoA dehydrogenase, MHBD (HSD10) ; *8*, 2-methylacetoacetyl-CoA thiolase; *9*, isobutyryl-CoA dehydrogenase; *10*, enoyl-CoA hydratase, ECHS1; *11*, 3-hydroxyisobutyryl-CoA deacylase or hydrolase, HIBCH; *12*, 3-hydroxyisobutyric acid dehydrogenase; *13*, methylmalonic semialdehyde dehydrogenase; *14*, acetyl-CoA carboxylase (cytosolic); *15*, propionyl-CoA carboxylase; *16*, methylmalonyl-CoA epimerase; *17*, malonyl-CoA decarboxylase; *18*, methylmalonyl-CoA mutase. Enzyme defects are indicated by **solid bars**

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Introduction

Branched-chain organic acidurias or organic acidaemias are a group of disorders that result from an abnormality of specific enzymes involving the catabolism of branched-chain amino acids (BCAAs; Catabolism of Branched-chain Amino Acids). Collectively, the most commonly encountered are maple syrup urine disease (MSUD), isovaleric aciduria (IVA), propionic aciduria

(PA) and methylmalonic aciduria (MMA). They can present clinically as a severe neonatal-onset form of metabolic distress, an acute and intermittent late-onset form, or a chronic progressive form presenting as hypotonia, failure to thrive, and developmental delay. Other rare disorders involving leucine, isoleucine, and valine catabolism are 3-methylcrotonylglycinuria, 3-methylglutaconic aciduria, short−/branched-chain acyl-CoA dehydrogenase deficiency, 2-methyl-3-hydroxybutyryl-CoA dehydrogenase deficiency, isobutyryl-CoA dehydrogenase deficiency, enoyl-CoA hydratase (ECHS1) deficiency, 3-hydroxyisobutyric aciduria (3-hydroxy-isobutyryl-CoA hydrolase or deacylase, HIBCH, deficiency), malonic aciduria (malonyl-CoA decarboxylase deficiency) and combined methylmalonic and malonic aciduria (ACSF3 deficiency). Most of these disorders can be diagnosed by identifying specific acylcarnitines and organic acid profiles in plasma and urine by tandem MS or by gas chromatography-mass spectrometry (GC-MS) and all can be detected by newborn screening using tandem MS. In MSUD, diagnostic confirmation relies on plasma aminoacids determination.

18.1 Maple Syrup Urine Disease, Isovaleric Aciduria, Propionic Aciduria, Methylmalonic Aciduria

18.1.1 Clinical Presentation

Children with maple syrup urine disease (MSUD), isovaleric aciduria (IVA), propionic aciduria (PA), or methylmalonic aciduria (MMA) have many clinical and biochemical features in common. There are three main clinical presentations:

1. A severe neonatal-onset form with acute metabolic decompensation and neurological distress.
2. An acute, intermittent, late-onset form also with recurrent episodes of metabolic decompensation.
3. A chronic, progressive form presenting as hypotonia, failure to thrive, and developmental delay.

In addition, prospective data gathered by newborn screening programmes, mainly using tandem MS and the systematic screening of siblings of affected subjects, have demonstrated the existence of milder or asymptomatic forms, especially for IVA.

18.1.1.1 Severe Neonatal-Onset Form

- **General Presentation**

The general presentation is that of a toxic encephalopathy with either ketosis or ketoacidosis (type I or II in the classification of neonatal inborn errors of metabolism in ▶ Chap. 1) in IVA, PA, MMA and in beta ketothiolase deficiency or without abnormalities in routine laboratory tests in MSUD. An extremely evocative clinical setting is that of a full-term infant born after a normal pregnancy and delivery who, after an initial symptom-free period, undergoes relentless deterioration with no apparent cause and is unresponsive to symptomatic therapy. The interval between birth and clinical symptoms may range from hours to weeks, depending on the severity of the defect, and may be related to the timing of the sequential catabolism of carbohydrates, proteins, and fats. Typically, the first signs are poor feeding and drowsiness, followed by unexplained progressive coma. There may be cerebral oedema with a bulging fontanelle, arousing suspicion of a central nervous system (CNS) infection. At a more advanced stage, neurovegetative dysregulation with polypnea/respiratory distress, hiccups, apnoeas, bradycardia, and hypothermia may appear. In this comatose state, most patients have characteristic changes in muscle tone and exhibit involuntary movements. Generalised hypertonic episodes with opisthotonus, boxing or pedalling movements (typical of MSUD), and slow limb elevations, spontaneously or upon stimulation, are frequently observed. Another pattern is that of axial hypotonia and limb hypertonia with large-amplitude tremors and myoclonic jerks, which are often mistaken for convulsions and are more frequently seen in MMA and PA. In contrast, true seizures occur late and inconsistently. The electroencephalogram may show a burst-suppression pattern. In addition to neurological signs, patients may present with dehydration and mild hepatomegaly.

- **Specific Signs**

Maple syrup urine disease

Concomitantly with the onset of the symptoms, the patient emits an intense (sweet, malty, caramel-like) maple-syrup-like odour. In general, neonatal (classic) MSUD does not lead to pronounced abnormalities seen on routine laboratory tests. Patients are not severely dehydrated, often present with elevated uric acid [1] have no metabolic acidosis, no hyperammonaemia or only a slight elevation (<150 μmol/l), no blood lactate accumulation, and the blood cell count is normal. The main laboratory abnormalities are greatly increased branched-chain amino acids (BCAAs) in plasma and the presence of 2-ketoacids rapidly detectable in urine with organic acid analysis. Mild ketonuria may also be present.

Isovaleric aciduria, propionic aciduria and methylmalonic aciduria

In contrast to MSUD, dehydration is a frequent finding in patients with IVA, PA, or MMA, and moderate hepatomegaly may be observed. They have metabolic acidosis (pH <7.30) with increased anion gap and ketonuria (Acetest 2–3 positive). However, ketoacidosis can be moderate and is often responsive to symptomatic

therapy. In MMA and PA, hyperammonaemia is a constant finding. When the ammonia level is very high (>500 µmol/l) it can induce respiratory alkalosis and lead to the erroneous diagnosis of a urea cycle disorder. Normal to low glutamine is a distinctive finding. Moderate hypocalcaemia (<1.7 mmol/l) and hyperlactataemia (3–6 mmol/l) are frequently found. Blood glucose can be normal, reduced, or elevated. When very high (20 mmol/l) and associated with glucosuria, ketoacidosis, and dehydration, it may mimic neonatal diabetes. Neutropenia, thrombocytopenia, non-regenerative anaemia, and/or pancytopenia can occur and are frequently erroneously ascribed to sepsis. Among these disorders, IVA is easily recognized by an unpleasant sweaty feet odour. In some cases, the combination of vomiting, abdominal distension, and constipation may suggest gastrointestinal obstruction. Cerebral haemorrhages have been described in a few neonates, a complication that may be linked to inappropriate correction of acidosis and may explain some poor long-term neurological outcomes.

18.1.1.2 Acute Intermittent Late-Onset Form

In approximately a quarter of patients, the disease presents after a symptom-free period, which is commonly longer than 1 year and sometimes lasts until adolescence or adulthood. Recurrent attacks may then be frequent but in between these episodes affected children may appear entirely normal. An acute attack may arise during catabolic stress induced by intercurrent illnesses, infections or following increased intake of protein-rich foods, but sometimes there may be no overt cause.

■ Neurological Presentation

Recurrent attacks of either coma or lethargy with ataxia are the main presentations of these acute late-onset forms. The most frequent variety of coma in MMA, PA and IVA is that presenting with ketoacidosis and mild hyperammonaemia, but in exceptional cases acidosis may be absent. There is no acidosis in MSUD.

Hypoglycaemia may rarely be a presenting sign in patients with MSUD. Although most recurrent comas are not accompanied by focal neurological signs, some patients may present with acute hemiplegia, hemianopsia, or symptoms and signs of cerebral oedema mimicking encephalitis, a cerebrovascular accident and stroke-like episodes, or a cerebral tumour. These acute neurological manifestations are freqently preceded by other premonitory symptoms that had been missed or misdiagnosed. They include acute ataxia, unexplained episodes of dehydration, persistent and selective anorexia, chronic vomiting with failure to thrive, hypotonia, progressive developmental delay and abnormal behaviour. Specific to MSUD, intermittent presentation include recurrent attacks of ataxia; between attacks amino acids may be normal and keto acids absent.

■ Haematological and Immunological Forms

Severe haematological manifestations are frequent, mostly concomitant with ketoacidosis and coma, and are sometimes the presenting problem. Neutropenia is regularly observed in both neonatal and late-onset forms of IVA, PA and MMA. Thrombocytopenia occurs mostly in infancy, and anaemia or overt pancytopenia occurs mostly in the neonatal period. Various cellular and humoral immunological abnormalities have been described in patients presenting with recurrent infections, leading to erroneous diagnosis and management.

18.1.1.3 Chronic, Progressive Forms

■ Gastrointestinal Presentation

Persistent anorexia, chronic vomiting, aversion to protein-rich food, failure to thrive, sometimes severe and osteopenia (evidence of a long-standing GI disturbance) are frequent manifestations. In infants, this presentation is easily misdiagnosed as gastro-oesophageal reflux, cow's milk protein intolerance, coeliac disease, late-onset chronic pyloric stenosis or hereditary fructose intolerance, particularly if symptoms start after weaning and diversification of food intake. Later in life, recurrent vomiting with ketosis may occur. These patients may remain undetected until an acute neurological crisis with coma leading to the diagnosis.

■ Chronic Neurological Presentation

Some patients present with severe hypotonia, muscular weakness and poor muscle mass that can simulate congenital neurological disorders or myopathies. Nonspecific developmental delay, psychiatric manifestations, seizures and movement disorders may also be observed during the course of the disease. However, these rather nonspecific findings are rarely the sole presenting symptoms. In MSUD, late onset chronic presentation includes hypotonia, spastic diplegia, developmental delay and failure to thrive.

18.1.1.4 Complications

■ Neurological and Psychiatric Complications

Maple syrup urine disease

Acute cerebral oedema is a well-recognized complication in newborns with MSUD and encephalopathy. Brain ultrasonography [2] and magnetic resonance imaging (MRI) display a characteristic pattern that may be of help in the diagnosis. In older patients metabolic decompensation may cause brain stem compression and unexpected death, particularly following intensive rehydration [3]; cerebral oedema may also develop slowly

due to long-standing elevations of BCAAs. Additionally, white matter changes can occur over time in those patients with poor biochemical control and persistently raised BCAAs. The areas most commonly affected are the periventricular white matter of the cerebral hemispheres, the deep cerebellar white matter, the dorsal part of the brain stem, the cerebral peduncles, the dorsal limb of the internal capsule and the basal ganglia. The severity of white matter changes does not correlate with signs of acute neurotoxicity, and the changes are reversible with appropriate treatment [4]. Acute axonal neuropathy may complicate late-onset decompensation [5]. Emerging long-term psychiatric complications are reported [6].

Propionic aciduria and methylmalonic aciduria

An increasing number of patients with PA and MMA have presented with an acute or progressive extrapyramidal syndrome associated with increased MRI signal within the basal ganglia (mostly the globus pallidus in MMA). The basal ganglia involvement may be due to oedema that evolves to necrosis. In addition, MRI studies indicate cerebral atrophy and delayed myelination [7]. These dramatic complications are arguments for adequate life-long dietary control even if the patient is free of symptoms. Even in well-treated patients with MMA or PA who are clinically and metabolically stable, brain lactate is elevated; this may indicate that aerobic oxidation and mitochondrial energy metabolism is persistently impaired from elevated intracellular propionic metabolites [7]. Late-onset optic neuropathy with visual dysfunction is another insidious complication in both MMA and PA [8]. In PA, late-onset psychiatric complications have been reported [9]. Autism spectrum disorder is often observed in PA [10]. Intellectual disability and other neuropsychiatric disturbances are also common. Long-term sensorineural hearing loss may be observed in MMA and PA patients [11].

Renal Complications

Renal tubular acidosis associated with hyperuricaemia may be an early and presenting sign in some late-onset patients with MMA. This condition partially improves with metabolic control. Chronic renal failure is frequent in MMA patients, starting from early childhood in those with severe disease but may occur later in life including adulthood [12, 13]. The renal pathology is a tubulointerstitial nephritis with type-4 tubular acidosis and adaptative changes secondary to a reduced glomerular filtration rate. The course of the renal disease is initially indolent, but end-stage renal failure may develop, and dialysis and kidney transplantation are likely to be necessary. As the nephropathy is a likely complication secondary to excessive MMA excretion [14] minimizing and deceleration of renal injury may require strict metabolic control. Of note, late-onset renal failure has also been reported in a number of patients with PA [15].

Skin Disorders

Large, superficial desquamation, alopecia, and corneal ulceration may develop in the course of late and severe decompensations in MSUD, PA or MMA. These skin lesions have been described as a staphylococcal scalded-skin syndrome with epidermolysis or as acrodermatitis enteropathica-like syndrome [16]. In many cases, these complications occur together with diarrhoea and can be ascribed to acute protein malnutrition, especially isoleucine deficiency.

Pancreatitis

Acute, chronic or recurrent pancreatitis may complicate organic acidaemias (MMA, PA and IVA). It has been the presenting illness in some patients with non-neonatal forms of IVA. The pathophysiological mechanisms are unknown. The condition may be difficult to diagnose and must be considered in the assessment of patients with acute deterioration. However, elevation of serum lipase often in the setting of abdominal pain and amylase alone does not confirm the diagnosis as pancreatitis being defined by inflammation on pancreas imaging. In contrast, isolated elevated lipase may normalize with correction of the metabolic status [17].

Cardiomyopathy and Disturbances in Cardiac Electrophysiology

Cardiomyopathy and prolonged QTc represent major complications in PA and, less prominently MMA, and may be responsible for rapid deterioration or death [18, 19]. Cardiomyopathy may develop as part of an acute decompensation or as a chronic deterioration even in patients who are metabolically stable or diagnosed on a systematic heart ultrasound without over clinical signs of heart dysfunction. Both dilated and hypertrophic types have been reported, with an estimated prevalence of 23% in one cohort [20]. In another PA cohort, 70% of patients beyond infancy were found to have developed disturbance in cardiac electrophysiology, particularly prolonged QTc, which could contribute to cardiac complications [19, 21]. The mechanism is uncertain but may result from energy deprivation and/or toxic accumulation. Investigation and follow-up may be useful to prevent irreversible damage and to help in decisions on therapeutic measures, as partial reversibility of cardiomyopathy with orthotopic liver transplantation has been described in rare cases [20, 22].

Liver Disease

This is an emerging long-term complication of both disorders [23]. Patients may exhibit liver fibrosis with

increase alfa-fetoprotein concentrations and a few liver cancers were reported in MMA [24]. Such liver findings are probably ascribable to mitochondrial hepatopathy [25] and warrant careful liver follow-up.

18.1.2 Metabolic Derangement

Maple Syrup Urine Disease

MSUD is caused by a deficiency of the branched-chain 2-ketoacid dehydrogenase (BCKD) complex, the second common step in the catabolism of the three BCAAs (Fig. 18.1, enzyme 1). Like the other 2-ketoacid dehydrogenases, BCKD is composed of three catalytic components (▶ Chap. 11, ▶ Fig. 11.2): a decarboxylase (E1), composed of E1α- and E1β-subunits and requiring thiamine pyrophosphate as a coenzyme, a dihydrolipoyl acyltransferase (E2) and a dihydrolipoamide dehydrogenase (E3). A deficiency of the E1 or E2 component can cause MSUD, whereas a deficiency of the E3 component produces a specific syndrome (dihydrolipoamide dehydrogenase [E3] deficiency) with congenital lactic acidosis, branched-chain 2-ketoaciduria and 2-ketoglutaric aciduria (▶ Chap. 11). However, E3 deficiency, particularly the neonatal-onset forms, may present with lactic acidaemia alone, with elevation of branched-chain amino acids and detectable alloisoleucine only becoming apparent weeks or months later. Variants forms affecting the phosphatase and kinase that regulate the BCKD complex have been described. BCKD phosphatase deficiency has been reported in a mild MSUD-like patient [26] and BCKD kinase deficiency in patients with syndromic autism with intellectual disability and low plasma BCAA levels [27]. Additionally, patients affected with disorders in the synthesis of lipoic acid could theoretically exhibit high levels of BCAA due to a secondary defect in E3. However, elevation of BCAA seems to be a rare finding in lipoic acid synthesis defects, which is occasionally found in E3 subunit deficiency but not in the other defects [28] (▶ Chap. 23). BCKD dysfunction results in marked increases in the branched-chain 2-ketoacids in plasma, urine and cerebrospinal fluid (CSF). Owing to the reversibility of the initial transamination step, the BCAAs also accumulate. Smaller amounts of the respective 2-hydroxy acids are formed. Alloisoleucine, a diastereomer of isoleucine, is invariably found in the blood of all patients with classic MSUD and in those with variant forms, at least in those still without dietary treatment.

Among the BCAA metabolites, leucine and 2-ketoisocaproic acid appear to be the most neurotoxic. In MSUD, these compounds are always present in approximately equimolar concentrations in plasma, and may cause acute brain dysfunction when their plasma concentrations rise above 1 mmol/l. Isoleucine and valine accumulation is of lesser clinical significance. Their 2-ketoacid to amino acid ratios favour the less toxic amino acids, and cerebral symptoms do not occur even when the blood levels of both amino acids are extremely high.

Isovaleric Aciduria

IVA is caused by a deficiency of isovaleryl-CoA dehydrogenase (IVD; Fig. 18.1, enzyme 2), an intramitochondrial flavoenzyme which, in a similar way to the acyl-CoA dehydrogenases (▶ Chap. 12, ▶ Fig. 12.1), transfers electrons to the respiratory chain via the electron transfer flavoprotein (ETF)/ETF-ubiquinone oxidoreductase (ETF-QO) system. Deficiencies of the ETF/ETFQO system result in multiple acyl-CoA-dehydrogenase deficiency (MADD; synonym: glutaric aciduria type II) (▶ Chap. 12). The enzyme defect results in the accumulation of derivatives of isovaleryl-CoA, including free isovaleric acid, which is usually increased in both plasma and urine (although normal levels have been reported), 3-hydroxyisovaleric acid (3-HIVA) and *N*-isovalerylglycine. This glycine conjugate is the major derivative of isovaleryl-CoA, owing to the high affinity of the latter for glycine *N*-acylase. Conjugation with carnitine (catalysed by carnitine *N*-acylase) results in the formation of isovalerylcarnitine.

Propionic Aciduria

PA is caused by a deficiency of the mitochondrial enzyme propionyl-CoA carboxylase (PCC; Fig. 18.1, enzyme 15), one of the five biotin-dependent enzymes. PCC is a multimeric protein composed of two different subunits, α- (which binds biotin) and β-PCC subunits. So far, all patients with isolated PA have been biotin resistant.

PA is characterised by greatly increased concentrations of free propionic acid in blood and urine and the presence of multiple organic acid by-products, among which propionylcarnitine, 3-hydroxypropionate and methylcitrate are the major diagnostic metabolites. The first is formed by acylation to carnitine. The second is formed by either β- or ω-oxidation of propionyl-CoA. Methylcitrate arises by condensation of propionyl-CoA with oxaloacetate, which is catalysed by citrate synthase in the Krebs cycle. During ketotic episodes, 3-HIVA is formed by condensation of propionyl-CoA with acetyl-CoA, followed by chemical reduction. High concentrations of organic acids derived from a variety of intermediates of the isoleucine catabolic pathway, such as tiglic acid, tiglylglycine, 2-methyl-3-hydroxybutyrate, 3-hydroxybutyrate and propionylglycine, can also be found. Owing to an abnormal biotin

metabolism, propionyl-CoA accumulation also occurs in multiple carboxylase deficiency (biotinidase deficiency, holocarboxylase synthetase [HCS] deficiency), resulting in defective activity of all four biotin-dependent carboxylases (► Chap. 27) also observed in carbonic anhydrase VA deficiency (► Chap. 19).

Methylmalonic Aciduria

MMA occurs where there is deficiency of methylmalonyl-CoA mutase activity (MCM; ◘ Fig. 18.1, enzyme 17), a vitamin B_{12}-dependent enzyme. This occurs with defects in the MCM-apoenzyme and because the apomutase requires adenosylcobalamin (AdoCbl) as a cofactor, also with disorders that affect AdoCbl formation (► Chap. 28).

Deficiency of MCM leads to accumulation of methylmalonyl-CoA, resulting in greatly increased amounts of methylmalonic acid in plasma and urine. Owing to secondary inhibition of PCC, propionic acid also accumulates, and other propionyl-CoA metabolites, such as propionylcarnitine, 3-hydroxypropionic acid, methylcitrate and 3-HIVA, are usually also found in urine. However, some mildly affected or asymptomatic patients, identified through urine organic acids screening in neonates but showing only slightly increased methylmalonic acid in blood and urine, have not shown constant excretion of metabolites derived from propionyl-CoA.

Recently, novel variants of MMA, also characterised by mild MMA, have been identified (below).

Vitamin B_{12} deficiency must be excluded in neonates presenting elevated propionyl-carnitine at newborn screening or when excessive urinary methylmalonic acid is found, particularly in a breast-fed infant whose mother either is a strict vegan or suffers from subclinical pernicious anaemia.

Secondary Metabolic Disturbances Common to PA and MMA

Accumulation of propionyl-CoA results in inhibitory effects on various pathways of intermediary metabolism, in increased levels of acylcarnitines (particularly propionylcarnitine) in blood and urine leading to a relative carnitine deficiency and in enhanced synthesis of odd-numbered long-chain fatty acids. Inhibition of various enzymes may explain some features such as hypoglycaemia, hyperlactataemia, hyperammonaemia and hyperglycinaemia. The increased ketogenesis that is a major cause of morbidity is not fully understood. Several pathomechanisms (e.g. accumulation of putatively toxic organic acids, inhibition of mitochondrial energy metabolism) have been suggested to explain acute and long-term organ damage [29].

Propionate, essentially in the form of propionyl-CoA, is produced in the body from three main sources: (1) catabolism of the amino acids isoleucine, valine, methionine and threonine, (2) anaerobic fermentation in the gut and (3) mobilization and oxidation of odd-chain fatty acids during prolonged fasting states. It has been estimated that catabolism of amino acids theoretically contributes approximately for 50% of the total propionate production, anaerobic gut bacteria 20%, and odd-chain fatty acids 30% [30]. These data, which are largely from stable isotope turnover studies, are based on a number of unproven assumptions and have not been reproduced in a more systematic manner. They are therefore open to question (for critical review, see [31]).

18.1.3 Genetics

Maple Syrup Urine Disease

MSUD is an autosomal-recessive disorder, with an incidence of 1 in 120,000 to 1 in 500,000. It is highly prevalent in the inbred Mennonite population in Pennsylvania, occurring in approximately 1 in 176 newborns [3]. MSUD is caused by mutations in *BCKDHA*, *BCKDHB* or *DBT* respectively coding for E1α, E1β and E2 subunits and accounting for 45%, 35% and 20% of MSUD patients respectively. In countries where consanguineous marriages are common, the frequency is also higher (about 1 in 50,000 in Turkey). About 75% of those affected suffer from the severe classic form, and the remainder suffers from the milder intermediate or intermittent variants. Over 150 different causal mutations scattered among the three genes, *E1α, E1β* and *E2*, give rise to either classic or intermediate clinical phenotypes [32].

Isovaleric Aciduria

IVA is an autosomal recessive disorder, with extreme clinical variability for unknown reasons. Reported mutations in *IVD* are highly heterogeneous, and generally no genotype-phenotype correlation has been established. However, children with IVA diagnosed by newborn screening and carrying a c.932C > T mutant allele can exhibit a milder, potentially asymptomatic phenotype [33].

Propionic Aciduria

PA is an autosomal recessive disorder with an incidence of less than 1 in 100,000. PA can result from mutations in *PCCA* or *PCCB* encoding the α- and β-subunits, respectively, of propionyl-CoA carboxylase. To date, more than 200 different allelic variations in *PCCB* and *PCCA* have been identified in different populations [34].

Following the introduction of the newborn screening programme in Japan a number of infants with an apparently mild phenotype and the Y435C mutation in *PCCB* have been reported. The natural history of this phenotype is not yet clarified [35].

Methylmalonic Aciduria

Isolated MMA can be caused by mutations in the *MUT* locus encoding the methylmalonyl CoA mutase (MCM) apoenzyme, or by those in genes required for provision of its cofactor, 5′-deoxyadenosylcobalamin (AdoCbl). Isolated MMA is classified into several genotypic classes and complementation groups. These are designated either mut^- or mut^0 (together termed mut), according to whether there is minimal or no apoenzyme activity in vitro, respectively, or cobalamin A, B or D-variant 2 (CblA/B/D-MMA) for cofactor defects (see ► Chap. 28 for further details). MMA is an autosomal recessive disorder with an overall incidence of about 1 in 50,000. Approximately one half to two thirds of patients have a mutase apoenzyme defect; the remaining patients have cobalamin variants. To date more than 200 disease-causing mutations in patients with $mut^{0/-}$ MMA have been identified at the *MUT* locus [36]. Mutations in *MMAA* and *MMAB* encoding the cblA and cblB proteins respectively have been identified in cblA and cblB patient cell lines (► Chap. 28).

Other variants with mild MMA elevation include defects in succinyl-CoA ligase and methylmalonyl-CoA epimerase. Succinyl-CoA ligase catalyzes the conversion of succinyl-CoA to succinate in the Krebs cycle (► Chap. 11). Its deficiency causes mild MMA, variable lactic acidosis, accumulation of succinylcarnitine and mitochondrial DNA depletion (► Chaps. 11 and 14).

A deficiency in methylmalonyl-CoA epimerase (MCEE) has been reported in individuals with mild MMA. This defect has a questionable clinical impact [37, 38]. It was reported in two patients in association with sepiapterin reductase deficiency and identical *MCEE* variants [37, 39]. More recently, MCEE deficiency was reported to present with the acute symptoms of a mild classical organic aciduria [40, 41].

18.1.4 Diagnostic Tests

Only MSUD can be diagnosed by using plasma amino acid alone. IVA, PA and MMA are diagnosed by their specific urinary organic acid profiles using GC-MS or abnormal acylcarnitines on tandem MS, while amino acid chromatography displays nonspecific abnormalities, such as hyperglycinaemia and hyperalaninaemia. Owing to acidosis and low 2-ketoglutarate production which impact on glutamine metabolism, hyperammonaemia associated with organic acidurias leads to normal or even low plasma glutamine levels [42]. Whatever the clinical presentation, the diagnosis can be made by sending filter-paper blood specimens, fresh or frozen urine samples or 1- to 2-ml samples of fresh or frozen plasma to an experienced laboratory for analysis. Specific loading tests are not necessary. Newborn screening for this group of organic acidurias can be performed by tandem MS. An increased leucine/isoleucine peak in blood spots taken at 24 or 36 h of age requires immediate notification (MSUD). Similarly, the abnormal acylcarnitine profile found in PA and MMA with propionylcarnitine (C3-carnitine) and that in IVA with isovalerylcarnitine (C5-carnitine) also requires immediate notification.

Molecular DNA testing is useful for diagnostic confirmation. Prenatal diagnosis is possible either by DNA testing in families in which the mutations are known in fresh or cultured chorionic villi. This can also be done in cultured amniotic cells. Alternatively, for MMA, PA and IVA, when the mutation is unknown, reliable prenatal diagnosis can be performed by the direct measurement of metabolites in amniotic fluid using GC-MS, stable-isotope dilution techniques, or tandem MS. For the 4 conditions, direct enzyme assay is also possible but rarely performed.

18.1.5 Treatment and Prognosis

CNS dysfunction can be prevented or at least minimised by early diagnosis (including newborn screening) and emergency treatment. Neonatal-onset forms frequently require early toxin removal (► Chap. 4). Thereafter dietary restriction, which is necessary to limit the production of organic acids and their metabolites and other specific treatments, is required both for survivors of the early-onset forms and for those with late-onset disease. For both groups it is essential that episodes of metabolic decompensation are recognised and treated sufficiently early; parents must be taught to recognise early warning signs and manage their child appropriately. Exhaustive recommendations on acute and long-term management of organic acidurias (MMA and PA) from the E-IMD consortium have been published [18, 43].

18.1.5.1 Principles of Treatment

Principles of Long-Term Dietary Treatment

Long-term dietary treatment is aimed at reducing the accumulation of toxic metabolites while, at the same time, maintaining normal physical development and nutritional status and preventing catabolism. Most patients require very specific food allowances, implying stringent dietary restrictions that will be necessary for

life. A few late-onset patients need only minimal restriction.

The cornerstone of treatment is the limitation of one or more essential amino acids which, if present in excess, are either toxic or precursors of toxic organic acids. Precise individual prescriptions are established for the daily intake of amino acids, protein and energy. The diet must provide the recommended daily allowance (RDA) and the estimated safe and adequate daily dietary intakes of minerals and vitamins and follow the principles of paediatric dietetics [44].

Protein/Amino Acid Prescriptions

Requirements for BCAAs and protein vary widely from patient to patient and in the same patient, depending on the nature and severity of the disorder, other therapies prescribed (stimulation of an alternate pathway), growth rate, state of health and feeding difficulties. Individual requirements must be estimated for each child by frequent monitoring of clinical and metabolic status. The balance between protein malnutrition and metabolic disequilibrium can be difficult to maintain in severe PA and MMA and needs to be kept under regular review, especially after an acute metabolic decompensation or after a change in the diet.

Within this group of organic acidurias, only in MSUD is the diet directly related to the intake of an amino acid, which is leucine in milligram amounts. Natural protein, which contains leucine, must be severely restricted in an age-dependent manner to only one tenth to a half of the normal recommended daily requirement. Consequently, in order to meet the protein RDA for the patient's age, a large supplement of BCAA-free amino acid mixture as a protein substitute is indispensable. In IVA it is generally sufficient to restrict natural protein to the recommended minimum daily requirements or just somewhat more; a special amino acid mixture free of leucine is sometimes needed. In neonatal PA and MMA dietary protein is generally restricted to the adequate age-related safe levels. Restriction of specific amino acids has not proved to be useful. Although controversial, a limited, relatively small, amount of an amino acid mixture free of valine, isoleucine, methionine and threonine can be added to the diet to supply additional nitrogen and other essential and nonessential amino acids in order to promote a protein-sparing anabolic effect. Selective aminoacid deficiency should be avoided and, when identified, treated by specific supplements. It has been reported that the BCAAs, particularly valine are necessary for the proliferation and maintenance of hematopoietic stem cells [45].

The prescribed amounts of leucine or natural protein are provided by natural foods. Breast milk or standard infant formula is used in young infants but breast milk should be preferentially used in the early infancy period. For toddlers and children solids are introduced, using serving lists and lists of amino acid content in foods. In all protein-restricted diets, high-protein foods (eggs, meat, dairy products), apart from milk, are generally avoided, since the lower percentage of amino acids in vegetable protein (compared with that in animal protein) makes it easier to satisfy the appetite of children.

Energy and Micronutrient Prescriptions

Energy and micronutrient prescriptions should follow the general rules common to all artificial medical diets, the model for which being PKU (► Chap. 16).

Evaluation of Clinical and Nutritional Status

The metabolic and nutritional statuses are both evaluated weekly during the first month of therapy, once a month during the first year, and later every 3–6 months [18]. In patients treated with a low-protein diet without an added amino acid mixture, measurement of urea excretion can be used to evaluate anabolism [46]. Regular assessment of developmental progress provides the opportunity for psychological support, as social and emotional needs are major issues of the overall therapy of the affected child and of the family's wellbeing.

18.1.5.2 Specific Adjustments

Maple Syrup Urine Disease

Acute phase management in the newborn

Exogenous toxin removal procedures such as haemodialysis and haemofiltration together with high-energy dietary treatment are usually recommended for the reversal of acute metabolic decompensation in symptomatic newborns with the classic form of MSUD [47]. With these measures the plasma leucine level is reduced to 1 mmol/l or less within hours. During the recovery interval, oral intake of BCAA-free formula (by tube feeding) should be started early and BCAA intake adjusted according to the plasma levels, which are monitored daily until the optimal equilibrium is achieved. During this stage, plasma concentrations of valine and isoleucine may fall below normal and become rate limiting for protein synthesis, a situation which requires generous valine and isoleucine supplements in doses of 300–400 mg/day. Newborn screening for MSUD by tandem MS allows for early diagnosis and intervention and in some cases obviates the need for extracorporeal detoxification. In affected newborns found positive on screening the oral intake of BCAA-free formula (tube feeding) with adequate calorie supply (glucose polymer) and supplementation with isoleucine and valine (300–400 mg/day) can be sufficient to stimulate protein synthesis and to normalise plasma leucine levels within 2–3 days [3, 48].

Long-term management

Management of MSUD comprises a life-long strict and carefully adjusted semisynthetic diet, as well as acute-phase treatment during episodes of catabolic stress. The dietary treatment of MSUD differs from that of other organic acidurias, since the condition results in elevated plasma BCAA levels. In that respect MSUD can be regarded as an aminoacidopathy, and the principles of dietary treatment are essentially those that apply to PKU (▶ Chap. 16). The diet consists of measured proportions of BCAA-containing foods (as natural protein) and a synthetic BCAA-free amino acid supplement, which in most preparations also contains the recommended requirements for minerals, vitamins and other essential nutrients. Additional fat and carbohydrate are provided by protein-free products and additional supplements. The aim of such treatments is to maintain the 2–3 h postprandial plasma BCAAs at near-normal concentrations (leucine: 80–200 μmol/l; isoleucine: 40–90 μmol/l; valine: 200–425 μmol/l). Since leucine is the most toxic precursor, the diet can be based on the leucine requirement, with frequent adjustment according to plasma leucine levels.

In newborns with the classic severe form of MSUD, the leucine requirement is 300–400 mg/day (80–110 mg/kg/day), which is not far from the leucine intake in healthy breast-fed newborns. MSUD patients may be kept on breast milk in early infancy. Minimum valine and isoleucine requirements are 200–250 mg/day. Apart from considerable inter-individual variation, children, adolescents and adults with the classic form of MSUD tolerate about 400–800 mg of leucine per day. Individuals with variant forms tolerate greater amounts, and some do well on a low-protein diet.

Serial monitoring of blood BCAA levels is essential in the treatment of MSUD, and intakes of BCAAs must frequently be titrated against plasma concentrations. Additional small amounts of free valine and isoleucine may be needed to those provided by natural protein, because the tolerance for leucine is lower than that for the other two. When the plasma leucine levels are high and those of valine and isoleucine low, a rapid fall of leucine can only be achieved by combining a reduced leucine intake with a temporary supplement of valine and isoleucine.

In MSUD, unlike other organic acidurias, no abnormal acylcarnitines are formed and there is no increased carnitine loss; consequently, no carnitine supplement is required. Although treatment with thiamine has often been advocated, its efficacy has not been confirmed in any form of MSUD (▶ Sect. 29.1.7).

Emergency regimen

During maintenance treatment minor illnesses such as fever, vomiting, or diarrhoea result in an increase in catabolism and amino acid release from muscle protein. Neurotoxic levels of BCAAs and BCKAs are reached within hours, and patients may present with apathy, ataxia, hallucinations and, eventually, with fasting hypoglycaemia (rare) and seizures. High energy intake and temporary removal of natural protein from the diet, and continuing supplements of BCAA-free amino acids (with the early addition of valine and isoleucine supplements) help to limit accumulation of the branched-chain compounds. Owing to its anabolic effect, intravenous insulin (0.05–0.20 IU/kg body weight/h) combined with large amounts of glucose and with continued enteral BCAA-free amino acids, can be successfully used to treat severe catabolic episodes. When enteral feeding is impossible, (gastric intolerance or enteral feeding refusal in adults), a parenteral BCAA-free amino acids mixture can be used [49]. Such therapy may prevent metabolic decompensation following major surgery and trauma and can obviate the necessity for extracorporeal toxin removal in critically ill children. The latter should be discussed in the event of very high leucine levels (>1100 μmol/l) and/or rapid onset of neurological symptoms.

Maternal MSUD

In pregnant women with MSUD, maintaining the plasma leucine level between 100 and 300 μmol/l and plasma valine and isoleucine in the upper normal ranges resulted in the delivery of healthy infants. Leucine tolerance increased progressively from the 22nd week of gestation from 350 to 2100 mg/day. The risk of metabolic decompensation in the mother during the catabolic postpartum period can be minimised by careful monitoring after delivery in a metabolic referral centre [50].

Liver transplantation

Liver replacement results in a clear increase in whole-body BCKD activity to at least the level seen in the very mild MSUD variant; liver transplantation allows removal of dietary restrictions, protection from acute decompensations during illness, arrest although not reversion of neurocognitive impairment progression, prevention of life-threatening cerebral edema, metabolic and clinical stability. Explanted livers of MSUD patients have been successfully used in domino transplantation [51, 52].

Prognosis

Treated patients with classic neonatal MSUD generally survive; they are usually healthy between episodes of metabolic imbalance, and some attend regular schools and have normal IQ scores. However, the average intellectual performance is clearly below that of normal subjects [48]. Recently, abnormal neurocognitive profile with higher verbal than performance abilities was reported in a cohort of 21 MSUD school-age patients [53]. The intellectual outcome is inversely related to how long after birth plasma leucine levels remained above 1 mmol/l and is dependent on the quality of long-term metabolic control [48]. This suggests that inclusion of

MSUD in neonatal screening programmes by tandem MS may improve the prognosis. Normal development and normal intellectual outcome and performance can be achieved at least in prospectively treated patients [3] and if average long-term plasma leucine levels are not more than 1.5–2 times normal. However, some patients may present mental health problems despite good metabolic control. Children may have inattention and hyperactivity, and older patients may show generalised anxiety, panic or depression, resulting in poor educational and social achievement [48, 54]. In addition, timely evaluation and intensive treatment of minor illnesses at any age is essential, as late death attributed to recurrence of metabolic crises with infections has occurred [3].

Isovaleric Aciduria

Acute phase management in the newborn

Intensive treatment with nonspecific measures (glucose infusion with appropriate electrolytes to provide calories and reduce endogenous protein catabolism,) including exogenous toxin (and ammonia) removal may be needed in newborns. Such infants are often in a poor clinical condition precluding the effective use of alternate pathways to enhance the removal of isovaleryl-CoA. In these circumstances, the administration of intravenous L-carnitine (100–400 mg/kg/day) and oral L-glycine (250–400 mg/kg/day) are effective means of treatment. Carbamylglutamate (oral loading dose of 50–100 mg/kg followed by 200 mg/kg/d in 4 divided doses) has been successfully used to treat hyperammonaemia in acutely ill patients with IVA [55].

Dietary therapy

The aim of treatment is to reduce the isovaleric acid burden to a minimum. Such a therapy consists of a low-protein diet with supplemental glycine and carnitine and should be started as soon as possible after birth. In most patients the amount of protein tolerated meets the official protein requirements; a special amino acid mixture free of leucine is sometimes needed. Excessive protein intake should be avoided.

Carnitine and glycine therapy

For supplemental therapy either oral L-carnitine (50–100 mg/kg/day) or oral L-glycine (150–300 mg/kg/day) can be used. Under stable conditions, the need for both supplementations is still controversial, but it can be useful during metabolic stress when toxic isovaleryl-CoA accumulation increases the need for detoxifying agents [56]. Supplementation with large doses of carnitine gives rise to an unpleasant odour in many IVA patients.

Prognosis

Prognosis is better than for the other organic acidurias. Even when a patient is compliant with treatment, metabolic crises can occur during catabolic stress, making a short hospitalisation for intravenous fluid (glucose/electrolytes/buffer) necessary. With puberty, metabolic crises rarely occur. Growth is normal; intellectual prognosis depends on early diagnosis and treatment and, subsequently, on long-term compliance [57]. According to this, inclusion of IVA into neonatal screening programmes by tandem MS should improve the prognosis. So far there is no evidence that uncomplicated maternal IVA has any adverse effect on the unborn child [58].

In asymptomatic individuals identified by newborn screening and showing a mild biochemical phenotype it is crucial to follow the course of the inherited metabolic disturbance prospectively, as far as possible without any therapeutic regimen in order to better define the natural history.

Propionic Aciduria and Methylmalonic Aciduria

Recommendations and treatment guidelines have been published by the EIMD consortium [18, 43].

Acute phase management in the newborn

The urinary excretion of propionic acid is negligible, and no alternate urinary pathway is sufficient to effectively detoxify newborns with PA. However, this does not mean that exogenous toxin removal procedures are inevitably required. Extracorporal detoxification such as haemo(dia)filtration and haemodialysis (peritoneal dialysis is far less efficient), together with measures to promote anabolism, should be considered when neonatal illness is accompanied by severe hyperammonaemia (>400 µmol/l). In contrast to PA, the efficient removal of toxin in MMA takes place via urinary excretion, because of the high renal clearance of methylmalonic acid (22 ± 9 ml/min per 1.73 m^2), which allows excretion of as much as 4–6 mmol MMA/day. Thus, in some cases not complicated by very high ammonia levels, emergency treatment may be limited to rehydration and promotion of anabolism [59].

When conservative measures with high energy supply are sufficient, hyperammonaemia (especially in PA) may be controlled by the use of sodium benzoate and/or carbamylglutamate [60]. The use of sodium phenylbutyrate is not recommended because in MMA and PA hyperammonemia is usually associated with decreased levels of glutamine [18, 42]. Metabolic decompensation in MMA and PA may be complicated by severe lactic acidosis due to thiamine deficiency, requiring vitamin supplementation [61].

Long-term management

The goal of treatment is to reduce the production of methylmalonic or propionic acid by means of

- Natural protein restriction
- Maintaining an optimal calorie intake
- Carnitine supplementation (100 mg/kg/day)
- Reduction of intestinal production of propionate by metronidazole

Dietary management

The aim of dietary treatment is to reduce the production of propionate by both the restriction of precursor amino acids using a low-protein diet and avoidance of prolonged fasting to limit oxidation of odd-chain fatty acids, which are liberated from triglyceride stores during lipolysis. The low-protein diet must provide at least the minimum amount of protein, nitrogen and essential amino acids to meet requirements for normal growth. Figures for estimates of safe levels of protein intake for infants, children and adolescents are available [44], which can be used as a guide for low-protein diets. In early childhood this is often 1–1.5 g/kg/day. To improve the quality of this diet it may be supplemented with a relatively small amount of synthetic amino acids free from the precursor amino acids. However, the long-term value of these supplements remains uncertain, and metabolic balance can often be achieved without them [44, 46]. Some studies have shown that the addition of a special amino acid mixture to a severely restricted diet has no effect on growth or metabolic status and that these amino acids are mostly broken down and excreted as urea [46].

Long fasts should be avoided. In order to prevent fasting at night nocturnal tube feeding may be required in the early years of management.

In children with severe forms of PA and MMA, anorexia and feeding problems are almost invariably present, and in order to maintain a good nutritional status, feeds have to be given via nasogastric tube or gastrostomy at some stage. This is essential to provide adequate dietary intake, to prevent metabolic decompensation and to help parents cope with a child who may be difficult to feed [44, 46].

Most patients with a late-onset form are easier to manage. Individual protein tolerance can be quite high. Even though this allows a less rigid protein restriction and leads to a lower risk of malnutrition, these patients must be taught to reduce their protein intake immediately during intercurrent illness to prevent metabolic imbalance.

Vitamin therapy

Every patient with MMA should be tested for responsiveness to vitamin B_{12}. Some late-onset forms (and, more rarely, neonatal-onset forms) are responsive to vitamin B_{12}; thus, parenteral vitamin therapy, starting with hydroxocobalamin 1000–2000 μg/day for about 10 days, must be carefully tried during a stable metabolic condition. During this period 24-h urine samples are collected for an organic acid analysis. Vitamin B_{12} responsiveness leads to a prompt and sustained decrease of propionyl-CoA by-products, mainly MMA. However, as biochemical results may be difficult to assess, B_{12} responsiveness must later be confirmed by molecular studies. Most B_{12} responsive patients need only mild protein restriction or none at all. Vitamin B_{12} is either given orally once a day or administered once a week (1000–2000 μg i.m.). In some cases, i.m. hydroxocobalamin therapy can be kept in backup for intercurrent infections.

Carnitine therapy

Chronic oral administration of L-carnitine (100 mg/kg/day) appears to be effective not only in preventing carnitine depletion but also in allowing urinary propionylcarnitine excretion and with subsequent reduction of propionate toxicity [18].

Metronidazole therapy

Microbial propionate production can be suppressed by antibiotics. Metronidazole, an antibiotic that inhibits anaerobic colonic flora, has been found to be specifically effective in reducing urinary excretion of propionate metabolites by 20–40% in MMA and PA patients. Long-term metronidazole therapy (at a dose of 10–20 mg/kg once daily for 10 consecutive days each month) may be of significant clinical benefit [18]. Regardless, metronidazole therapy remains questionable. Of note, reversible axonal peripheral neuropathy ascribed to metronidazole has been reported in 2 PA sibs [62].

Growth hormone

Growth hormone (GH) induces protein anabolism. It is contraindicated in the acutely ill patient but potentially useful in the long term for those in whom growth is poor. There is a place for recombinant human GH treatment as an adjuvant therapy in some patients with MMA and PA, mainly in those with reduced linear growth, but controlled long-term studies are needed [18].

Biochemical monitoring

During the course of decompensation, plasma ammonia, blood gases, electrolytes, calcium, phosphate, lactate, glucose, uric acid, lipase and ketones in urine (or blood) should be monitored. Some groups prefer also to measure urea and urea to MMA molar ratio in urine [46]. Regular amino acid analysis (all essential amino acids, and in particular isoleucine) is important. Furthermore, MMA in plasma or urine should be controlled in order to define the lowest possible level in each individual patient on treatment. There may be little practical use for the measurement of acylcarnitines and of odd-chain fatty acids in terms of directing clinical management. Regarding propionic acid metabolites in urines, there is no consensus on which specific metabolites would be more specific. Determination of plasma methylcitric acid and FGF21 could be of help in predicting disease burden, long-term complications and the impact of transplantation in PA and MMA [63]. Therefore, intra-individual variations in a given patients associated with clinical and laboratory parameters are to be considered altogether.

Prognosis

Around 15% of patients with MMA are vitamin B_{12} responsive and have mild disease and a good long-term outcome [12, 64]. Conversely, both vitamin B_{12}-unresponsive patients with MMA and those with PA have severe disease and many encephalopathic episodes, mainly due to intercurrent infections [65]. Among all patients with all forms of MMA, mut^0 patients have the poorest prognosis, and vitamin B_{12}-responsive cblA and mut^- patients, the best [12, 18, 66]. Owing to earlier diagnosis and better treatment, outcomes for PA and MMA patients have improved [46, 64, 65]. Survival rates into early and mid-childhood can now exceed 70%. However, morbidity, in terms of cognitive development, remains high, with a majority of patients having DQ/IQ in the mildly to moderately retarded range [67, 68]. With improved management, the frequency of growth retardation has decreased, and now most patients with PA and MMA have growth curves within the normal range [46]. Abnormal neurological signs (mainly movement disorders, chorea, dystonia) continue to increase with age [12, 65]. Chronic progressive impairment of renal function is a frequent and serious complication that manifests in patients with high MMA excretion [12, 69]. Including PA and MMA into newborn screening programmes by tandem MS may make it possible to identify the non-neonatal forms of the diseases in the newborn period and contribute to a further improvement in the outcomes in this group. Decreased early mortality, less severe symptoms at diagnosis and more favourable short-term neurodevelopmental outcomes were recorded in patients identified through expanded newborn screening. However, the short duration of follow-up so far does not allow drawing final conclusions about the effects of newborn screening on long-term outcome [65].

There are only a few reports of female patients with MMA who have carried a pregnancy to term [70]. The outcome was favourable despite high MMA levels in blood and urine However, the majority of pregnancies can be complicated by cesarean delivery and increased risk of prematurity. Among 17 reported pregnancies, only one was associated with mild metabolic decompensation in the mother [70].

Liver/kidney transplantation

In MMA, liver transplantation or combined liver-kidney transplantation eradicates episodes of hyperammonaemia and has resulted in excellent long-term survival in some patients suggesting stabilization of neurocognitive development [71]. However, some patients have developed acute decompensation and basal ganglia necrosis years after liver transplantation and while on a normal diet. Today, it is recommended that such patients be maintained on a mild protein restricted diet and with continued carnitine supplementation. Long-term follow-up will be mandatory to evaluate whether patients who undergo early liver transplant [72] need kidney transplantation later in life. Such an early liver transplant appears a reasonable choice for treating severe MMA in an attempt to prevent renal failure and the need of kidney transplant [73]. In the setting of kidney failure, the best option is probably a combined liver-kidney transplantation [74]. Though initially viewed as a successful option in MMA patients in end-stage renal failure, with significant improvement in their metabolic control [69], isolated kidney transplantation should rather be reserved for adult cblA patients with end-stage renal failure. For PA, cardiomyopathy, when present, may be partially reversible following liver transplantation [20, 22]. Liver transplantation experience in PA is still limited. Some studies reported clinical improvement and improved dietary protein tolerance [75, 76]. However, others have reported a high mortality risk as well as high morbidity especially worsening of pre-existing renal failure [77].

Management of intercurrent decompensations

Acute intercurrent episodes are prevented or minimised by awareness of the situations that may induce protein catabolism. These include intercurrent infections, trauma, anaesthesia and surgery, and dietary indiscretion. In all cases, the main response comprises a reduction in protein intake. All patients should have detailed instructions (sick day protocol), including information on a semi-emergency diet, in which natural protein intake is reduced by half, and an emergency diet, in which it is stopped. In both, energy supply is augmented using carbohydrates and lipids, such as solutions based on protein-free formula base powder or a mixture of glucose polymer and lipids diluted in an oral rehydration solution. For children treated with specific amino acid mixtures the usual supplements can be added, though one should be aware that they increase osmolarity and that their taste renders nasogastric tube feeding often unavoidable. Their use is contraindicated in MMA and PA in cases of severe hyperammonaemia. At home, the solution is given in small, frequent drinks during day and night or by nasogastric tube [44]. After 24–48 h, if the child is doing well the usual diet is resumed within 2 or 3 days.

In cases of clinical deterioration with anorexia and/or gastric intolerance or if the child is obviously unwell, the patient must be hospitalised to evaluate the clinical status, to search for and treat intercurrent disease and to halt protein catabolism. Emergency therapy depends on the presence of dehydration, acidosis, ketosis and hyperammonaemia. Most often, intravenous rehydration for

12–24 h results in sufficient clinical improvement to allow for progressive renutrition with continuous enteral feeding. During this renutrition step enough natural protein to at least cover the minimal dietary requirements should be introduced into the feeds. The energy intakes are supplied with carbohydrates and lipids. During this stage of management, close metabolic evaluation is recommended, as the condition is labile and may deteriorate, requiring adjustment of the therapy. Conversely, if the patient's condition improves quickly the usual diet should be initiated without delay.

During periods when enteral feeding is contraindicated or poorly tolerated, as can occur with severe or prolonged decompensation, the use of total parenteral nutrition may be an effective mean for improving metabolic control and preventing further deterioration [18, 43].

18.2 3-Methylcrotonyl Glycinuria

18.2.1 Clinical Presentation

The clinical phenotype described in 3-methylcrotonyl-CoA carboxylase (3-MCC) deficiency (MCCD) has been highly variable ranging from neonatal onset with severe neurological involvement and even death to a complete lack of symptoms in adults [78]. In the past 15 years family studies and newborn screening have identified a number of totally asymptomatic newborn infants, siblings and mothers with MCCD who have very low carnitine concentrations in blood. Many symptoms and signs in consanguineous families, initially attributed to MCCD, are most likely due to rare homozygous disease causing mutations in other disease genes [79]. However, in a small number of affected individuals, MCCD does appear to cause metabolic decompensation with hypoglycaemia, ketonaemia and severe metabolic acidosis.

18.2.2 Metabolic Derangement

3-MCC is one of the four biotin-containing carboxylases known in humans (◘ Fig. 18.1, enzyme 3). Its deficiency leads to accumulation of 3-methylcrotonyl-CoA and 3-methylcrotonic acid. Most of the 3-methylcrotonyl-CoA is conjugated with glycine to form 3-methylcrotonylglycine (MCG) whereas acylation with carnitine leading to the formation of 3-hydroxyisovaleryl carnitine appears to be only a minor pathway. 3-Hydroxyisovalerate (3-HIVA), another major metabolite, is derived through the action of a crotonase on 3-methylcrotonyl-CoA and the subsequent hydrolysis of the CoA-ester.

18.2.3 Genetics

3-MCC is a heteromeric enzyme consisting of α- (biotin-containing) and β-subunits. MCCD results from loss of function mutations in *MCCC1* and *MCCC2* respectively encoding these subunits. More than 50 mutations have been identified in both genes [78, 80]. They are associated with an almost total lack of enzyme activity in fibroblasts. The apparent biochemical severity of all the *MCC* mutations contrasts with the variety of the clinical phenotypes. The introduction of tandem MS into newborn screening has revealed an unexpectedly high prevalence of this disorder, which in certain areas appears to be the most frequent organic aciduria found [81].

18.2.4 Diagnostic Tests

The diagnosis relies on a characteristic urinary profile of organic acids, with huge excretion of 3-HIVA and 3-methycrotonylglycine and without the lactate, methylcitrate, and tiglylglycine found in multiple carboxlase deficiency (MCD) (▶ Chap. 27). Supplementation with pharmacological doses of biotin does not alter this pattern. Total and free carnitine concentrations in plasma are extremely low. The presence of 3-hydroxyisovaleryl carnitine (C5OH) in plasma and in dried blood spots is characteristic for MCCD. However, diagnostic approach based solely on detection of C5OH may lead to overdiagnosis. In view of it's generally benign nature, it is debatable whether or not MCCD should be included in newborn screening programmes [82].

18.2.5 Treatment and Prognosis

Asymptomatic individuals most probably do not require treatment. In those with metabolic crisis glycine and carnitine therapies directed at increasing the excretion of glycine and carnitine conjugates are complementary rather than competitive means of detoxification. Glycine supplementation (175 mg/kg/day) increases the excretion of 3-MCG. Carnitine supplementation (100 mg/kg/day) corrects the very low plasma carnitine levels and increases the excretion of 3-HIVA. Long-term treatment of symptomatic infants based on a mildly protein-restricted diet is debatable.

18.3 3-Methylglutaconic Aciduria

Primary 3-methylglutaconic aciduria caused by 3-methylglutaconyl-CoA hydratase deficiency (*AUH mutations*) has only been identified in very few individuals, who presented with a wide spectrum of clinical signs of a neurometabolic disease ranging from no symptoms (at 2 years of age) to mild neurological impairment, severe encephalopathy with basal-ganglia involvement, quadriplegia, athetoid movement disorder, severe psychomotor retardation and leukoencephalopathy in a 61-year-old woman. 3-Methylglutaconyl (MGC)-CoA is metabolised to 3-hydroxy-3-methylglutaryl-CoA by 3-MGC-CoA hydratase (Fig. 18.1, enzyme 4). Defective activity is characterised by urinary excretion of 3-MGC and 3-methylglutaric acids. Both metabolites derive from accumulated 3-methylglutaconyl-CoA, through hydrolysis and dehydrogenation, respectively. The combined urinary excretion of 3-MGC and 3-methylglutaric acids range from 500 to 1000 mmol/mol creatinine, of which 3-methylglutaric acid represents about 1%. The metabolic pattern also includes 3-HIVA, which differentiates it from the other secondary causes (below). 3-MGC-CoA hydratase activity can be measured in fibroblasts. The role of the human 3-MGC-CoA hydratase in leucine metabolism has been elucidated, and different mutations in *AUH* have been identified. No clear therapeutic regimen has been described. Carnitine supplementation may have beneficial effects.

Secondary 3-MGC acidurias are a relatively common finding in a number of metabolic disorders, particularly mitochondrial disease. In most the excretion of 3-MGC acid is only slightly increased and accompanied by other disease specific metabolites. However, there are some disorders where 3-MGC aciduria is a more significant and consistent finding with urinary excretion >40 μmol/mmol creatinine. Previously, 3-MGC acidurias had been classified into types I to V 3-MGC aciduria, but they are now reclassified and named according to their pathological mechanism and defective protein or historical name (Table 18.1) [83]. There remain, however, disorders for which the underlying pathological mechanism is still unclear [84]. For example, mutations in *CLPB* were found in individuals with intellectual disability, congenital neutropenia, progressive brain atrophy, movement disorder, cataracts, and 3-MGC aciduria without any obvious mitochondrial respiratory chain dysfunction [85].

Table 18.1 Classification of disorders with significant 3-methylglutaconic aciduria

	Defect	Name	Gene	Previous classification	Reference
Primary	Leucine catabolism	3 HMG CoA hydratase deficiency	AUH	Type I	This chapter
Secondary	Phospholipidpar remodelling	TAZ defect or Barth syndrome	TAZ	Type II	▶ Chapter 35
		SERAC1 defect or MEGDEL syndrome	SERAC1	Type IV	
		Sengers syndrome	AGK	Type IV	
	Mitochondrial membrane associated disorder	OPA3 defect or Costeff syndrome	OPA3	Type III	▶ Chapter 10
		DNAJC19 defect or DCMA syndrome	DNAJC19	Type V	
		TMEM70 defect	TMEM70	Type IV	
	Component of the MICOS complex	QIL1 deficiency	QIL/ MIC13		
	Mitochondrial DNA deletion	Pearson/Kearns-Sayre			
	Subunit of mitochondrial import machinery	TIMM50 deficiency	TIMM50		
	Unknown	CLPB defect	CLPB		This chapter
		NOS 3-MGC-Aciduria	Unknown	Type IV	

NOS, not otherwise specified

18.4 Short/Branched Chain Acyl-CoA Dehydrogenase Deficiency

Isolated 2-methylbutyrylglycinuria, caused by 2-methylbutyryl-CoA dehydrogenase deficiency (MBD) and encoded by *ACDSB* (Fig. 18.1, enzyme 6), is an autosomal recessive disorder of isoleucine metabolism [86]. A few patients have been diagnosed following various clinical symptoms, and a set of asymptomatic subjects of Hmong descent were identified through newborn screening with elevated C5-acylcarnitine concentrations in blood spots. Detection of MBD deficiency in newborn screening is not limited to this population, and an increasing number of asymptomatic patients have been extensively investigated. Clinical relevance of this disorder remains in doubt and requires careful long-term follow-up of affected individuals. Theoretically, valproic acid should be avoided, as valproyl-CoA could be a substrate of MBD.

18.5 2-Methyl-3-Hydroxybutyryl-CoA Dehydrogenase Deficiency

Only a few patients with 2-methyl-3-hydroxybutyryl-CoA dehydrogenase (MHBD) deficiency (HSD10 disease) have been described. All male patients had an unusual neurodegenerative and progressive disease, and some affected females had psychomotor retardation and speech delay. Related women (mothers and grandmothers of patients) have shown mild to moderate developmental delay. In early childhood the severe neurodegenerative symptoms included rigidity, dystonic posturing, spastic diplegia, dysarthria, choreoathetoid movements, restlessness, cortical blindness, myoclonic seizures, brain atrophy, periventricular white matter and basal ganglia abnormalities. The majority of patients identified so far have had a severe progressive neurological phenotype rather than ketoacidotic attacks, in contrast to patients with a defect in the next step of isoleucine degradation attributable to 2-methylacetoacetyl-CoA thiolase deficiency. Nevertheless, one 6-year old boy has been diagnosed with MHBD deficiency in the course of a severe ketoacidotic crisis in the absence of any neurological symptoms thus mimicking 2-methylacetoacetyl-CoA thiolase deficiency [87].

MHBD deficiency (Fig. 18.1, enzyme 7) is a defect in the degradation of isoleucine but also in neurosteroid metabolism. MHBD is a multifunctional protein with an additional non-enzymatic role required for mitochondrial integrity and cell survival [87–89]. Laboratory findings include marked elevations of urinary 2-methyl-3-hydroxybutyrate and tiglylglycine without elevation of 2-methylacetoacetate. The organic acid excretion is more pronounced after a 100-mg/kg oral isoleucine challenge. Enzyme studies have shown markedly decreased activity of MHBD in fibroblasts and lymphocytes. MHBD deficiency is caused by mutations in *HADH2* on the X-chromosome. A short-term stabilisation of neurological symptoms and a biochemical response to an isoleucine-restricted diet have been observed in some patients [88, 89].

The deficiency of 2-methyl-acetoacetyl-CoA thiolase (Fig. 18.1, enzyme 8), also known as 3-ketothiolase or T2, is discussed in ▶ Chap. 13.

18.6 Isobutyryl-CoA Dehydrogenase Deficiency

The mitochondrial enzyme isobutyryl-CoA dehydrogenase (IBD) catalyses the third step in the degradation of valine (Fig. 18.1, enzyme 9). It is encoded by *ACAD8* [90]. Fewer than 20 patients with IBD deficiency have been described. Only the first patient, a 2-year-old, was diagnosed following the investigation of anaemia and dilated cardiomyopathy. Other patients have been identified following the expansion of newborn screening [90, 91]. This disorder can be detected on the basis of elevated butyrylcarnitine/isobutyrylcarnitine (C4-carnitine) concentrations in newborns' blood spots analysed by tandem MS. The presence of this metabolite, which is also present in short-chain acyl-CoA dehydrogenase deficiency, requires further investigation for precise diagnosis [91]. The possible clinical implication of this enzyme defect is not known, and to date most of the identified patients have remained asymptomatic. However, a few patients have moderate speech delay and careful follow-up is necessary.

18.7 3-Hydroxyisobutyric Aciduria

A few patients with increased excretion of 3-hydroxyisobutyric acid (3-HIBA), an intermediate of the catabolic pathways of valine and thymidine, have been identified. This condition may be linked to various enzymatic defects. Unfortunately, in most cases described, the enzymatic diagnosis has been speculative.

Clinical presentation is heterogeneous. Some patients present in infancy, with acute metabolic episodes with ketoacidosis, hypoglycaemia or hyperlactataemia. Muscle involvement and hypertrophic cardiomyopathy have been reported. CNS involvement is highly variable, ranging from normal development to brain dysgenesis observed in neonates.

Several enzyme defects may underlie 3-hydroxyisobutyric aciduria. However, only combined

deficiency of malonic, methylmalonic and ethylmalonic semialdehyde dehydrogenase (MMSDH) (◘ Fig. 18.1, enzyme 13) [92] and 3-hydroxyisobutyryl-CoA deacylase also called hydrolase deficiency (◘ Fig. 18.1, enzyme 11) have been identified [93]. Mutations in *ALDH6A1* encoding MMSDH were found in two unrelated developmentally delayed patients with 3-hydroxyisobutyric aciduria [94]. 3-hydroxy-isobutyryl-CoA hydrolase or deacylase, HIBCH (◘ Fig. 18.1, enzyme 11), deficiency was reported in patients presenting Leigh-like disease with elevated hydroxy-C4-carnitine and multiple mitochondrial respiratory chain defects and mutations in *HIBCH* [95] (► Chap. 10).

18.8 Malonyl-CoA Decarboxylase Deficiency

Malonyl-CoA decarboxylase (MLYCD) deficiency is a rare condition, with fewer than 30 cases reported, in which there is excessive excretion of malonic acid. MLYCD is usually expressed in fibroblasts or leukocytes, and various mutations have been reported in *MLYCD* [96]. A neonatal form has been described presenting with progressive lethargy, hypotonia, hepatomegaly, metabolic acidosis, and mild hyperammonaemia, variously associated with hypoglycaemia and/or hyperlactacidaemia. Cardiac failure due to cardiomyopathy was present in some patients at birth. In the late-onset forms, most have presented with acute metabolic episodes secondary to intercurrent infections. Some patients were previously known to be affected with a mild and nonspecific psychomotor retardation. Other children have been diagnosed following systematic screening for mental retardation and hypotonia. Cardiomyopathy was present in about 40%.

The physiological role of MLYCD, a cytosolic enzyme, could be in the regulation of cytoplasmic malonyl-CoA abundance and, thus, of mitochondrial fatty acid uptake and oxidation (◘ Fig. 18.1, enzyme 16). Patients with MLYCD deficiency display a number of phenotypes that are reminiscent of mitochondrial fatty acid oxidation disorders [96]. However, in contrast to these, dicarboxylic aciduria together with ketonuria is found during catabolic episodes and the patients exhibit normal ketogenesis on acute fat-loading tests.

The disorder is autosomal recessive. More than 20 mutations in *MLYCD* have been reported. No hotspot mutations have been identified. No genotype-phenotype relationship was detected, and siblings may have different presentations [96].

Total and free carnitine concentrations in plasma are low. Documented accumulation of malonylcarnitine would allow tandem MS screening of newborn blood spots. MLYCD activity has been found to be reduced in cultured fibroblasts and/or leukocytes of most defective cell lines, with residual activity of less than 10% of control [96].

No rules for treatment and prognosis have been established. Carnitine supplementation corrects the carnitine deficiency and may improve the cardiomyopathy and muscle weakness. Conversely, some patients have worsened despite carnitine supplementation and have recovered with a long-chain triglyceride-restricted/medium-chain triglyceride-supplemented diet [97]. Long-term prognosis is unknown. Except for the two patients who developed extrapyramidal signs following an acute crisis, most patients have residual mild developmental delay. There are subjects identified by newborn screening who remained asymptomatic at least during preschool age.

18.9 ACSF3 Deficiency

ACSF3 deficiency is a rare disorder presenting with combined malonic and methylmalonic aciduria (CMAMMA) due to mutations in *ACSF3*, which encodes a putative methylmalonyl-CoA and malonyl-CoA synthetase, a member of the acyl-CoA synthetase family [98]. Diagnosis relies on a characteristic profile of urinary organic acids, in which malonic and methylmalonic acids are constant findings. Abnormal succinic aciduria has been found in about half the cases, as have various dicarboxylic and glutaric acidurias. The disorder has been detected in a number of asymptomatic infants in the Quebec newborn urine screening programme and appears to be benign [98].

18.10 Short-Chain Enoyl-CoA Hydratase 1 (ECHS1) Deficiency

Short-chain enoyl-CoA hydratase (ECHS1) (◘ Fig. 18.1, enzyme 10), a mitochondrial matrix enzyme, active in valine catabolism and short-chain fatty acid β-oxidation, is immediately upstream of HIBCH in the valine pathway (◘ Fig. 18.1, enzyme 11). A recent literature review of 45 cases of ECHS1 deficiency caused by mutations in *ECHS1*, identified 4 main phenotypes: a severe rapidly fatal neonatal form with white matter abnormalities; a severe infantile form with developmental delay, pyramidal and extrapyramidal signs, optic atrophy, feeding difficulties, and degeneration of the deep gray nuclei; a similar but less severe infantile form with slower progression, and lastly a disorder characterised by paroxysmal exercise-induced dystonic attacks and isolated pallidal degeneration on MRI. Urine metabolite testing can distinguish between ECHS1 and HIBCH deficiencies. Blood acylcarnitine profile has been found normal in ECHS1 deficiency [99].

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Disorders of the Urea Cycle and Related Enzymes

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Contents

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Urea Cycle and Related Enzymes

The urea cycle is the main route for ammonia detoxification (Fig. 19.1). Its defects generally cause hyperammonaemia. The complete cycle is found only in periportal hepatocytes and involves two mitochondrial and three cytosolic enzymes as well as the mitochondrial ornithine/citrulline antiporter and the activating mitochondrial enzyme N-acetylglutamate synthase, which can turn the cycle on or off. In addition, liver mitochondrial carbonic anhydrase Va, the hepatic aspartate/glutamate mitochondrial antiporter citrin, and the intestinal enzyme Δ^1-pyrroline-5-carboxylate synthetase supply the cycle with, respectively, bicarbonate, aspartate, and, if needed, with ornithine made *de novo*. In extrahepatic tissues urea cycle enzymes make arginine from citrulline produced from ornithine or made from arginine by nitric oxide synthase. Thus, UCDs can also impact on arginine-derived functions including those of the nitric oxide system.

Introduction

Urea cycle disorders (UCDs) are genetic defects causing loss of function of any of the urea cycle (UC) enzymes carbamoyl phosphate synthetase 1 (CPS1), ornithine transcarbamylase (OTC), argininosuccinate synthetase (ASS), argininosuccinate lyase (ASL) and arginase (ARG1), the mitochondrial ornithine/citrulline antiporter (ORC1) and the CPS1-activating enzyme N-acetylglutamate synthase (NAGS). Their frequency is about 1:35,000 births, with at least 25% presenting in newborns [1]. Deficiencies of the mitochondrial enzymes NAGS, CPS1 and OTC primarily cause hyperammonaemia. Those of the extramitochondrial enzymes ASS, ASL and ARG1, and of the ORC1 antiporter also produce specific alterations in the levels of some amino acids that may be important in disease pathogenesis and are valuable for diagnosis. Deficiencies of the hepatic mitochondrial carbonic anhydrase Va (CAVA) and citrin aspartate/glutamate antiporter, as well as those of the bifunctional enzyme Δ^1-pyrroline-5-carboxylate synthetase (P5CS) can also cause hyperammonaemia by restricting the supply to the UC of bicarbonate, aspartate and *de novo* made ornithine, respectively. Inheritance of UCDs is autosomal recessive except P5CS deficiency, which can be dominant or recessive, and OTC deficiency, which is X-linked.

Acute hyperammonaemia, a clinical emergency caused by most UCDs, usually first manifests soon after birth with irritability, food refusal, vomiting, vegetative instability, muscular hypotonia, convulsions, somnolence, lethargy, coma and death or neurological sequelae if untreated. Partial UC enzyme deficiencies can result in a later onset of hyperammonaemia and/or poor appetite, vomiting, failure to thrive, developmental delay, cognitive impairment, abnormal behaviour or frank neurologic and/or psychiatric manifestations, as well as hepatomegaly and increased liver enzymes in plasma, or even acute liver failure. ARG1 deficiency rarely causes acute hyperammonaemia. Instead, it produces developmental delay, seizures and spastic diplegia. Increased plasma ammonia and glutamine and low citrulline characterise NAGS, CPS1 and OTC deficiencies. Amongst these, a high urinary excretion of orotic acid is only observed in OTC deficiency. ASS, ASL and ARG1 deficiencies present with a high plasma citrulline and characteristic plasma and urinary amino acid profiles.

Treatment of UCDs aims at rapidly lowering ammonia by minimizing ammonia production by using protein restriction and prevention of catabolism; and by maximizing ammonia removal by attempting to enhance residual UC function with arginine or citrulline where appropriate, by nitrogen scavenging using alternate pathway therapy with benzoate and/or phenylacetate or phenylbutyrate and by employing dialytic measures. Liver transplantation is curative or nearly so for most of these disorders but probably not for ASL deficiency. Administration of N-carbamylglutamate can replace the missing N-acetylglutamate in NAGS deficiency, virtually curing this deficiency. Citrin deficiency dramatically differs from other UCDs in that carbohydrates should be limited and high amounts of protein given.

19.1 Mitochondrial Urea Cycle Disorders

These comprise CPS1, OTC and NAGS deficiencies. Since the exclusive role of NAGS is to produce the essential activator of CPS1, N-acetyl-L-glutamate (NAG), NAGS deficiency is clinically indistinguishable from CPS1 deficiency. OTC deficiency is the most frequent urea cycle error (generally about 60% of UCD patients) whereas CPS1 and NAGS deficiency are very rare (respectively, 1:1,300,000 and <1:2,000,000 live births) [1].

Clinical Presentation

The main complication of any UCD in all age groups is acute hyperammonaemia, which clinically presents with encephalopathy. Since CPS1 and OTC catalyse the initial two steps of the UC, in which ammonia is converted to carbamoyl phosphate and then is incorporated into citrulline, these defects and NAGS deficiency tend to produce the most marked hyperammonaemia among all the UCDs [2].

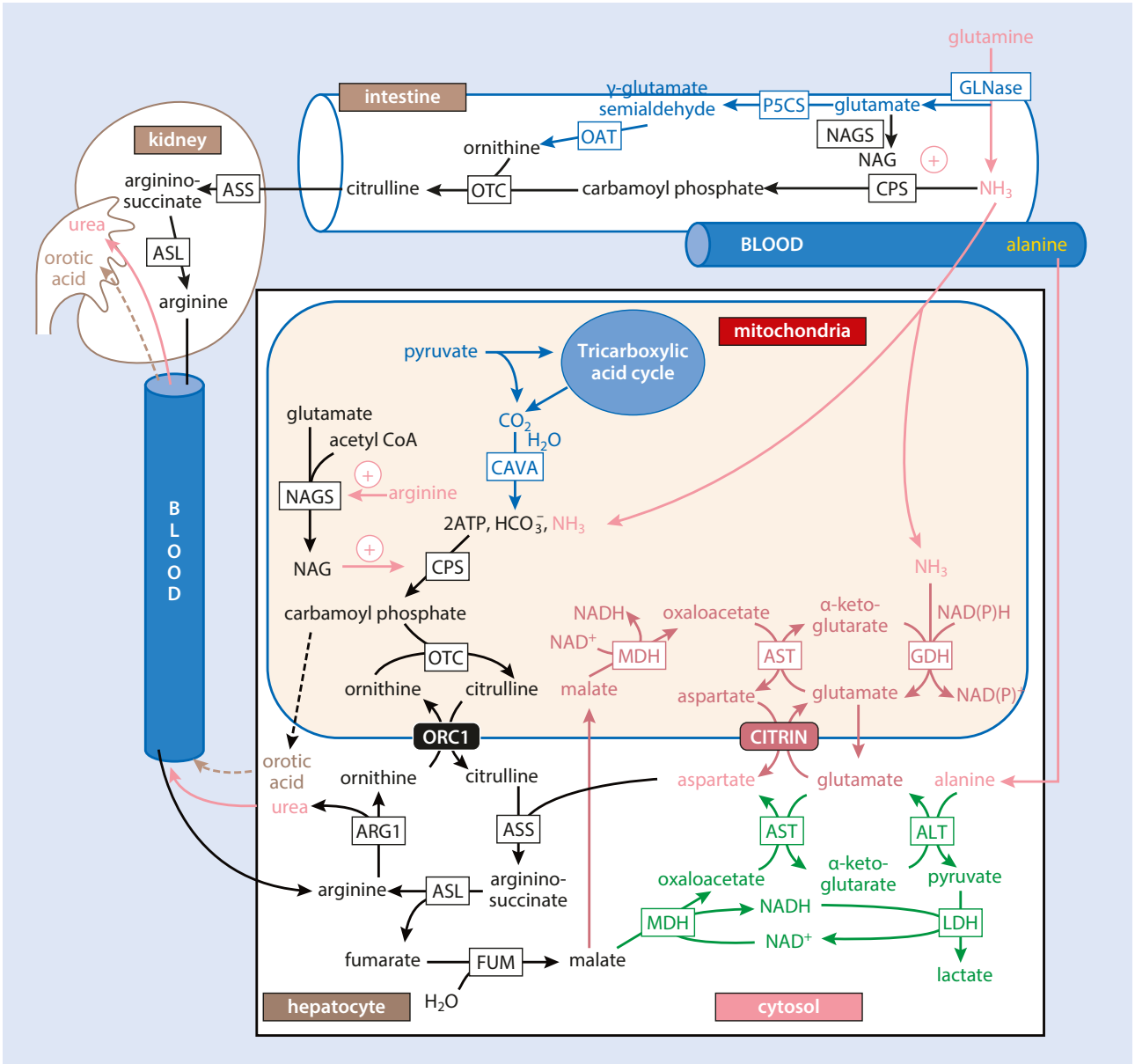

Fig. 19.1 The urea cycle. Metabolic scheme of the urea cycle (in *black*) and ancillary reactions (*coloured*). For simplicity not all the substrates and product of each reaction are shown. The contributions of the adult intestine and kidney to arginine synthesis are also shown in a highly simplified way. The major nitrogenous sources of the urea cycle (ammonia, glutamine, alanine and aspartate), as well as ornithine, and the product, urea, are coloured *red*. Provision and excretion reactions for these compounds are symbolized with red arrows. The reactions involved in the provision of cytosolic aspartate by the mitochondria are coloured *dark red*. The reactions of the aspartate cycle used to convert fumarate to aspartate in the cytosol are coloured *green*. ALT alanine aminotransferase, ARG1 arginase 1 (arginase 2 is extrahepatic; therefore it is not shown), ASL argininosuccinate lyase, ASS argininosuccinate synthetase, AST aspartate aminotransferase, CPS1 carbamoyl phosphate synthetase 1, CAVA carbonic anhydrase Va, CITRIN, aspartate/glutamate antiporter, FUM fumarase, GDH glutamate dehydrogenase, GLNase glutaminase, LDH lactate dehydrogenase, MDH malate dehydrogenase, NAGS N-acetylglutamate synthase, OAT ornithine ω-aminotransferase, ORC1 ornithine/citrulline antiporter, OTC ornithine transcarbamylase, P5CS Δ^1-pyrroline-5-carboxylate synthetase. The encircled plus signs (in *orange*) indicate the allosteric stimulations of *CPS1* by N-acetylglutamate (NAG) and of NAGS by arginine. The dotted line from carbamoyl phosphate indicates that several metabolic steps in the cytosol are required for orotic acid (in *brown*) production. In addition to citrin, other mitochondrial carriers exist for glutamate; they have not been specified. Malate can also access the mitochondria in several ways and, again, a specific carrier is not shown for it. The full malate-aspartate shuttle is not shown either (► Chap. 11). Note that CPS1, NAGS, and OTC are found exclusively in periportal hepatocytes and in enterocytes; that Citrin and CAVA are hepatic and that ARG1 is found in the liver and in red cells. *ASS* and ASL are more widespread and can be assayed in the liver, the kidney and in fibroblasts. P5CS is present in the intestine and fibroblasts, and ORC1 in the liver and in fibroblasts. For clarity, enzyme abbreviations are in italic lettering. However, in the text of this chapter, abbreviations in italics refer to the corresponding genes

Newborns

Newborn patients appear healthy at birth but may already present by day 2 with a rapidly progressing encephalopathy [3]. The clinical course is very similar to bacterial sepsis, which can lead to significant delay in the start of specific management for hyperammonaemia. Patients show vomiting, refusal to feed, somnolence/stupor/coma, muscular hypotonia, seizures, hyper- or hypoventilation, and hypo- or hyperthermia. Respiratory alkalosis is a common initial finding. In some patients with CPS1 or OTC deficiency signs of acute liver failure, including coagulopathy, may be found. High blood pressure may be a red flag for neonatal hyperammonemia that is not found in bacterial sepsis [4].

Children, adolescents and adults

Outside the newborn period the presentation may be variable. In most patients manifestations occur during or shortly after an intercurrent infection or other catabolic situations (e.g. fever, vomiting, diarrhoea, postpartum, surgery, rapid weight loss, treatment with steroids, chemotherapy), or following a high-protein meal (e.g. a barbecue). The symptoms are likewise highly variable, usually nonspecific and may be subtle or only episodic, although most commonly there is an unexplained change in consciousness (e.g. reduced vigilance, somnolence) or novel neurological signs (e.g. tremor, irritability, seizures) often mistaken for encephalitis, drug intoxication or brain tumour. In OTC deficiency, coagulopathy and other signs of acute liver failure are frequently present [5]. Patients, even of early age, may self-select a low-protein diet, refusing high-protein containing foods (e.g. meat, fish, dairy products). This contrasts with citrin deficiency, a UCD in which patients tend to crave protein and avoid carbohydrate.

Female carriers of OTC deficiency form a particular subgroup. Because of the variable individual inactivation of the X-chromosome hosting the mutant *OTC* (X-inactivation or lyonization phenomenon), they have highly variable, sometimes very low, residual OTC function. Thus, some are asymptomatic, others report symptoms over many years that are likely explained by recurrent undiagnosed hyperammonaemia [6], being often detected only after a male offspring is diagnosed, whereas still others present with frank deficiency.

Metabolic Derangements

In NAGS, CPS1 and OTC deficiencies citrulline production is reduced or abolished and this amino acid is consequently low or virtually absent from plasma. The failure to incorporate ammonia into carbamoyl phosphate (CP) explains the hyperammonaemia of CPS1 and NAGS deficiencies; in OTC deficiency the hyperammonaemia may be due to CPS1 inhibition by CP [7] that accumulates in the mitochondria [8]. Some CP leaks out of the mitochondria, leading to excessive pyrimidine biosynthesis and overproduction of orotic acid and uracil (▶ Chap. 32). The high urinary excretion of orotic acid and uracil differentiates conditions in which CP accumulates, such as OTC deficiency and the cytosolic UCDs, from those with expectedly low CP, such as CPS1 and NAGS deficiencies [9]. Of note, ornithine aminotranferase deficiency may mimic OTC deficiency in early infancy before ornithine increases (▶ Chap. 21). 3-Methylglutaconic aciduria has been reported to be found frequently in CPS1 deficiency [10]. This trait of mitochondrial dysfunction may not be specific for CPS1 deficiency since it has also been found, although with lower frequency, in other UCDs.

In UCDs, excessive ammonia is available for use by glutamine synthetase (present in perivenous hepatocytes, skeletal muscle and glial cells), and thus glutamine levels increase in plasma, CSF and tissue, provided 2-ketoglutarate is available (see also ▶ Chap. 24 ▶ Fig. 24.1). This increase can occur before, and can persist after, clinically manifest hyperammonaemia. Other non-essential amino acids that can be made from ammonia, such as glycine, serine, glutamate and, particularly, alanine, are also increased in plasma.

High ammonia levels are neurotoxic. Ammonia in its non-ionic form NH_3 crosses membranes and enters the brain, where it is in pH-dependent equilibrium with its more abundant non-permeable ionic form NH_4^+. Acute severe hyperammonaemia can cause seizures and coma with cerebral oedema and, if prolonged, permanent neurological sequelae. Cortical atrophy, dilated ventricles and demyelination [11], with neurocognitive delay and even cerebral palsy, are classical neurological deficits associated to hyperammonaemia. Chronic but more modest hyperammonaemia can cause impairment of executive functions, behavioural alterations and decreased IQ values. Increased brain ammonia causes excessive glial glutamine synthesis and accumulation, which may be a key element in astrocyte swelling [12] that is considered an important causative factor of the brain oedema that occurs in acute hyperammonaemia. Other ammonia-triggered water and electrolytic changes affecting glia [13] may also be involved in this swelling. Excess brain glutamine by itself may have neurotoxic effects, as well as ammonia, which interferes with K^+ buffering of astrocytes, leading to increased extracellular K^+ [14]. Many other ammonia-associated neural cell derangements have been postulated [15].

Genetics

Except *OTC*, which is X-linked, all other UCD genes are autosomal. These genes and their intronic regions vary widely in size, potentially contributing to the heterogeneity of possible genetic alterations. Disease-causing mutations in regulatory regions of some of these genes have been described and should be included in the diagnostics (see below).

Of the few reported *NAGS* mutations, c.1450T>C was found in several independent families, causing the change p.Trp484Arg and possibly in addition a splicing defect, with resultant severe NAGS deficiency [16]. In CPS1 and OTC deficiencies >260 mutations and >500 mutations have been found in the corresponding *CPS1* and *OTC* genes, with <10% recurrence [17, 18]. The Leiden Open (source) Variation Database (LOVD) is freely available and displays DNA variations for most UCD genes (► http://grenada.lumc.nl/LSDB_list/lsdbs).

Diagnostic Tests

Figure 19.2

Biochemical Assays and Enzyme Studies

The hallmark of these UCDs is hyperammonaemia, generally in the absence of hypoglycaemia, lactic acidosis or ketonuria. Plasma ammonia levels at presentation are usually in excess of 400–500 µmol/l. In severe encephalopathy they often exceed 1000 µmol/l (normal <150 µmol/l in newborns and <50 µmol/l outside the newborn period). An introduction to the

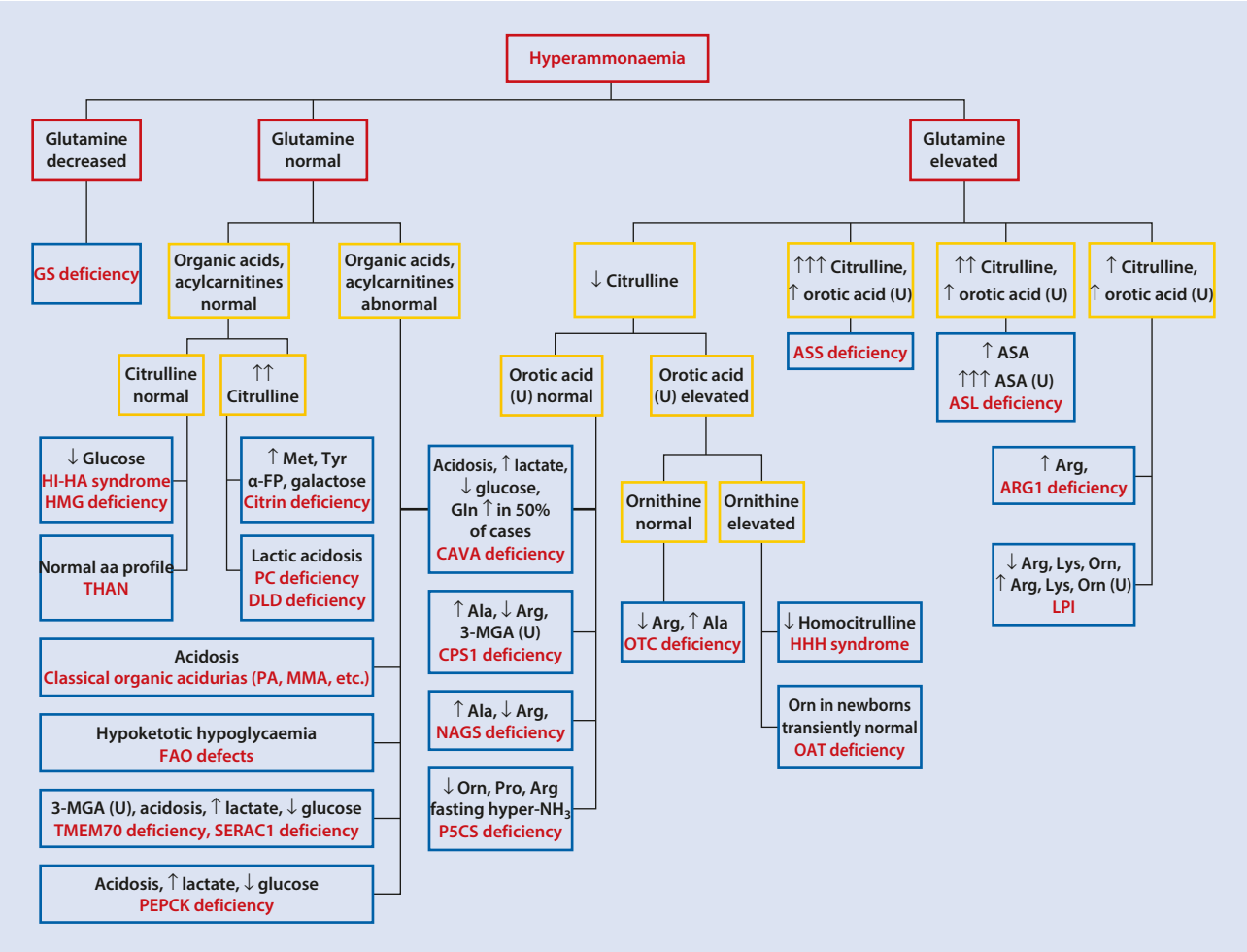

Fig. 19.2 Diagnostic algorithm that can be applied to any hyperammonaemic patient (see also ► Sect. 1.4.15 and ► Table 1.4). **Unless indicated, plasma is used for the analytical determinations.** α-FP alpha-fetoprotein, Ala alanine, ASA argininosuccinic acid, ASL argininosuccinate lyase, Arg arginine, ARG1 arginase 1, ASS argininosuccinate synthetase, CAVA carbonic anhydrase Va, CPS carbamoyl phosphate synthetase, DLD dihydrolipoamide dehydrogenase, FAO fatty acid oxidation, Gln glutamine, GS glutamine synthetase, HHH hyperornithinaemia-hyperammonaemia-homocitrullinuria, HI-HA hyperinsulinism-hyperammonaemia, HMG 3-hydroxy-3-methylglutaryl-CoA lyase, LPI lysinuric protein intolerance, Met methionine, 3-MGA 3-methylglutaconic aciduria, MMA methylmalonic aciduria, NAGS N-acetylglutamate synthase, OAT ornithine aminotransferase, Orn ornithine, OTC ornithine transcarbamylase, PA propionic acidaemia, PC pyruvate carboxylase, P5CS Δ^1-pyrroline-5-carboxylate synthetase, Pro proline, SERAC1 serine active site-containing protein 1, THAN transient hyperammonaemia of the newborn, TMEM70 transmembrane protein 70, Tyr tyrosine, U urine. (Figure modified from [3])

patient with hyperammonaemia is given in ▶ Sect. 1.4.15 and ▶ Table 1.4).

In addition there are characteristic alterations of the plasma amino acid profile in the absence of specific pathologic changes in the urine organic acids or blood acylcarnitine profiles; concentrations of glutamine (often >1000 μmol/l) and alanine (often >600 μmol/l) are increased while citrulline (often <10 μmol/l) and arginine (often <30 μmol/l) are decreased. For NAGS and CPS1 deficiencies there is no additional biochemical marker while in OTC deficiency increased urinary excretions of orotic acid and uracil are frequently found. A test with L-carbamyl-L-glutamate has been proposed to detect NAGS deficiency early [19] but this should not postpone the start of other treatment modalities. Although enzyme studies in a liver or intestinal biopsy (or even in plasma for OTC [20]) can confirm a diagnosis, such confirmation is now generally done by genetic means [3]. **Mutation analysis** is the gold standard for diagnosis and prenatal testing. It is crucial for distinguishing between NAGS and CPS1 deficiencies. Although sequencing is straightforward for NAGS deficiency since *NAGS* is small, it is recommended to include the 5′-upstream enhancer region, where one clinically relevant mutation was reported [21]. Conventional sequencing of *CPS1* exons is more difficult given the large number of exons. In addition, several intronic mutations are known that would be missed. cDNA sequencing in fibroblasts and lymphocytes is a less laborious and more sensitive alternative, although special techniques are necessary [22]. Many laboratories offer DNA sequencing of *OTC*, however, the mutation detection rate, even in patients with a clear biochemical diagnosis, is only about 85–90% [23], possibly because of the occurrence of promoter and enhancer mutations as well as due to the large intronic regions of the gene. Therefore, looking for mutations in promoter and enhancer, and other methods may be used to improve the diagnostic yield.

Allopurinol testing appears to have limited value [24] and has now been mostly superseded by molecular analysis.

Newborn screening is currently not available for mitochondrial UCDs [25] although combined use of low and high citrulline and orotate, respectively, in dry blood spots are used in Israel for OTC deficiency [26].

19

Treatment and Prognosis

Emergency Management

In acute hyperammonaemia, treatment must be initiated immediately. Even the need to confirm an elevated ammonia level must not delay the start of treatment. The principles of management are [3] (see also ▶ Chap. 4):

- Stop exogenous protein supply.
- Prevent endogenous protein catabolism by ensuring a high energy supply and avoiding deficiency of essential amino acids.
- Reduce ammonia, either with drugs alone, or together with dialysis (depending on the level of hyperammonaemia).

Stopping exogenous protein supply (for a maximum of 24–48 hours) requires concomitant provision of all the energy needed to prevent catabolism of endogenous proteins. Severe deficiency of essential amino acids triggers endogenous protein degradation and hence can sustain the hyperammonaemia, consequently it is undesirable to extend total protein restriction by more than 1–2 days. To achieve a sufficient energy supply, 10% glucose should be given. If central venous access is available, higher glucose concentrations can be infused. The aim in newborn patients is to supply at least 10 mg/kg/min of glucose (always with sodium and potassium to avoid free water infusion).

Parallel to the start of glucose infusions, intravenous short infusions of drugs to detoxify ammonia should be given. Sodium benzoate and/or sodium phenylacetate should be given at a dose of 250 mg/kg in 90–120 min followed by a maintenance infusion of these drugs at a dose of 250–500 mg/kg/d. Repeated short infusions, if needed, should be administered with caution to avoid drug toxicity [3]. In addition to nitrogen scavenging drugs, L-arginine should be given at a dose of 250 mg/kg in 90–120 min, followed by a maintenance dose of 250 mg/kg/d. Blood or plasma ammonia must be determined at least every 3 hours until the acute situation is successfully managed. If at any time during the crisis the ammonia level escalates to >500 μmol/l or if the patient is encephalopathic (seizures, severely reduced consciousness or coma), dialysis should be started as soon as possible [3, 27]. The method will depend on the experience of the local metabolic centre but haemodiafiltration or haemodialysis have been proven most efficient while peritoneal dialysis with currently used solutions may be considered only if a more effective dialytic technique cannot be applied or for bridging to this more effective technique. Any patient with acute hyperammonaemia should be transferred to a metabolic centre experienced with UCDs and capable of undertaking dialysis, but treatment with the available measures should be initiated in the primary center and should be maintained during transfer.

Maintenance Treatment

NAGS deficiency is the only UCD for which drug treatment is almost curative: N-carbamyl-L-glutamate (also known as carglumic acid), a synthetic analogue of the physiological activator of CPS1, NAG, given orally

activates CPS1 and thereby urea cycle function. The recommended maintenance treatment is 100 mg/kg/d in 3 doses before meals. However, it may be possible to reduce the amount given by titrating the required dose in each patient. Some patients may need as little as 10 mg/kg/d. Most patients will remain stable with this treatment but they should still avoid excess protein; additional nitrogen scavenging drugs may be needed during catabolic events (intercurrent diseases, fever, etc.).

Patients with severe CPS1 and OTC deficiencies are prone to recurrent hyperammonaemic crises. Those with complete enzyme deficiency, with very low protein tolerance or frequent metabolic crises despite treatment, should undergo liver transplantation as soon as it is possible and safe (>3 months of age and/or >5 kg body weight). Until then, aggressive conservative measures must be employed aimed to preserve mental function [3].

Conservative treatment requires a low-protein diet in most patients, although trying to approach the FAO/WHO recommendations for daily protein intake [26, 28], thus making necessary the individual titration of protein tolerance. To avoid dietary deficiencies, essential amino acids, vitamins, and trace elements should be monitored regularly and supplemented as needed. In addition, most patients will require nitrogen scavenging drugs. Oral sodium benzoate (an unlicensed medication, although it is used in low amounts in the food industry and as a pharmaceutical excipient) and/or oral sodium phenylbutyrate or oral glycerol phenylbutyrate (a more palatable alternative to standard preparations of sodium phenylbutyrate; there is a sugar-encapsulated sodium phenylbutyrate preparation that is taste-neutral), are recommended, each given in an amount of 200–250 mg/kg/d divided in 3 doses (in the case of glycerol phenylbutyrate 5–12.4 g/m^2/d divided in 3 doses) [3, 29]. To maximize the residual urea cycle function, L-arginine and/or L-citrulline (both chemicals or food supplements and not licensed drugs) are given in a daily oral dose of 100–200 mg/kg, divided in 3 doses [3].

Outcome

Patients with mitochondrial UCDs manifested during the newborn period have a significant risk of death [30] or, if they survive, of learning difficulties [31]. Both survival and neurocognitive outcome largely depend on the duration and the extent of the hyperammonaemia. Several strategies have been proposed to improve outcome, including education of health care professionals for increasing awareness, establishment of metabolic centres, and automatic 'red flags' in the emergency departments for certain situations [32], in addition to ensuring the availability of routine and rapid determination of ammonia in an emergency setting [3]. Liver transplantation, although preventing further hyperammonaemia, will not restore mental function if lost [33, 34].

Pregnancy and Postpartum Period

Most female OTC patients go through pregnancy without any problems but their increasing extra protein needs (first trimester 1 g/day; second trimester 10 g/day; third trimester 31 g/day) should be met. The postpartum period also requires special attention for all UCDs, as several case reports on fatal crises highlight the particular risk during this period of severe protein catabolism [35, 36].

19.2 Cytosolic Urea Cycle Disorders

This group of disorders is the second most frequent among the UCDs and includes ASS and ASL deficiencies, each representing about 15% of the UCD patients, and ARG1 deficiency, representing 3% [1].

Clinical Presentation

Newborns

Newborn presentations of ASS and ASL deficiencies closely resemble those of mitochondrial UCDs (► Sect. 19.1), with hyperammonaemic encephalopathy of similar severity, although peak plasma ammonia may not be as high and the onset delayed to day 6–7 of life or even later [3]. At the other end of the clinical spectrum are patients who may have been detected by newborn screening but who are asymptomatic [37, 38]. ARG1 deficiency only rarely presents in the newborn period, either with neonatal hyperammonaemia and/or cholestasis [reviewed in 39].

Children, adolescents and adults

Outside the newborn period, the symptoms in patients with ASS and ASL deficiencies are similar to those of mitochondrial UCDs (► Sect. 19.1). ASS deficiency has been reported presenting with acute liver failure [40], treated in some patients with liver transplantation, although other patients recovered with conservative management. ASS deficient patients are at particular risk of developing acute hyperammonaemia in the post-partum period [36] and in other severe catabolic circumstances. In contrast, ASL deficient patients are less prone to recurrent hyperammonaemic decompensation but can still develop intellectual disability, seizures and chronic hepatopathy [30]. Marked hepatomegaly can be a presenting sign mimicking hepatic glycogenosis. Arterial hypertension is also sometimes found in adolescents and adults with ASL deficiency [41]. Brittle hair due to trichorrhexis nodosa is almost pathognomonic for ASL deficiency, resulting

from arginine deficiency and responding well to arginine administration. The clinical picture of patients with ARG1 deficiency is entirely different, being characterised primarily by developmental delay with neurological and intellectual impairment, growth retardation and spastic tetra- or diplegia [42]. This last manifestation starts in late infancy and is progressive if plasma arginine levels remain elevated. Many patients with ARG1 deficiency have seizures and may even develop status epilepticus.

Metabolic Derangements

The impairment of the UC at the level of ASS, ASL and ARG1 explains the characteristically elevated plasma and urinary levels of citrulline, argininosuccinate and arginine in the corresponding deficiency of each of these enzymes, and the decreased level of arginine in ASS and ASL deficiency (prior to treatment with L-arginine). Inhibition of ASS by argininosuccinate or by arginine [43] accounts for the increased citrulline levels in ASL and ARG1 deficiencies, although the increase is generally lower than in ASS deficiency. The normal or increased citrulline levels differentiate extramitochondrial from mitochondrial UCDs. The high renal clearance of argininosuccinate explains the lower relative increase in the plasma levels of this amino acid in ASL deficiency than the increase of citrulline in ASS deficiency (typically 1000-fold increase). In ARG1 deficiency, the presence in extrahepatic tissues of a second arginase (ARG2) may explain the relatively modest increase (about 15-fold) of plasma arginine, the normal or near-normal plasma ornithine, and the presence of urea, which, nevertheless, is generally decreased [42].

Citrulline and argininosuccinate include in their molecular structure one molecule of ornithine and, respectively, one and two atoms of waste nitrogen. Consequently, the abundant urinary excretion of these intermediates in ASS and ASL deficiencies effectively removes waste nitrogen, although with simultaneous loss of two (ASS deficiency) or one (ASL deficiency) ornithine molecules per urea equivalent. This renders the supply of ornithine an essential determinant of how much waste nitrogen is excreted in ASS and ASL deficiencies, justifying the administration of arginine (converted to ornithine upon cleavage by arginase) [44]. In line with the poorer waste nitrogen-carrying capacity of citrulline than that of argininosuccinate, hyperammonaemic crises are more frequent in ASS deficiency than in ASL deficiency and appear to be due to a secondary impairment of OTC because of the poor availability of ornithine, which is reflected in the frequent observation of increased orotic acid excretion during these crises. Interestingly, orotic acid excretion is also frequently elevated in ARG1 deficiency [42], possibly reflecting increased CP production because of overactivation of the NAGS-CPS1 axis (arginine is a NAGS activator [45]), compounded with decreased ornithine availability for the OTC reaction in the liver.

ASS and ASL have a paramount role in the recycling to arginine of the citrulline produced when nitric oxide (NO) is made by NO synthase. ASL also belongs to an intracellular membrane-bound protein complex that channels exogenous arginine to NO synthase, and its mutations can prevent such channelling [46]. Inadequate NO synthesis and other toxic factors, perhaps argininosuccinate or its derivative guanidino compounds such as guanidinosuccinate, may be involved in the pathogenesis of ASL deficiency and explain the more important neurocognitive alterations than in other UCDs [47]. Animal studies and preliminary data in humans suggest that drugs that supply NO might be beneficial in ASL deficiency [48].

In ARG1 deficiency, the mild and sporadic hyperammonaemia does not account for the spastic diplegia and the seizures, suggesting that central nervous system toxicity of increased arginine or its metabolites (polyamines, guanidino compounds, NO, agmatine) is a crucial pathogenic factor. Spastic paraplegia is a common feature of ARG1 deficiency, of P5CS deficiency (▶ Sect. 21.3) and of the HHH syndrome (▶ Sect. 21.2), all 3 processes in which ornithine delivery to the mitochondria or arginine/ornithine balance is impaired [49]. Seizures could be due to either accumulation of guanidinoacetate, a known proepileptogenic compound [50], and/or to the secondary imbalance between glutamate and γ-amino-butyric acid (GABA), given the important interconnections between ornithine and glutamate metabolism [51] (▶ Chap. 30).

Genetics

DNA is generally used for genetic analysis of these disorders. The existence of pseudogenes restricts cDNA studies of *ASS1* to fibroblasts and the liver. One common mutation has been described in classical ASS deficiency (c.1168G>A, p.Gly390Arg) in patients from all ethnic backgrounds [52]. Common mutations in mild ASS deficiency (most frequent, c.535T>C, p.Trp179Arg and c.1085G>T, p.Gly362Val) have mainly been found in Turkish patients [52]. There are few recurrent *ASL* mutations associated with a severe phenotype (c.857A>G, p.Gln286Arg is the most frequent) or with milder clinical course (e.g. c.532G>A, p.Val178Met) [53]. Intragenic complementation [54] complicates determination of disease-causality of individual mutations. Mutations in *ARG1* (>60 reported) are mainly private, with few being recurrent [55]. See ▶ http://grenada.lumc.nl/LSDB_list/lsdbs for listing of mutations.

Diagnostic Tests

Biochemical assays

The mainstay of biochemical diagnosis of cytosolic UCDs is the plasma amino acid profile. Markedly elevated citrulline levels are highly suggestive of ASS deficiency, with few alternatives to consider in the differential diagnosis, namely the deficiencies of ASL, citrin, pyruvate carboxylase, and dihydrolipoamide dehydrogenase (see also ► Chap. 3, ► Table 3.2). Plasma citrulline levels >500 µmol/l are pathognomonic for ASS deficiency. Similarly, the presence of argininosuccinate (and/or its two anhydrides) in plasma and urine is pathognomonic for ASL deficiency. In ARG1 deficiency elevation of arginine in plasma is characteristic. Levels are often not very high in newborns but they increase during infancy and often reach >500 µmol/l in untreated patients.

Enzyme studies

In ASS deficiency, ASS activity is rarely assayed. In ASL and ARG1 deficiencies, red blood cells are an easily accessible source for direct enzyme assays but conflicting results for ASL have been reported [56]. Overall, enzyme studies are currently not standard for confirmation of the diagnosis of a cytosolic UCD but are a valuable tool if mutation analysis fails.

Mutation analysis

Mutation analysis is now used to confirm the diagnosis and to offer future prenatal testing. In ASS deficiency, it is performed with a high success rate by sequencing the 14 coding *ASS1* exons and flanking intronic regions. To improve the detection rate, RNA from fibroblasts (but not from blood cells due to the expression of pseudogenes) can be used. The same approach with a similar excellent detection rate can be applied in ASL deficiency and in ARG1 deficiency, where RNA studies using lymphocytes further improve the diagnostic yield.

Newborn screening

Newborn screening for ASS and ASL deficiencies can be incorporated in the amino acid profile determined routinely by tandem-MS/MS. Elevation of citrulline and the presence of argininosuccinate respectively suggest ASS and ASL deficiency; elevations of arginine are suggestive for ARG1 deficiency but not present in all patients. Based on the experience in some newborn screening programs, there are concerns that patients with mild or asymptomatic disease might be subjected to unnecessary treatment [37, 56], rendering further studies essential.

Treatment and Prognosis

Emergency management follows the same principles as for mitochondrial UCDs, with identical dosages of infusions and medications (► Sect. 19.1), except for ASL deficiency, for which a short infusion of L-arginine is given at up to 400 mg/kg over 90–120 min, followed by maintenance infusion of up to 400 mg/kg/day, since the response of some patients is very rapid and renders additional drugs unnecessary.

Maintenance treatment for ASS and ASL deficiencies is as for CPS1 and OTC deficiencies, although, particularly in patients with ASL deficiency, there is a lower risk of recurrent metabolic crises. Nevertheless, a low-protein diet is required in most patients including its supplementation with essential amino acids, vitamins, and trace elements. Nitrogen scavenging drugs are usually needed for metabolic stability: 200–250 mg/kg/day sodium benzoate and/or sodium phenylbutyrate or glycerol phenylbutyrate), distributed in 3 equal doses. To enhance partial urea cycle function, L-arginine (but not L-citrulline) should be given at a dose of 100–300 mg/kg/day in 3 dosages. To minimize potential argininosuccinate toxicity in ASL deficiency a reduced dose (in comparison to earlier literature) of arginine is recommended (100–300 mg/kg/day) [3].

Liver transplantation prevents hyperammonaemia but it does not reverse neurological damage. It should be considered for patients with poor metabolic control despite compliance with conservative therapy, or those with liver failure. In ARG1 deficiency L-arginine and L-citrulline must not be given, and the aim is to lower plasma arginine to <200 µmol/l, although this goal is difficult to achieve because of the strict protein restriction required. Recently, clinical trials testing recombinant pegylated human arginase for treatment of ARG1 deficiency were started (► ClinicalTrials.gov Identifier: NCT03921541). Although the experience is limited, liver transplantation in ARG1 deficiency appears not only to normalize urea cycle function but also to prevent further progression of neurological disease [57].

Outcome

Survival is better than for mitochondrial UCDs. However, especially in ASL deficiency, neurocognitive morbidity is similarly poor [2] and executive functions may be impaired despite good metabolic control [34]. Patients with ARG1 deficiency have an important risk of progressive spastic paraplegia [42].

19.3 Urea Cycle Mitochondrial Transporter Defects

Two mitochondrial transporter defects can cause disruption of the urea cycle.

19.3.1 Hyperornithinemia, Hyperammonaemia and Homocitrullinuria (HHH) Syndrome

See ▶ Chap. 21, ▶ Sect. 21.2.

19.3.2 Citrin Deficiency

Citrin deficiency (also known as AGC2 deficiency) is due to lack of function of the hepatic mitochondrial aspartate/glutamate antiporter citrin, which can supply cytosolic aspartate for the ASS reaction. Citrin deficiency, initially identified in Japan as citrullinaemia type II (CTLN2) [58], is largely a Far East disease with a heterozygosity frequency in Japan of about 1/40. It is now considered a panethnic disorder with patients having been found in other populations (see for example [59]), although at much lower frequency (<1:2,000,000 live births in the West) [1].

Clinical Presentation

There are two main age-dependent clinical presentations: Neonatal Intrahepatic Cholestasis Caused by Citrin Deficiency (NICCD) and citrullinaemia type II (CTLN2) which occurs in adolescents and adults. A third, less common, clinical phenotype, Failure To Thrive and Dyslipidemia Caused by Citrin Deficiency (FTTDCD) may also occur in childhood. Both are linked to *SLC25A13* mutations.

Newborns

A large proportion of newborns with citrin deficiency develop neonatal intrahepatic cholestasis with persistent jaundice, failure to thrive, hepatomegaly and cholestasis [60]. Anaemia, low plasma albumin and total protein, impaired coagulation with prolonged prothrombin time, raised serum AST, hypoglycaemia, galactosaemia and galactosuria are frequent findings. Plasma citrulline is moderately increased (levels are often in the range of 100–500 μmol/l) and other amino acids are also raised (methionine, threonine, tyrosine, serine and/or phenylalanine). These may be detected by newborn screening, although some patients with NICCD have had negative screening results. For most patients liver disease and biochemical abnormalities resolve by 12 months of age.

Childhood

Many children remain well during childhood and have normal plasma amino acids and liver function. After 1 year of age, most patients develop a characteristic craving for protein-rich/fat-rich foods and avoidance of carbohydrate-rich foods and sugars. A proportion of those over a year of age develop FTTDCD. Poor appetite, fatigue, growth retardation, low quality of life scores, hypercholesterolaemia affecting both HDL- and LDL-cholesterol, hypoglycaemia, and modest increases of plasma citrulline and ornithine are the main manifestations. Some patients develop recurrent pancreatitis or hepatoma.

Adolescents and adults

CTLN2 can follow a period of normal health lasting several decades (usual age of onset, 20–50 years, although cases have presented in the 11–79 years range), and predominantly affects males (2.4:1 male/female ratio in Japan). It is characterised by neuropsychiatric symptoms of sudden onset, recurrent ammonia intoxication that can lead to coma and death, and high plasma citrulline levels (100–500 μmol/l). Alcohol, sugars (carbohydrate toxicity) or catabolic insults such as surgery or infection may be triggering events. Glycerol administration for cerebral oedema leads to further deterioration. Steatohepatitis and some liver fibrosis are frequent, while hypertriglyceridaemia, chronic pancreatitis and hepatoma are potential complications. Food preferences are as in FTTDCD.

Metabolic Derangements

The exchange of mitochondrial aspartate for cytosolic glutamate and the malate/aspartate shuttle (MAS) (◘ Fig. 19.1 and ▶ Chap. 11 ▶ Fig. 11.1), of which citrin is a part, are both affected [61]. There is insufficient supply of mitochondrial aspartate for ASS within hepatocytes. Furthermore, the cytosolic conversion of the fumarate released by ASL to form aspartate is impaired, due to the low cytosolic NAD resulting from MAS disturbance. The low cytosolic aspartate decreases liver ASS activity, resulting in citrulline accumulation, and also impairs protein and pyrimidines synthesis of liver cells, since these two processes also are cytosolic and use aspartate. Protein synthesis impairment results in liver failure explaining the hypoalbuminaemia and hypoproteinaemia of NICCD while pyrimidine synthesis defect explains the lack of urinary orotic acid that differentiates citrin deficiency from classical ASS deficiency. The high cytosolic NADH/NAD ratios in the liver explain the hypoglycaemia and the galactosaemia that are frequently observed in NICCD, since cytosolic NAD is needed both for gluconeogenesis from lactate and for UDP-galactose to UDP-glucose conversion. Sugars, glycerol and alcohol are toxic in citrin deficiency because their metabolism in liver cells increases the cyto-

solic NADH/NAD ratio, magnifying the cytosolic NAD deficiency [61].

Hyperammonaemia develops in CTLN2 but not in NICCD. Liver ASS is significantly decreased (for unknown reasons) in CTLN2, whereas it is normal in NICCD [58, 61]. Classically, it has been believed that this decrease in ASS, combined with the poor aspartate supply, leads to dysfunction of the UC [61]. However, a new viewpoint has been put forward [62] in which the lack of a functional MAS compromises energy production in liver cells, leading to low hepatocyte ATP, which also impairs ASS activity and glutamine synthetase activity, (▶ Sect. 24.1 on glutamine synthetase deficiency). In any case, decreased glutamine synthetase activity can be the reason for the absence of glutamine increase in CTLN2 in the presence of hyperammonaemia (◘ Fig. 19.2). Arginine levels are normal or even elevated in CTLN2 and NICCD, probably reflecting normal extrahepatic ASS activity and a supply of aspartate by another mitochondrial carrier that catalyzes the aspartate/glutamate exchange (aralar or AGC1) and that is not expressed in the liver [63] (▶ Sect. 11.11).

In agreement with the role of decreased energy production in the liver in citrin deficiency, energy provision by medium chain triglycerides (MCTs) appears the most effective therapy of citrin deficiency [62]. Metabolism by the liver of MCT escapes the tightly regulated carnitine palmitoyltransferase I pathway and does not need cytosolic NAD. If there is secondary galactosaemia, lactose should be eliminated from food. Pyruvate administration is also used for therapy [61], based on the ability of pyruvate to decrease the NADH/NAD ratio and to be an energy source that, in contrast to glucose, does not need cytosolic NAD to be utilised. Arginine also appears beneficial in CTLN2. Perhaps the ornithine produced in the liver from arginine speeds the OTC reaction, increasing the intrahepatic citrulline level, secondarily lowering the apparent K_m for aspartate of human ASS [64], thus improving ASS activity at suboptimal aspartate concentrations.

Genetics

Citrin deficiency is due to mutations in *SLC25A13* and shows recessive inheritance. Many of the >100 disease-associated *SLC25A13* variants reported (▶ http://www.hgmd.cf.ac.uk/ac/gene.php?gene=SLC25A13) are splicing mutations, frameshifts and premature stops that truncate or ablate parts of the citrin molecule [58]. A few mutant alleles predominate in given populations such as c.1177+1G>A and c.851-854del in Japan (70% of the disease alleles) or c.851-854del, c.615+5G>A, IVS16ins3kb (c.1750+72_1751-4dup17ins), and c.1638_1660dup23 among Chinese (>80% of the alleles in a large patient cohort). A number of alleles are shared by different Far East populations, while the p.Arg360* mutation appears to be widespread [59].

A frequency of 1/17,000 homozygotes or compound heterozygotes for disease-causing alleles has been estimated for Japan, similar to the NICCD frequency in that country, indicating full penetrance for this clinical form, but much more frequent than CTLN2 (1/100,000–1/230,000 in Japan) [58]. A lower penetrance among females than among males can account for the lower frequency of CTLN2 in women than in men.

Diagnosis

Biochemical assays

In newborns with intrahepatic cholestasis the finding of increased plasma citrulline without significant hyperammonaemia, with normal or elevated levels of arginine and without urinary orotic acid, particularly with a high plasma level of alpha-fetoprotein and/or increased galactose in blood and urine, is strongly suggestive of NICCD [59]. In patients identified by neonatal screening with increased blood citrulline, it is important to first exclude ASS deficiency. Plasma ammonia and glutamine and urinary orotic acid are high in severe ASS deficiency but not in NICCD, and the reverse is true for arginine. Alpha-fetoprotein is increased in NICCD only. If tyrosine and alpha-fetoprotein are elevated, succinylacetone should be assayed to exclude tyrosinaemia type 1.

A specific diagnosis of FTTDCD is difficult to make unless NICCD had been diagnosed previously. The presence of dyslipidemia is the paramount chemical indicator of FTTDCD, although increased citrulline levels and high lactate/pyruvate ratios can also be suggestive biomarkers. Nevertheless, blood citrulline levels can be increased by other factors such as by eating watermelon [65] or in renal failure [66]. CTLN2 can be differentiated from classical ASS deficiency by the lack of increase in the level of plasma glutamine (◘ Fig. 19.2) and the normal or somewhat increased arginine level in citrin deficiency, these levels being respectively high and low in ASS deficiency, and by the absence of urinary orotic acid in CTLN2.

Protein studies

Western blots of lymphocytes or cultured fibroblasts using antibodies that recognize the N-terminal moiety of citrin generally detect little or no cross-reactive immune material in most patients with citrin deficiency (but see [67]).

Mutation analysis

Mutation detection in both copies of *SLC25A13* is the gold standard for diagnosis. In populations with prevalent mutations, the affected alleles can be searched first. RNA analysis is possible in cultured fibroblasts and peripheral blood lymphocytes [68].

Newborn screening

NICCD can be suspected in newborns presenting in the screening with elevated citrulline or galactosaemia, hypermethionaemia, tyrosinaemia or hyperphenylalaninaemia, particularly in regions with a high carrier ratio (Far East countries) [58]. Recently, a pilot study done in China on 237,630 newborns combined metabolic screening for citrulline levels with genetic screening for 28 *SLC25A13* mutations in those newborns with normal-high or high citrulline levels (about 30,000 newborns). They used a high-throughput iPLEX genotyping assay, identifying five NICCD patients [69].

Treatment and Prognosis

Emergency management of hyperammonaemic episodes in CTLN2 should avoid carbohydrate or glycerol infusions, because they worsen the hyperammonaemia [58, 61]. Mannitol infusion to combat brain oedema appears safe. Measures to rapidly decrease ammonia such as haemodialysis can be used, as well as administration of intravenous sodium benzoate and sodium phenylacetate. Arginine appears beneficial and should be administered. Energy should be provided while restricting the carbohydrate supply by using MCTs and amino acids. In NICCD the initial treatment may require packed red blood cells [59] and albumin for anaemia and hypoproteinaemia, respectively, and supportive measures for coagulopathy and liver insufficiency when present and severe. It is also essential to provide a lactose-free formula particularly if galactosaemia is observed, with some reduction in the fraction of calories provided by sugars in favour of MCTs and protein. Levels of fat-soluble vitamins and Zn should be monitored and supplemented when needed.

Maintenance treatment of NICCD involves the use of lactose-free and MCT-enriched formula, relaxing this use with clinical improvement or at the end of the first year. When introduced, other foods should be protein-rich and fat-rich, such as eggs or fish. In CTLN2, the diet should follow the patient preferences for high protein/fat and low carbohydrate. Arginine (5–15 g/day reported) and sodium pyruvate (3–6 g in three 2 g-dosages reported) can be given [58], although presently the mainstay for maintenance therapy is MCTs administration providing about 20% of the daily caloric needs. Alcohol must be prohibited. Vigilance for hepatocellular carcinoma is necessary. If steatohepatitis and neuropsychiatric manifestations do not improve, liver transplantation provides a permanent cure. For FTTDCD a protein and MCT-rich and carbohydrate-poor diet with no lactose is recommended. Sodium pyruvate may improve growth. It is a chemical, although calcium pyruvate is sold as a food supplement.

Outcome of NICCD is generally good with little intervention. The prognosis for CTLN2 has been poor without liver transplantation. Presently the awareness of avoiding glucose or glycerol infusions and the introduction of MCTs and possibly also of arginine and pyruvate appear to have improved the outcome of conservative treatment.

19.4 Urea Cycle Defects Due to Deficiencies of Ancillary Enzymes

19.4.1 Δ^1-Pyrroline-5-Carboxylate Synthetase (P5CS) Deficiency

P5CS, encoded by *ALDH18A1*, catalyses the first common step of *de novo* ornithine and proline synthesis. Its deficiency has been reported to cause a fasting hyperammonaemia and cutis laxa syndrome with global developmental delay (De Barsy syndrome), and/or spastic paraplegia, with the peculiarity of having dominant or recessive inheritance and frequent *de novo* mutations (reviewed in [51]). This deficiency is described in ▶ Chap. 21.

19.4.2 Carbonic Anhydrase Va (CAVA) Deficiency

There is little experience on this disease as only 20 patients have been reported thus far [70–75]. However, the disorder may be more frequent than appreciated, since it was found in 10 out of 96 patients with early-onset hyperammonaemia in whom genetic diagnosis of other UCDs had failed [71].

Clinical Presentation

Most patients have developed neonatal symptoms identical to those with neonatal onset UCDs, presenting with hyperammonaemic encephalopathy within the first days after birth. In contrast to other neonatally presenting UCDs, all but one patient survived without sequelae, including those patients requiring haemodialysis. Surprisingly, the patient who died [73] presented brain oedema with increased intracranial pressure and brain herniation when the blood biochemical parameters had normalized, casting doubts on the reasons for the brain oedema, as arterial hypertension was reported in this 18-month infant. The majority of patients have had only a single hyperammonaemic crisis; those who had a second one had a milder episode [70, 71]. No symptoms have yet been described in children, adolescents or adults.

Metabolic Derangement

Bicarbonate cannot cross the mitochondrial membrane, and the spontaneous conversion of CO_2 to bicarbonate can be too slow for the needs of urea synthesis. CAVA accelerates this conversion within liver mitochondria, supplying the bicarbonate used intramitochondrially by CPS1, pyruvate carboxylase, propionyl CoA carboxylase and 3-methylcrotonyl CoA carboxylase. Therefore, CAVA deficiency impairs the urea cycle, gluconeogenesis and branched-chain amino acid metabolism, yielding an unusual combination of biochemical findings [76]. These include hyperammonaemia, decreased plasma citrulline and absence of urinary orotic acid, hypoglycaemia, metabolic acidosis, high plasma lactate and urinary ketone bodies, and a urinary profile of organic acids containing carboxylase-related metabolites (► Chap. 27). The apparently successful empirical use of carbamylglutamate could be justified if the affinity of CPS1 for bicarbonate were increased when the enzyme is saturated by its essential allosteric activator, acetylglutamate (of which carbamylglutamate is an analogue).

Genetics

CAVA is caused by mutations in *CA5A*. A deletion of exon 6 (c.619-3421_774+502del) may be prevalent in patients from the Indian subcontinent [71, 76]. A locus specific homepage can be found at: ► http://databases.lovd.nl/shared/genes/CA5A.

Diagnostic Tests

Biochemical assays should include plasma ammonia, blood lactate and urine ketone bodies, in addition to blood glucose and plasma amino acid profiles. Organic acids in the urine should be analysed to search for carboxylase metabolites. Blood acylcarnitine profiles are normal in this disorder [76].

Enzyme studies would require analysis of liver tissue and thus mutation analysis is used to confirm CAVA deficiency.

Mutation analysis. The first mutations were reported in 2014 [70]. *CA5A* is small (only seven coding exons) but requires careful design of oligonucleotide primers because of highly homologous sequences of pseudogenes. As the gene is mainly expressed in the liver, use of RNA for analysis would require invasive sampling and therefore has not been reported.

Treatment and Prognosis

Emergency management for CAVA deficiency is mainly symptomatic, focusing on treating hyperammonaemia as for intramitochondrial UCDs (► Sect. 19.1). N-carbamyl-L-glutamate (100 mg/kg as a single oral dose, possibly repeated after 4–6 hours) can be considered as some patients apparently responded well. Otherwise, management is standard, including sufficient fluid and energy substitution, balance of acid-base status and of glucose homeostasis, and protein restriction during hyperammonaemia.

Maintenance treatment was not required in the patients reported in the literature as all interventions could be reduced soon after recovery from the metabolic crisis. Nevertheless, an emergency plan should be provided along with appropriate family counselling.

Outcome in CAVA deficiency generally appears excellent but observations in more patients are needed.

19.5 Transient Hyperammonaemia of the Newborn (THAN)

The term THAN refers to a condition mostly described in premature infants (but sometimes also in term newborns), which was often found together with respiratory distress syndrome. The cause of THAN is not clear but some authors suggest shunting of blood via the open ductus venosus (transporting high levels of ammonia up to 300 µmol/l) and thus escaping clearance by the hepatic UC [77, 78]. In this condition ammonia may be greatly increased (>3000 µmol/l), whilst glutamine is usually in the reference range resulting in a plasma ratio of glutamine/ammonia <1.6 [79]. Therapy of asymptomatic patients might not be necessary; in symptomatic patients, extracorporeal detoxification and drug therapy has been described but the benefit from this is not clear [78, 80]. If additional complications are not present, outcome is much better than in classical UCD patients with similar extent of hyperammonaemia.

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Disorders of Sulfur Amino Acid Metabolism

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Contents

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Sulfur Amino Acid Metabolism

The essential amino acid methionine (Met) is converted by two methionine adenosyltransferases (MAT I/III and MAT II) to S-adenosylmethionine (AdoMet); a small proportion is catabolized by the transamination pathway via the intermediate 2-keto-4-methylbutyrate (◘ Fig. 20.1). The methyl group of AdoMet is used in numerous biologically important methylation reactions, yielding S-adenosylhomocysteine (AdoHcy); excess methyl groups of AdoMet are removed from the cycle by glycine N-methyltransferase (GNMT). AdoHcy is cleaved by S-adenosylhomocysteine hydrolase (SAHH) to homocysteine and adenosine, which is a substrate for various reactions including phosphorylation by adenosine kinase (ADK). Homocysteine can be converted back to methionine by the folate/vitamin B_{12}-dependent remethylation pathway or by betaine-homocysteine methyltransferase (BHMT) using betaine as a methyl-group donor. Alternatively, homocysteine is irreversibly metabolized to sulfate, starting with the transsulfuration pathway. Homocysteine is condensed with serine to form cystathionine, which is subsequently cleaved to form cysteine, α-ketobutyrate and ammonia; these reactions are catalysed by cystathionine β-synthase (CBS) and cystathionine γ-lyase (CTH), respectively, which can both also act on cysteine and/or homocysteine to synthesize the signalling molecule hydrogen sulfide (H_2S). Cysteine is used for the synthesis of glutathione (GSH; ▶ Chap. 31) and taurine (via cysteine sulfinate, catalysed by cysteine dioxygenase, CDO). Cysteine can also be converted to pyruvate and hydrogen sulfide by aspartate aminotransferase (AST) and 3-mercaptopyruvate sulfurtransferase (MPST). Hydrogen sulfide produced in these reactions or by gut microbiota is oxidized in mitochondria. Oxidation starts with formation of glutathione persulfide, catalysed by sulfide:quinone oxidoreductase (SQOR), followed by oxidation to sulfite by persulfide dioxygenase (PDO, a product of the *ETHE1* gene) and interconversions between sulfite and thiosulfate involving PDO and thiosulfate transferase (TST) [1]. Finally, sulfite is oxidised to sulfate by sulfite oxidase (SUOX), which requires the molybdenum cofactor (MoCo). This is synthesised via cyclic pyranopterin monophosphate (cPMP) by enzymes encoded by the molybdenum cofactor synthesis 1, 2 and 3 genes (*MOCS1*, *MOCS2* and *MOCS3*) and by the gene for gephyrin (*GPHN*). Inorganic sulfur released from cysteine residues by a series of reactions is also used in the formation of mitochondrial iron-sulfur (FeS) cluster cofactors (see ▶ Chap. 10 for details).

Introduction

Disorders of sulfur amino acid metabolism can affect methionine demethylation, homocysteine (Hcy) remethylation (▶ Chap. 28), Hcy transsulfuration (including classical homocystinuria), glutathione synthesis (▶ Chap. 31) or cysteine/hydrogen sulfide oxidation. There may be altered blood or urinary concentrations of methionine, AdoMet, sarcosine, AdoHcy, total Hcy and cysteine, cystathionine, hydrogen sulfide, sulfite, thiosulfate or adenosine.

The commonest methionine demethylation disorder is MAT I/III deficiency: this is often benign but can cause neurodevelopmental problems. Other features of demethylation disorders include liver disease (GNMT deficiency), myopathy (SAHH and ADK deficiencies), facial dysmorphism (ADK deficiency) or halitosis (MTO and MAT I/III deficiency). Methionine restriction may be beneficial in some patients with MAT I/III or ADK deficiency. CBS deficiency (classical homocystinuria) is the commonest disease in this group. Its severity ranges from a multisystemic childhood condition (with lens dislocation, osteoporosis, marfanoid features, central nervous system and vascular complications) to an isolated thromboembolic disease in adults. Treatment is primarily with pyridoxine in pyridoxine-responsive patients and a low-methionine diet ± betaine in non-responders. Treatment can prevent most complications even in pyridoxine non-responsive patients if they are diagnosed by neonatal screening.

The other transsulfuration disorder, CTH deficiency, appears to be benign.

Disorders of cysteine and hydrogen sulfide oxidation include SQOR deficiency, ethylmalonic encephalopathy, isolated SUOX deficiency and combined SUOX/xanthine oxidase deficiency due to impaired molybdenum cofactor (MoCo) synthesis. These are severe disorders with early-onset seizures or neurological complications; other problems may include diarrhoea, petechiae, acrocyanosis, lens dislocation or urolithiasis. MoCo deficiency type A can be treated with a synthetic precursor of the cofactor and ethylmalonic encephalopathy may profit from liver transplantation.

20.1 Methionine S-Adenosyltransferase Deficiency (Mudd's Disease)

20.1.1 Clinical Presentation

A few patients have presented with neurodevelopmental problems or malodorous breath but most patients have been detected by newborn screening programs for cystathionine beta-synthase (CBS) deficiency using methio-

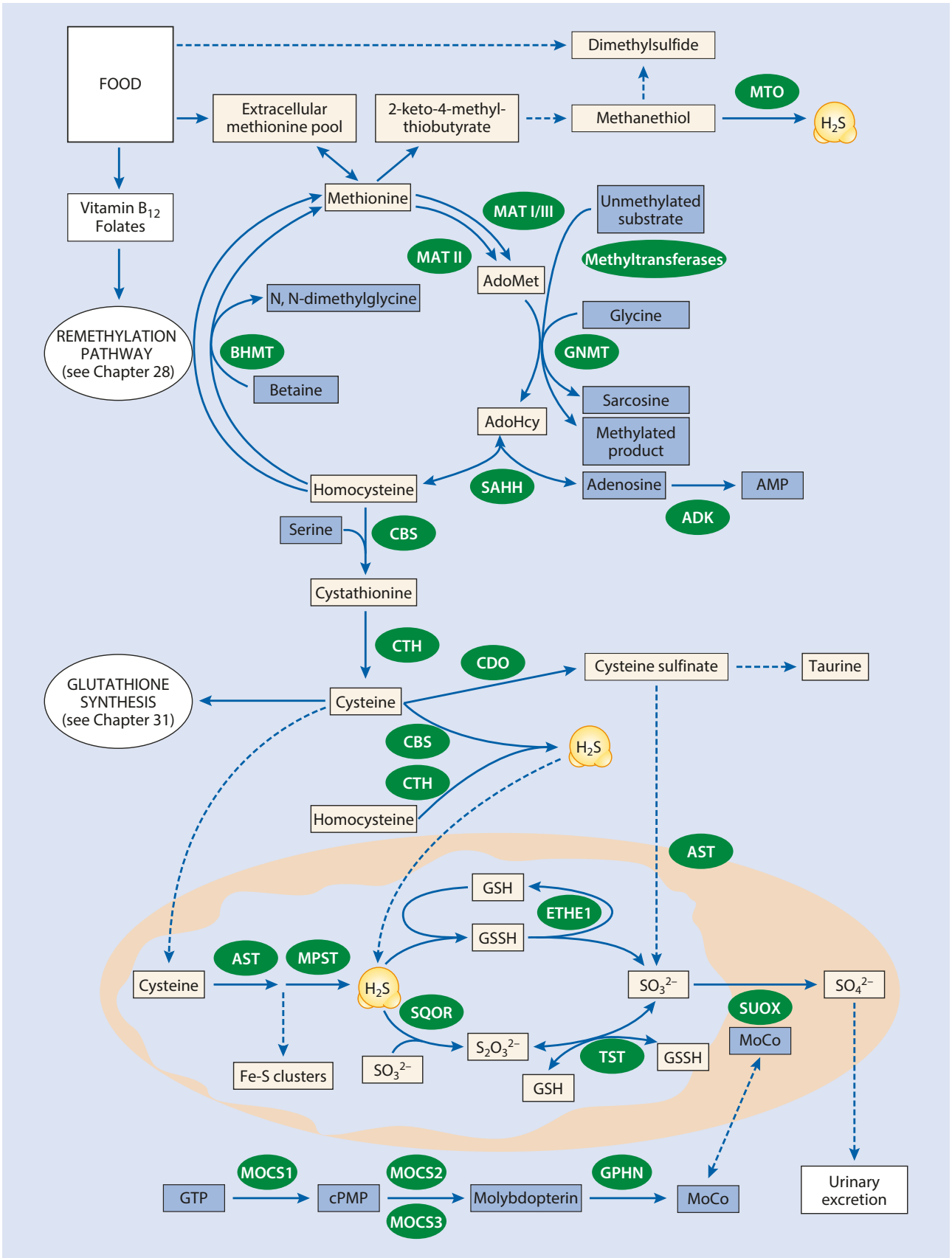

Fig. 20.1 Sulfur Amino Acid Metabolism. Sulfur-containing metabolites are marked in yellow, enzymes or genes in green, full lines and curves with arrows indicate typical direction of metabolite fluxes, dashed lines indicate transport across membranes or multi-step reactions . See text for abbreviations

nine as a marker. Some of these patients have developed neurological symptoms, such as learning difficulties or dystonia, or hypomyelination on neuroimaging. Neurological abnormalities have occurred in most patients with plasma methionine concentrations above 800 µmol/L, whereas they have been rare in subjects with lower levels [2, 3].

20.1.2 Metabolic Derangement

Methionine S-adenosyltransferase converts Met to AdoMet using ATP. MAT exists in 3 enzyme forms. MAT I and III are encoded by the same gene; they are tetrameric and dimeric forms, respectively, and are both liver-specific. MAT II is encoded by a different gene and converts methionine to AdoMet throughout the body, explaining why MAT I/III deficiency is relatively benign.

20.1.3 Genetics

MAT I/III deficiency is inherited as an autosomal recessive trait. Several mutations (e.g. p.R264H) exhibit a dominant negative effect; hypermethioninemia in these cases is inherited as an autosomal dominant trait and is benign.

20.1.4 Diagnostic Tests

In MAT I/III deficiency, the plasma methionine concentration ranges from 50 to >2000 µmol/L. Excess Met is transaminated to the malodorous compound dimethylsulfide [3]. Other causes of hypermethioninaemia include liver disease of various etiology, prematurity and, less often, CBS, SAHH and ADK deficiencies or an excessive intake of methionine (for details see the Table 20.1 and [3]). CBS deficiency can usually be dis-

Table 20.1 Biochemical Findings in Inborn Errors of Sulfur Amino Acid Metabolism

Condition-enzyme deficiency	Concentrations in plasma/serum					Other tests
	Methionine µmol/L	tHcy µmol/L	AdoMet nmol/L	AdoHcy nmol/L	Cystathionine* nmol/L	
Typical low and high limits of reference ranges	L:12–15 H:40–45	L:5–7 H:10–15	L:50–80 H:120–170	L:10–20 H:40–80	L:50–80 H:350–500 (1000 in newborns)	
Defects of methionine demethylation						
MAT I/III deficiency	↑↑	n-↑	n-↓	n	n-↑	P-sarcosine n-↑ dimethylsulfide in exhaled air ↑
MTO deficiency	n	N/A	N/A	N/A	N/A	Dimethylsulfoxide, dimethylsulfone, dimethylsulfide, methanethiol in exhaled air and/or body fluids ↑-↑↑
GNMT deficiency	↑-↑↑	n-↑	↑↑	n	↑	P-sarcosine normal despite ↑↑ AdoMet
SAHH deficiency	n-↑↑	n-↑	↑↑	↑↑	n	S-creatine kinase ↑, P-sarcosine ↑
ADK deficiency	n-↑↑	n-↑	↑-↑↑	↑-↑↑	N/A	U-adenosine ↑
Defects of transsulfuration						
CBS deficiency	n-↑↑	↑-↑↑	↑-↑↑		n-↓	Met/cystathionine ratio ↑, S-sarcosine ↑, P-tCys ↓, P-folate n-↓, P-H_2S n-↑, P,U-homolanthionine ↑ – ↑↑, P,U-thiosulfate and sulfite n-↑
CTH deficiency	n	n-↑	n	n	↑↑	P-H_2S normal
Defects of cysteine and H_2S metabolism						
SQOR deficiency	N/A	n-↑[a]	N/A	N/A	n-↑[a]	Findings in episodes: B, U-lactate↑, U-ketone bodies↑, S-creatine kinase ↑, B-glucose ↓; findings outside of episodes[a]: P-sulfite n- ↑, P-H_2S n-↑

(continued)

Table 20.1 (continued)

Condition-enzyme deficiency	Concentrations in plasma/serum					Other tests
	Methionine µmol/L	tHcy µmol/L	AdoMet nmol/L	AdoHcy nmol/L	Cystathionine* nmol/L	
Ethylmalonic encephalopathy	n	n-↓	n	n	n-↑	P-tCys, P,U- sulfite and thiosulfate↑↑, B-butyryl- and glutaryl-carnitines↑, P- H_2S ↑-↑↑
SUOX deficiency	n	↓-↓↓	n	↑	n-↑	P-tCys↓-↓↓, P,U-sulfite, S-sulfocysteine and thiosulfate ↑↑, P- H_2S n-↑; P,U-hypotaurine ↑, P,U-taurine n-↑
Molybdenum cofactor synthesis defects	n	↓-↓↓	n	n	n-↑	P-tCys ↓-↓↓ P,U-sulfite, S-sulfocysteine and thiosulfate ↑↑, P,U-taurine n-↑, P- H_2S n-↑, S-uric acid, ↓↓ U-xanthine and hypoxanthine ↑-↑↑
Inborn errors in the remethylation pathway (for details see ► Chap. 28)						
MTHFR deficiency	n-↓	↑-↑↑	n-↓	↑-↑↑	n – ↑↑	P-folate n-↓, CSF-folate ↓
cblE, cblG, cblD- var.1	n-↓	↑-↑↑	n-↓	↑-↑↑	n – ↑↑	U-formininoglutamic acid and AICAr n- ↑-↑↑
Combined cbl defects: cblC, cblD, cblF, cblJ	n-↓	↑-↑↑	n-↓	↑-↑↑	n – ↑↑	B-propionylcarnitine ↑ – ↑↑, B-methylmalonylcarnitine n – ↑, B,S,U-MMA ↑↑, S-vitamin B_{12} n-↓
Secondary disorders of sulfur amino acid metabolism						
Vitamin B_{12} deficiency	n-↓	↑ – ↑↑	n-↓	↑	n – ↑↑	S-vitamin B_{12} and holoTCII ↓-↓↓; B-propionylcarnitine ↑ – ↑↑, B-methylmalonylcarnitine n – ↑, B,S,U-MMA ↑ – ↑↑
Folate deficiency	n-↓	↑ – ↑↑	n-↓	↑	n – ↑↑	S-folate↓-↓↓, P-sarcosine n-↑↑, U-formininoglutamic acid and AICAr n-↑-↑↑
NRF2 superactivity	N/A	↓	N/A	N/A	N/A	See ► Chap. 31
Liver disease including IEMs affecting liver function	n – ↑↑	n-↑	n-↑	n-↑	n-↑	e.g. U-4-hydroxy-phenylpyruvate and 4-hydroxy-phenylacetate ↑↑ in tyrosinemia type I
Renal failure	n	↑ – ↑↑	↑ – ↑↑	↑↑	↑ – ↑↑	Serum 2-methylcitrate ↑↑ > ↑ MMA

B blood, *P* plasma, *S* serum, *U* urine, *CSF* cerebrospinal fluid, *N/A* data not available, ↓ metabolite decreased, ↑ and ↑↑ metabolite increased and markedly elevated, *,↓ and ↑ cystathionine concentrations detectable only by sensitive LC-MS or GC-MS methods (↑↑ elevated cystathionine levels detectable also by amino acid analyzer), *AICAr* 5-Aminoimidazole-4-carboxamide riboside, *cbl* cobalamin complementation group, *cblD-var.1* remethylation defect cblD- variant without methylmalonic aciduria, *holoTCII* holotranscobalamin II (active vitamin B_{12}), *NRF2* nuclear factor erythroid 2–related factor 2, *MMA* methylmalonic acid, *MTHFR* methylenetetrahydrofolate reductase, *tHcy* total homocysteine, *tCys* total cysteine; other abbreviations as in the legend to Fig. 20.1

[a]unpublished data (V.Kožich, A.Kuster et al, manuscript under revision)

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tinguished by measuring the plasma total homocysteine (tHcy) though, surprisingly, tHcy is often slightly increased in MAT I/III, SAHH and ADK deficiencies. Plasma AdoMet and AdoHcy levels are usually normal in MAT I/III deficiency, whereas both these metabolites are increased in SAHH, ADK and CBS deficiencies. The diagnosis is generally confirmed by mutation analysis because the enzyme assay requires a liver biopsy.

20.1.5 Treatment and Prognosis

A methionine- or protein-restricted diet is recommended in patients with plasma methionine levels above 800 µmol/L, aiming to achieve methionine levels around 500–600 µmol/L. As yet, however, there is limited clinical evidence of benefit. Improved myelination has also been reported after treatment with oral AdoMet [2].

20.2 Methanethiol Oxidase Deficiency

20.2.1 Clinical Presentation

Methanethiol oxidase deficiency was described in five patients with a cabbage-like smelling breath from three sibships [4]. The odour was observed in all five patients. Additional neurological problems were observed in patients from two consanguineous families but the role of MTO deficiency in the neurological complications is uncertain. This condition is probably under-diagnosed due to the limited availability of the specialized metabolite assays.

20.2.2 Metabolic Derangement

Methanethiol (methylmercaptan, CH_3SH) is a malodorous gas produced in catabolism of methionine by human tissues and by intestinal microbiota. It is absorbed into the circulation and converted by MTO to formaldehyde, H_2O_2 and H_2S. Deficient MTO activity results in accumulation of methanethiol and its metabolites, dimethylsulfide, dimethylsulfoxide and dimethylsulfone, in body fluids [4]; due to their volatility these malodorous compounds appear in the breath.

20.2.3 Genetics

MTO deficiency is an autosomal recessive trait due to biallelic pathogenic variants in the selenium-binding protein 1 gene (*SELENBP1*).

20.2.4 Diagnostic Tests

Diagnosis of extraoral halitosis due to MTO deficiency requires specialized assays demonstrating increased concentrations of methanethiol and dimethylsulfide in exhaled breath and of dimethylsulfone and dimethylsulfoxide in urine, and/or demonstrating MTO deficiency in cultured fibroblasts. Analysis of *SELENBP1* may now be the initial diagnostic test for MTO deficiency.

20.2.5 Treatment and Prognosis

Metronidazole reduces the concentration of malodorous compounds and dietary methionine restriction may be another treatment option.

20.3 Glycine N-Methyltransferase Deficiency

20.3.1 Clinical Presentation

The five published patients with glycine N-methyltransferase (GNMT) deficiency [5] showed mild to moderate elevation of plasma aminotransferases, although values fluctuated and were sometimes within the reference range. Two siblings had mild hepatomegaly and a liver biopsy in one of them showed mild centrilobular fibrosis. Liver histology was normal in a patient without hepatomegaly. There were no other consistent symptoms.

20.3.2 Metabolic Derangement

GNMT is a liver enzyme involved in the degradation of excess methionine, in particular removing excess AdoMet by donating its methyl group to glycine forming sarcosine. Its deficiency causes the accumulation of methionine and AdoMet while plasma sarcosine is normal.

20.3.3 Genetics

GNMT deficiency is inherited as an autosomal recessive trait.

20.3.4 Diagnostic Tests

GNMT deficiency can be distinguished from other causes of hypermethioninaemia by demonstrating grossly increased plasma AdoMet levels with normal sarcosine and AdoHcy and a raised AdoMet/AdoHcy ratio. Assay of GNMT activity is complicated by its liver specificity. Molecular genetic analysis of *GNMT* is recommended to confirm the diagnosis.

20.3.5 Treatment and Prognosis

The biochemical abnormalities in one patient resolved on a low methionine diet (300 mg/d) [5] but the three other patients were healthy on a normal diet at 14, 16 and 17 years of age (personal communications R. Cerone and P. Augoustides-Savvopoulou). Thus, the human disorder appears to be largely benign and not to warrant dietary treatment. In line with MAT I/III deficiency,

very high methionine levels, e.g. above 1000 μmol/L may be a reason to initiate a methionine restricted diet.

The GNMT knockout mouse develops steatosis that progresses to steatohepatitis, cirrhosis and hepatocellular carcinoma. Nicotinamide is an acceptor for the methyl-group of AdoMet; its administration lowered AdoMet levels and prevented steatosis and liver fibrosis in these mice. Patients with GNMT deficiency should, therefore, have follow-up with monitoring of liver function. Treatment with nicotinamide may be considered if they develop complications.

20.4 S-Adenosylhomocysteine Hydrolase Deficiency

20.4.1 Clinical Presentation

S-Adenosylhomocysteine hydrolase (SAHH) deficiency has been reported in more than 10 patients [6, 7]. All had a severe myopathy, with hypotonia and usually with raised plasma creatine kinase values; several patients died of respiratory failure in infancy. The survivors all had delayed psychomotor development and some had strabismus; neuroimaging usually showed hypomyelination. Most patients had hepatomegaly and/or liver disease with coagulopathy, and this was probably responsible for fetal hydrops in two siblings. Hepatocellular carcinoma may be an additional complication [8].

20.4.2 Metabolic Derangement

SAHH activity is present in all cells and converts AdoHcy to homocysteine and adenosine. In SAHH deficiency, AdoHcy accumulates and inhibits a number of essential methyltransferase reactions.

20.4.3 Genetics

SAHH deficiency is inherited as an autosomal recessive trait.

20.4.4 Diagnostic Tests

Patients with SAHH deficiency have increased plasma AdoMet and grossly elevated AdoHcy levels. Plasma methionine concentrations are also usually raised, with slightly increased tHcy. The diagnosis can be confirmed by enzyme analysis in red blood cells or fibroblasts or by molecular genetic analysis of *AHCY*.

20.4.5 Treatment and Prognosis

A methionine- or protein-restricted diet will decrease and sometimes even normalize plasma AdoMet and AdoHcy [6, 7, 9] and may be beneficial if started early in life. Phosphatidylcholine and creatine supplements have been given because AdoHcy is formed during the synthesis of these molecules. Successful treatment with liver transplantation has also been reported [9].

20.5 Adenosine Kinase Deficiency

Adenosine kinase (ADK) deficiency has been reported in 27 patients [8, 10, 11, 12]. ADK converts adenosine to AMP (adenosine monophosphate). In ADK deficiency, adenosine accumulation leads to increased urinary adenosine excretion, raised plasma AdoMet and AdoHcy concentrations, usually accompanied by increased plasma methionine, and slightly increased tHcy levels. Raised AdoHcy levels are likely to inhibit essential methyltransferase reactions and AMP deficiency will impair ADP and ATP synthesis (for details see ► Chap. 32).

20.6 Cystathionine β-Synthase Deficiency

20.6.1 Clinical Presentation

This disorder is also known as classical homocystinuria. Its clinical spectrum was extensively studied by Mudd [13] and has been reviewed recently with data from the international E-HOD registry [14]. The severity and age at presentation vary markedly and correlate with the patient's response to pyridoxine. Responses can be classified as partial, full or extreme, based on the fall in homocysteine and the pyridoxine dose required [14]. Pyridoxine non-responders have a severe childhood-onset multisystem disease, whereas patients extremely responsive to pyridoxine usually present as adults with an isolated thromboembolic event; those with a lesser degree of responsiveness have an intermediate phenotype. The clinical features predominantly involve four organ systems:

- **Eye**

Dislocation of the lens (ectopia lentis) is the most characteristic finding. In untreated pyridoxine non-responsive patients, it usually occurs between 2 and 12 years of age; it occurs later in pyridoxine responders. It may be preceded by severe (>5 dioptres) or progressive lenticular myopia, which is unusual outside this condition [15]. Following dislocation of the lens, movement of the eye may cause the iris to tremble (iridodonesis) and there is a risk of glaucoma.

Skeleton

Most pyridoxine non-responsive patients have excessive growth, particularly around puberty, with elongation and thinning of long bones, enlarged epiphyses (especially at the knee) and arachnodactyly. This is often called a 'marfanoid' habitus but the joints are stiff, in contrast to Marfan syndrome. Other deformities include genu valgum, pes cavus, and pectus excavatum or carinatum. Adults have premature osteoporosis, which may lead to scoliosis and pathological fractures.

Brain

Approximately half the patients have learning difficulties. Problems usually become apparent in early to mid-childhood. Patients are often clumsy and some have progressive dystonia or seizures. Psychiatric problems are common even in those treated from birth and correlate with low IQ [16].

Vascular System

Deep venous thrombosis is the commonest vascular problem in adults and may lead to pulmonary embolism [13]. Cerebral venous sinus thrombosis is particularly frequent in children with homocystinuria but can occur at any age. In adults, there is also an increased risk of carotid and renal artery thrombosis; the risk of coronary heart disease is increased to a lesser degree.

Rarer complications include spontaneous pneumothoraces and pancreatitis. The skin and hair may show hypopigmentation that is reversed by treatment.

It is likely that many extremely pyridoxine-responsive patients remain asymptomatic throughout life, particularly if they have a relatively high pyridoxine intake. This includes c.833T>C (p.I278T) and c.341C>T (p.A114V) homozygotes [14]. These patients may have been under-represented in Mudd's review of the natural history of CBS deficiency.

20.6.2 Metabolic Derangement

Cystathionine beta-synthase — a cytosolic tetrameric enzyme — is expressed predominantly in liver, pancreas, kidney and brain. Its activity can also be determined in cultured fibroblasts and in plasma due to its release from the liver [17]. Its catalytic domain binds heme and the cofactor pyridoxal 5′phosphate (PLP) in addition to its substrates; the regulatory domain binds the allosteric activator AdoMet.

The pathophysiology is not fully understood. CBS deficiency leads to the accumulation of AdoHcy and homocysteine, enhanced remethylation to methionine and AdoMet, and depletion of cystathionine and cysteine. Accumulated homocysteine itself may elicit endoplasmic reticulum stress, alters intracellular signalling and modifies sulfhydryl groups on proteins; increased AdoHcy impairs methylation reactions; and decreased cystathionine and cysteine are associated with apoptosis, oxidative stress and alterations of structural proteins. Decreased concentration of hydrogen sulfide was not seen in patients′ plasma, and in contrast signs of enhanced compensatory synthesis of H_2S from accumulating Hcy by CTH were reported [18].

The elevated homocysteine is thought to be responsible for thromboembolism and vascular disease. The connective tissue abnormalities are often also attributed to elevated homocysteine but, as they are rare in disorders of homocysteine remethylation, decreased cysteine is probably at least partly responsible [19]. Animal models with *CBS* deletions are lethal due to severe hepatopathy but the knock-in models recapitulate in part the human phenotype, including connective tissue involvement [20].

20.6.3 Genetics

CBS deficiency is inherited as an autosomal recessive disease and occurs worldwide. Its prevalence is 1:1800 in Qatar, ~1:100,000–200,000 in populations of European descent and much lower in African and Asian populations [21, 22]. More than 160 mutations are included in the CBS Mutation Database [23]. Mutations associated with a severe pyridoxine non-responsive phenotype include c.572 T>C (p.T191M) in patients from Iberian Peninsula and South America, c.919G>A (p.G307S) in patients of Irish and British ancestry, c.1006C>T (p.R336C) in Qatari patients, c.1224-2A>C, (p.W409_G453del or deletion of exon 12) in Central Europe and Turkey, and c.969G>A (p.W323X) in Saudi Arabs. Mutations associated with pyridoxine responsiveness include the pan-ethnic mutation c.833T>C (p.I278T), as well as the c.341C>T (p.A114V) and c.146C>T (p.P49L) variants that were observed in patients with the extremely pyridoxine responsive form of the disease [24, 14].

CBS mutations affect in vivo activity by various molecular mechanisms including decreased stability of mutant enzymes due to misfolding and dysregulated activation by AdoMet. The molecular mechanism of pyridoxine responsiveness remains unclear, although stabilization of the mutant enzyme by the chaperoning effect of PLP seems most plausible. Other small osmolytes and ligands, including heme, have chaperone activity and may lead to novel forms of treatment [25].

20.6.4 Diagnostic Tests

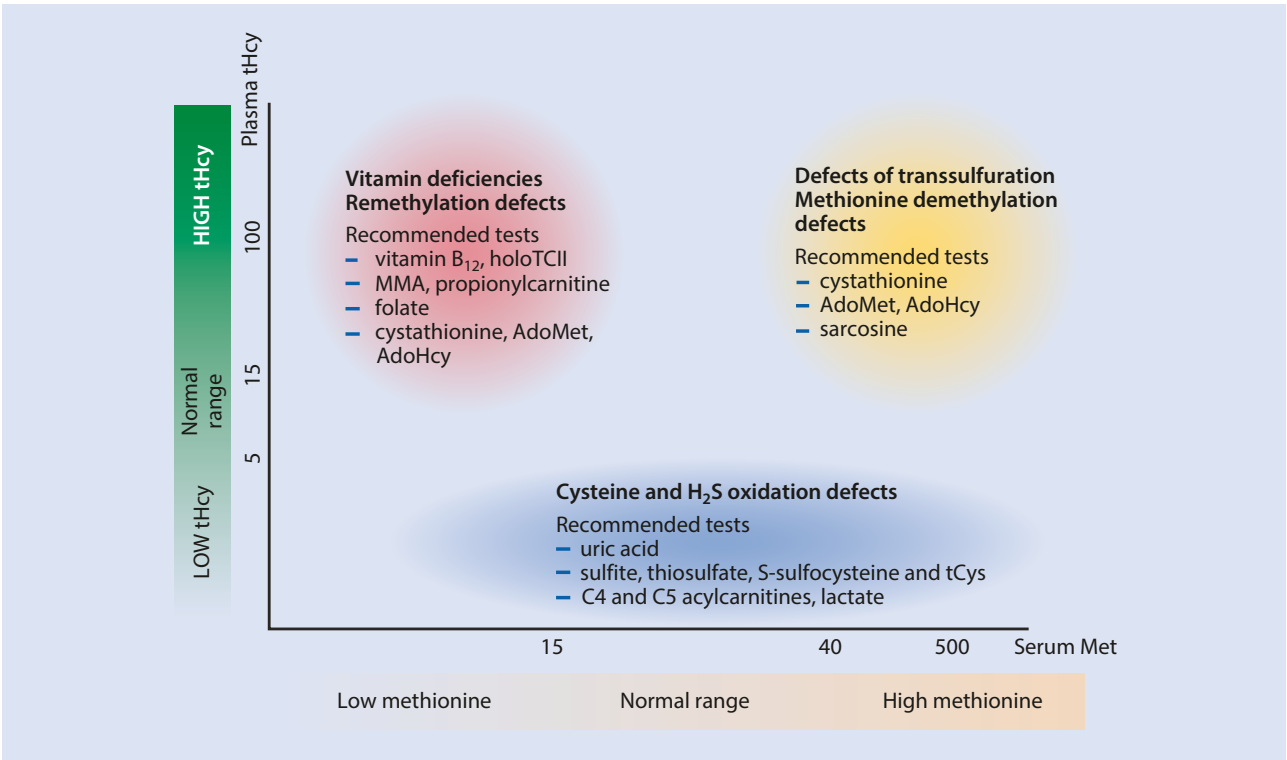
The principal test for CBS deficiency is determination of the plasma total homocysteine (tHcy) concentration. Similar median values of 226-262 μmol/L have been observed in all four categories of responsiveness, although occasionally values even lower than 50 μmol/L have been reported, especially in patients presenting in adulthood [14]. Plasma should ideally be separated from whole blood within 1 hour of venepuncture [26]. Due to low sensitivity and reproducibility, and demanding pre-analytical requirements, measurement of free homocystine by amino acid analysers is not recommended. To avoid misdiagnosis in pyridoxine responsive patients, pyridoxine supplements including multivitamins should be avoided for at least 2 weeks prior to testing. The diagnosis of CBS deficiency is further supported by elevated plasma methionine (median 440 μmol/L in non-responders while only 60 μmol/L in extreme responders) and low to low-normal plasma cystathionine levels determined by sensitive mass spectrometric methods, with an increased methionine-to-cystathionine ratio [24, 14]. Other causes of hyperhomocysteinemia include inborn errors of homocysteine remethylation, folate or vitamin B_{12} deficiencies (▶ Chap. 28), renal insufficiency and drugs (■ Fig. 20.2, see ■ Table 20.1 for biochemical findings in other conditions) [26]. CBS deficiency can be confirmed by enzyme assay in cultured fibroblasts or plasma, and/or mutation analysis of *CBS*; each technique may yield normal results in a few patients.

Newborn screening for homocystinuria generally uses methionine and the methionine-to-phenylalanine ratio as primary markers [27]. Sensitivity is low, however, for pyridoxine responsive patients and limited for pyridoxine non-responsive homocystinuria, with an inverse relationship to the cut-off used. Analysis of the E-HOD registry data showed that among 43 patients detected by newborn screening, 39 were non-responsive and 4 were partially responsive; no fully or extremely responsive patients were identified [14]. Use of tHcy in dried blood spots as a second tier marker increases the specificity of newborn screening but its use as a primary marker has seldom been reported due to the need of an additional analytical assay [27].

Prenatal testing is best done by molecular genetic analysis, if the mutations in both parents are known. Preimplantation diagnosis is also feasible. If needed, enzymatic analysis can be done in cultured amniocytes.

20.6.5 Treatment and Prognosis

The main forms of treatment are pyridoxine, a methionine-restricted diet and betaine. Treatment aims



■ **Fig. 20.2** Changes of blood methionine and tHcy in disorders of sulfur amino acid metabolism. Graph showing the most likely groups of disorders as a function of plasma total homocysteine and serum methionine concentrations, and some recommended diagnostic tests. See ■ Table 20.1 for lists of the individual disorders and results of the recommended tests

to prevent complications by lowering the plasma tHcy concentration, whilst maintaining normal growth and avoiding abnormally low methionine concentrations. Plasma tHcy levels can be kept below 50 μmol/L in fully pyridoxine responsive patients. In pyridoxine non-responsive patients, good outcomes have been achieved by keeping the lifetime median free homocystine concentration below 11 μmol/L [28], suggesting that tHcy levels below approximately 100 μmol/L are acceptable [29].

Approximately 50% patients with CBS deficiency respond to pharmacological doses of pyridoxine. Vitamin B_{12} and folate deficiencies are common in CBS deficiency and must be corrected before assessing the response to pyridoxine. Once a stable baseline tHcy concentration has been established, we recommend a trial of pyridoxine 10 mg/kg daily (minimum 100 mg, maximum 500 mg) for 2–6 weeks, with serial tHcy monitoring [29]. Patients detected by newborn screening are seldom responsive but we recommend a trial of approximately 30 mg/kg/d (maximum 100 mg) for 2 weeks. Rhabdomyolysis and episodes of apnoea have been reported in a few neonates given doses of 200 mg/d or more [30]. Patients are said to be non-responsive if tHcy falls by less than 20%, partially responsive if it falls by more than 20% but remains above 50 μmol/L and fully responsive if tHcy falls below 50 μmol/L; if they can maintain tHcy below 50 μmol/L on less than 1 mg/kg/d pyridoxine, we refer to them as extremely responsive [14].

For long-term treatment, the pyridoxine dose should be the lowest that achieves maximum lowering of homocysteine. It should not exceed 10 mg/kg/day (maximum 500 mg/day) as peripheral neuropathy has been reported after long-term high doses (generally >900 mg/day) [31].

Dietary treatment should be considered in all patients who do not achieve satisfactory tHcy levels with pyridoxine alone. Most patients require a low-methionine diet, analogous to that used in PKU. This can achieve excellent tHcy levels but the diet is difficult, particularly if started after infancy. Moreover, life-long treatment is needed and compliance often deteriorates during adolescence.

Infants with severe CBS deficiency need a methionine restriction of about 120–180 mg/day to achieve plasma tHcy concentrations below 100 μmol/L; this increases to 160–300 mg/day in children, with a small rise in adolescence. Patients 'tolerate' more methionine if they have some residual CBS activity or are also on betaine. Babies are given a combination of breast milk or normal infant formula and a special methionine-free infant formula; the proportions are adjusted according to the plasma methionine and tHcy concentrations. Older patients are given small, measured amount of foods containing methionine each day with low-protein foods and supplements of a methionine-free, cystine-enriched amino acid mixture, vitamins and minerals [32]. Cysteine is a conditionally essential amino acid in CBS deficiency but it is uncertain how much is needed. Anthropometric measurements and nutritional status should be assessed regularly.

Late-diagnosed patients who cannot manage a low-methionine diet may still profit from a milder protein restriction that is above the minimum safe intake, without supplements of an amino acid mixture. This may lead to acceptable tHcy levels in some partially pyridoxine responsive patients.

Betaine acts as a methyl donor for the remethylation of homocysteine to methionine, catalysed by the enzyme, betaine homocysteine methyltransferase. Treatment with betaine leads to a fall in plasma tHcy accompanied by a rise in methionine concentrations. The starting dose is 100 mg/kg/day in children and 6 g/day in adults, divided into two daily doses as the half-life is >14 hours. The dose is adjusted according to response but doses above 150–200 mg/kg/day are unlikely to confer additional benefit [33]. Betaine is generally well tolerated but some patients dislike the taste and high doses are associated with a fishy odour. There have been a few reports of acute cerebral oedema in patients taking betaine. This appears to be due to methionine toxicity; the lowest plasma methionine concentration associated with cerebral oedema was 972 μmol/L but there must be other factors as many patients with higher levels have not developed this complication [34]. Betaine is best used as adjunctive treatment in patients who are partially pyridoxine-responsive or who cannot comply adequately with dietary treatment. If used alone, betaine seldom achieves target tHcy levels and increases of methionine concentrations above 1000 μmol/L should be avoided. As well as promoting remethylation of homocysteine, betaine may act as a chaperone for the mutant CBS enzyme [35].

Vitamin B_{12} and folate deficiencies may occur in patients with CBS deficiency, probably due to increased flux through the remethylation pathway and inhibition of MTHFR by high AdoMet concentrations. Vitamin B_{12} deficiency should be corrected and all patients should be given folic acid.

Thromboembolism is a major cause of death. Antithrombotic drugs, such as aspirin or dipyridamole, may be justified in poorly controlled patients. Dehydration should be avoided. Biochemical control must be optimized before elective surgery. Perioperative low molecular weight heparin, inflation/compression stockings and early mobilisation are recommended.

Maternal homocystinuria does not appear to cause foetal malformations. It is essential to continue treatment, including betaine, during pregnancy with regular biochemical and dietary monitoring. Low molecular weight heparin should be given for at least the third trimester and 6 weeks post-partum [36].

Several forms of enzyme therapy are under development, all acting in blood to lower metabolite levels; a phase I/II trial is in progress for two of these [37] and ClinicalTrials.gov Identifier: NCT05154890. Taurine supplements may complement other forms of treatment but, in a brief trial, the only observed benefit was increased flow-mediated dilatation in those patients with initial low values [38].

A number of untreated patients used to die by 20 years of age, mostly pyridoxine non-responders. Treatment greatly improves the prognosis: all the major complications can be prevented if patients are diagnosed by newborn screening and comply with treatment [28]. Even with imperfect control, treatment greatly reduces the vascular risk [39].

20.7 Cystathionine γ-Lyase Deficiency

20.7.1 Clinical Presentation

Cystathioninuria was originally described in patients with cognitive impairment, however further studies in families revealed asymptomatic CTH deficient individuals which shows that there was an ascertainment bias. The available data do not provide convincing evidence that cystathionine γ-lyase (CTH) deficiency is associated with adverse clinical outcomes [40].

20.7.2 Metabolic Derangement

Cystathionine and N-acetylcystathionine accumulate in plasma and grossly increased amounts are excreted in urine. Hypertension has been reported in a mouse model of CTH deficiency and attributed to reduced formation of the vasodilator, H_2S. In humans, however, neither hypertension nor decreased plasma H_2S concentrations were found [41].

20.7.3 Genetics

CTH deficiency is inherited as an autosomal recessive trait.

20.7.4 Diagnostic Tests

Plasma and urinary cystathionine are markedly elevated and detectable even by conventional amino acid analysis that is unable to determine physiological submicromolar concentrations of cystathionine in plasma; plasma tHcy may be slightly elevated. The differential diagnosis for elevated cystathionine includes prematurity in newborns, vitamin B_6 deficiency, defects of homocysteine remethylation and neuroblastoma. The diagnosis is confirmed by *CTH* mutation analysis since enzyme assay in cultured fibroblasts is not reliable.

20.7.5 Treatment and Prognosis

Some individuals may respond biochemically to pyridoxine administration, however, treatment appears unnecessary.

20.8 Sulfide:Quinone Oxidoreductase Deficiency

20.8.1 Clinical Presentation

SQOR deficiency was described in three patients from two consanguineous families [42]. The patients developed normally without serious health concern until the age 4–8 years when they presented with coma and encephalopathy after prolonged fasting and/or diarrhoea. Brain imaging revealed basal ganglia lesions typical for Leigh syndrome. Two of the patients died.

20.8.2 Metabolic Derangement

SQOR deficiency is expected to lead to accumulation of H_2S and subsequent inhibition of cytochrome c oxidase, which was confirmed in cultured fibroblasts. Inhibition of the respiratory chain caused severe lactic acidosis, and patients also showed hypoglycaemia and ketonuria. Analysis of three siblings from family B (reported in [42]) when they were well revealed elevated plasma H_2S in only one individual but a borderline high or increased plasma sulfite and S-sulfocysteine in all patients (V. Kožich, A.Kuster et al, manuscript under revision).

20.8.3 Genetics

SQOR deficiency is caused by biallelic pathogenic variants in *SQOR* and inherited as an autosomal recessive disease.

20.8.4 Diagnostic Tests

Brain imaging, metabolite analysis and enzymatic testing for oxidative phosphorylation disorders do not allow SQOR deficiency to be distinguished from other causes of Leigh syndrome. The primary diagnostic test is DNA analysis.

20.8.5 Treatment and Prognosis

Three treatment modalities have been proposed but not yet evaluated: reduction of H_2S precursors in diet (e.g. onion, garlic and cruciferous vegetables), reduction of H_2S produced by intestinal microbiota using metronidazole, and scavenging H_2S by hydroxocobalamin. Death due to multiorgan failure was reported in two patients; the third patient experienced repeated episodes of coma with neurological sequelae after each decompensation.

20.9 Ethylmalonic Encephalopathy

20.9.1 Clinical Presentation

Ethylmalonic encephalopathy (EE) is a progressive multisystem disease. It presents in the first months of life with hypotonia, chronic diarrhoea, orthostatic acrocyanosis, a recurrent petechial rash and bruising (with normal platelets). Other features include developmental regression, microcephaly, seizures, episodes of coma, poor growth and hyperlactataemia. Most patients die in early childhood. A few mildly affected patients have presented later in childhood with spasticity, learning difficulties or episodes of encephalopathy [43]. Cerebral imaging shows necrotic lesions in the putamen, caudate nuclei and periaqueductal region, sometimes with abnormalities in the subcortical white matter or brainstem and occasionally malformations.

20.9.2 Metabolic Derangement

EE is caused by deficiency of the mitochondrial persulfide dioxygenase necessary for the conversion of GSH persulfide to sulfite and GSH [44]. Hydrogen sulfide is synthesized endogenously (Fig. 20.1) and also formed by bacterial anaerobes in the large intestine. In EE, the accumulating H_2S inhibits cytochrome c oxidase and short-chain fatty acid oxidation; the latter results in ethylmalonic aciduria and raised butyryl- and glutarylcarnitines in blood. H_2S also has vasodilating and vasotoxic effects; damage to small blood vessels causes bleeding into the skin. Production of H_2S by gut bacteria probably contributes to the severe, persistent diarrhoea.

20.9.3 Genetics

EE is a rare autosomal recessive disorder caused by mutations in *ETHE1*. No genotype-phenotype correlation has been established.

20.9.4 Diagnostic Tests

Ethylmalonic acid and butyrylglycine are consistently present in urine, with raised butyryl- and glutarylcarnitines in blood. Plasma H_2S may be elevated and total homocysteine decreased; urinary sulfite and thiosulfate are also markedly elevated [41]. The diagnosis is confirmed by demonstrating *ETHE1* mutations.

20.9.5 Treatment and Prognosis

Treatment has been undertaken with metronidazole (to reduce bacterial H_2S production) and N-acetylcysteine (a precursor of glutathione, which can accept the sulfur atom of H_2S). Although this leads to some clinical and biochemical improvement [45], the prognosis remains poor. Recently, liver transplantation has led to neurodevelopmental progress in 3 patients, though one subsequently had a metabolic stroke triggered by gastroenteritis [46].

20.10 Molybdenum Cofactor Deficiency

20.10.1 Clinical Presentation

Patients usually present soon after birth with intractable seizures, poor feeding, hypotonia and exaggerated startle reactions, resembling hypoxic ischaemic encephalopathy. This leads to microcephaly, profound psychomotor retardation and severe spasticity. Neuroimaging abnormalities first appear in late pregnancy; within days of birth, restricted diffusion in the cortex and basal ganglia and abnormal white matter progresses to multicystic

leukoencephalopathy. Dislocation of the ocular lens occurs during infancy and xanthine renal stones can develop later. There is often mild dysmorphism, with puffy cheeks, a long philtrum and a small nose; cerebral malformations, such as polymicrogyria, may be present.

A few patients present later in childhood with developmental delay, extrapyramidal or pyramidal signs (which may appear suddenly after an infection) and/or seizures. Some develop dislocated lenses or a marfanoid habitus. Neuroimaging usually shows abnormalities in the basal ganglia. These patients may walk and acquire language but can deteriorate, even as adults [47]. Very mild or even asymptomatic cases have been also reported [48].

20.10.2 Metabolic Derangement

Molybdenum cofactor (MoCo) synthesis involves three steps. MoCo deficiency type A affects the conversion of GTP to cyclic pyranopterin monophosphate (cPMP) (◘ Fig. 20.1). Patients with MoCo deficiency type B cannot convert cPMP to molybdopterin. MoCo deficiency type C affects gephyrin, which catalyses adenylation of molybdopterin and insertion of molybdenum to form the cofactor. (Bottom ◘ Fig. 20.1).

The molybdenum cofactor is needed for sulfite oxidase (SUOX), aldehyde oxidase, the mitochondrial amidoxime reducing component and xanthine dehydrogenase. Decreased SUOX activity leads to abnormalities in sulfur containing metabolites as described in ▶ Sect. 20.11. Sulfite accumulation is probably responsible for the neurotoxicity and lens dislocation. Deficiency of xanthine oxidase causes raised xanthine and hypoxanthine, and low urate concentrations (see also ▶ Chap. 32).

20.10.3 Genetics

MoCo deficiency is an autosomal recessive disorder. Most patients have mutations in *MOCS1*, which by alternative splicing encodes two proteins that catalyse the conversion of GTP to cPMP [49]. Many other patients have mutations in *MOCS2*, which encodes both subunits of molybdopterin synthase heterotetramer. After forming molybdopterin, this enzyme needs to be reactivated by molybdopterin synthase sulphurase, encoded by *MOCS3*; one mildly affected patient with *MOCS3* mutations has been reported [50]. Mild phenotypes have also been reported with certain *MOCS1* and *MOCS2* mutations including a MOCS2 allele that is prevalent in the Roma community [48]. Homozygous mutations in *GPHN* (the gene for gephyrin) have been found in two patients with severe MoCo deficiency. The parents of these children were asymptomatic but a heterozygous *GPHN* mutation appears to have caused epileptic encephalopathy in one man [51] and other heterozygous mutations may also predispose to epilepsy. In addition to its role in MoCo synthesis, gephyrin is involved in the formation of inhibitory synapses and this may be responsible for the problems in heterozygotes.

20.10.4 Diagnostic Tests

Abnormalities of sulfur-containing analytes are as described below for SUOX deficiency. Elevated sulfite can be detected in fresh urine using dipsticks while plasma tHcy is very low and free cystine is usually undetectable [41]. The plasma urate concentration is initially normal but decreases after a few days and remains low (<60 μmol/L) while xanthine and hypoxanthine are elevated in urine. The diagnosis is confirmed by mutation analysis, which is generally used for prenatal diagnosis, though sulfite oxidase activity can be assayed in chorionic villi.

20.10.5 Treatment and Prognosis

Without treatment, most patients have profound handicap and early death. Several patients with MoCo deficiency type A have now been treated with daily intravenous infusions of cPMP [52] and some remain seizure-free with near-normal development after more than 6 years. To avoid irreversible damage, treatment generally needs to be started within 24 hours of birth. There is no effective treatment for severe MoCo deficiency types B or C. Patients with a mild phenotype may profit from a diet low in cysteine and methionine [50].

20.11 Isolated Sulfite Oxidase Deficiency

20.11.1 Clinical Presentation

The clinical features and neuroimaging resemble those in MoCo deficiency except for the absence of renal stones. Most patients present within 3 days of birth with seizures, poor feeding and axial hypotonia, followed by spasticity, microcephaly and severe psychomotor impairment [53]. A few patients present at 6–24 months of age with a movement disorder, stroke or developmental regression, often after an infection or minor head injury [54]. Many patients develop dislocated lenses.

20.11.2 Metabolic Derangement

Sulfite derived from cysteine and H_2S is normally oxidised to form sulfate. In sulfite oxidase deficiency, accumulating sulfite is converted to thiosulfate and reacts with cysteine to form S-sulfocysteine. Sulfite damages the brain and probably causes lens dislocation by disrupting cystine cross-linkages in the suspensory ligament. Secondary PLP deficiency may contribute to pathogenesis of the disorder [55].

20.11.3 Genetics

Sulfite oxidase deficiency is an autosomal recessive disorder caused by mutations in *SUOX*.

20.11.4 Diagnostic Tests

Elevated sulfite can be detected in fresh urine using dipsticks but the test is unreliable because urine is seldom fresh when reaching the laboratory. Specific quantitative methods show markedly raised sulfite in plasma and urine samples frozen at -80 °C within about 30 minutes of collection. Elevated S-sulfocysteine is a stable biomarker and can be demonstrated by standard amino acid analysis or, more reliably, by LC-MS/MS. Plasma cystine, total cysteine and tHcy are very low, while plasma taurine concentrations may be raised. Thiosulfate concentrations in plasma and urine are markedly increased and sulfate is decreased [41]. Urate and xanthine concentrations are normal. The diagnosis is confirmed by mutation analysis; the enzyme can be assayed in fibroblasts or chorionic villi but this is now seldom needed.

20.11.5 Treatment and Prognosis

The prognosis for neonatal-onset cases is poor. Treatment with a diet low in cysteine and methionine may help patients with mild forms of sulfite oxidase deficiency [54].

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Disorders of Ornithine and Proline Metabolism

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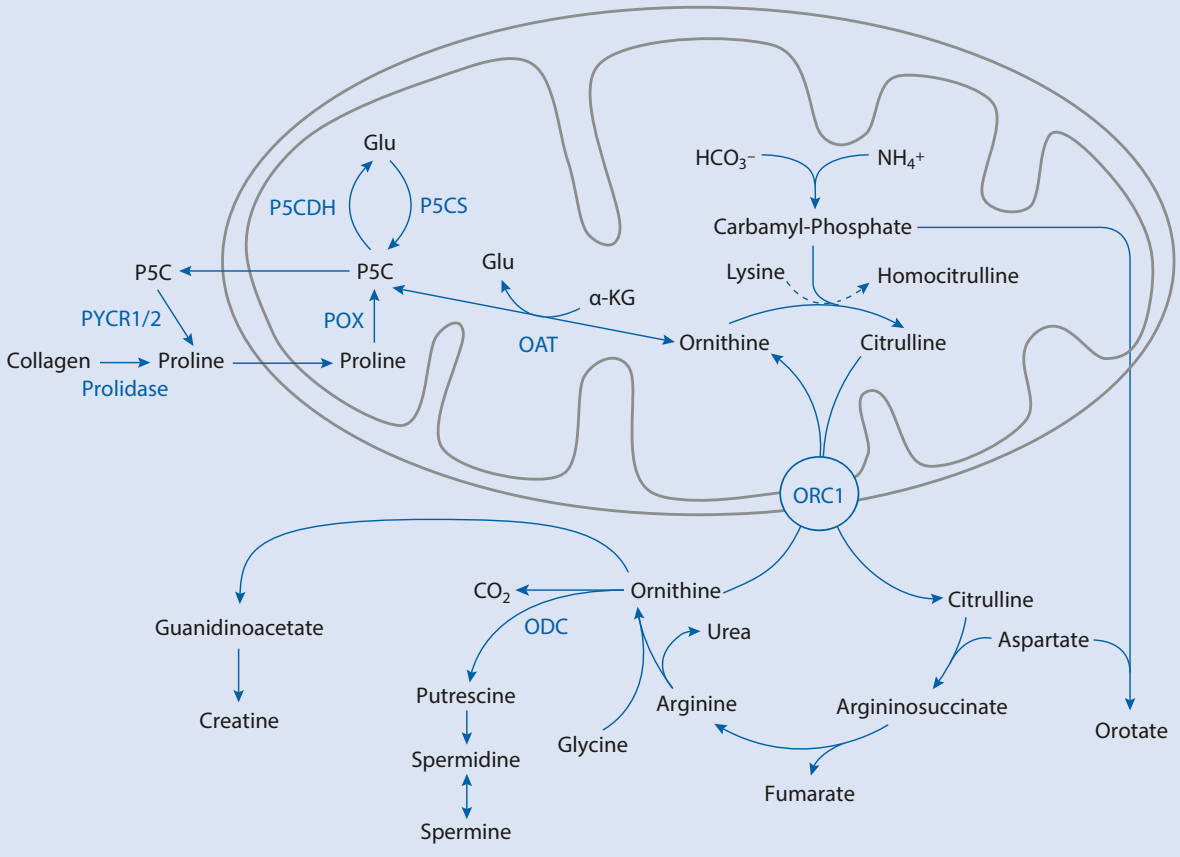
Ornithine and Proline Metabolism

Ornithine is an intermediate in metabolic pathways involving the urea cycle, proline metabolism and the biosynthesis of creatine and polyamines. Ornithine-δ-aminotransferase (OAT) is a pyridoxal phosphate-requiring, mitochondrial matrix enzyme that plays a pivotal role in these pathways. The OAT reaction is freely reversible: during the neonatal period it plays an anapleurotic function for the urea cycle with the net flux in the direction of ornithine and, via the urea cycle, arginine biosynthesis, while after a few months of age the net flux reverses to favour arginine disposal via the synthesis of Δ1-pyrroline-5-carboxylate (P5C), an intermediate in proline and glutamate synthesis. Ornithine also plays an essential role, serving as the substrate upon which urea is assembled (◘ Fig. 21.1). Since both OAT and ornithine transcarbamoylase (OTC) are mitochondrial matrix enzymes, ornithine produced in the cytoplasm from arginine must be transported into the mitochondrial matrix by a specific energy-requiring transport system involving ORNT1 (SLC25A15), an antiporter in the inner mitochondrial membrane, which exchanges cytosolic ornithine with mitochondrial citrulline. In the cytoplasm ornithine is decarboxylated to putrescine which is then converted to spermine.

Proline, unlike all other amino acids (except hydroxyproline), has no primary amino group (it is termed as an imino acid) and uses, as a consequence, a specific set of enzymes for its metabolism. P5C, the product or precursor of the OAT reaction, is both the immediate precursor and the degradation product of proline. P5C synthetase, a bifunctional ATP- and NADPH-dependent mitochondrial enzyme that is highly active in the gut and also expressed in brain catalyses the reduction of glutamate to P5C. The P5C/proline cycle transfers reducing/oxidizing potential between cellular organelles. Owing to its pyridinoline ring, proline (together with hydroxyproline) contributes to the structural stability of proteins, particularly collagen (see also iminoglycinuria ▶ Sect. 25.2).



◘ **Fig. 21.1** **Ornithine and proline metabolic pathways**. P5C Δ1-pyrroline 5-carboxylate, Glu glutamate, α-KG α-ketoglutarate, OAT ornithine-δ-aminotransferase, ODC ornithine decarboxylase, ORC1 ornithine/citrulline antiporter, P5CS Δ1-pyrroline-5-carboxylate synthetase, P5CDH Δ1-pyrroline-5-carboxylate dehydrogenase, PYCR1/2 Δ1-pyrroline-5-carboxylate reductase, PRODH proline dehydrogenase; the step indicated by the broken line, lysine transcarbamylase, is not well defined

Introduction

Hyperornithinaemia due to ornithine aminotransferase (OAT) deficiency results in gyrate atrophy of the choroid and retina (GA) and leads to progressive visual loss. Treatment includes an arginine-restricted diet and a trial of pyridoxine (vitamin B_6) which, in some patients, can slow visual loss and chorioretinal degeneration. Rarely, neonates with OAT-deficiency present with hyperammonaemia and require treatment with arginine supplementation.

In the *hyperornithinaemia, hyperammonaemia, and homocitrullinuria (HHH) syndrome* clinical manifestations are variable and may be related to intermittent episodes of hyperammonaemia. Progressive spastic paraparesis is often a late complication. Deficient transport of ornithine into the mitochondria impairs the urea cycle and results in episodic hyperammonaemia, hyperornithinaemia and increased urinary excretion of homocitrulline and orotic acid. Treatment includes protein restriction, citrulline or arginine supplementation and in some cases ammonia scavengers.

P5C synthetase (P5CS) deficiency causes two distinct phenotypes: a rare recessive neurocutaneous syndrome with cutis laxa, developmental delay, joint laxity and cataracts and, depending on the specific residues affected by the mutation(s), may also cause autosomal dominant or autosomal recessive adult onset spastic paraplegia. The metabolic phenotype in some cases with neurocutaneous cutis laxa may include mild fasting hyperammonaemia, hypoornithinaemia, hypocitrullinaemia, hypoargininaemia and hypoprolinaemia.

Deficiency of P5C reductase (P5CR) associated to mutations in *PYCR1* causes autosomal recessive cutis laxa with progeroid features, while mutations in *PYCR2,* a paralog of *PYCR1,* cause microcephaly and hypomyelination. Both disorders show no apparent metabolic phenotype.

The phenotypic consequences of *Hyperprolinaemia type I* are uncertain, while *Hyperprolinaemia type II* appears to be associated with a disposition to recurrent seizures.

21.1 Hyperornithinaemia Due to Ornithine Aminotransferase Deficiency (Gyrate Atrophy of the Choroid and Retina)

21.1.1 Clinical Presentation

The initial visual symptoms include myopia followed by night blindness and usually begin in early to mid-childhood [1]. Additional ophthalmological findings include constricted visual fields, posterior subcapsular cataracts with onset in the late teens, elevated dark adaptation thresholds and reduced or nondetectable electroretinographic (ERG) responses. Retinopathy can be detected before the patient notes visual disturbances. The fundoscopic appearance of the chorioretinal atrophy in gyrate atrophy is highly specific and is illustrated in Fig. 21.2.

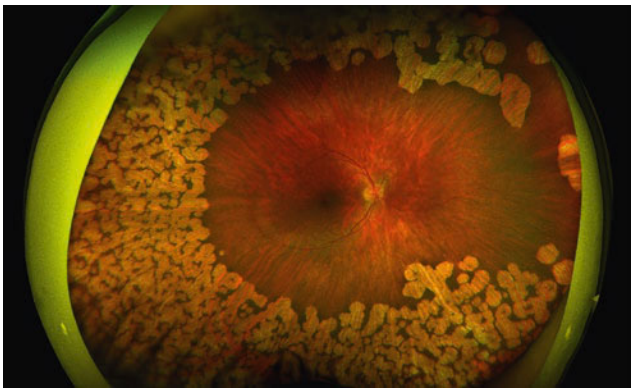


Fig. 21.2 Fundoscopic appearance of the chorioretinal atrophy in a 7 year old child. (Courtesy of Dr. Jaume Català (Ophthalmology Department) and the Neurometabolic Unit of Sant Joan de Déu Hospital, Barcelona)

The chorioretinal degeneration in gyrate atrophy is progressive, and most patients become virtually blind between the ages of 45 and 65. A few patients demonstrate a significant reduction in plasma ornithine levels in response to pharmacological doses of vitamin B_6 and usually have a milder course and maintain central visual function at older ages. In general, intrafamilial variation in the extent and progress of the chorioretinal degeneration is much less than interfamilial variation. Vitreous haemorrhage causing sudden loss of vision is a rare complication. Most patients have normal intelligence consistent with other family members, although one report suggests an increased incidence of intellectual disability [1, 2].

A few patients, typically premature infants, have presented in the neonatal period with poor feeding, failure to thrive, symptomatic hyperammonaemia and orotic aciduria mimicking OTC deficiency (see metabolic derangement, below) (► Chap. 19) [3, 4].

Post-mortem histopathological study of the retina in a pyridoxine-responsive patient showed focal areas of photoreceptor atrophy with adjacent retinal pigment epithelial hyperplasia [1]. Electron microscope studies revealed abnormal mitochondria in the corneal endothelium and the non-pigmented ciliary epithelium and similar, but less severe, abnormalities in the photoreceptors. In an *Oat* knockout mouse model with progressive retinal degeneration, the first morphologic abnormalities are in the retinal pigment epithelium, a cell type that normally expresses high levels of OAT [4]. In addition to the ocular findings, systemic abnormalities have been

reported in some patients. These include diffuse slowing on EEG in about 50%, abnormal muscle ultrastructure, muscle weakness, abnormal ultrastructure of hepatic mitochondria and peculiar fine, sparse, straight hair with microscopic abnormalities [1]. Early degenerative and atrophic brain changes that were not age related were found by magnetic resonance imaging (MRI) of the brain in about 70%, and evidence of peripheral nervous system involvement was noted in half the patients studied [5, 6].

21.1.2 Metabolic Derangement

Beyond the neonatal period, gyrate atrophy patients on an unrestricted diet develop hyperornithinaemia (fasting plasma ornithine averages 915 μM with a range of 400–1200 μM, see reference 1) due to a deficiency of OAT activity. The enzyme deficiency has been demonstrated in liver, muscle, hair roots, cultured skin fibroblasts and lymphoblasts. The pathophysiological mechanism of the retinal degeneration is unclear. OAT requires pyridoxal phosphate (PLP) as a cofactor. In a few patients (<10%), fibroblast OAT activity increases significantly when assayed in the presence of high concentrations of PLP. Most of these patients also show a partial reduction (>30% of baseline fasting values on a constant protein intake diet) of plasma ornithine when given pharmacological doses of pyridoxine (vitamin B_6).

Neonates who have presented with increased blood ammonia have low levels of plasma ornithine, citrulline and arginine and orotic aciduria in their first weeks of life, with hyperornithinaemia developing later in life [3, 4]. One infant with OAT deficiency, diagnosed prenatally, had normal plasma ornithine and arginine in cord blood but developed reduced levels of these amino acids at 2–4 months of age on a normal diet, with concomitant increases in plasma ammonia and glutamine [4]. Arginine administration corrected the low plasma arginine and hyperammonaemia, but produced hyperornithinaemia. This human phenotype is similar to, but less severe than, that of mice homozygous for targeted disruption of the *Oat* gene, which require arginine supplementation to survive the neonatal period [4]. These observations indicate that the net flux in the OAT reaction in the newborn period is in the direction of ornithine synthesis rather than ornithine degradation. Disruption of the anapleurotic function of the OAT reaction for the urea cycle, especially in patients whose dietary arginine is less than that required for growth, can lead to insufficient levels of citrulline and arginine, inadequate ureagenesis and consequent hyperammonaemia. The role played by arginase 2, an intramitochondrial arginase expressed beyond weaning in enterocytes, is not clear.

Children and adults with OAT deficiency have reduced levels of creatine in blood, urine, muscle and brain [5] as a result of ornithine inhibition of glycine transamidinase and the subsequent reduction of creatine biosynthesis (Fig. 21.1). Subnormal levels of serum creatinine reflect the reduction in total body creatine. In contrast to other series, which find normal intellect in adults [1], a series of seven French paediatric patients revealed a high prevalence of neurological impairment [2]. The authors speculated that these phenotypic features could be related to secondary brain creatine deficiency. This possibility should be carefully evaluated in future studies to consider possible complications of neonatal hyperammonaemia as an alternative explanation.

21.1.3 Genetics

OAT deficiency is an autosomal recessive disorder and has been described in patients from various ethnic backgrounds, but its incidence is highest in the Finnish population [1]. Intermediate levels of OAT activity are observed in skin fibroblasts from obligate heterozygotes for both pyridoxine-nonresponsive and pyridoxine-responsive variants.

More than 70 mutations have been defined in patients of various ethnic origins [1]. In Finns, one mutant allele, *OAT-L402P*, accounts for >85% of all *OAT* alleles and has only been described in individuals of Finnish origin [1]. Several other *OAT* alleles have been shown to be characteristic of specific populations [1].

21.1.4 Diagnostic Tests

The most prominent biochemical abnormality in those ingesting an unrestricted diet is a 5- to 20-fold elevation of plasma ornithine. Patients with the pyridoxine-responsive variant tend to have lower levels than those with the pyridoxine-nonresponsive variant, although this distinction is unreliable. Urinary excretion of ornithine and that of lysine, arginine and cystine is increased when plasma ornithine is 400 μmol/l or greater. These changes are secondary to competitive inhibition by ornithine of the common renal transport shared by these amino acids. Plasma ornithine levels in gyrate atrophy are usually higher than those in the HHH syndrome, and the characteristic presence of homocitrulline in the urine in HHH differentiates these two hyperornithinaemic conditions. Neonatal OAT deficiency can be difficult to distinguish from OTC deficiency, as plasma levels of ornithine, arginine, and citrulline are reduced in both disorders and orotic acid is also increased. Since hyperornithinaemia is not present in all (or perhaps any)

neonates with GA, newborn screening using this as a marker will be unreliable.

For confirmation of the diagnosis, molecular genetic analysis of *OAT* or direct assay of OAT activity can be performed in extracts of cultured skin fibroblasts or lymphoblasts. When the mutation is known, molecular analysis is appropriate for prenatal diagnosis and carrier detection.

21.1.5 Treatment and Prognosis

The goal of treatment has been to reduce plasma ornithine levels to less than 200 μM. Reduction of plasma ornithine can be achieved by dietary restriction of arginine (the precursor of ornithine in foods) [1]. On average, food proteins contain 4–6% arginine (nuts and seeds have higher arginine content). To limit arginine intake sufficiently to reduce ornithine accumulation, it is usually necessary to limit natural protein severely and supplement the diet with a mixture of essential amino acids to provide adequate nutrition. Care must be taken to avoid excessive arginine restriction, which will result in hypoargininaemia with associated poor growth and skin rash, and even hyperammonaemia, especially if total nitrogen intake is high. Thus, successful management of an arginine-restricted diet requires careful monitoring of growth, physical examinations, nutritional status and plasma amino acid levels.

Arginine is an essential amino acid in patients with OAT deficiency. Infants with symptomatic hyperammonaemia or evidence of impaired waste nitrogen metabolism (hyperglutaminaemia, orotic aciduria) should be supplemented with arginine. Arginine intake in patients less than 3–4 months of age should not be restricted until plasma ornithine begins to increase.

Pharmacological dosage of pyridoxine HCl has resulted in plasma ornithine reduction in a small number of patients where doses between 200 and 500 mg a day lowered levels by between 25% and 60% [1]. A 2- to 4-week trial of pyridoxine treatment (300–500 mg/day) with no change in dietary protein intake and comparison of fasting plasma ornithine levels pre- and post-pyridoxine is recommended for all newly diagnosed patients, to determine their responsiveness.

Over 30 patients have been given a low-arginine diet in the long term, some in combination with pharmacological doses of pyridoxine. Compliance with diet restriction and long-term commitment and motivation are important factors influencing the outcome. A series of 17 patients on an arginine-restricted diet had plasma ornithine levels in the range of 400–500 μmol/l and showed slower loss of visual function after 13.9 years than 10 patients not on the diet [7]. Long-term substantial reduction of plasma ornithine levels started at an early age may be beneficial in slowing the progression of chorioretinal lesions and loss of retinal function. In a study of two sets of siblings with GA who were treated with an arginine-restricted diet for 16–17 years, each younger sibling, who was prescribed the diet at an earlier age, demonstrated a dramatic reduction in progression of lesions compared with the older sibling [8]. One patient was unable to tolerate the semisynthetic low-arginine diet and was treated with a natural food low-protein diet (0.8 g/kg/day) for 26 years, with moderate reduction of plasma ornithine levels and delayed progression of chorioretinal degeneration [9].

The effects of the above therapeutic measures on vision late in life have yet to be assessed. A study of a knockout mouse model for OAT deficiency has shown that a trial of dietary arginine restriction completely prevented the appearance of retinopathy at the age when untreated mice developed GA [4]. This observation validates the efficacy of reduction in ornithine accumulation by arginine restriction and emphasizes the importance of early diagnosis and early treatment.

Other therapeutic approaches applied in small numbers of patients have included supplementation of proline [7], creatine [10] and lysine [1]. Creatine supplementation corrected the muscle histopathology and phosphocreatine deficiency as measured by NMR, but did not have an obvious symptomatic effect; nor did it halt the progression of retinal degeneration.

Children born to women with OAT deficiency on an unrestricted diet appear to have no adverse effects of exposure to hyperornithinaemia. In multiple instances, it has been possible to manage an arginine-restricted diet successfully over a pregnancy in affected women [7]. As in other disorders with amino acid accumulation, these women will have an increasing requirement for the restricted amino acid (arginine) in the last trimester and must be followed carefully with weight checks, nutritional measures and plasma amino acid levels. Hyperammonaemia in neonates with OAT deficiency responds to standard treatment, and particularly to arginine supplementation.

21.2 Hyperornithinaemia, Hyperammonaemia and Homocitrullinuria (HHH) Syndrome

21.2.1 Clinical Presentation

The clinical manifestations in the HHH syndrome cover a broad spectrum, with some related to episodic hyperammonaemia (■ Table 21.1) [11]. Intolerance to protein feeding, vomiting, seizures and developmental delay

Table 21.1 Differential diagnosis of disorders involving ornithine and proline metabolism

	OAT	HHH	P5CS Cutis laxa	P5CS HSP	PYCR1	PYCR2	PRODH	P5CDH
Inheritance	AR	AR	AR/AD	AD/ AR	AR	AR	AR	AR
Retinal degeneration	+ gyrate atrophy	+/–						
Cataract			+	+/–				
Episodic lethargy/coma	Neonates only	+						
Liver dysfunction		+						
Developmental delay/MR	+/–	+	+	+/–	+	+	+/–	+/–
Seizures		+				+/–	+/–	+
Cerebellar ataxia		+						
Hypomyelination						+		
Thin corpus callosum			+/–	+/–	+/–	+		
Pyramidal signs/spastic paraparesis		+	+/–	+	+/–	+/–		
Dysmorphisms			+	+/–	+	+/–	+/–	
Progeroid appearance			+		+			
Microcephaly			+/–		+/–	+		
Lax and wrinkled skin			+		+			
Joint laxity			+		+	+/–		
Visible veins			+		+			
Plasma ammonia	↑ neonates only	↑ +/–	↑ +/– (only with fasting)					
Plasma ornithine	↑ +	↑ +	↓ (+/–)					
Plasma proline			↓ +/–				↑ +	↑ +
Plasma citrulline/arginine		↓ +/–	↓ (+/–)					
Homocitrullinuria	+/–	+						
Orotic aciduria	Neonates only	+/–						
Brain MRS glycine						↑		

OAT ornithine aminotransferase deficiency, *HHH* hyperornithinaemia, hyperammonaemia, homocitrullinuria syndrome, *PC5S* Δ1-pyrroline-5-carboxylate synthase deficiency, *PYCR1* Δ1-pyrroline-5-carboxylate reductase 1 deficiency, *PYCR2* Δ1-pyrroline-5-carboxylate reductase 2 deficiency, *PRODH* proline dehydrogenase deficiency, *P5CDH* Δ1-pyrroline 5-carboxylate dehydrogenase deficiency, *MR* mental retardation, *HSP* hereditary spastic paraplegia, + characteristic finding, +/– frequently seen, (+/–) less frequently seen, ↑ elevated, ↓ reduced

from infancy are common complaints. Neonatal onset of lethargy, hypotonia and seizures, with progression to coma and death has been observed in the most severe form [11]. Persistent or recurrent liver dysfunction was the presenting symptom in over a third of patients in a series of French-Canadian patients [12]. Also, severe but reversible hepatocellular necrosis and acute hepatitis-like episodes and coagulopathy, especially factor VII and X deficiencies, have been reported [11, 12], sometimes in the absence of overt hyperammonemia, suggesting that HHH syndrome should be added to the list of metabolic disorders causing liver failure. HHH

syndrome may also present a more chronic and slowly progressive course, characterized by an aversion to protein rich foods, variable intellectual impairment or mental regression and signs of motor dysfunction with no obvious relationship to compliance with treatment [11]. Regardless of the age and type of onset, most patients present with progressive neurological dysfunction, mainly characterized by pyramidal tract signs with spastic gait, associated with cerebellar symptoms; seizures, mainly myoclonic, are also frequently observed [11]. Abnormal neuroimaging findings include cortical, cerebellar and spinal cord atrophy, thin corpus callosum and stroke-like lesions [13]. Retinal abnormalities have been reported in 2 of >70 HHH patients described [14, 15]. Mildly affected adult patients may have apparently normal intelligence but may display behavioural or psychiatric disturbances with protein intolerance.

21.2.2 Metabolic Derangement

Patients with the HHH syndrome have a marked elevation of plasma ornithine associated with hyperammonaemia and increased urinary excretion of homocitrulline. The HHH syndrome is a disorder of metabolic compartmentation, with impaired transport of ornithine into the mitochondria (◘ Fig. 21.1), resulting in a functional deficiency of both OTC and OAT activities. The intramitochondrial deficiency of ornithine leads to utilisation of carbamoylphosphate by OTC, using lysine (homoornithine) to form homocitrulline and further homoanalogues (homoarginine) (◘ Fig. 21.1) and formation of orotic acid secondary to excess flux down the pyrimidine biosynthetic pathway (► Chap. 32, ► Fig. 32.2).

21.2.3 Genetics

The HHH syndrome is a panethnic disease with over 100 patients reported [16]. The disease has been reported to be more frequent in Canada, as a result of a founder mutation in Quebec [17], in Italy and in Japan. Inheritance is autosomal recessive. The gene (*SLC25A15*) encodes the ornithine transporter protein ORC1. The common mutant allele in patients of French-Canadian origin is F188Δ, a 3-bp inframe deletion [17]. Even in homozygotes for this deletion there is considerable phenotypic variability, and also for other patients there is no clear-cut genotype-phenotype correlation [12]. The R197X mutation has been reported in multiple Japanese patients. Obligate heterozygotes are clinically normal and cannot be identified by biochemical studies.

21.2.4 Diagnostic Tests

The HHH syndrome can be differentiated from other hyperammonaemic syndromes by laboratory findings (◘ Table 21.1). The triad of hyperornithinaemia, hyperammonaemia and homocitrullinuria is pathognomonic. Homocitrulline can be mistaken for methionine in some amino acid analysers (◘ Table 21.1). The plasma ornithine concentration is elevated to 3–10 times normal and tends to be somewhat lower than that seen in OAT deficiency. Despite a functional deficiency of OTC activity, plasma citrulline reduction is less pronounced than in OTC deficiency.

In addition to homocitrullinuria, urine amino acid screening shows increased ornithine and hyperdibasic amino aciduria when the plasma ornithine concentration is above 400 μmol/l. At lower plasma ornithine concentrations, homocitrullinuria may be the only urine amino acid abnormality. Furthermore, excessive homocitrulline excretion is observed in infants ingesting certain artificial formulas and may also be formed during heating of milk [18]. Persistent homocitrullinuria without a dietary source is abnormal and has also been detected in hyperlysinaemia. Orotic aciduria is common in HHH and can be induced by allopurinol challenge (► Chap. 3), as in patients with primary OTC deficiency (► Chap. 19).

In a few countries, HHH syndrome is part of the expanded newborn screening program. However, it may be missed because some affected neonates may not show elevated plasma ornithine levels in the first days of life [19].

The method of choice for prenatal diagnosis in couples of known genotype is mutation analysis.

21.2.5 Treatment and Prognosis

Treatment is aimed at preventing ammonia toxicity and, during episodes with hyperammonaemia, follows the principles outlined for the urea cycle disorders (► Chap. 19). In general, a low-protein diet combined with citrulline or arginine supplementation have been effective in achieving biochemical control for most patients. In some patients, ammonia scavengers such as sodium benzoate and/or sodium phenylbutyrate are used to improve metabolic control. Treatment may not prevent the late development of spastic gait, although the authors' personal experience includes multiple patients who have been treated with citrulline supplementation and mild dietary protein restriction for more than 20 years with no progression of neurological abnormalities.

Prognosis is variable, ranging from mild neurological involvement to a severely disabling disease; mortality is relatively low and treated patients are usually metabolically stable and do not experience relapses of hyperammonaemia [11].

Successful pregnancies have been reported in women with HHH syndrome [11]. However, it is advisable to exercise caution in the dietary management during pregnancy and in the postpartum period when there is a potential risk of hyperammonaemia. Offspring of both women and men with HHH syndrome have been apparently normal.

21.3 Δ1-Pyrroline-5-Carboxylate Synthetase Deficiency

21.3.1 Clinical Presentation

The first two patients aroused clinical attention in early infancy because of developmental delay, lax skin and joints, muscular hypotonia and failure to thrive [20, 21]. In the last 15 years at least 30 additional patients with this neurocutaneous syndrome now called cutis laxa type III have been described occurring both in the original autosomal recessive (ARCL3A) as well as in a sporadic autosomal dominant way (ADCL3) [22, 23]. Typical features included failure to thrive, hypotonia and severe developmental delay with cognitive impairment, associated with progeroid features, cutis laxa, joint laxicity, hip dislocation, adducted thumbs, cataracts, short stature, spasticity and often microcephaly [22–24]. Cutis laxa is not an obligate sign and may improve or disappear with age. Other less consistent features included intrauterine growth retardation, corneal clouding, retinitis pigmentosa, visible skin veins, kinky tortuosity of brain vessels, abnormal fat pads and cerebellar atrophy.

In 2015 a spastic paraplegia form of the disease (spastic paraplegia SPG9) with some traits of the neurocutaneous syndrome but without report of cutis laxa, joint laxity, or herniae, was associated with monoallelic or biallelic *ALDH18A1* mutations and, respectively, autosomal dominant (SPG9A) and recessive (SPG9B) inheritance [22, 25, 26]. To date, at least 50 SPG9 patients have been reported, about two thirds of which carry monoallelic mutations. SPG9 patients are characterised by an upper motor neuron syndrome generally of later onset, with variable degrees of weakness and progressive spastic limb paresis. Particularly in the recessive form there is also learning disability, growth retardation, dysmorphic features, microcephaly and/or cyclic vomiting, and bilateral cataracts, all symptoms reminiscent of the more severe neurocutaneous syndrome.

It thus has been proposed that the neurocutaneous and the spastic paraplegia syndromes represent, respectively, higher and lower degrees of severity of the same disorder corresponding to higher and lower degrees of loss of P5CS function (SPG9A < SPG9B < ADCL3 ≤ ARCL3A) [22].

21.3.2 Metabolic Derangement

The metabolic phenotype described in many but not in all patients includes hypoornithinaemia, hypocitrullinaemia, hypoargininaemia, hypoprolinaemia and mild hyperammonaemia (Table 21.1), a pattern of metabolic abnormalities consistent with impaired proline and ornithine synthesis due to deficiency of Δ^1-pyrroline-5-carboxylate synthetase (P5CS). This enzyme catalyses an essential step in the pathways by which proline, ornithine and arginine are synthesised from glutamate (Fig. 21.1). Abnormal profiles were only reported in patients with mutations affecting the glutamate-5-kinase domain or with a complete abolition of protein expression. In autosomal dominant families the most striking anomaly were very low citrulline levels regardless of the domain affected by the mutation. As in the original patients, glutamine loading tests confirmed a metabolic block at the level of P5CS in vivo in fibroblast cultures from two related subjects with the dominant form of the disease [20, 25]. In connective tissue there is a high proline requirement for collagen synthesis. Deficient proline synthesis may impair protein synthesis in the lens epithelium and/or fibrocytes, and it is also possible that P5C metabolism contributes to the antioxidant defence of the lens. P5CS activity is present in the brain, and impaired local proline production in the nervous system may have a predominant role in the neurocognitive alterations, as the two pure disorders of proline synthesis, PYCR1 and PYCR2 (discussed below) present with severe mental disability. Thus, although proline is traditionally considered a non-essential amino acid, impaired synthesis of proline is consistent with many of the clinical abnormalities, such as lax joints and skin, cataracts and neurodegeneration [21, 23]. Regarding the pyramidal signs that appear in the course of the autosomal recessive disease and are the hallmark of the dominant form, it has been speculated that a decrease in the mitochondrial pool of ornithine may be responsible for motor neuron degeneration [22]. This pathomechanism may be shared with HHH syndrome and arginase deficiency, both of which are also associated

with low mitochondrial ornithine and spastic paraplegia in adults.

The paradoxical fasting hyperammonaemia reported in one of the original patients is consistent with a relative deficiency of ornithine limiting ureagenesis and ammonia detoxification in the liver. Following a meal, arginine derived from dietary protein temporarily corrects this deficit by producing ornithine through arginase and thus enhancing urea cycle function, with the result that plasma ammonia decreases despite the nitrogen load in the meal. Notably, in this special situation, arginine becomes an essential amino acid. Ammonia was either normal or not measured in the other patients.

21.3.3 Genetics

Biallelic mutations in *ALDH18A1* encoding P5CS cause the early onset autosomal recessive neurocutaneous form (ARCL3A) [22]. Sporadic de novo mutations cause autosomal dominant cutis laxa syndrome (ADCL3). Biallelic or monoallelic *ALDH18A1* mutations affecting specific residues are associated with spastic paraplegia, either inherited in an autosomal dominant (SPG9A) or recessive (SPG9B) way [22].

21.3.4 Diagnostic Tests

Since the abnormal metabolite profile is corrected in the fed state, the metabolic phenotype of P5CS deficiency is easily missed. The combination of low fasting levels of ornithine, citrulline, arginine and proline plus a tendency to paradoxical fasting hyperammonaemia or one of the above together with a clinical phenotype of mental retardation, connective tissue manifestations and/or cataracts should suggest this disorder (◘ Table 21.1). The diagnosis is confirmed by mutation analysis. In the autosomal dominant adult form of the disease, citrulline may be a potential trait biomarker as the associated phenotype is not distinct from other causative genes.

21.3.5 Treatment and Prognosis

Supplementation of the deficient amino acids seems to be a reasonable therapeutic approach. However, administration of ornithine in the two reported siblings at a late stage of the disease did not result in any clinical improvement. Early recognition would allow the opportunity for a therapeutic trial with a combination of amino acids, such as citrulline, arginine, ornithine and proline.

21.4 Δ1-Pyrroline-5-Carboxylate Reductase Deficiency 1 (PYCR1) and 2 (PYCR2)

Mutations in the gene encoding P5C reductase (*PYCR1*) cause autosomal recessive cutis laxa with progeroid features and psychomotor retardation supporting the assumption of a significant role for proline biosynthesis in connective tissue and in normal intellectual development [27, 28]. Clinical features include lax and wrinkled skin apparent at birth, joint hyperlaxity, distal arthrogryposis, characteristic facial features including a triangular face, short nose, long philtrum, and large ears, mild to moderate microcephaly, and mental retardation [29]. Cells and tissues from these individuals display increased apoptosis in response to oxidative stress [27]. Serum proline levels in these patients were normal.

Mutations in *PYCR2,* encoding an isoenzyme of PYCR1 have been described in children with failure to thrive, progressive microcephaly and hypomyelinating leukodystrophy [30, 31]. Metabolomic studies in the *PYCR2* mutant mouse model showed elevation of cerebral glycine concentrations with, at Western blot and immunohistochemical analyses, increased intramitochondrial levels of SHMT2, a key enzyme for glycine and serine metabolism in brain [32]. In vivo MRS study confirmed the elevation of brain glycine levels in two sib patients with *PYCR2* deficiency, indicating that a perturbation of glycine metabolism may contribute to the phenotypic manifestations of the disease [32].

21.5 Proline Dehydrogenase (Proline Oxidase) Deficiency (Hyperprolinaemia Type I)

21.5.1 Clinical Presentation

Hyperprolinaemia type I is a rare disorder which appears to be well tolerated in some individuals, but in others may contribute to risk for schizophrenia or other psychiatric, cognitive or behavioural abnormalities [33].

21.5.2 Metabolic Derangement

Hyperprolinaemia type I is caused by a deficiency of proline dehydrogenase (proline oxidase), a mitochondrial inner-membrane enzyme, which catalyses the conversion of proline into P5C (◘ Fig. 21.1). Hence, in hyperprolinaemia type I, there are increased levels of proline in plasma (usually not above 2000 μM; normal range 100–450 μM), urine and cerebrospinal fluid (CSF).

Hyperprolinaemia (as high as 1000 μM) is also observed as a secondary phenomenon in hyperlactataemia, possibly because proline oxidase is inhibited by lactic acid. Remarkably, and in contrast to hyperprolinaemia type II, heterozygotes may have mild hyperprolinaemia.

Of note, neonatal hyperprolinaemia mimicking hyperprolinaemia type I has also been found in mild forms of glutaric aciduria type 2 [34]. Hyperprolinaemia was also a feature in 3 cases with *SLC25A22* mutations [35] (▶ Chap. 30).

21.5.3 Genetics

The mode of inheritance is autosomal recessive. *PRODH*, the gene encoding proline dehydrogenase, maps to the region deleted in the velocardiofacial syndrome/DiGeorge syndrome. Numerous missense mutations have been identified, not all of which are associated with enzyme deficiency [33, 36].

21.5.4 Diagnostic Tests

The diagnosis is made by amino acid analysis. Direct enzyme assay is not possible, since the enzyme is not expressed in leukocytes or skin fibroblasts. Mutation analysis is thus necessary to confirm the diagnosis.

21.5.5 Treatment and Prognosis

Since the prognosis is generally excellent, dietary treatment is not indicated.

21.6 Δ1-Pyrroline-5-Carboxylate Dehydrogenase Deficiency (Hyperprolinaemia Type II)

21.6.1 Clinical Presentation

This is a relatively benign disorder, though a predisposition to recurrent seizures is highly likely [37] and has recently also been described in a 64 year old adult [38]. Only about 50% of patients with hyperprolinemia type II present with seizures during infancy or childhood. These seizures may be triggered by fever and may respond to common anticonvulsants. Most individuals have accompanying learning disabilities. This disease was first identified in the Irish Traveller population and may remain largely undiagnosed outside of this inbred community.

21.6.2 Metabolic Derangement

Hyperprolinaemia type II is caused by a deficiency of pyrroline 5-carboxylate (P5C) dehydrogenase, a mitochondrial inner-membrane enzyme involved in the conversion of proline into glutamate (◘ Fig. 21.1). Hence, in hyperprolinaemia type II there are increased levels of proline in plasma (usually exceeding 2000 μM; normal range 100–450 μM), urine and CSF, as well as of P5C. Heterozygotes do not have hyperprolinaemia. Evidence has been presented that the accumulating P5C is a vitamin B_6 antagonist (owing to adduct formation) and that the seizures in this disorder may be due at least in part to vitamin B_6 inactivation [39] (▶ Chap. 29).

21.6.3 Genetics

This autosomal recessive disease is caused by *ALDH4A1* mutations and has been reported in only very few patients [33, 39, 40].

21.6.4 Diagnostic Tests

The accumulation of P5C in physiological fluids is used to differentiate between type II and type I hyperprolinaemia. This compound can be qualitatively identified by its reactivity with ortho-aminobenzaldehyde and can be quantitatively measured by several specific assays [33]. P5C dehydrogenase activity can be measured in skin fibroblasts and leukocytes.

21.6.5 Treatment and Prognosis

The benign character of the disorder does not justify dietary treatment (which, in any case, would be very difficult). Seizures are B_6 responsive.

21.7 Polyamine Synthetic Defects

Polyamines, (spermidine, $H_2N(CH)_4NH(CH)_3NH_2$; and spermine, $H_2N(CH)_3NH(CH)_4(CH)_3NH_2$), are small polycations with diverse biological functions including regulation of cell growth and differentiation. Polyamines bind DNA, RNA, and certain proteins affecting the structure and stability of these key macromolecules [41]. Ornithine is the precursor of the three-step polyamine biosynthetic pathway that involves the sequential action of ornithine decarboxylase (ODC), spermidine synthase and spermine synthase (◘ Fig. 21.1). ODC is a highly

regulated homodimeric enzyme that catalyzes the conversion of ornithine to putrescine ($H_2N(CH)_4NH_2$). ODC has a remarkably short half life (~ 10 minutes) and its expression is regulated at several levels including protein stability mediated by a 37 amino acid C-terminal domain which is the binding site for one of three protein regulators or antizymes, designated AZ1, AZ2, and AZ3, each encoded by paralogous genes, *OAZ1, OAZ2,* and *OAZ3*, respectively. Binding of one of the three antizymes targets ODC for ubiquitin-independent proteosomal degradation [41, 42].

21.8 Ornithine Decarboxylase (ODC) Superactivity Syndrome

In 2018, two groups independently reported a total of five unrelated patients with a history of polyhydramnios, developmental delay, relative macrocephaly, sparse scalp hair, absent eyebrows, sparse eyelashes, a tall forehead and MRI abnormalities of the central nervous system including periventricular cysts and abnormalities of the corpus callosum [43, 44, reviewed in 45]. All affected individuals were heterozygous for unique de novo C-terminal truncating variants in the final exon of *ODC1*. These variant alleles encode a mutant ODC with full catalytic activity but insensitive to inhibition mediated by antizyme binding to the C-terminus resulting in a net gain-of-ODC function. In support of this model, one patient had elevated N-acetyl putrescine detected on a metabolomics assay of serum while another had increased RBC ODC by immunoblot and a twofold increase in RBC polyamines [43, 44]. Moreover, a transgenic mouse model overexpressing ODC in hair follicle keratinocytes demonstrated a hair phenotype orthologous to that in humans [46]. These observations provide compelling evidence for ODC "superactivity" resulting from variants that disrupt antizyme binding as the cause of this clinical phenotype.

ODC regulation is an important and complicated biological system that likely harbors additional inborn errors, yet to be recognized. There are 5′ and 3′ translational regulators of ODC mRNA, the 3 parologous antizymes that promote degradation ODC protein by binding to the C-terminus as well as an ODC-related protein (AzI) which blocks the ODC inhibitory activity of the antizymes [47]. Importantly, potential treatment is possible using 2-difluoromethylornithine (DFMO), a specific ODC inhibitor has been used to treat certain malignancies [42]. Although some damage clearly occurs in utero, DFMO may well have some beneficial effect for patients with ODC superactivity syndrome, especially if instituted early. DFMO administration to the mouse model restored hair growth [46].

Standard metabolic tests on these patients including plasma amino acids and urine organic acids were normal. Thus, diagnosis of additional patients will require informed clinical suspicion leading to metabolomic and/or molecular testing.

21.9 Spermine Synthase Deficiency (Snyder Robinson Syndrome)

Snyder Robinson syndrome is an X-linked disorder characterised by moderate to severe intellectual disability, unsteady gait, hypotonia and variable dysmorphism with osteoporosis [48]. It is due to deficiency of spermine synthase, an enzyme in the ornithine decarboxylase pathway leading from putrescine to spermine (◘ Fig. 21.1). Spermine synthase deficiency leads to excessive spermidine catabolism, which generates toxic metabolites that have been shown to cause lysosomal defects and oxidative stress. Consequently, autophagy-lysosome flux and mitochondrial function are compromised in the Drosophila nervous system and SRS patient cells [49]. An evocative metabolic signature including elevation of spermidine, isoputreanine, ornithine and N8-acetylspermidine, a novel potential biomarker, has recently been identified by metabolomics [48].

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Cerebral Organic Acid Disorders and Other Disorders of Lysine Catabolism

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Contents

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Catabolism of Lysine, Hydroxylysine, and Tryptophan

Species-, organ- and organelle-specific differences in the enzymes involved in the catabolism of lysine, hydroxylysine and tryptophan are not yet completely unravelled, and this synopsis is therefore partially hypothetical as far as human metabolism is concerned. Lysine, hydroxylysine, and tryptophan are thought to be degraded within the mitochondrium, initially via separate pathways, which converge into a common pathway at the point of 2-aminoadipic-6-semialdehyde (hydroxylysine catabolism and pipecolic acid pathway of lysine catabolism) and at the point of 2-oxoadipic acid (tryptophan catabolism; ◘ Fig. 22.1). The major route of lysine catabolism in most tissues is via the saccharopine pathway starting with the bifunctional enzyme 2-aminoadipic-6-semialdehyde synthase (enzyme 1). A small amount of lysine is catabolised via pipecolic acid and the peroxisomal key enzyme pipecolic acid oxidase (enzyme 2); this pipecolic acid pathway, however, might be an important route of lysine catabolism in the brain. Unlike in bacteria, however, the human origin of pipecolic acid is not yet fully understood. An orthograde production of pipecolic acid from lysine but also retrograde production from 2-aminoadipic-6-semialdehyde was shown. In addition, the microbiome should be considered as an alternative source. Hydroxylysine enters the pathway after phosphorylation by hydroxylysine kinase (enzyme 3. 2-Aminoadipic-6-semialdehyde is converted into 2-aminoadipic acid by 2-aminoadipic-6-semialdehyde dehydrogenase (antiquitin, enzyme 4, which is then converted to 2-oxoadipic acid by 2-aminoadipate aminotransferase (enzyme 5. 2-Oxoadipic acid is primarily converted to glutaryl-CoA by the dehydrogenase E1 and transketolase domains-containing protein 1 (DHTKD1), an enzyme with 2-oxoadipic dehydrogenase activity, forming the 2-oxoadipate dehydrogenase complex (OADHc, enzyme 6a) together with dihydrolipoamide S-succinyltransferase (DLST) and dihydrolipoamide dehydrogenase (DLD) similar to the 2-oxoglutarate dehydrogenase complex of the Krebs cycle. The E1 subunit of the 2-oxoglutarate dehydrogenase complex, 2-oxoglutarate dehydrogenase (OGDH), has a higher affinity for 2-oxoglutarate, but can alternatively accept 2-oxoadipic acid as a substrate (enzyme 6b). DHTKD1 and OGDH, which display substrate overlap, form a mitochondrial megacomplex with DLST and DLD. Glutaryl-CoA is dehydrogenated and decarboxylated to crotonyl-CoA by glutaryl-CoA dehydrogenase (enzyme 7). This enzyme transfers electrons to flavin adenine dinucleotide (FAD) and hence to the respiratory chain via electron transfer protein (ETF)/ETF-dehydrogenase (ETF-DH). Crotonyl-CoA is subsequently converted to 3-hydroxybutyryl-CoA by short-chain enoyl-CoA hydratase 1 (ECHS1, enzyme 8, ► Chap. 18). This enzyme is multispecific and also acts as a crotonase in the degradative pathways of valine, isoleucine, and short-chain fatty acids. 3-Hydroxybutyryl-CoA is converted to acetoacetyl-CoA by 3-hydroxyacyl-CoA dehydrogenase (enzyme 9, ► Chap. 13). Glutaric acid, which may derive from the intestinal microbiome, spontaneous disintegration of glutaryl-CoA or other sources, is reactivated by succinyl-CoA-dependent conversion of succinate-hydroxymethylglutarate CoA transferase to glutaryl-CoA (enzyme 10). From the six distinct enzyme deficiencies identified in the degradation of lysine, only enzymes 4, 6b, 7, and 8 have clinically proven relevance as metabolic disorders. Glutaric aciduria type I is caused by deficient glutaryl-CoA dehydrogenase (enzyme 7). Glutaric aciduria type II, caused by ETF/ETF-DH deficiencies, is discussed in ► Chap. 12. Pipecolic acid oxidase (enzyme 2) is discussed in the context of peroxisomal disorders in ► Chap. 42, 2-aminoadipic-6-semialdehyde dehydrogenase (antiquitin, enzyme 4) deficiency in ► Chap. 29, 2-oxoglutarate dehydrogenase deficiency (enzyme 6b) in ► Chap. 11, and ECHS1 deficiency (enzyme 8) in ► Chap. 18, since its major pathogenic effect is located in the valine catabolic pathway. Finally, several recent findings point to new functions for different short-chain lysine acylations of mitochondrial proteins (non-enzymatic acylations) and histones (enzyme-mediated) as important posttranslational modifications that regulate various cellular processes. Human inborn errors of these processes are yet to be discovered.

L-2- and D-2-Hydroxyglutaric aciduria type I are caused by deficiencies of specific FAD-dependent dehydrogenases, whereas D-2-hydroxyglutaric aciduria type II is caused by gain-of-function mutations of mitochondrial isocitrate dehydrogenase 2 and D-2-/L-2-hydroxyglutaric aciduria by inherited deficiency of the mitochondrial citrate carrier which mediates transport of dicarboxylic metabolites between the mitochondrion and the cytosol (◘ Fig. 22.2). Aspartoacylase (aminoacylase 2) irreversibly splits *N*-acetylaspartic acid (NAA), a brain-specific compound where its concentration reaches approximately 20 mM, into acetate and aspartate in oligodendrocytes (not illustrated). Deficiency of this enzyme causes *N*-acetylaspartic aciduria (Canavan disease). Deficiency in *N*-acetyltransferase (NAT), which catalyses NAA synthesis, causes hypoacetylaspartia.

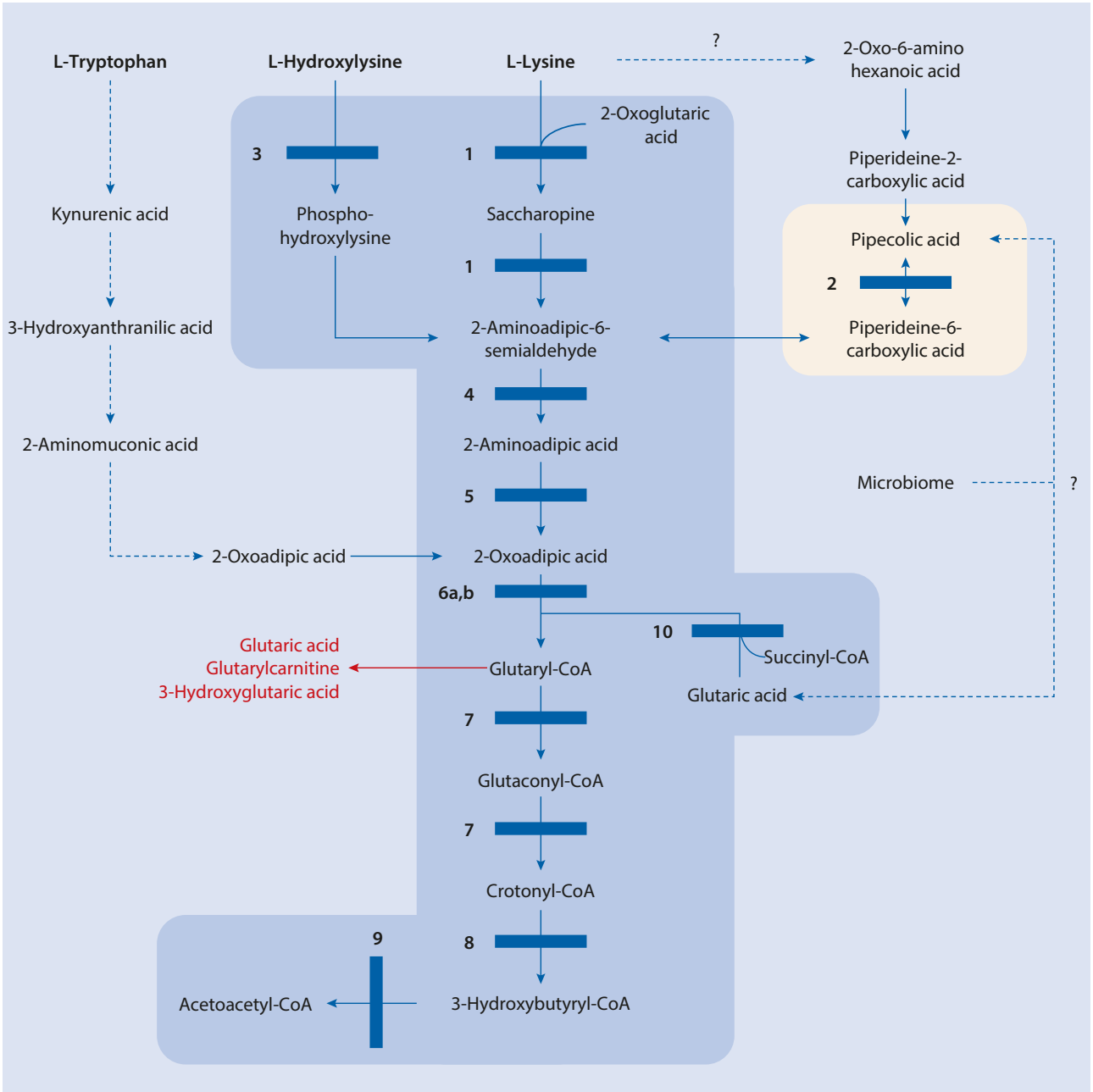

Fig. 22.1 Tryptophan, hydroxylysine and lysine catabolic pathways. *1*, 2-aminoadipic-6-semialdehyde synthase; *2*, pipecolic acid oxidase; *3*, hydroxylysine kinase; *4*, 2-aminoadipic-6-semialdehyde dehydrogenase (antiquitin); *5*, 2-aminoadipate aminotransferase); *6a*, 2-oxoadipate dehydrogenase complex with DHTKD1 as E1 subunit; *6b*, 2-oxoglutarate dehydrogenase complex, an enzymatic complex of the Krebs cycle, accepts 2-oxoadipic acid as an alternative substrate to 2-oxoglutarate; *7*, glutaryl-CoA dehydrogenase; *8*, short-chain enoyl-CoA hydratase 1 (crotonase); *9*, 3-hydroxyacyl-CoA dehydrogenase; *10*, succinate-hydroxymethylglutarate-CoA transferase. Enzyme deficiencies are indicated by solid bars across the arrows. Question marks indicate current uncertainties in the human lysine catabolic pathway. Note that the pathways are compartmentalized. The blue shaded area depicts the mitochondrial part, while the yellow box contains the peroxisomal part and the colourless area the cytosolic part of the pathways (with minor simplifications). Red coloured metabolites are elevated in glutaric aciduria type I

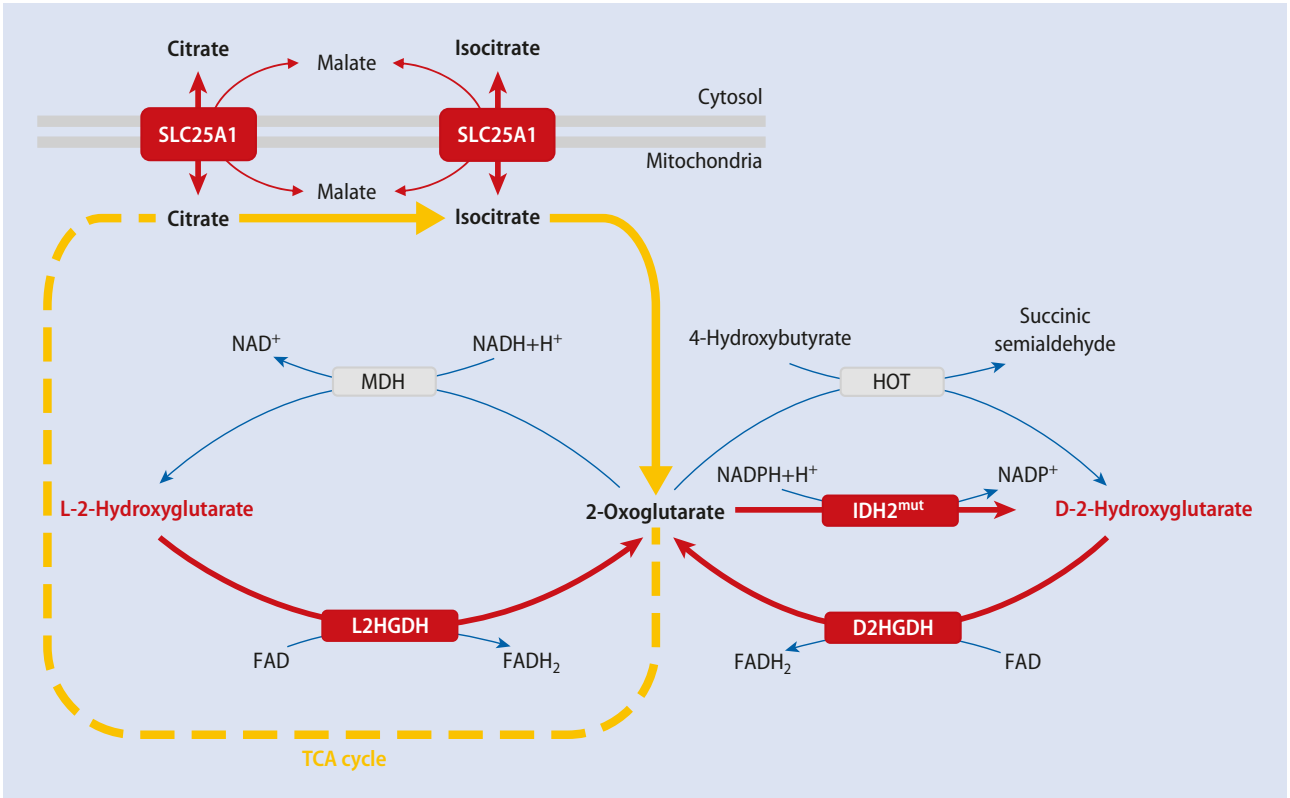

Fig. 22.2 Molecular origin of 2-hydroxyglutaric acidurias. The tricarboxylic acid (TCA) cycle intermediate 2-oxoglutarate is key to the understanding of 2-hydroxyglutaric acidurias. L-2-hydroxyglutarate is formed from 2-oxoglutarate by a side reaction of mitochondrial L-malate dehydrogenase (MDH) and, subsequently, this "faulty" metabolite is reconverted by L-2-hydroxyglutarate dehydrogenase (L2HGDH), a proof-reading enzyme, deficient in L-2-hydroxyglutaric aciduria. 2-Oxoglutarate is also used as a substrate by hydroxyacid-oxoacid transhydrogenase (HOT) for the conversion of 4-hydroxybutyrate to succinic semialdehyde, forming D-2-hydroxybutyrate and hence coupling TCA cycle and GABA metabolism. D-2-hydroxyglutarate is reconverted to 2-oxoglutarate by D-2-hydroxyglutarate dehydrogenase (D2HGDH), which is deficient in D-2-hydroxyglutaric aciduria type I. Although this enzyme is functional in D-2-hydroxyglutaric aciduria type II its capacity is exceeded by the conversion of 2-oxoglutarate to D-2-hydroxyglutarate by the neomorphic isocitrate dehydrogenase 2 due to a gain-of-function mutation. The above discussed enzymes are all functional in combined L-2- and D-2-hydroxyglutaric aciduria, which is caused by inherited deficiency of the mitochondrial tricarboxylate transporter, also called mitochondrial citrate carrier (SLC25A1), mediating the exchange transport of malate against citrate and isocitrate between mitochondria and cytosol. Deficiency of this transporter increasingly shunts mitochondrial citrate and isocitrate towards 2-oxoglutarate, the substrate for both L-2- and D-2-hydroxyglutarate synthesis by MDH and HOT, respectively

Introduction

Twelve inborn errors of metabolism are described in this chapter. Glutaric aciduria type I, L-2-hydroxyglutaric aciduria, D-2-hydroxyglutaric aciduria (type I and II), D-2-/L-2-hydroxyglutaric aciduria, N-acetylaspartic aciduria, and hypoacetylaspartia are all associated with neurological disease of varying severity whereas hyperlysinaemia/saccharopinuria, hydroxylysinuria, 2-aminoadipic and 2-oxoadipic aciduria, aminoacylase 1 deficiency and glutaric aciduria type III are likely non diseases or have an unclear clinical significance.

A group of organic acid disorders predominantly presents with (progressive) neurological symptoms of ataxia, epilepsy, myoclonus, pyramidal symptoms reflecting white matter disease, extrapyramidal symptoms due to basal ganglia lesions, and macrocephaly [1]. The core cerebral organic acid disorders are glutaric aciduria type I, D-2-hydroxyglutaric aciduria (types I and II), L-2-hydroxyglutaric aciduria, D-/L-2-hydroxyglutaric aciduria, succinic semialdehyde dehydrogenase deficiency (▶ Chap. 30), and *N*-acetylaspartic aciduria. Strikingly, in all these disorders the pathological compounds that accumulate either are odd-chain dicarboxylic acids (D-2-, L-2-, 3-hydroxyglutarate, glutarate) sharing the same carbon backbone with the excitatory amino acid glutamate (2-amino-glutarate), or have been suggested to be neurotransmitters/–modulators (γ-hydroxybutyrate, *N*-acetylaspartylglutamate). Evidence is accumulating from in vitro and in vivo studies showing that these acyl-CoA esters and accompanying carbonic acids indeed interfere with important pathways of cerebral metabolism, including glutamatergic

or gamma amino butyric acid (GABA)-ergic neurotransmission, cerebral energy metabolism, and myelin metabolism. Delayed myelination or progressive white matter disease, basal ganglia injury and cerebellum pathology, the main pathologies in cerebral organic acid disorders, are also characteristic of mitochondrial disorders, suggesting at least partial common pathological mechanisms. In L-2-hydroxyglutaric aciduria, the risk of developing cerebral neoplasms is increased.

Among this disease group, only glutaric aciduria type I forms characteristic acylcarnitines (i.e. glutarylcarnitine), which can be used for mass screening of newborns by tandem mass spectrometry. Metabolic hallmarks such as hypoglycaemia, metabolic acidosis, lactic acidaemia, or hyperammonaemia, the usual concomitants of branched-chain, 'classic' organic acid disorders (▶ Chap. 18), are generally absent, and hence the correct diagnosis requires an increased awareness of referring physicians and biochemists. Diagnostic clues can be derived from neuroimaging findings (◘ Figs. 22.3, and 22.4). Progressive disturbances of myelination, cerebellar atrophy, cortical atrophy, signal changes and/or atrophy of the basal ganglia and any symmetrical (fluctuating) pathology apparently independent of defined regions of vascular supply are suggestive.

In contrast to the cerebral organic acid disorders and pyridoxine-dependent epilepsy due to 2-aminoadipic-6-semialdehyde dehydrogenase deficiency (▶ Chap. 29), the other known defects of lysine and hydroxylysine degradation all appear to be rare biochemical variants of human metabolism with low clinical significance.

Increasing evidence points to a close link between metabolism and cell signalling via short-chain lysine acylations of metabolic proteins and histones such as acetylation, succinylation, malonylation, and glutarylation. Lysine acylation modifies mitochondrial function, enzyme activity, and enables concerted adaptation to environmental changes and hence is considered an important posttranslational modification.

22.1 Hyperlysinaemia (2-Aminoadipic Semialdehyde Synthase Deficiency)/Saccharopinuria

22.1.1 Clinical Presentation

About half of the identified individuals were detected incidentally and were healthy [2]. Symptoms include developmental delay, epilepsy, spasticity, ataxia, short stature, joint laxity, and spherophakia, respectively. Overall, the associated phenotypes appeared to be random rather than causally linked.

22.1.2 Metabolic Derangement

Hyperlysinaemia/saccharopinuria is caused by deficiency of the bifunctional protein 2-aminoadipic semialdehyde synthase (enzyme 1 in ◘ Fig. 22.1). This is the first enzyme of the mitochondrial saccharopine pathway, which is the main route of lysine degradation in most tissues [3]. The two functions of this enzyme, lysine:2-oxoglutarate reductase and saccharopine dehydrogenase, may be affected differently by gene variations. Most often, both activities are decreased, resulting in predominant hyperlysinaemia and hyperlysinuria with relatively mild saccharopinuria (hyperlysinaemia I). In hyperlysinaemia II/saccharopinuria, saccharopine dehydrogenase activity is more decreased than lysine:2-oxoglutarate reductase activity, resulting in a predominant excretion of saccharopine.

Failure to remove the ε-amino group is thought to result in an overflow of the minor lysine degradation pathway, with removal of the α-amino group by oxidative deamination. The oxoacid cyclises and is reduced to pipecolic acid. As a consequence, hyperpipecolataemia is regularly observed in hyperlysinaemia. Unlike in bacteria, however, this orthograde production of pipecolic acid from lysine is not generally accepted, since the enzyme initiating this pathway has not yet been identified in man.

Hyperlysinuria can also result from impaired renal tubular transport, often as part of a genetic transport defect of dibasic amino acids (▶ Chap. 25). In this situation it occurs without hyperlysinaemia.

22.1.3 Genetics

Hyperlysinaemia/saccharopinuria is caused by bi-allelic variations in *AASS* [3].

22.1.4 Diagnostic Tests

The initial observation in individuals with hyperlysinaemia/saccharopinuria is an impressive lysinuria with up to 15,000 mmol/mol creatinine (controls <70). Detailed amino acid analysis reveals additional accumulation of saccharopine, homoarginine, 2-amino adipic acid, and pipecolic acid. Elevations of the same metabolites can be documented in other body fluids, such as plasma and cerebrospinal fluid (CSF), with high lysine as the predominant abnormality (up to 1700 μmol/l in plasma, controls <200, and up to 270 μmol/l in CSF, controls <28). Secondary hyperlysinaemias due to mitochondrial shortage of 2-oxoglutarate are also observed in urea cycle disorders, pyruvate carboxylase deficiency,

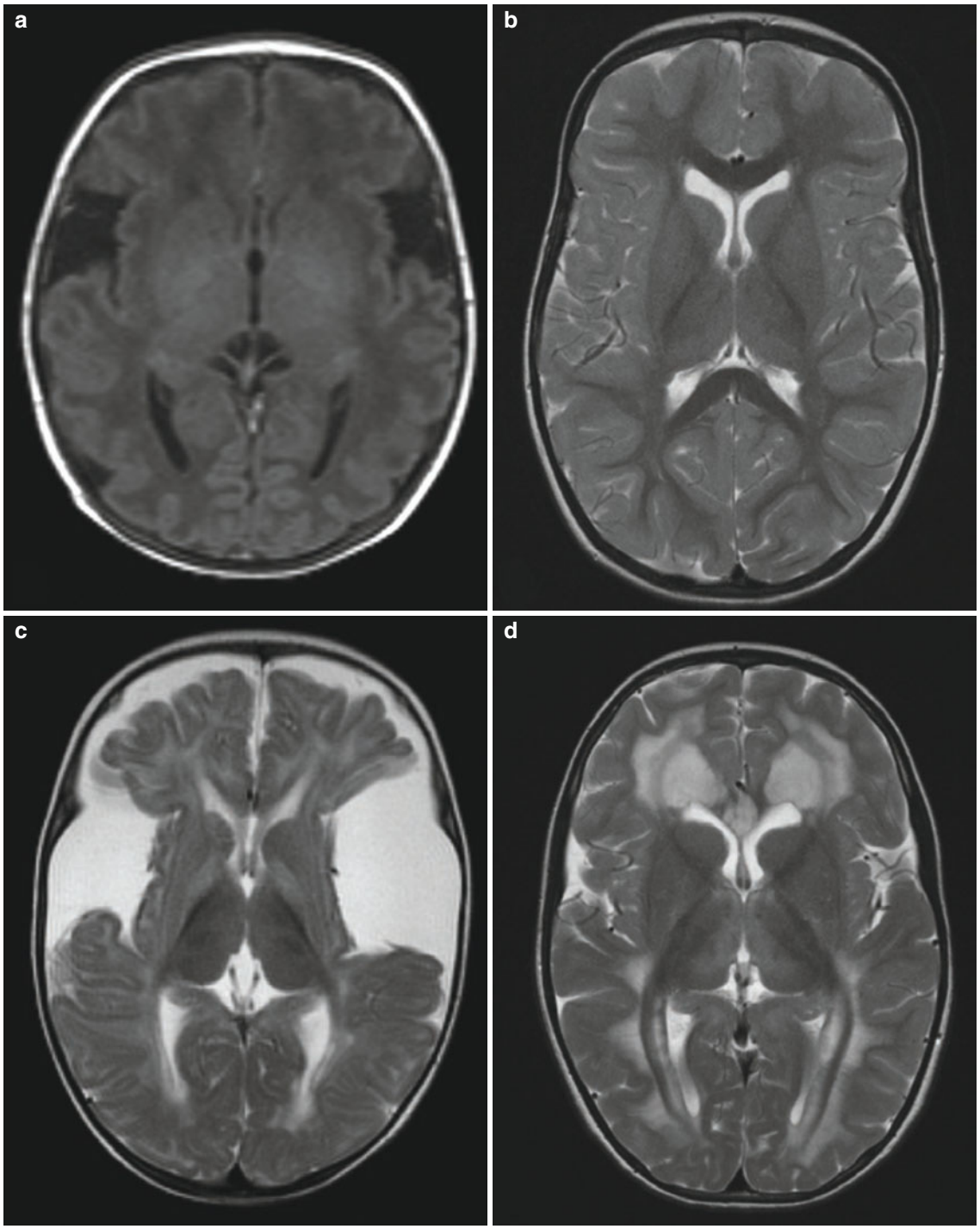

Fig. 22.3 MRI findings in patients with glutaric aciduria type I. **a,** T1-weighted axial MRI of an asymptomatic male newborn with glutaric aciduria type I, showing enlargement of temporopolar and frontopolar CSF spaces and an immature gyration pattern. **b,** T2-weighted axial MRI of an asymptomatic 2-year-old girl identified by newborn screening. Previously dilated external CSF spaces and temporal hypoplasia have normalised. There is no pathology of the basal ganglia or anywhere else. **c,** T2-weighted axial MRI at age 7.5 months showing striatal atrophy and markedly dilated temporopolar and frontopolar CSF spaces. Signal abnormalities of globus pallidus, thalamus, and supratentorial white matter are also found. This child presented with moderate axial hypotonia, which progressed after a delay in the start of emergency treatment during an infectious disease. After a further 4 weeks, the child developed dystonia of all extremities. **d,** T2-weighted axial MRI of a girl at age 11 years with suspected late-onset disease variant showing marked hyperintensity of the supratentorial white matter sparing the U fibres and mild to moderate signal changes of the caudate, thalamus, and dentate nuclei (not shown). The girl presented with nausea and vertigo at 10 years of age, which has improved following the start of carnitine supplementation and a protein-controlled diet. Motor and cognitive function is normal. (By courtesy of Dr. Inga Harting and Dr. Angelika Seitz)

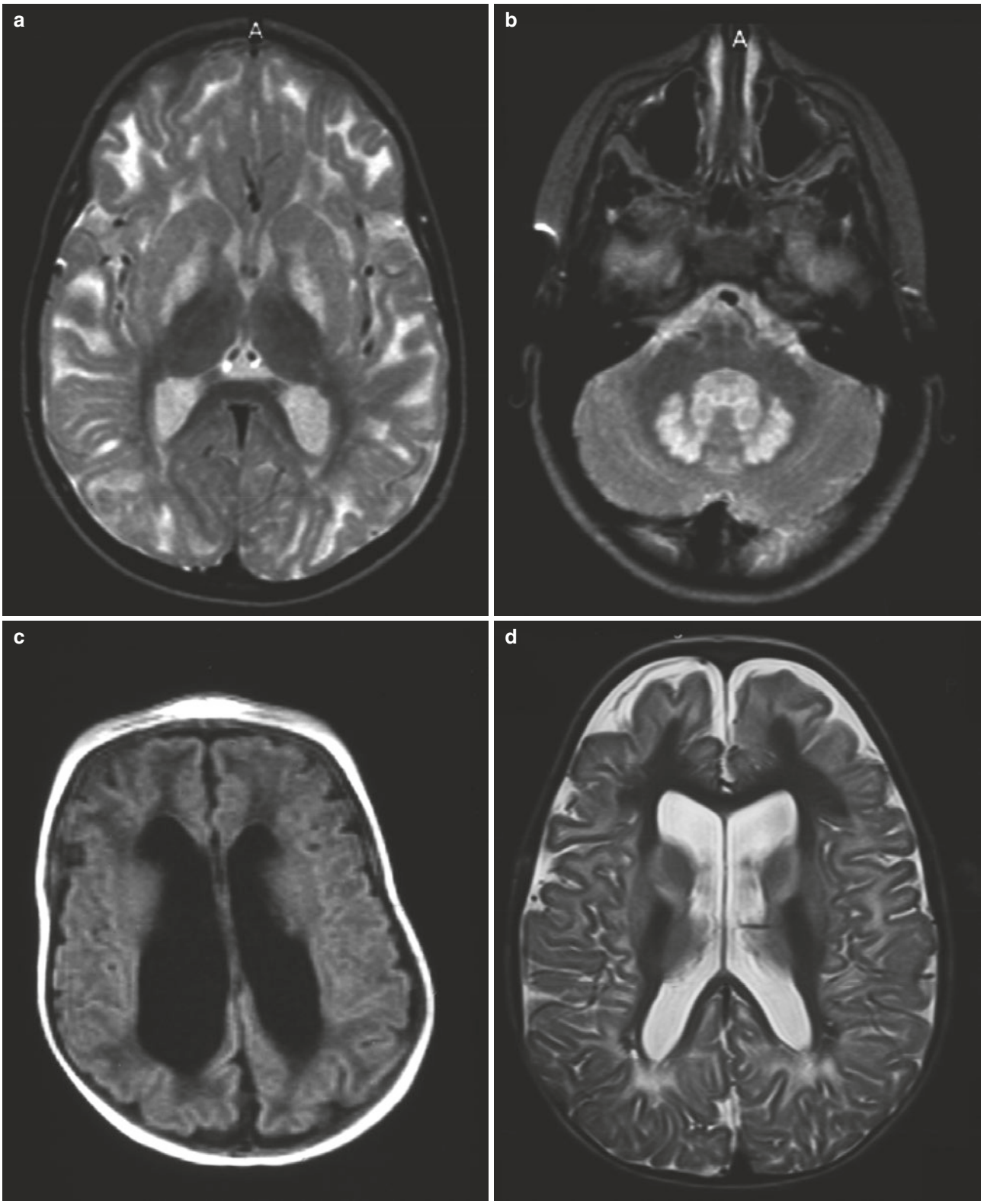

Fig. 22.4 MRI findings in patients with other cerebral organic acidurias. **a & b,** Axial T2-weighted MRI of a 8.5-year-old boy with L-2-hydroxyglutaric aciduria, illustrating characteristic involvement of subcortical white matter (also affecting the U fibres) and globus pallidus (**a**), and symmetrical involvement of the dentate nuclei (**b**). **c,** Axial MRI of a 2-month-old girl with D-2-hydroxyglutaric aciduria type I. Note the delayed myelination and occipitally pronounced enlargement of lateral ventricles. **d,** Axial fast spin echo image of a 6.5-year-old girl suffering from *N*-acetylaspartic aciduria. Note the marked discrepancy between the severely affected subcortical white matter and the relatively spared central white matter, at least frontally

methylmalonic and propionic acidurias [4], and L-2-hydroxyglutaric aciduria.

The deficiency of 2-aminoadipic semialdehyde synthase can be confirmed by molecular genetic studies and by determining the overall degradation of [1-^{14}C] lysine to $^{14}CO_2$ or the specific activity of lysine:2-oxoglutarate reductase and saccharopine dehydrogenase, respectively, in fibroblasts [3].

22.1.5 Treatment and Prognosis

Long-term dietary restriction of lysine has no proven benefit. As affected individuals do not suffer from metabolic decompensations, specific interventions during intercurrent illnesses are not recommended. Hyperlysinaemia/saccharopinuria has not been associated with an increased risk of mortality.

22.2 Hydroxylysinuria (Hydroxylysine Kinase Deficiency)

Hydroxylysinuria and concomitant hydroxylysinaemia has been identified in a few patients, all of whom showed some degree of cognitive disability [5]. No further clinical and/or biochemical studies were reported. The abnormality can be assumed to be caused by a defect of hydroxylysine kinase (enzyme 3 in ◘ Fig. 22.1).

22.3 2-Aminoadipic and 2-Oxoadipic Aciduria (DHTKD1 Deficiency)

22.3.1 Clinical Presentation

2-Aminoadipic and 2-oxoadipic aciduria is thought to be of low or even no clinical significance. Over 20 individuals are known, more than half of whom are asymptomatic [6]. Symptoms include psychomotor retardation, muscular hypotonia, epilepsy, ataxia, and failure to thrive, but it is likely that these are coincidental findings. However, a heterozygous nonsense mutation in *DHTKD1* (p.Tyr485Xaa) has been associated with Charcot-Marie-Tooth disease type 2Q in a large Chinese pedigree [7].

22.3.2 Metabolic Derangement

The metabolic profile is heterogeneous, with most patients showing elevations of 2-aminoadipic, 2-oxoadipic and 2-hydroxyadipic acid, whereas some excrete only 2-aminoadipic acid. Isolated excretion of 2-aminoadipic acid may be caused by antiepileptic therapy with vigabatrin, which inhibits 2-aminoadipate aminotransferase. 2-Aminoadipic acid is deaminated to 2-oxoadipic acid by a mitochondrial 2-aminoadipate aminotransferase. 2-Oxoadipic acid is also formed from the degradation of tryptophan, but this is not yet fully understood in humans. 2-Oxoadipic acid is further metabolised to glutaryl-CoA via two distinct enzyme complexes: The major pathway involves the 2-oxoadipate dehydrogenase complex (OADHc) which contains DHTKD1 as E1 subunit, a close homolog to 2-oxoglutarate dehydrogenase (OGDH). DHTKD1 has a high affinity for 2-oxoadipic acid. Alternatively, 2-oxoadipic acid can be handled by the oxoglutarate dehydrogenase complex (OGDHc), containing OGDH as E1 subunit which prefers 2-oxoglutaric acid as a substrate but can alternatively handle 2-oxoadipic acid if DHTKD1 is blocked or deficient. Evidence is increasing that OGDH, DHTKD1, DLST, and DLD can form a hybrid 2-oxo acid dehydrogenase complex [8].

22.3.3 Genetics

Autosomal recessive inheritance is implied by the pedigrees and by the finding that parents cannot be biochemically differentiated from controls. In 2012, pathogenic mutations in *DHTKD1* localized on 10p14 were identified as molecular cause of 2-aminoadipic and 2-oxoadipic aciduria [9].

22.3.4 Diagnostic Tests

Affected individuals are diagnosed by demonstrating variable elevations of 2-aminoadipic acid on amino acid chromatography and/or of 2-oxoadipic and 2-hydroxyadipic acids on urinary organic acid analysis. Plasma lysine may be twofold elevated and urinary glutaric acid up to 50 mmol/mol of creatinine (controls <9). The suspected diagnosis is confirmed by identification of two disease-causing *DHTKD1* variants.

22.3.5 Treatment and Prognosis

Autosomal recessive 2-aminoadipic and 2-oxoadipic aciduria is likely to be a non-disease, while individuals with a specific heterozygous nonsense variant may develop Charcot Marie Tooth disease type 2Q. Individuals with 2-aminoadipic and 2-oxoadipic aciduria as well as with Charcot Marie Tooth disease type 2Q do not suffer from metabolic decompensations,

and specific interventions during intercurrent illnesses do not appear necessary. Administration of pharmacological doses of vitamins B_1 (thiamine) and B_6 (pyridoxine) as well as low lysine diet had no effect on the levels of pathological metabolites and no proven clinical benefit.

22.4 Glutaric Aciduria Type I (Glutaryl-CoA Dehydrogenase Deficiency)

22.4.1 Clinical Presentation

Glutaric aciduria type I should be seriously considered in the differential diagnosis of any infant who has macrocephaly combined with hypoplasia of the temporal cortex and concomitantly enlarged Sylvian fissures, striatal lesions (putamen > caudate > > globus pallidus), and variable signal changes of periventricular white matter on magnetic resonance imaging (MRI) (Fig. 22.3) and/or a complex movement disorder with predominant dystonia, orofacial dyskinesia and dysarthria superimposed on axial hypotonia. Chorea may also be observed [10, 11]. In 75% of patients, macrocephaly is present at or shortly after birth, peaking at the age of 3–6 months, preceding the severe neurological disease and possibly a clue to diagnosis in countries without extended newborn screening programs. Furthermore, affected babies often present with potentially reversible muscular hypotonia which may slow motor development in the first year of life. Neuroimaging studies have been performed in a number of asymptomatic newborns and infants, revealing the characteristic findings of temporal hypoplasia (95% of all patients; (Fig. 22.3), wide anterior temporal and sylvian CSF spaces, an immature gyration pattern, delayed myelination, and isolated T_2 hyperintensity in the globus pallidus [12]. These non-striatal MRI abnormalities may completely resolve if treatment is started in the newborn period (Fig. 22.3). The clinical significance of enlarged subdural fluid spaces in infants with glutaric aciduria type I is the unprotected crossing of these spaces by bridging veins. Such infants are prone to suffer acute subdural haemorrhages, which may be accompanied by retinal haemorrhages, after only minor head trauma, particularly around the first birthday when starting to walk. Parents of children with glutaric aciduria type I have been wrongly accused for abusive head trauma [13]. Alternatively, vascular abnormalities have been explained by altered haemodynamics and endothelial dysfunction [14].

At a median age of 9–10 months, the majority of untreated patients suffer an acute brain injury, usually associated with febrile infectious disease, but this acute encephalopathic crisis may also be precipitated by any other episode that induces catabolism, including undesirable reactions following routine immunisations [11]. MRI reveals striatal injury spreading in a dorsoventral direction (Fig. 22.3), starting at the dorsolateral aspects of the putamen. Almost all reported encephalopathic crises have occurred before 36 months of age. They have not yet been described at school age, during adolescence, or in adulthood. Acquired motor skills are often acutely lost, including the ability to sit, pull up to standing, to suck and swallow, and head control. The infants appear alert with profound muscular hypotonia of the trunk and a mobile dystonia of the extremities which develops over days to months following striatal injury. Usually there are no metabolic derangements as in "classic" organic acidurias. If the underlying metabolic disorder remains undiagnosed, additional cerebral systems are slowly but progressively affected. Impaired chewing and swallowing, vomiting and aspiration, plus increased energy demand due to increased muscle tone frequently results in failure to thrive and malnutrition. Kyphoscoliosis and chest wall dystonia can cause restrictive lung disease. Early death (40–50% of symptomatic patients by the age of 20 years) may occur in the course of pneumonia and respiratory failure, during hyperpyrexic crises, or suddenly without apparent cause [11].

Although the majority of patients present with characteristic symptoms and disease course, the natural history of glutaric aciduria type I can be variable even within families. With the implementation of extended newborn screening programs a growing number of patients with a so-called insidious onset disease variant have been identified. These patients develop dystonia over weeks and months without a preceding episode that is known to precipitate striatal injury; however, more subtle symptoms like hand tremor and mild oral dyskinesia could also be the first manifesting signs. Striatal injury and dystonia tend to be less severe than in individuals with acute encephalopathic crises. In neonatally screened individuals, the insidious-onset variant is most frequently found in individuals not receiving or adhering to the recommended low lysine diet [15, 16]. A few individuals, mainly diagnosed in adolescence or adulthood during family studies, or previously undiagnosed women with glutaric aciduria type I, identified through false positive newborn screening results of their non-affected children, have not developed striatal injury despite never having been treated. Finally, previously unaffected adolescent and adult patients can present with progressive signal changes in the white matter but unaffected basal ganglia [12]. Although this was initially suggested to represent a late-onset disease variant, recent studies have not supported the notion of a distinct disease course (Fig. 22.3). Recent MRI studies

demonstrate that white matter changes are a common finding in glutaric aciduria type I. They progress with age and are commonly found in high excretor patients [16, 17], highlighting the risk of long-term neurotoxicity due to cerebral accumulation of glutarate and 3-hydroxyglutarate and concomitantly progressive neuroaxonal compromise.

Chronic kidney disease has recently been detected as the first non-neurologic presentation in a growing number of patients, even in those identified by newborn screening, and does not seem to be impacted by recommended therapy [16].

Finally, three patients with glutaric aciduria type I with poor adherence or late start of recommended therapy and malignant brain tumors have been reported [18]. More information is required and careful clinical and neuroradiological follow-up is required to understand whether patients with glutaric aciduria type I bear an increased risk of developing brain neoplasms similar as in L-2-hydroxyglutaric aciduria.

22.4.2 Metabolic Derangement

Glutaric aciduria type I is caused by a deficiency of glutaryl-CoA dehydrogenase, a mitochondrial flavin adenine dinucleotide (FAD)-requiring enzyme, which catalyses the dehydrogenation of glutaryl-CoA as well as the subsequent decarboxylation of glutaconyl-CoA to crotonyl-CoA (enzyme 7 in ◘ Fig. 22.1). In glutaric aciduria type I, part of the accumulating glutaryl-CoA in mitochondria is esterified with carnitine to glutarylcarnitine by carnitine acyltransferase, leading to an increased ratio of acylcarnitines to free carnitine in plasma and urine. Glutarylcarnitine is excreted, contributing to secondary carnitine deficiency.

The mechanisms of age-specific destruction of specific cerebral structures in glutaric aciduria type I is complex. Evidence points to impaired brain energy metabolism and production of reactive oxygen species induced by accumulating glutaric acid, 3-hydroxyglutaric acid, and glutaryl-CoA: glutaryl-CoA inhibits the 2-oxoglutarate dehydrogenase complex, glutaric acid impairs the dicarboxylic acid shuttle and hence the metabolic coupling between astrocytes and neurons, and 3-hydroxyglutaric acid promotes excitotoxic mechanisms [19]. Accumulation of these putatively dicarboxylic neurotoxins in the brain is facilitated by the low permeability of the blood-brain barrier for dicarboxylic acids, causing ‘entrapment’ of these metabolites in the brain compartment of patients [20]. It has been suggested that disturbed cerebral haemodynamics, such as disturbed autoregulation and regional perfusion pressure gradients, adds to the metabolic toxicity of this disease [14]. Recently, enhanced glutarylation of lysine residues and concomitantly impaired function of mitochondrial proteins, particularly of glutamate dehydrogenase and carbonic anhydrase 5b, was demonstrated in glial cells [21]. The functional consequence of this finding needs to be further elucidated.

22.4.3 Genetics

Glutaric aciduria type I is an autosomal recessive disorder caused by pathogenic variants in *GCDH*. Results of newborn screening programs in various regions and cohorts worldwide give an estimated mean frequency of about 1:125,000 [16]. The disease is much more frequent in certain communities, such as the Amish people in Pennsylvania (homozygous for p.Ala421Val, incidence of 1 in 300–400 newborns), the Oji-Cree First Nations in Canada (homozygous for the splice site mutation IVS-1 + 5 g > t, incidence of 1 in 300 newborns), and the Irish travellers (homozygous for p.Glu365Lys).

About 200 different disease-causing mutations in *GCDH* have been identified so far [22]. There is a correlation between genotype and biochemical phenotype in that specific *GCDH* variations with residual enzyme activity of 3–30% are associated with low excretions of metabolites, while loss of enzymatic activity predicts a high excretor phenotype. However, no correlation between genotype and acute or insidious onset of striatal injury until age 36 months has yet been found [11], while white matter changes are much more prevalent in patients with the high excretor phenotype [17]). Single common founder mutations have been identified in high-risk populations (see above), but glutaric aciduria type I is, in general, genetically quite heterogeneous with a high frequency of private mutations: the most frequent mutation in Caucasians, p.Arg402Trp, has been identified in 10–20% of alleles [22].

22.4.4 Diagnostic Tests

Before the introduction of extended newborn screening programs, patients with glutaric aciduria type I have been diagnosed by (quantitative) urinary organic acid analysis [23]. Since urinary concentrations may be (intermittently) normal in low excreting patients, the diagnosis has been challenging or even unsuccessful in some, and successful diagnosis often required repetitive metabolic testing. Additional diagnostic hints are carnitine deficiency with concomitantly increased acylcarnitines due to elevated glutarylcarnitine (C5DC) in plasma and urine. Increased C5DC concentrations can be specifically detected by tandem mass spectrometry

(MS/MS) [24], which has led to the inclusion of glutaric aciduria type I into MS/MS-based newborn screening programs in a growing number of countries. While patients with a high excretor status can be reliably identified by C5DC screening, this test has a lower sensitivity for patients with a low excretor phenotype [16].

Elevated urinary excretion of glutaric acid is found in a number of other disease states, such as glutaric aciduria type II and III, mitochondrial dysfunction, and renal failure. Quantitative analysis of 3-hydroxyglutaric acid in urine has a high sensitivity including patients with the low excretor phenotype and those having secondary carnitine depletion [23]. However, it is known that 3-hydroxyglutaric acid is also elevated in patients with short-chain 3-hydroxyacyl-CoA dehydrogenase deficiency (▶ Chap. 12) and severe ketosis.

Loading tests, e.g. with lysine, or prolonged fasting tests provoking catabolism may be extremely harmful and should be avoided. Demonstration of two known pathogenic *GCDH* variants or significantly decreased glutaryl-CoA dehydrogenase activity in leukocytes (or cultured skin fibroblasts) ultimately confirms glutaric aciduria type I. These confirmatory tests are particularly important in diagnostically problematic cases and are also recommended for individuals with high clinical and/or neuroradiological suspicion but unremarkable metabolic test results. Evidence-based recommendations for the diagnosis of glutaric aciduria type I have been published and revised twice [25, 26].

22.4.5 Treatment and Prognosis

More than 700 patients have been identified worldwide and major progress has been achieved in the prevention of striatal necrosis occurring within the first 36 months of age. A favorable neurologic outcome critically depends on two factors: (1) newborn screening (or any other diagnostic approach that allows the identification of asymptomatic individuals) and (2) adherence to recommended, evidence-based therapy. However, up to one-third of neonatally screened individuals still do not or only partially benefit from early diagnosis and start of therapy [10, 16], due to differences in the therapeutic management. In addition, low-excreting individuals missed by newborn screening are still confronted with high mortality and morbidity, as in the pre-screening era, since post-symptomatic start of metabolic therapy cannot reverse striatal damage.

The following therapeutic measures are recommended and should be introduced and carefully evaluated and adapted by an experienced multi-professional team to minimize the risk of neurological impairment [25, 26].

Emergency Treatment

Emergency treatment during every intercurrent illness must start immediately and before the onset of neurological signs. Gastrointestinal infections are especially dangerous. Treatment should consist of frequent high carbohydrate feeds and increased carnitine supplementation. If feeds are not tolerated high-dose intravenous glucose and carnitine must be given [25, 26]. If lysine-free amino acid supplements are used, these are offered orally, in addition. If the temperature rises above 38.5 °C (101 °F) antipyretics should be administered liberally. All patients should be supplied with an emergency card. Frequent visits and regular information and training of parents may help to prevent lapses or mistakes. This concept should be strictly followed for the first 6 years of life. After this age emergency treatment is individually adjusted. Emergency treatment is thought to be the most effective component of current treatment strategies to prevent acute striatal injury [16].

Oral Supplementations with Carnitine and Riboflavin

Carnitine should be supplemented lifelong to prevent secondary carnitine depletion and to foster the formation of non-toxic C5DC. The lack of carnitine supplementation has been associated with increased mortality [11]. Riboflavin responsiveness and the therapeutic benefit of riboflavin remain unproven. Furthermore, there is no standardised protocol to test for riboflavin responsiveness.

Dietary Treatment

Application of a low lysine diet aims to reduce the quantitatively most relevant precursor amino acid of the putatively neurotoxic glutaric and 3-hydroxyglutaric acids. Dietary treatment involves reduced intake of natural protein, preferably with supplementation of lysine-free, tryptophan-reduced, arginine-fortified amino acid mixtures, at least until age 6 years (◘ Table 22.1), to minimise the risk of malnutrition. To continue low lysine diet is not generally recommended beyond the vulnerable period for striatal injury, i.e. the first 6 years of life [25, 26]. After age 6 years, protein-controlled diet with avoidance of protein excesses is recommended. Special efforts to supply adequate calories are often necessary in patients with dystonia and swallowing difficulties. This may require nasogastric or gastrostomy feeding.

Treatment of the Complex Movement Disorder

The complex movement disorder is difficult to treat, and the efficacy of a drug cannot be predicted precisely for an individual patient [25, 26]. Baclofen (1–2 mg/kg daily) and/or diazepam (0.1–1 mg/kg

Table 22.1 Maintenance therapy in patients with glutaric aciduria type I

		Patient age				
Treatment		0–6 mo	7–12 mo	1–3 y	4–6 y	>6 y
1. Low lysine diet						
Lysine (from natural protein)[1]	mg/kg/d	100	90	80–60	60–50	Controlled protein intake using natural protein with a low lysine content and avoiding lysine-rich food
Amino acid supplements (protein)[2]	g/kg/d	1.3–0.8	1.0–0.8	0.8	0.8	
Energy	kcal/kg/d	100–80	80	94–81	86–63	
2. Micronutrients	%	≥100	≥100	≥100	≥100	>100
3. Carnitine	mg/kg/d	100	100	100	100–50	50–30

After [25, 26]
[1]Using natural protein with a low lysine content. [2]Lysine-free, tryptophan-reduced, arginine-fortified. Consider an individualisation of treatment if normal growth is not achieved

daily) are commonly used to reduce involuntary movements and improve motor function, mostly through muscle relaxation. In some patients their use and dosage are limited by worsening of axial hypotonia and sedative effects. Trihexiphenidyl may improve dystonia, especially in adolescent and adult patients, but it may also be effective in children if the dosage is increased slowly. Botulinum toxin type A may help to prevent hip dislocation and reduce limb dystonia. Antiepileptics such as vigabatrin, carbamazepine, and valproate (which is even contraindicated) as well as L-DOPA, and amantadine are ineffective to control movement disorders in glutaric aciduria type I. The long-term benefits of intrathecal baclofen administration and neurosurgical interventions such as pallidotomy and deep brain stimulation (globus pallidus internus) are uncertain and, since they involve a significant risk of neurological deterioration, these interventions should be decided upon very cautiously and individually.

Although early diagnosis by newborn screening in combination with metabolic treatment has significantly improved the neurologic outcome by decreasing the frequency of striatal injury and untimely death [15, 16], up to one-third of screened patients still develops neurological symptoms of variable degree. Furthermore, longitudinal observational studies of screened individuals unravelled progressive white matter disease with unclear clinical significance, particularly in high excretor patients, the development of chronic kidney disease which does not seem to be impacted by recommended therapy, and the manifestation of malignant brain tumours in a few patients with poor adherence to or late start of therapy. This highlights the need for safe and more effective therapies and careful long-term follow-up.

22.5 Glutaric Aciduria Type II (Multiple Acyl-CoA Dehydrogenase Deficiency)

For historical reasons, multiple acyl-CoA dehydrogenase deficiency, is still termed glutaric aciduria type II; however, the name-giving finding of glutaric aciduria/acidemia is rather a biochemical epiphenomenon than a pathomechanistic explanation of this complex disease involving electron transfer in the mitochondrial respiratory chain. A detailed description of the disease is found in ▶ Chap. 12.

22.6 Glutaric Aciduria Type III (Succinate Hydroxymethylglutarate CoA-Transferase Deficiency)

22.6.1 Clinical Presentation

Glutaric aciduria type III is an autosomal recessive metabolic abnormality with unknown incidence. It is likely a clinically benign condition [27].

22.6.2 Metabolic Derangement

Individuals with glutaric aciduria type III present with isolated glutaric acid accumulation, without the elevated levels of 3-hydroxyglutaric acid and glutarylcarnitine that are found in glutaric aciduria type I. This indicates absence of elevated glutaryl-CoA. Deficiency of succinate hydroxymethylglutarate CoA-transferase causes glutaric aciduria type III (enzyme 10 in ◘ Fig. 22.1). This enzyme converts glutaric acid to glutaryl-CoA using succinyl-CoA as a coenzyme donor [28]. The origin of glutaric acid as substrate for this enzyme remains to be elucidated. Bacterial production in the intestine and spontaneous breakdown of glutaryl-CoA might be considered as a source.

22.6.3 Genetics

Bi-allelic variations in *C7orf10* located on 7p14.1 are causative for glutaric aciduria type III [29].

22.6.4 Diagnostic Tests

Patients with glutaric aciduria type III are diagnosed by urinary organic acid analysis; mutation analysis of *C7orf10* can confirm the diagnosis.

22.6.5 Treatment and Prognosis

Since this is a biochemical abnormality with minor or even no clinical significance, there is no indication for treatment. The prognosis of affected individuals is likely to be favourable.

22.7 L-2-Hydroxyglutaric Aciduria (L-2-Hydroxyglutaric Dehydrogenase Deficiency)

22.7.1 Clinical Presentation

Most patients with L-2-hydroxyglutaric aciduria follow a characteristic disease course [30]. In infancy and early childhood mental and psychomotor development appears normal or only slightly retarded. Thereafter seizures, progressive ataxia, pyramidal tract signs, slight extrapyramidal signs, and progressive mental retardation become the most obvious clinical findings. Progressive macrocephaly is present in about half of the patients. Cognitive disability is frequent and is often significant, with an IQ of about 40–50 in adolescents and adults. Sometimes mental deterioration is rapidly progressive, and a fatal neonatal outcome has been rarely described.

In L-2-hydroxyglutaric aciduria the pattern of neuroradiologic abnormalities is pathognomonic [30]. The subcortical white matter appears mildly swollen with some effacement of gyri. The progressive loss of arcuate fibres is combined with severe cerebellar atrophy and increased signal densities of dentate nuclei and globi pallidi (◘ Fig. 22.3) on T_2-weighted images. Patients with this disease bear an increased risk for developing malignant brain tumours, such as medulloblastoma, glioblastoma multiforme, astrocytoma, and primitive neuroectodermal tumour [31].

22.7.2 Metabolic Derangement

The disorder is caused by an inherited deficiency of FAD-linked 2-hydroxyglutarate dehydrogenase, a mitochondrial enzyme converting L-2-hydroxyglutarate to 2-oxoglutarate [32] (◘ Fig. 22.2). L-2-hydroxyglutarate is increased in CSF, plasma, and urine [33]. In addition, a number of hydroxydicarboxylic acids (glycolate, glycerate, 2,4-dihydroxybutyrate, citrate, and isocitrate) are elevated in CSF. Another consistent biochemical finding is an increase of lysine in blood and CSF.

L-2-Hydroxyglutarate has no known functions, but its formation results from a side reaction of L-malate dehydrogenase on 2-oxoglutarate, the structural homologue of oxaloacetate, to L-2-hydroxyglutarate, which is converted back to 2-oxoglutarate by L-2-hydroxyglutarate dehydrogenase [34]. 2-Oxoglutarate is an important Krebs cycle intermediate but has many other functions. It is also required for the first step of mitochondrial lysine oxidation, i.e. the formation of saccharopine, which explains elevated lysine concentrations. L-2-Hydroxyglutaric aciduria is considered a disease of the growing group of disorders of metabolite proofreading.

22.7.3 Genetics

L-2-Hydroxyglutaric aciduria is caused by bi-allelic variants in *L2HGDH* [35].

22.7.4 Diagnostic Tests

L-2-Hydroxyglutarate is found elevated in all body fluids [30]. In addition, lysine is slightly increased in CSF, as is protein, the latter occurring in the absence of pleocytosis. Confirmation of the suspected diagnosis is usually done genetically.

22.7.5 Treatment and Prognosis

Riboflavin has led to a partial improvement of neurological symptoms in a few patients and reduced urinary excretion of L-2-hydroxyglutarate in some [36] but not in others (G. F. Hoffmann, personal observation). Epilepsy can generally be controlled by standard medications. No causal therapy is currently known which could stop or prevent the progression of neurological symptoms and tumorigenesis, and thus the prognosis is usually poor.

22.8 D-2-Hydroxyglutaric Aciduria Type I (D-2-Hydroxyglutarate Dehydrogenase Deficiency) and Type II (Isocitrate Dehydrogenase 2 Deficiency)

22.8.1 Clinical Presentation

Two genetic causes of D-2-hydroxyglutaric aciduria, type I and II, have been delineated [37, 38]. Although both types share some clinical overlap, patients with type I are usually less severely affected and develop a more variable clinical phenotype than those with type II. Frequent clinical findings are developmental delay, muscular, hypotonia, and epilepsy. Some individuals with type I develop an attenuated phenotype with mild developmental delay or even remain asymptomatic. In contrast, type II patients more often present with a neonatal onset with epileptic encephalopathy, severe muscular hypotonia, lack of psychomotor development, cortical blindness, (dilated) cardiomyopathy), and early death.

In the severely affected individuals, neuroimaging uniformly reveals disturbed and delayed gyration, myelination and opercularisation, ventriculomegaly, more pronounced of the occipital horns, and cysts over the head of the caudate nucleus (◘ Fig. 22.4).

22.8.2 Metabolic Derangement

Patients show moderately (type I) to highly (type II) elevated levels of D-2-hydroxyglutarate in all body fluids. In addition, Krebs cycle intermediates are found to be elevated in the urine of some patients, as well as GABA in CSF. Type I is caused by deficient D-2-hydroxyglutarate dehydrogenase, an enzyme that converts D-2-hydroxyglutarate to 2-oxoglutarate [37], while type II originates from mutated mitochondrial isocitrate dehydrogenase 2 [38] (◘ Fig. 22.2). The neomorph enzyme gains the ability to convert 2-oxoglutarate into D-2-hydroxyglutarate, which is in contrast to its normal function, the NADPH-producing oxidative decarboxylation of D-isocitrate to 2-oxoglutarate and hence the control of mitochondrial redox balance and mitigation of cellular oxidative defense.

A similar mechanism like in D-2-hydroxyglutaric aciduria type II explains D-2-hydroxyglutaric aciduria observed in patients with malignant gliomas and acute myeloid leukaemia due to somatic mutations in isocitrate dehydrogenase 1 (cytosolic) or 2 (mitochondrial).

22.8.3 Genetics

Bi-allelic mutations in *D2HGDH* (2q37.3) are the molecular cause of D-2-hydroxyglutaric aciduria type I [37], while autosomal dominant variations of *IDH2* located on 15q26.1 [38] cause D-2-hydroxyglutaric aciduria type II, with a high frequency of de novo variations.

22.8.4 Diagnostic Tests

Elevation of 2-hydroxyglutaric acid can be identified by conventional gas chromatography/mass spectrometry analysis, but cannot be differentiated in the L-2- and D-2-isomers, which requires chromatographic separation using derivatisation with a chiral reagent or a chiral stationary phase. D-2-hydroxyglutaric acid is found elevated in urine, plasma, and CSF. In addition, GABA is often elevated in CSF, and intermediates of energy metabolism are elevated in urine (lactic, succinic, malic, and 2-oxoglutaric acids). The suspected diagnosis is usually confirmed genetically.

D-2-Hydroxyglutaric acid can also be elevated in multiple acyl-CoA dehydrogenase deficiency (glutaric aciduria type II), and – rarely – in patients with glutaric aciduria type I and succinic semialdehyde dehydrogenase deficiency, but these can be readily distinguished by the urine organic acid profile (► Chaps. 12 and 30).

22.8.5 Treatment and Prognosis

To date there is no rational therapy for D-2-hydroxyglutaric aciduria type I and II; riboflavin and L-carnitine supplementation has not been of benefit. Seizures can be very difficult to control, and patients have died early with profound developmental delay. The clinical phenotype does not appear to progress rapidly in type I disease, if affected children do not develop an early onset epileptic encephalopathy. The course of type II disease is usually progressive with early death in

childhood in about 50%. Specific inhibition of the neomorphic isocitrate dehydrogenase 2 by a small molecule rescued cardiomyopathy and improved survival in a mouse model for this disease. However, it remains to be elucidated whether this strategy is safe and effective in affected individuals [39].

22.9 D-2- and L-2-Hydroxyglutaric Aciduria (Mitochondrial Citrate Carrier or SLC25A1 Deficiency)

22.9.1 Clinical Presentation

D-2- and L-2-Hydroxyglutaric aciduria has been described as a devastating neurometabolic disorder in more than 50 patients. Affected individuals display a severe clinical phenotype with neonatal onset metabolic encephalopathy, infantile epilepsy refractory to antiepileptic drug therapy, severe global developmental retardation, muscular hypotonia, cortical blindness, and early death [40]. Recently, a milder disease variant presenting primarily with a neuromuscular junction defect causing congenital myasthenic syndrome type 23 has been reported, extending the clinical phenotype.

22.9.2 Metabolic Derangement

The major biochemical finding is accumulation of D-2- and L-2-hydroxyglutaric acids in body fluids with a predominance of D-2-hydroxyglutaric acid. Krebs cycle intermediates including 2-oxoglutarate, malate, fumarate, and succinate are also elevated accompanied by decreased concentrations of citrate and isocitrate [41]. SLC25A1, the mitochondrial citrate carrier, exchanges cytosolic malate for mitochondrial citrate and isocitrate (◘ Fig. 22.2). If this exchange transport is disrupted this has negative consequence for mitochondrial and cytosolic metabolism: (1) In mitochondria, concentrations of Krebs cycle intermediates downstream of isocitrate, such as 2-oxoglutarate, are permanently increased, resulting in increased formation of both D-2- and L-2-hydroxyglutarate, whose synthesis requires 2-oxoglutarate. (2) In the cytosol, concomitant citrate depletion leads to decreased formation of NADPH+H and fatty acid and sterol synthesis.

22.9.3 Genetics

Bi-allelic variations in *SLC25A1* (located on 22q11.21) encoding the mitochondrial citrate carrier, cause D-2- and L-2-hydroxyglutaric aciduria [42] and congenital myasthenic syndrome 23 [41].

22.9.4 Diagnostic Tests

The metabolic work-up is performed in analogy to D-2- and L-2-hydroxyglutaric acidurias (see above).

22.9.5 Treatment and Prognosis

The prognosis in the hitherto described patients was poor. Treatment with citrate may result in biochemical and clinical improvement to some extent (◘ Fig. 22.5) [43].

22.10 N-Acetylaspartic Aciduria (Aspartoacylase or Aminoacylase 2 Deficiency) (Canavan Disease)

22.10.1 Clinical Presentation

N-Acetylaspartic (NAA) aciduria mostly manifests at 2–4 months of age with head lag, muscular hypotonia and macrocephaly, progressing to marked developmental delay, seizures, optic nerve atrophy, progressive spasticity, and opisthotonic posturing [44]. At birth the head circumference may not be remarkably increased; however, in the majority of cases it increases pathologically after 6 months of age, crossing the percentiles with obvious macrocephaly by 1 year. In the second year of life seizures often develop, together with irritability and sleep disturbance. Muscular hypotonia gives way to spasticity reminiscent of cerebral palsy. Impaired chewing and swallowing, problems with gastro-oesophageal reflux, vomiting and aspiration can result in recurrent infections and failure to thrive.

The most consistent findings on MRI studies are diffuse abnormalities of white matter [45]. Although not always present and not uniform, MRI usually shows symmetric diffuse low signal intensity on T_1-weighted images and high signal intensity on T_2-weighted images (◘ Fig. 22.4).

The neuropathology of Canavan disease is characterised by a progressive loss of myelinated arcuate fibres [45]. Detailed histopathological descriptions at autopsy have elucidated that white matter is characteristically soft and gelatinous. The spongy or vacuolisation changes are clearly seen in the lower layers of the grey matter and in the subcortical white matter, with the more central white matter relatively spared.

Most patients follow the disease course described above, which is also termed the infantile form. Rare clinical

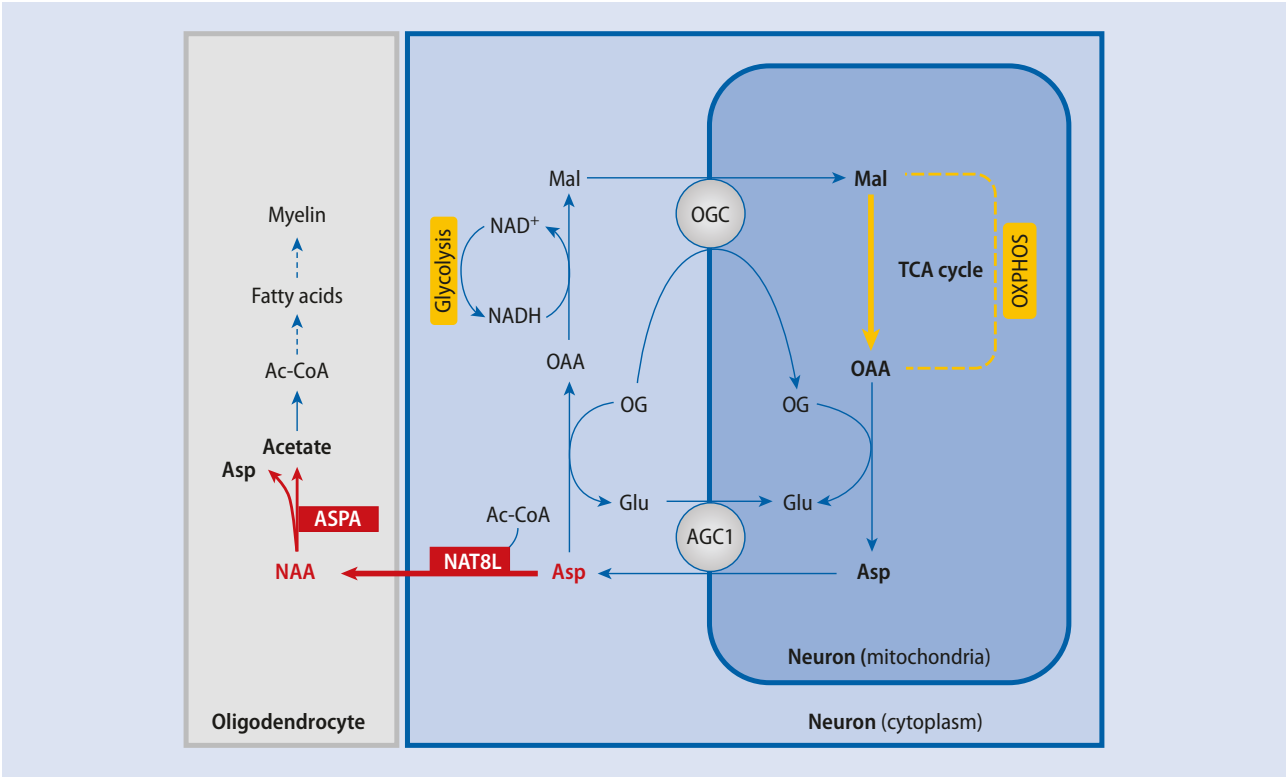

Fig. 22.5 Cerebral *N*-acetylaspartate (NAA) metabolism. The discrepant compartmentation of NAA synthesis by L-aspartate *N*-acetyltransferase (NAT8L) in neurons, deficient in individuals with hypoacetylaspartia, and its subsequent hydrolysis by aspartoacylase (ASPA) in oligodendrocytes, deficient in individuals with Canavan disease, is a mechanism for channeling NAA-associated acetate from neurons to oligodendrocytes, providing a major substrate for myelin synthesis. NAA metabolism is coupled to the cerebral malate (Mal)-aspartate (Asp) shuttle, which affects the redox status of cytoplasm and mitochondria and thus regulates energy production in these compartments. Maintaining a low ratio of NADH to NAD^+ in the cytoplasm, and a high ratio in the mitochondria, provides a driving force for the respiratory chain (OXPHOS) in mitochondria, whereas it favors glycolysis in the cytoplasm. Ac-CoA acetyl-CoA, AGC1 aspartate–glutamate carrier 1, Glu glutamate, OAA oxaloacetate, OG 2-oxoglutarate, OGC oxoglutarate carrier, TCA tricarboxylic acid

variants with different disease courses have been described as congenital, i.e. presenting at or shortly after birth, or as juvenile forms, i.e. presenting after 5 years of age.

22.10.2 Metabolic Derangement

The disease is caused by aspartoacylase (aminoacylase 2) deficiency leading to the accumulation of NAA in brain, CSF, plasma, and urine. In the brain, aspartoacylase is exclusively located in oligodendrocytes hydrolysing its natural substrate NAA, which is formed in neurons from L-aspartate and L-acetate (Fig. 22.5). Defective NAA catabolism is thought to result in reduced brain acetate levels and myelin lipid synthesis. This has been demonstrated in aspartoacylase-deficient mice showing a 30% decrease in total myelin lipids at the time of peak postnatal myelination in the brain [46]. Besides acetate depletion, NAA may also act as an efflux molecular water pump between neurons and oligodendrocytes enabling the removal of neuronal metabolic water produced by glucose oxidation. Decreased NAA catabolism might result in osmotic dysregulation of the brain and, subsequently, spongiform leukodystrophy [47].

22.10.3 Genetics

N-Acetylaspartic aciduria is an autosomal recessive disease caused by bi-allelic variations in *ASPA*. It is a pan-ethnic disease with a much higher frequency among Ashkenazi Jews, most of whom carry two specific mutations, a missense mutation, p.Glu285Ala, accounting for 84% of mutant alleles, and a nonsense mutation, p.Tyr231X, accounting for 13% [48]; the frequency of these two mutations makes carrier screening possible. In non-Jewish patients the mutations are diverse and mostly private.

22.10.4 Diagnostic Tests

The diagnosis is established by determining NAA in the urine by organic acid analysis. Hundredfold elevations are pathognomonic but the disorder should be confirmed by molecular tests and/or enzyme analysis.

22.10.5 Treatment and Prognosis

No effective treatment exists for *N*-acetylaspartic aciduria. Lithium citrate, which induces a mild decrease in brain NAA levels of affected children, is safe but not clinically effective [49]. Because acetate in the form of acetyl-CoA is a building block for lipids, it has been proposed that dietary acetate supplementation with glyceryl triacetate might be a therapeutic option. Although the results of a low-dose safety study were published, the therapeutic efficacy of glyceryl triacetate remains unproven [50]. Recent AAV-ASPA gene therapy studies in aspartoacylase-deficient mice aim to target astro- or oligodendroglia, redirecting NAA metabolism in the brain and normalizing myelination [51]. It remains to be elucidated whether this innovative strategy can be translated to a disease-changing therapy for affected individuals with Canavan disease.

The prognosis for most affected individuals remains very poor, with death usually occurring in the first decade of life although there may be survival into the second decade in a (near) vegetative state.

22.11 Aminoacylase 1 Deficiency

Aminoacylase 1 deficiency is a rare disease with less than 20 patients reported. The clinical relevance of this disorder has not yet been fully elucidated. Although initially considered a non-disease, some patients present with a heterogeneous clinical spectrum including intellectual and motor disability, delayed speech development, muscular hypotonia, and autistic features. However, since detection of aminoacylase 1 deficiency was part of selective screening in symptomatic patients with suspected metabolic disease, a strong selection bias of this cohort is likely.

N-Acetylation of a protein extends its half-life; 50–80% of proteins show formylated or acetylated *N*-termini. Free amino acids can be recycled after protein breakdown by hydrolysis of N-acetylated amino acids using aminoacylases. Aminoacylase 1 catalyzes the release of free amino acids from a variety of *N*-acetylated precursors – except for *N*-acetylaspartate (▶ Sect. 22.10). It has a high tissue-specific activity in kidney and brain. Enzyme deficiency results in increased formation and urinary excretion of acetylated amino acids [52].

Aminoacylase 1 deficiency is an autosomal recessive disorder caused by homozygous or compound heterozygous mutations in *ACY1* [52].

22.11.1 Diagnostic Tests

Elevated urinary exretion of *N*-acetylated amino acids including derivatives of methionine, glutamine, alanine, leucine, glycine, valine, and isoleucine can be detected by gas chromatography/mass spectrometry or NMR spectroscopy [52]. Decreased aminoacylase 1 activity in lymphoblasts or bi-allelic variations of *ACY1* confirms the diagnosis.

22.11.2 Treatment and Prognosis

Since the clinical significance of aminoacylase 1 deficiency remains unclear, there is no clear-cut indication for treatment. Studies systemically evaluating the effect of metabolic treatment are pending. The prognosis of affected individuals is likely to be favourable.

22.12 Hypoacetylaspartia (L-Aspartate N-Acetyltransferase Deficiency)

A single patient with hypoacetylaspartia has been described with marked developmental delay and secondary microcephaly with truncal ataxia, seizures, and behavioural abnormalities on follow-up, in whom ^{1}H-MRS had revealed the absence of NAA signal [53]. A defect of L-aspartate N-acetyltransferase, the enzyme that is required for the synthesis of NAA, was suspected, and a neuron-specific protein, NAT8L, encoded by *NAT8L*, was found to be responsible for NAA synthesis, and was mutated in the patient [54] (◘ Fig. 22.5).

22.13 Malate-Aspartate Shuttle Defects

Recently, defects in 5 of the 6 components of the malate-aspartate shuttle were described. They are presented in ▶ Chap. 11.

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Nonketotic Hyperglycinaemia and Lipoate Deficiency Disorders

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Contents

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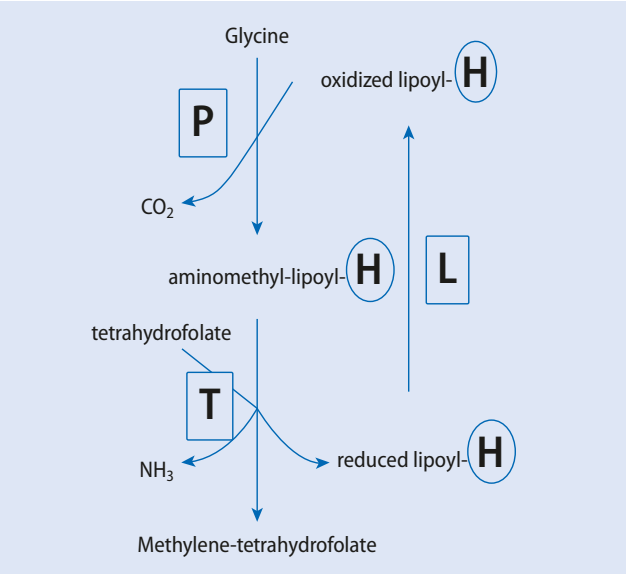


Fig. 23.1 The glycine cleavage enzyme is a four protein complex with the H-protein carrying a lipoyl-group as the central core. The P-protein contains pyridoxal-5′-phosphate and decarboxylates glycine with transfer of the aminomethyl-group onto lipoate of the H-protein. The T-protein releases ammonia and transfers the remaining methyl-group onto tetrahydrofolate making 5,10-methylenetetrahydrofolate. The resulting reduced lipoate on the H-protein is reoxidized by the L-protein

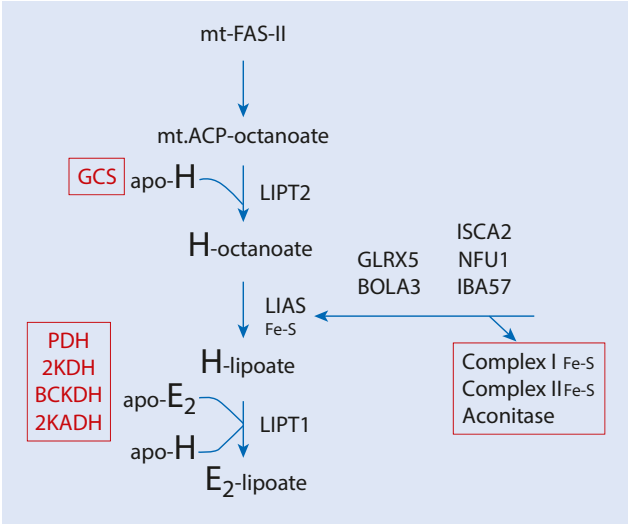


Fig. 23.2 Octanoate is synthesized by intramitochondrial fatty acid synthesis (mt.-FAS II) on the mitochondrial acylcarrier protein (mt.ACP) and transferred to apo-H protein by lipoyltransferase 2 (LIPT2). To make lipoyl-H, lipoate synthase (LIAS) donates sulfur atoms from its iron-sulfur cluster, which were synthesized through multiple genes including *GLRX5*, *BOLA3*, *NFU1*, *ISCA2* and *IBA57*. They also create iron-sulfur clusters for respiratory chain complex I and II and aconitase. Lipoyltransferase 1 (LIPT1) transfers the lipoate group from the H-protein to the E2 component of pyruvate dehydrogenase (PDH), 2-ketoglutarate dehydrogenase (2KDH), branched chain ketoacid dehydrogenase (BCKDH) and 2-ketoadipate dehydrogenase (2KADH). LIPT1 deficiency causes dysfunction of the E2 components; LIPT2, LIAS, and GCSH add deficiency of the glycine cleavage enzyme (GCS). Some disorders of iron sulfur cluster biogenesis genes result in deficient activity of complexes I and II of the respiratory chain, which carry iron sulfur clusters (▶ Chap. 10)

Glycine Metabolism

Figures 23.1 and 23.2.

Introduction

Nonketotic hyperglycinaemia (NKH) is caused by defective glycine cleavage enzyme activity. Classic NKH is caused by mutations in *GLDC* and *AMT*. Disorders of lipoate synthesis and transport are caused by mutations in *LIAS, BOLA3, NFU1, GLRX5, ISCA2, IBA57, LIPT1* and *LIPT2*. When these affect glycine metabolism, they are referred to as variant NKH. Patients with severe form of classic NKH present with neonatal epileptic encephalopathy followed by minimal developmental progress, spasticity and therapy-resistant epilepsy. Patients with the attenuated form of classic NKH make variable developmental progress and present with attention deficit, hyperactivity, chorea and episodic lethargy. A specific pattern of diffusion restriction on brain MRI is always present in the first months of life and is useful to distinguish from non-genetic causes of elevated CSF glycine. High levels of CSF glycine, hydrocephalus, and a short and thin corpus callosum are indicative of severe classic NKH, whereas less elevated CSF:plasma glycine ratios, onset after 4 months, or absence of epilepsy are indicators of attenuated classic NKH. Current treatment consists of reduction of glycine levels with benzoate, sometimes dietary glycine restriction, or ketogenic diet, and blocking the excitatory effect of glycine on NMDA-receptors with either dextromethorphan or ketamine. Therapy is most effective when initiated early in patients with attenuated NKH where it improves development.

23.1 Definition

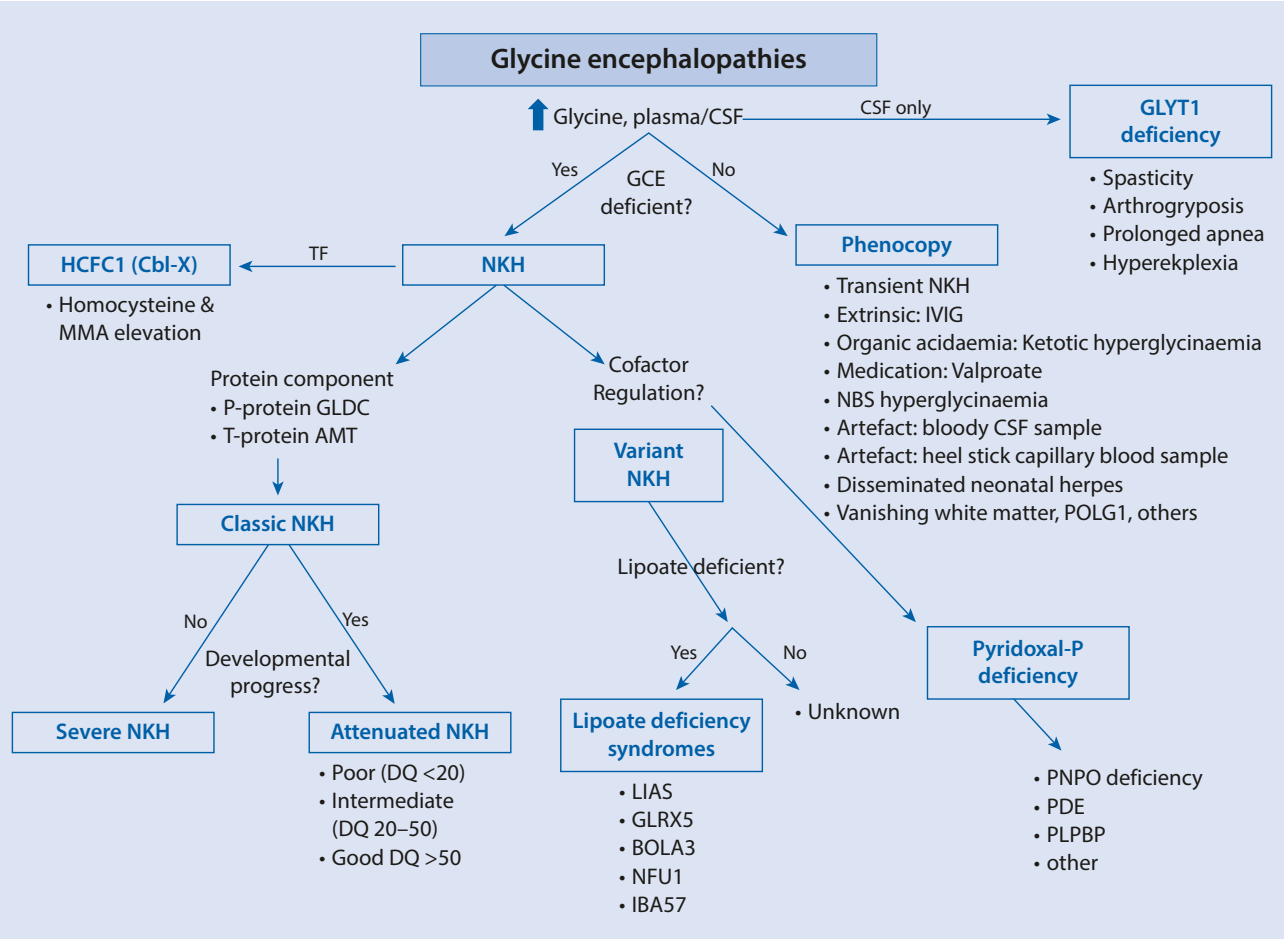
Glycine encephalopathies are genetic disorders causing cerebral dysfunction due to disorders of transport of glycine, such as the glycine transporter GLYT1 (▶ Chap. 30), or due to a defect in the metabolism of glycine. Nonketotic hyperglycinaemia (NKH) is a genetic disorder characterized by deficient activity of the glycine cleavage enzyme. Classic NKH is caused by mutations in genes that encode the protein components of the glycine cleavage enzyme system (GCS). Based on the clinical severity and developmental outcome, classic NKH is further divided into severe classic NKH and

attenuated classic NKH [1, 2]. Variant NKH has an overlapping phenotype to classic NKH and is caused by mutations affecting the biosynthesis of the main cofactor lipoic acid, and fits into the larger group of the lipoate synthesis disorders. Lipoate is not only cofactor for the GCS but also for pyruvate dehydrogenase, 2-ketoglutarate dehydrogenase (▶ Chap. 11) and branched chain ketoacid dehydrogenase (▶ Chap. 18). These disorders are also referred to as multiple mitochondrial dysfunction syndromes (▶ Chap. 10). Disorders affecting active pyridoxal-5-phosphate lead to reduced activity of a large number of pyridoxal-P-dependent reactions including the GCS, such as pyridox(am)ine 5′-phosphate oxidase deficiency (resulting from PNPO mutations) (▶ Chap. 29). Phenocopies of NKH, historically called transient NKH, are non-genetic secondary causes of elevated plasma and CSF glycine levels. Atypical NKH is a heterogeneous historic term, which should no longer be used. A scheme for the differential diagnosis of glycine encephalopathies is given in ◘ Fig. 23.3.

23.2 Clinical Presentation

23.2.1 Severe Classic NKH

Severe classic NKH presents with epileptic encephalopathy during the neonatal period with a few patients presenting in early infancy. Initial signs and symptoms include hypotonia, increasing lethargy, coma, apnoea, myoclonic jerks, seizures and frequent hiccupping, which may have already started during the prenatal period, and sometimes pin-point pupils. Patients typically require ventilatory support for the first 10–20 days, although not in the 15% of patients who do not develop



◘ **Fig. 23.3** Nosology and diagnostic considerations for elevated glycine levels. CSF cerebrospinal fluid, NKH nonketotic hyperglycinaemia, GCE glycine cleavage enzyme, TF transcription factor, DQ developmental quotient, GLYT1 glycine transporter 1, PNPO deficiency pyridox(am)ine oxidase deficiency, PDE pyridoxine dependent epilepsy, PLPBP pyridoxal-phopshate binding protein; Lipoate deficiency syndromes are caused by multiple genes, not all listed here see text; IVIG intravenous gammaglobulin, NBS hyperglycinaemia, newborn screening identified hyperglycinaemia, POLG1 mitochondrial polymerase gamma

apnoea. Seizures include myoclonic movements or hiccupping. The initial EEG pattern often includes burst-suppression, which can progress in infancy to hypsarrhythmia followed by multifocal epilepsy. Coma and apnoea are rarely described in the patients who present after the neonatal period. At least 15–30% of patients with classic NKH die during the neonatal period often due to withdrawal of intensive care and ventilatory support [1–3]. A few patients with severe classic NKH present with congenital malformations including club feet and cleft lip/cleft palate.

Brain MRI consistently shows a typical pattern of diffusion restriction in the corticospinal tract, posterior tegmental tracts and cerebellar white matter in all patients with NKH regardless of severity which over time evolves into a diffuse pattern affecting the entire supratentorial white matter [4] (Fig. 23.4). The corpus callosum is short and thin and fails to grow appropriately in infancy in severe NKH. Brain malformations characterized by retrocerebellar cysts with hydrocephalus, and cerebellar hypoplasia occur in 3–5% of severe patients [1, 2]. An elevated glycine peak is present in the white and grey matter at 3.5 ppm on magnetic resonance spectroscopy (MRS), separated from myoinositol at long echo time [4].

All children with severe classic NKH have profound developmental delay with a developmental age of approximately 6 weeks [2]. Patients can learn to smile, coo, roll over but do not learn to sit, reach and grasp objects or to communicate. During the first year, patients usually develop spastic quadriparesis and truncal hypotonia. Seizures gradually worsen in infancy and progress into intractable seizures, requiring multiple anticonvulsant treatment [1, 2]. Cortical blindness is very frequent and microcephaly often develops over time. Further long-term problems include feeding difficulties requiring tube feeding, gastroesophageal reflux often requiring Nissen fundoplication, and orthopaedic problems in childhood such as hip dislocation and scoliosis often requiring surgical intervention [1]. Airway maintenance becomes poor over time, ultimately resulting in the child's death.

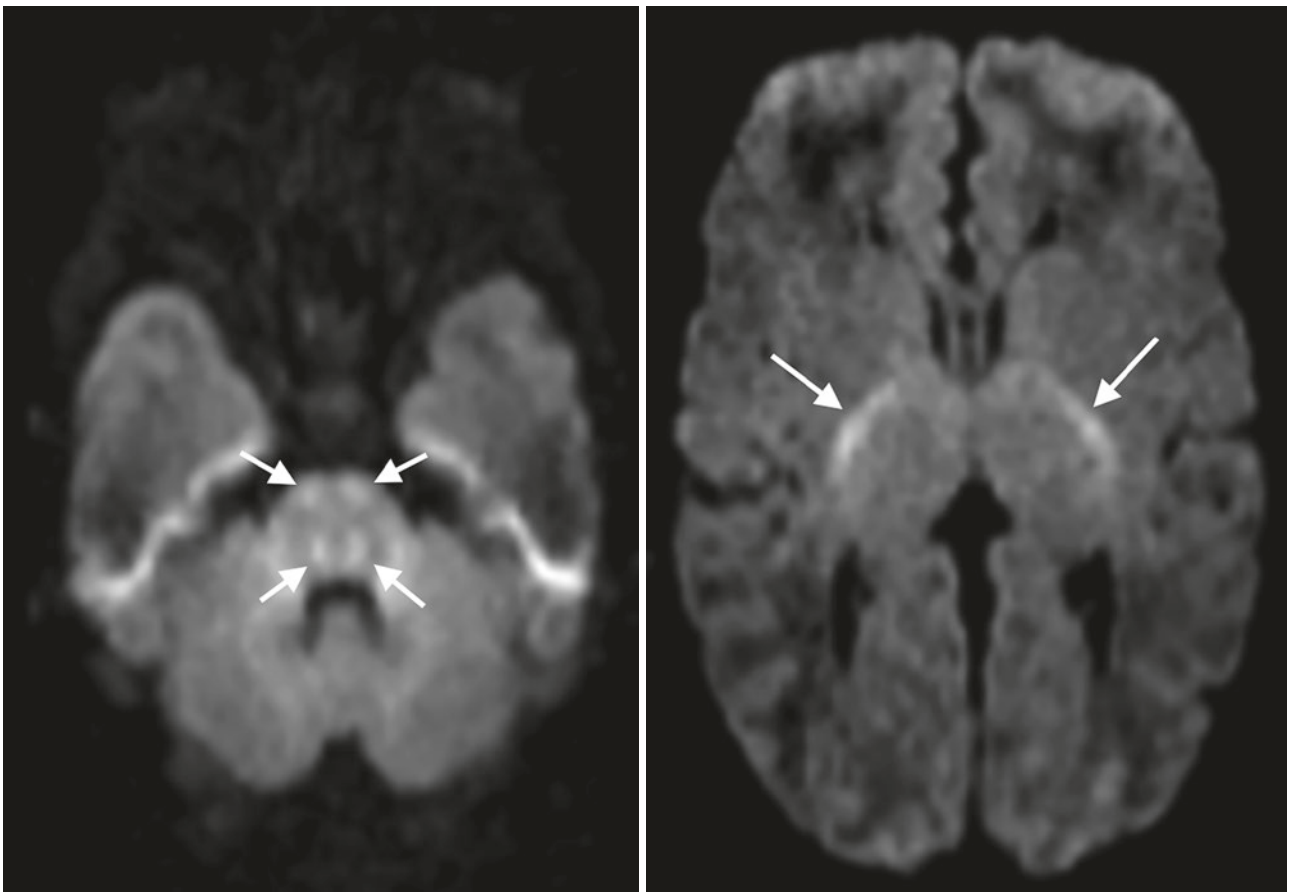


Fig. 23.4 Brain MRI with diffusion restriction of a newborn with classic nonketotic hyperglycinaemia. Diffusion restriction is seen in the corticospinal and central tegmental tract in the brain stem and in the posterior limb of the internal capsule

23.2.2 Attenuated Classic NKH

Patients with attenuated classic NKH attain developmental milestones with varying degrees of developmental and intellectual progress. Patients may present in the neonatal period, resembling severe classic NKH, or later in infancy with hypotonia, lethargy, coma and seizures [1, 2]. Developmental progress has been reported in 15–20% of patients with neonatal onset and in 50% of patients presenting in infancy [1–3]. A similar pattern of diffusion restriction is recognized as in severe NKH [4]. However, patients with attenuated classic NKH do not have congenital brain malformations [1, 2], the size of the corpus callosum and its growth is significantly more preserved and the elevation of the glycine peak on MRS is less and sometimes not detectable [4].

Children with attenuated classic NKH develop hyperactivity, which is often severe, as well as chorea, behavioural problems and intermittent episodes of lethargy and ataxia [1, 2]. Patients often ambulate and achieve various motor skills. Expressive speech is much more delayed than receptive speech. Seizures may occur but often less severe requiring treatment with only one or even no anticonvulsant [2]. The EEG pattern of burst-suppression occurs less frequently [1]. The degree of intellectual impairment in attenuated classic NKH is variable: depending on the intellectual outcome, patients may be differentiated into attenuated good, intermediate, or poor classic NKH [2]. Patients with attenuated good NKH reach a DQ of 50 to 80 and have mostly hyperactivity and behavioural issues with no epilepsy. They can have late onset and intermittent episodes of chorea only [2, 5]. Patients with attenuated intermediate NKH have a DQ between 20 and 50 and have severe hyperactivity, chorea, and poor to absent speech with mild to no seizures. Patients with poor attenuated NKH make some developmental progress but have a DQ <20 and exhibit seizures manageable with medications, autistic features, absent speech, and moderate spasticity.

Rarely, patients with treated classic NKH, both severe and attenuated, can exhibit systemic phenotypic features, the cause of which is not yet known. These include gastric problems with delayed emptying, severe intestinal dysmotility up to pseudo-ileus, sudden life-threatening electrolyte imbalances such as hypokalaemia or hypernatremia and unexplained prolonged crying [6].

23.2.3 Lipoate Disorders Including Variant NKH

Variant NKH is caused by a deficiency of the cofactor lipoate and combines the clinical findings of severe NKH with mitochondriopathies, such as leukoencephalopathy, optical atrophy, cardiomyopathy, and sometimes episodes of severe lactic acidosis. Some patients formerly classified as atypical NKH must retrospectively be reclassified as variant NKH. Our knowledge of the clinical phenotype of the newly described group of lipoate disorders is still evolving.

Patients with lipoate synthase deficiency, due to mutations in *LIAS*, presented during the neonatal period with a clinical picture resembling severe NKH including seizures, myoclonus, burst-suppression pattern on EEG, hypotonia, and apnoea, as well as leukodystrophy and Leigh-like lesions on cerebral MRI. Two patients presenting with intractable seizures died during infancy. Another mildly affected patient had stable developmental delay and transient seizures only [7, 8]. A patient with LIPT2 deficiency showed encephalopathy with cortical atrophy, hypotonia and spasticity [9]. Glycine and lactate were both increased. Patients with LIPT1 deficiency have elevated lactate but do not have increased glycine. Some patients presented neonatally with severe fatal disease of hypertonia, dystonia, pulmonary hypertension. Other developed Leigh disease and psychomotor regression, spasticity and an extrapyramidal syndrome, and on MRI cerebral and cerebellar atrophy, thalamic lesions and white matter disease.

Several iron sulfur cluster biogenesis disorders impair lipoate synthesis (mutations in *BOLA3, NFU1, ISCA2, GLRX5, IBA57*) (◘ Fig. 23.2; ▶ Chap. 10; ▶ Fig. 10.2 and ▶ Table 10.5). The clinical features can be grouped into neonatal, infantile and late onset presentations.

Only a dozen individuals with BOLA3 deficiency have been reported. A neonatal presentation in two siblings consisted of muscular hypotonia and respiratory insufficiency and hypertrophic cardiomyopathy. Brain MRI showed leukodystrophy. Both died at the age of 3 months after rapid neurological deterioration characterized by seizures, spasticity and extrapyramidal signs. The majority of BOLA3-deficient children presented in infancy with regression of motor skills, followed by hypotonia, poor feeding, hypertrophic cardiomyopathy, seizures, spasticity, extrapyramidal signs and optic atrophy [7]. This was followed by neurodegeneration over the course of months to years. Brain MRI showed leukodystrophic signal abnormalities in deep white matter sparing subcortical fibres, which in some became cavitating in the centrum semiovale. Most children succumbed in the first 2 years, but one patient had a more protracted course over 11 years with myoclonus, tonic-clonic seizures, recurrent status epilepticus, spasticity, ataxia, choreoathetoid movements and loss of speech [7]. One patient presented atypically at age 18 months with a stroke-like event resulting in acute right hemiparesis,

ataxia and loss of speech and language skills [10]. MRI revealed extensive, bilateral signal abnormalities in deep cerebral white matter, corpus callosum, basal ganglia, brainstem and cerebellum. Over the following years, he gradually regained motor function and speech. At the age of 8 years, he had attention deficit disorder but otherwise normal motor and cognitive function.

Twenty individuals with NFU1 deficiency have been reported. Neonatal presenting patients exhibited bradycardia and apnoea and signs of arterial pulmonary hypertension and died by 4 months of age. Most patients had infantile onset with failure-to-thrive, poor feeding, muscular hypotonia, developmental delay, loss of motor skills and apnoea, and in several patients pulmonary artery hypertension [11]. It was usually fatal before age 2 years. Some late presenting patients had a more protracted course, evolving to stable spastic paraparesis or tetraparesis [12, 13]. Cerebral MRI showed leukodystrophy, often cavitating.

ISCA2 deficiency has been reported in 21 individuals. A neonatal presentation included a rapidly progressive severe course with leukoencephalopathy and death at age of 3 months [14]. The majority of ISCA2 deficient individuals were infantile onset and presented between the age of 3–7 months after an initial normal development with loss of motor skills and hypotonia, and in half of the cases with nystagmus [15]. Almost all had optic atrophy with progressive loss of vision. Later, spasticity and hyperreflexia, progressive motor and cognitive regression evolved and some developed seizures. Brain MRI showed periventricular leukodystrophy sparing the U-fibres. Affected children evolved into a vegetative state within 1–2 years, and most died in early childhood.

Three individuals with GLRX5 deficiency presented in childhood between 2 and 7 years of age with gait abnormalities or worsening clumsiness evolving to spastic diplegia [7]. Cognitive development was either intact or only slightly affected (mild learning difficulties, poor concentration). Some also had visual disturbance caused by optic atrophy. Brain MRI showed signal abnormalities involving the frontal and parietal white matter, periventricular, and lower medulla oblongata extending into the mid-thoracic spinal cord. All three were still alive into young adulthood. An infantile presenting patient presented with rapid, severe developmental and motor regression, cavitating leukodystrophy and intractable epilepsy. In contrast, adult GLRX5 deficient individuals (aged 44–64 year) had sideroblastic anaemia and no neurological impairment. Sideroblastic anaemia has not been present in any other iron sulfur cluster disorder described here.

Twenty eight subjects with IBA57 deficiency have been reported. In two neonatal presenting siblings intra-uterine growth retardation, polyhydramnios, microcephaly and enlarged cerebral ventricle system was identified prenatally [16]. At birth, they presented with severe hypotonia, generalized muscle weakness, absent primitive reflexes and dysmorphic features, including retrognathia, high arched palate, widely spaced nipples, and arthrogryposis of multiple joints. The condition was rapidly fatal. Cerebral MRI revealed hypoplasia of the corpus callosum and medulla oblongata, bilateral frontoparietal polymicrogyria, severely enlarged lateral ventricles and cytotoxic edema in some cortical areas. In the most common infantile presentation (15 subjects) infants presented between 4 and 15 months of age with loss of motor and mental skills. Extensive white matter lesions were seen in cerebrum, cerebellum, mesencephalon and in the upper spinal cord, evolving into cavitating leukodystrophy in one individual [17]. A late onset phenotype was reported in 11 individuals with spastic paraplegia becoming symptomatic between 3 and 12 years, and variably associated with optic nerve atrophy and motor and sensory peripheral neuropathy (abbreviated 'SPOAN') [18]. All affected subjects led an independent adult life without cognitive impairment. Cerebral MRI was often unremarkable but in one subject showed, besides bilateral optic nerve atrophy, scattered white matter alterations.

Disorders of pyridoxal-P metabolism such as PNPO deficiency may also resemble NKH but with a much more complex metabolic profile that includes a mildly elevated CSF glycine (► Chap. 29).

23.3 Metabolic Abnormalities

Glycine is an amino acid involved in multiple intersecting biochemical pathways in its synthesis and use, including synthesis of creatine (*AGAT*), purines (*GARS-AIRS-GART*), porphyrines (*ALAS*), sarcosine (*GNMT*), acylglycine synthesis including hippurate, and via glycine-C-acetyltansferase to methylglyoxal. The most important pathway for the synthesis of glycine is from serine through the serine-hydroxymethyltransferases. Serine itself is synthesized from the glycolytic pathway (► Chap. 24). The most important catabolic pathway of glycine is the GCS. The glycine content of protein in food differs significantly. The glycine content per gram of total protein is low in milk, higher in soy, but particularly high in meat and gelatin [19]. When excluding the GCS, the excess flux of glycine synthesized in the body over its degradation is far larger than the amount of glycine taken in from food. Thus, the contribution of dietary glycine is only of limited impact in NKH management [19].

The GCS breaks down glycine with tetrahydrofolate into carbon dioxide, ammonia, and the generation of 5,10-methylenetetrahydrofolate (■ Fig. 23.1) and constitutes a major route for the generation of one-carbon units [20, 21]. It is a four protein complex located in the mitochondrial inner membrane with the H-protein carrying a lipoyl-group as the central core. The lipoyl-group is synthesized from mitochondrial octanoate (see full description in legend to ■ Fig. 23.2).

The GCS is expressed in liver, brain and in placental villi in the syncytiotrophoblast. In the brain it is highly expressed in astrocytes of the cerebrum and the cerebellum, but only lowly expressed in the brain stem and not in the spinal cord. It is also expressed in neural progenitor cells, in radial glial cells, and in neural stem cells [22]. One study of single cell RNA sequencing has identified it in astrocytes, as well as in corticospinal and corticostriatal neurons and in certain interneuron populations, a finding that still needs confirmation [23]. Deceased neonates with classic NKH exhibit myelin spongiosis in the corticospinal tract, optic radiation and brain stem, explaining the diffusion restriction seen on MRI. On electron microscopy, there is splitting of the myelin lamellae [24]. In contrast, patients with variant NKH have shown cavitating leukodystrophy and hypertrophic cardiomyopathy at autopsy [7, 11]. Absence of the GCS results in increased levels of glycine in all body fluids. High concentrations of glycine outside the brain such as seen with excessive provision of glycine do not result in symptoms. In NKH, glycine accumulates particularly in the brain. Glycine is an inhibitory neurotransmitter of glycinergic receptors in brain stem and spinal cord, possibly contributing to apnoea and hypotonia. However, in neuronal stem cells the glycinergic receptor may be excitatory rather than inhibitory (see also ▶ Sect. 30.4). In addition, glycine is an allosteric activator of the excitatory N-methyl-D-aspartate (NMDA) type glutamate receptor NR1/NR2 type. Increased levels of glycine result in overactivation of this NMDA receptor. D-serine, also an NMDA receptor activator, is synthesized from glycine in the brain and decreased levels are documented in the cortex of children with NKH [25]. Glycine is also an activator of excitatory NR1/NR3 receptors, the function of which is unclear. Furthermore, glycine is a trophic factor for cerebellar Purkinje cells. In mice with deficient GCS, there is a deficiency of methylated folates in the brain [26, 27]. Replenishment of the one methyl-group metabolism improves symptoms and survival in these mice [26, 27]. Although there is a slight increase in CSF homocysteine levels of children with classic NKH, CSF methylfolate levels are not reduced.

Patients with lipoate deficiency whether primary or secondary to iron-sulfur cluster disorders have deficient activity of the lipoate bearing enzymes pyruvate dehydrogenase and 2-ketoglutarate dehydrogenase, and all, except LIPT1, also have deficient GCS activity. Most iron sulfur cluster disorders also affect complexes I and II of the respiratory chain (▶ Chap. 10).

23.4 Genetics

Classic NKH is an autosomal recessive disorder with biallelic pathogenic variants identified in *GLDC* encoding the P-protein in 80% of cases or in *AMT* encoding the T-protein in 20% of cases. Recently mutations have been identified in *GCSH* encoding the H-protein in patients with variant NKH. There is extensive intragenic heterogeneity in *GLDC* with over 200 mutations identified, most of them private [2, 28]. The most common pathogenic variants are missense mutations, followed by RNA splicing mutations and insertion/deletion mutations (indels), and 20% of disease-causing alleles are intragenic copy number variants (deletions or duplications). Recurrent pathogenic variants include the severe mutations p.Arg515Ser (comprising 10% of all disease-causing alleles), IVS19-1G > A and IVS22 + 1G > C and the residual activity retaining variants p.Ala389Val and p.Ala802Val [2, 28]. In *AMT*, the majority of pathogenic variants are missense mutations followed by indels and RNA splicing mutations, but no intragenic copy number variants have been reported to date. Recurrent pathogenic variants include p.Arg320His which is present in 15% of all disease-causing alleles and p.Arg222Cys and p.Arg94Trp present in >5% of disease-causing alleles. A few variants in the 5'UTR of *AMT* around −55 to −66 were recognized as pathogenic and this often ignored area needs to be examined.

Testing of *GLDC* and *AMT* is negative in approximately 5% of patients with deficient GCS activity. These cases comprise the group of disorders referred to as variant NKH [7]. Variant NKH results from biallelic mutations in the genes involved in lipoate synthesis. Mutations have been identified in *LIAS, BOLA3, GLRX5, NFU1, ISCA2, LIPT1, LIPT2*, and *IBA57* in the patients with lipoate disorders [7–9, 11, 15, 16, 18] (▶ Chap. 10). Due to large genetic heterogeneity and the rarity of these conditions, a genotype-phenotype correlation has not yet been established in the genes causing lipoate deficiency syndrome.

Both classic and variant NKH are inherited in an autosomal recessive pattern. Parents of children with NKH are assumed to be heterozygous carriers of NKH. *De novo* mutations were documented in approximately 1% of individuals with classic NKH [2]. Thus, the heterozygote state should be confirmed in parents as a *de novo* mutation dramatically reduces recurrence risk.

23.5 Diagnostic Tests

Increased levels of glycine are found in plasma, urine, and CSF. Increased plasma glycine levels are usually the first indication of a glycine disorder but have incomplete sensitivity and low specificity and always require confirmatory testing. Most infants identified on newborn screening with highly elevated blood glycine levels did not have NKH and remained asymptomatic [29]. Excessive glycine intake such as seen with glycine buffered intravenous gamma globulin infusion can cause very elevated plasma glycine levels. Extremely high glycine levels are also seen in neonatal disseminated herpes virus infection. Branched chain organic acidurias often cause elevated plasma glycine levels and must be excluded. Hyperglycinuria without hyperglycinaemia is noted in disorders of the renal carrier of glycine, proline and hydroxyproline.

Increased levels of CSF glycine are highly indicative of nonketotic hyperglycinaemia. Normal CSF glycine values are 3–20 μM in the neonatal period and 3–12 μM after age 6 months. The lowest CSF glycine found in a series of 124 patients with classic NKH was 40 μM [2]. An elevated CSF:plasma glycine ratio is a further indication of NKH, but is only valid if the CSF glycine level is elevated. Normal CSF:plasma glycine ratio are <0.02, with all patients with severe classic NKH have a ratio >0.08. Contamination of CSF with blood or serum, as evidenced by increased CSF protein levels, can cause false elevation of both CSF glycine and the CSF:plasma glycine ratio and should not be relied upon for diagnosis [30]. Valproate inhibits the GCS and raises both the plasma and CSF glycine levels and the CSF:plasma glycine ratio. Very rare patients with attenuated NKH had normal CSF glycine levels. The sensitivity of elevated CSF glycine is >99%, making it the preferred diagnostic test.

In the first months of life, when most patients become symptomatic, brain MRI studies show a consistent pattern of diffusion restriction in the pyramidal tract most notable in the posterior limb of the internal capsule and the anterior brain stem, in the posterior tegmental tract and cerebellum [4]. This combination is highly suggestive of NKH and distinguishes the presentation from the transient NKH and from variant NKH, which tends to show leukodystrophy or involvement of basal ganglia.

Mutation analysis of the genes involved in the GCS is an excellent confirmatory test, but the frequent variants of unknown significance makes that it is best done in conjunction with biochemical and radiological findings. Using both sequencing and deletion/duplication analysis of *GLDC* and *AMT*, 98% of the alleles are identified. A database of mutations in over 500 families is available (▶ https://databases.lovd.nl/shared/genes/ [28]). In new cases identified in recent years, >90% of missense alleles were already previously identified [28]. For prenatal diagnosis, mutation analysis can be used once the familial pathogenic variants are known, or if only one allele is known and thus the gene is identified, intragenic linkage of intragenic polymorphisms can be used for the unknown allele. Preimplantation diagnosis has also been performed successfully in some families with a known molecular genetic diagnosis.

Measurement of GCS activity is possible in liver tissue and placenta. Enzymatic analysis may be used for prenatal diagnosis, when the mutation in the family is unknown. This test has at least a 1% false negative diagnostic rate, making it less recommended [31]. Due to cycling over the P-protein, the patients with T-protein defects show ~10% residual activity in measurements of whole GCS activity. The glycine exchange assay is deficient in defects of the P-protein or H-protein but is normal in defects of T-protein. A whole body glycine metabolism analysis is done by measuring exhaled carbon dioxide ^{13}C isotope enrichment after administration of an enteral dose of 1-^{13}C-glycine [32].

Transient NKH is a phenocopy. In this clinical setting, neonates present with acute neurological symptoms and have elevated CSF glycine with often elevated CSF:plasma glycine levels. These elevated CSF glycine levels disappear spontaneously over the next days to weeks. There are no mutations present in the genes of the glycine cleavage enzyme and the GCS activity is normal. This feature can be seen in a variety of clinical settings, most commonly in hypoxic ischemic injury in a neonate [33]. The absence of the typical pattern of diffusion restriction of NKH indicates a phenocopy [4].

Most patients with variant NKH manifest only mild elevations of glycine in plasma and CSF and may also have an increase of plasma alanine and serum lactate and pyruvate concentrations with 2-ketoglutarate in urine organic acids. Patients with NFU1 may in addition have elevated 2-aminoadipic acid and 2-ketoadipic acid. Enzymatic analyses reveal deficient GCS activity, but also consistently a deficiency in PDH activity, and in certain disorders of iron sulfur cluster synthesis (NFU1, ISCA2, IBA57, BOLA3) a deficiency of complexes I and II of the respiratory chain. Western blot analysis can demonstrate reduced amounts of lipoylated H-protein, E2 of PDH, and 2KDH [2, 3, 5, 7–9, 11, 15, 16]. To exclude PNPO deficiency, CSF pyridoxal-P levels should be measured, and other amino acids such as threonine may show abnormalities (▶ Chap. 29). Identification of the correct genetic defect is important since treatment and prognosis of classic NKH are different from that of variant NKH or PLP disorder.

23.6 Treatment

Withdrawal of intensive care in the neonatal period is an ethical consideration given the very poor outcome in severe classic NKH [34]. Correct distinction between severe and attenuated NKH can aid in this decision making [2]. Once breathing resumes, children with classic NKH will only rarely die, likely of sudden death in epilepsy (SUDEP), and adequate supportive care must be provided.

First principle of medical treatment in classic NKH is reduction of plasma glycine levels by benzoate with 250–750 mg/kg/day in 3–6 daily doses, with higher doses in severely affected patients (500–750) compared to attenuated patients (250–500) [1, 2, 19]. Monitoring of benzoate treatment should include regular measurement of plasma glycine obtained 1 hour after a dose (goal of treatment ≤300 μmol/L), and benzoate levels if glycine is low (non-toxic level ≤ 2.5 mmol/L) [1, 19]. Side effects of benzoate are incompliance due to unpalatability often requiring administration via G-tube, esophagitis or gastritis, which should always be avoided by prophylactic treatment with proton pump inhibitors, and carnitine deficiency, which should be monitored for [35]. Overdosage of benzoate may result in toxicity, beginning as nausea, vomiting, lethargy and hypocalcaemia leading to coma, seizures, acidosis, hypernatremia, hypokalaemia, and death [19]. Dosing over 750 mg/kg/day can cause renal dysfunction syndrome. Children with severe NKH requiring high doses of benzoate can benefit from a glycine- and serine-restricted diet, which can be assisted by a glycine-free amino acid formula [19]. Dietary gelatin and high protein intake should always be avoided. Benzoate treatment results in a reduction of seizure frequency and improvement of alertness for all [1, 35]. In severe NKH, it does not ameliorate developmental delay. Ketogenic diet markedly reduces the glycine pool, and decreases plasma and CSF glycine levels, and hence concomitant benzoate dosing must be reduced. The clinical effect of this form of glycine restriction is only partially described, and includes a decrease of seizures, increased alertness with variable effect on the EEG pattern [36].

The second principle of medical treatment in classic NKH is the use of receptor antagonists to block the effects of glycine at the neurotransmitter receptors. Dextromethorphan blocks the glutamate binding site of the excitatory NMDA receptor. High doses, 3–15 mg/kg/day, are needed to achieve neurological effect where it reduces seizures and improves alertness. High dosing should take into account pharmacogenomic differences at *CYP2D6*, and to a lesser effect *CYP3A4* and *UGT1A1*. Its importance is most striking in attenuated NKH where early treatment together with benzoate has resulted in substantially increased cognitive outcome [37], and late treatment has at times resulted in resolution of chorea, increased alertness and improved school function. In patients with severe NKH, dextromethorphan is less effective or ineffective and may result in recurrent pneumonia, presumably due to decreased coughing [1]. An alternative lipophilic non-competitive NMDA receptor blocker is ketamine, used in oral application at daily doses of 15 mg/kg in neonates, reduced to 9 mg/kg/day in infants (range: 1–32 mg/kg) with improved cognitive outcome in case of early treatment in attenuated mutation carriers [5]. Treatment with strychnine, a competitive antagonist of inhibitory GlyR receptor, resulted in some clinical improvement in a few historical cases.

Symptomatic epilepsy treatment with anticonvulsants is challenging for patients with severe classic NKH and multiple anticonvulsants are required. A systematic study of anticonvulsant effectiveness is not available. Benzodiazepines (clonazepam, clobazam) are most effective for treating myoclonic seizures in early infancy. Levetiracetam, topiramate and phenobarbital are most commonly used in older children. Felbamate, an NMDA-receptor-blocker, may be considered for treating recalcitrant seizures, although serious side effects limit its wider use. Vigabatrin can cause rapid deterioration [38]. ACTH treatment has not been effective in the treatment of West syndrome and is associated with worsening clinical status including inducing coma. Valproate is contraindicated in the patients with NKH. It inhibits residual GCS enzyme activity increasing brain glycine levels leading to encephalopathy with chorea, paradoxical increase in seizures and coma [39]. A vagal nerve stimulator has been effective in several older patients with severe NKH [40]. In attenuated NKH, hyperactivity is difficult to control medically. Hyperactivity and behavioural problems respond best to Applied Behavioural Analysis therapy, resulting in substantial developmental progress in some children. Scoliosis does not respond to bracing and surgical options for scoliosis and hip dysplasia have to be weighted with the quality of life [41]. Respiratory secretion management is challenging in older severe NKH patients and careful management can be helpful in avoiding recurrent pneumonia, often a terminal complication. The role of one methylgroup donors has not been proven in humans, in contrast to mice with NKH, but folinic acid treatment has not shown improvement. Patients with certain rare mutations could potentially benefit from co-factor therapy with pyridoxalphopsphate (*GLDC*) or folinic acid (*AMT*).

No effective treatment currently exists for variant NKH and lipoate synthesis defects. Benzoate and dextromethorphan have no apparent effect. Avoidance of

catabolism can aid in preventing regressive episodes in patients with *BOLA3* mutations. High dose lipoate can be tried but results are generally disappointing. Ketogenic diet can cause massive acidosis. Exogenous ketone therapy and triheptanoin have shown early positive indication. In PNPO, seizures are primarily pyridoxal-P responsive.

23.7 Prognosis

Prognostic indicators of severe outcome include high CSF glycine level (>230 μM), hydrocephalus, and short and thin corpus callosum on brain MRI; whereas prognostic indicators of attenuated NKH are a lower CSF:plasma glycine ratio (<0.08), late onset (≥4 months), normal sized corpus callosum and absence of epilepsy [2, 4]. Frequent hiccupping and EEG patterns of burst suppression and hypsarrhythmia tend to indicate severe outcome [1]. In classic NKH, a genotype-phenotype correlation has been established following the hypothesis that residual enzyme activity was associated with an improved developmental outcome [2]. The presence of two mutations without residual activity always predicts severe NKH. At least one mutation with residual activity is required for attenuated NKH. Patients with two residual activity conferring mutations have the best neurocognitive outcome [2]. Early treatment in attenuated patients with a mutation with residual activity is paramount for achieving the optimal developmental outcome, such as seen in two siblings studies treated with benzoate and ketamine or dextromethorphan from very early on [5, 37]. This emphasizes the importance of early genotyping of the patients for an accurate prognosis. Survival of patients with severe NKH varies from months when untreated, possibly due to SUDEP, to over two decades when seizure management and supportive care are done effectively, and for individual patients prediction on survival is not possible. Patients with attenuated NKH often survive for many decades.

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Disorders of Glutamine, Serine and Asparagine Metabolism

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Contents

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Glutamine Metabolism

Glutamine, the most abundant amino acid in the human organism, is synthesized de novo from glutamate and ammonia by the cytosolic enzyme glutamine synthetase. Glutamine synthetase is ubiquitously expressed within the human body with high levels in brain (astrocytes), liver and skeletal muscle. Its reaction is the only known way to produce glutamine in the human body and there is no net absorption from the intestine under physiological conditions. Glutamine plays a pivotal role in the mammalian metabolism as constituent, precursor, and amino moiety donor for many proteins, purines and pyrimidines, nicotinamide adenine dinucleotide, adenosine-monophosphate, amino acids and glucose. It is also a substrate for transamination reactions, required for the production of alpha-ketoglutarate, closure of the methionine salvage pathway, and salvage of alpha-keto acids. The mitochondrial enzyme glutaminase is likewise ubiquitously expressed and catalyses the reverse reaction of glutamine into glutamate, the main excitatory neurotransmitter in the central nervous system, and ammonia (◘ Fig. 24.1).

Serine Metabolism

Serine is a non-essential amino acid and has important functions besides its role in protein synthesis. It is a precursor of a number of compounds (partly illustrated in ◘ Fig. 24.2), including D-serine, glycine (► Chap. 23), cysteine, serine phospholipids (► Chap. 35), sphingomyelins, and cerebrosides (► Chap. 40) that play an essential role in neuronal development and function. It must be synthesized within the brain because of its poor permeability by the blood-brain barrier. This synthesis is confined to astrocytes, and its shuttle to neuronal cells is performed by a dedicated neutral amino acid transporter, ASCT1. Moreover, it is a major source of N^5,N^{10}-methylene-tetrahydrofolate (THF) and of other one-carbon donors that are required for the synthesis of purines and thymidine. It is also involved with NADH and cellular respiration [1]. Serine is synthesized de novo from a glycolytic intermediate, 3-phosphoglycerate and can also be synthesized from glycine by reversal of the reaction catalyzed by serine hydroxymethyltransferase, which thereby converts N^5,N^{10}-methylene-THF into THF (► Chap. 28). Recently it has also been found that low serine levels may occur as a secondary marker with *GOT2* mutations that cause a treatable malate-aspartate shuttle-related encephalopathy (► Chap. 11) [2].

Asparagine Metabolism

Asparagine is synthesized de novo by asparagine synthetase (ASNS) which catalyzes the transfer of ammonia from glutamine to aspartic acid via a β-aspartyl-AMP intermediate. Nutritional intake is another source of asparagine. Because of the poor transport of asparagine across the blood-brain barrier (the level of cerebrospinal asparagine is only 1 to 10% of the plasma level), the brain depends on local de novo synthesis. Thus, asparagine synthesis is essential for the development and function of the brain but not of other organs. ASNS is expressed at low levels in most tissues except the brain that exhibits a brain-specific splice variant. Besides its role as a constituent of proteins, asparagine is a precursor of the glucogenic amino acid L-aspartate, itself a precursor of the neurotransmitter D-aspartate. It also plays an important role in ammonia detoxification and in asparagine-linked protein glycosylation (◘ Fig. 24.1).

Introduction

One disorder of glutamine synthesis is known, due to glutamine synthetase deficiency. It has been reported in only a few patients who mostly presented as newborns with epileptic encephalopathy, and in a single late onset case with seizures and microcephaly. The outcome is very poor, and it is not yet known whether early start of glutamine substitution therapy can improve the clinical prognosis. Glutaminase deficiency due to loss of function mutations or short tandem repeat expansion in *GLS* in patients with early onset neurological disease, has been recently reported [3, 4].

Four disorders of serine metabolism are known. Three are in its biosynthesis: namely, 3-phosphoglycerate dehydrogenase deficiency, phosphoserine aminotransferase deficiency and phosphoserine phosphatase deficiency. Most patients with 3-phosphoglycerate dehydrogenase deficiency have a severe infantile phenotype with congenital microcephaly, intellectual disability and intractable seizures or Neu-Laxova syndrome, a severe dysmorphism syndrome with, as a rule, perinatal lethality. In patients with the severe, infantile form, the treatment is oral L-serine, supplemented with glycine in case of unsatisfactory clinical response. Phosphoserine aminotransferase deficiency has been reported in six patients and in Neu-Laxova syndrome. One patient was treated from birth on with serine and glycine, and this resulted in a normal outcome at the age of 15 years. Phosphoserine phosphatase deficiency has been described in nine patients. It is also a cause of Neu-Laxova syndrome. Mutations have recently

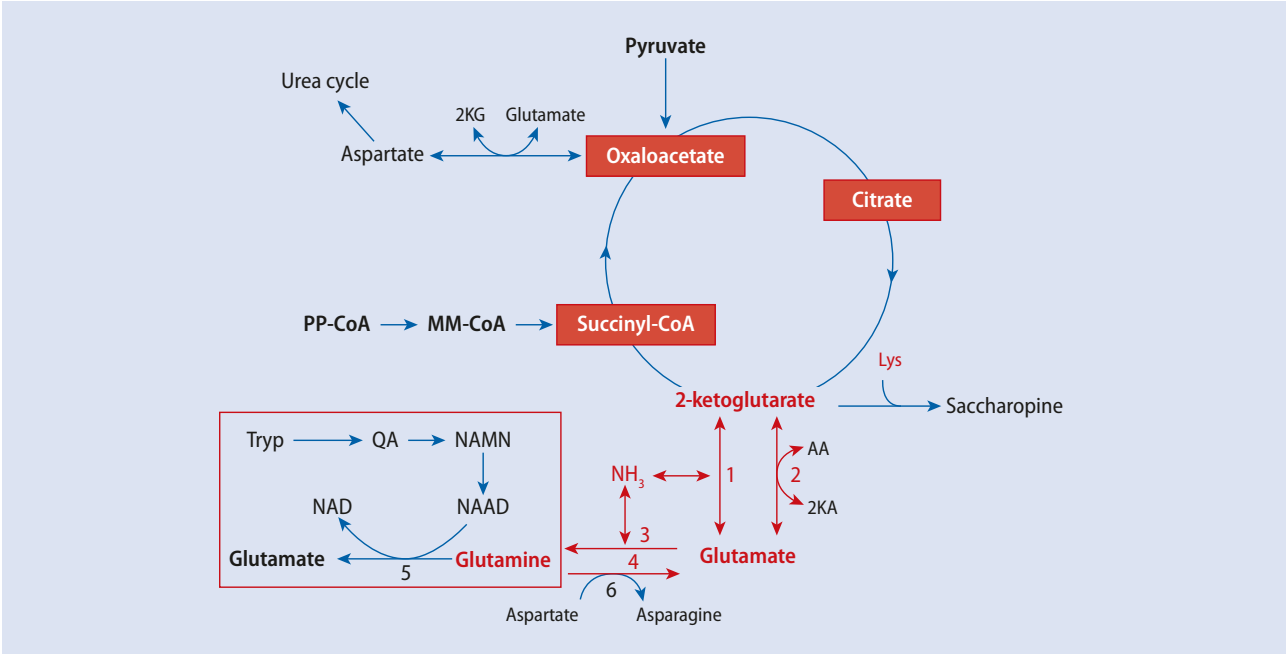

Fig. 24.1 2-Ketoglutarate/ Glutamate/ Glutamine pathways. In red are the 4 mitochondrial reactions involved in 2-Ketoglutarate/ Glutamate/Glutamine/NH3 metabolism: (1) Glutamate dehydrogenase, (2) Glutamate pyruvate transaminase (alanine transaminase, SGPT), (3) Glutamine synthetase and (4) Glutaminase. 2-ketoglutarate is also the key compound for the first step of lysine metabolism. In cases of low 2-ketoglutarate availability, lysine accumulates as observed in most causes of hyperammonaemia. Glutamine levels are usually high in urea cycle defects whereas they are normal to low in pyruvate carboxylase deficiency and organic acidaemias (where there is a low 2-ketoglutarate production) and very low in glutamine synthetase deficiency. Glutamine is also involved in the synthesis of NAD+ by giving its amino group to NAAD in the irreversible reaction, NAD synthetase (reaction 5). There are also alternative routes for NAD+ generation starting from nicotinate, nicotinamide, nicotinamide riboside, or tryptophan (not shown in the figure). Asparagine synthetase (reaction 6) involves a complex process which allows asparagine synthesis from aspartate using glutamine as an amino group donor. 2KA 2-ketoacid, 2KG 2-Ketoglutarate, AA amino acid, MM-CoA methylmalony-CoA, NAAD nicotinic acid adenine dinucleotide, NAD nicotinamide adenine dinucleotide, NAMN nicotinic acid mononucleotide, PP-CoA propionyl-CoA, QA quinolinic acid, Tryp tryptophan

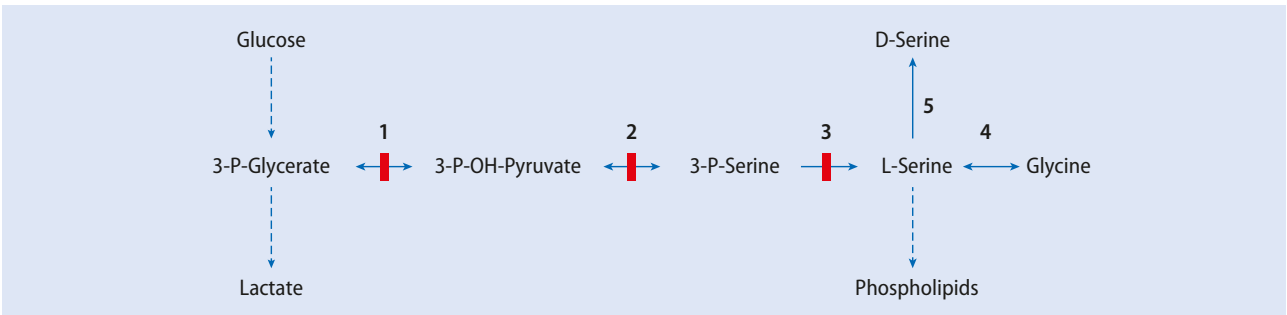

Fig. 24.2 Pathway of de novo serine synthesis. P, phosphate; 1, 3-phosphoglycerate dehydrogenase; 2, phosphoserine aminotransferase; 3, 3-phosphoserine phosphatase; 4, serine hydroxymethyltransferase (utilizes tetrahydrofolate); 5, serine racemase. Glycine is synthesized from serine, but also from other sources. Red bars across arrows indicate the known defects in serine synthesis

been reported in *SLC1A4*, encoding the brain serine transporter. They are associated with a phenotype that closely resembles that of severe 3-phosphoglycerate dehydrogenase deficiency.

One disorder of asparagine synthesis has been reported, due to asparagine synthetase deficiency. Thirty-three patients are known. Most showed severe psychomotor/intellectual disability, progressive

microcephaly, limb hypertonia, hyperreflexia and mostly also intractable epilepsy. Six patients died within the first year of life. The poor permeability of the blood-brain barrier for asparagine leaves little hope for a therapeutic effect of substitution. A milder form was reported in one family.

The conditions treated in this chapter share a similar clinical presentation. In general, they are severe early-onset neurodevelopmental disorders with microcephaly, epilepsy and brain MRI abnormalities consisting of hypomyelination and/or migration disturbances.

24.1 Inborn Errors of Glutamine Metabolism

24.1.1 Glutamine Synthetase Deficiency

Glutamine synthetase (GS) deficiency (enzyme 3 ◘ Fig. 24.1) is an ultra-rare disease, first described in 2005 [5].

▪ Clinical Presentation

GS deficiency has only been reported in a few patients, most of them with Turkish and one with Sudanese origin [5, 6]. Most of them presented as newborns with epileptic encephalopathy, as the only sign or in combination with other symptoms including diarrhoea, erythematous skin rash, and multiorgan failure. Two patients died during the newborn period while one patient survived until age six years. This patient was affected by chronic epileptic encephalopathy with severe mental retardation. One lately reported patient presented at five months with seizures and was diagnosed with GS deficiency at age 30 months with additional developmental delay, microcephaly and muscular hypotonia [7]. In early onset patients, the disease started prenatally as illustrated by brain magnetic resonance imaging (MRI) which showed cerebral and cerebellar atrophy associated with an almost complete agyria [8].

▪ Metabolic Derangement

The main biochemical findings in GS deficiency are the low glutamine concentrations in all body fluids. Remarkably, plasma glutamine concentrations were initially only borderline low in two patients but continuously decreased during infancy [5, 6]. All other amino acids including glutamate are normal. The only other constant finding is chronic moderate hyperammonaemia with most ammonia levels between 100–200 μM [6]. This reflects the failure to clear, within the perivenous hepatocytes, ammonia that escaped the urea cycle in periportal hepatocytes. Pathophysiology of GS deficiency is not completely understood. It is however likely that, adding to the developmental abnormalities caused by glutamine deficiency during embryonic and fetal stages, a combination of systemic glutamine and nicotinamide adenine dinucleotide (NAD^+) deficiency (▶ Sect. 24.1.2), and chronic hyperammonemia lead to the severe phenotype [9].

▪ Genetics

Defects in *GLUL* cause GS deficiency. The disease is transmitted in an autosomal-recessive mode. The mutations known in the patients were all missense changes presumably allowing some, albeit little, residual GS activity and it can be assumed that a complete loss of GS function would be lethal during embryonic or fetal development. Prenatal diagnosis is feasible by genetic means.

▪ Diagnostic Tests

GS deficiency is characterized by decreased levels of glutamine in all body fluids, but the decrease can be moderate in less severely affected patients. Moreover, glutamine levels may be only slightly decreased in the newborn period and infancy but may become later as low as <10 μM in plasma. Thus, already any slight decrease in plasma glutamine concentration in an infant with epileptic encephalopathy should be taken seriously and glutamine concentrations in other body fluids should be investigated. Since GS is ubiquitously expressed, enzyme studies can be done in many tissues but are not routine tests.

Confirmation of the diagnosis should be by mutation analysis. Newborn screening is currently not available and would require reliable assays for glutamine in dried blood spots.

▪ Treatment and Prognosis

Patients with GS deficiency are not at risk of acute metabolic decompensations. Glutamine deficiency and hyperammonemia are chronic and difficult to treat. In two patients, enteral glutamine substitution (given every two hours or as continuous gastric tube feeding at a dose of up to 1020 mg/kg/day) led to correction of glutamine in plasma and partly also in the CSF, and to some clinical stabilization [10]. Whether early start of glutamine therapy can improve the overall clinical prognosis is however unknown. In addition to glutamine substitution, correction of systemic NAD^+ deficiency by nicotinamide treatment may be beneficial but this has been tested only in a single patient with no conclusive results yet [7, 11].

Based on the patients reported so far, the overall outcome of GS deficiency is poor with death in the newborn period or in early childhood in some patients. It is

however unknown whether early treated or mildly affected patients may survive longer.

24.1.2 NAD Synthesis Defect

A defect of nicotinamide adenine dinucleotide (NAD) de novo synthesis caused by bi-allelic variants in *NADSYN1*, encoding NAD synthetase 1, has recently been described in five individuals from four unrelated families (enzyme 5 ◘ Fig. 24.1) [12]. The patients had presented with multiple overlapping congenital malformations affecting the skeletal system, heart, kidneys and other organs. There is, however, no data on therapy in humans but an NAD precursor-rich dietary supplementation was successfully given to mice with defects in the NAD-synthesis pathway [11].

24.1.3 Glutaminase Deficiency

Glutaminase deficiency (enzyme 4 ◘ Fig. 24.1) due to loss of function mutations or short tandem repeat expansion in *GLS* has recently been reported [3, 4]. The patients presented with lethal early neonatal encephalopathy [3] or with early-onset delay in overall development and progressive ataxia [4]. In contrast to defects in glutamine synthetase, patients had a substantial and persistent elevation in plasma glutamine of around 2000 µmol/L [4]. There is currently no therapy available.

24.1.4 Glutaminase Hyperactivity

A de novo gain-of-function variant in *GLS* encoding GLS has recently been described in a single patient with infantile onset of cataracts, profound developmental delay, axial hypotonia, cataract and self-injurious behaviour. In keeping with GLS hyperactivity, increased glutamate and decreased glutamine concentrations were measured in urine and fibroblasts. Using MRS, brain glutamate was extremely high and glutamine almost undetectable [13].

24.2 Inborn Errors of Serine Metabolism

24.2.1 3-Phosphoglycerate Dehydrogenase Deficiency

▪ Clinical Presentation

Fifty-three (reported and non-reported) patients (from 38 families) are known, with four different clinical presentations [14–22]. The most severe end of the known clinical spectrum is *Neu-Laxova syndrome* first reported in 1971. It is characterized by severe fetal growth restriction, microcephaly, a distinct facial dysmorphism, ichthyosis, skeletal anomalies and perinatal lethality. Patients with a defect in one of the other serine biosynthesis steps can also have this syndrome (see further). The majority of the patients with 3-phosphoglycerate dehydrogenase deficiency had the *severe, infantile phenotype* characterized by congenital microcephaly, intractable seizures in the large majority, and pronounced intellectual disability. From birth on, these patients suffer from feeding difficulties with vomiting and from irritability with inconsolable crying. Therapy-resistant convulsions appear in the first weeks to months of life and show great variation. The psychomotor development of these patients is extremely poor; the reported patients (aged up to 24 years) had a developmental age of less than one year. Hypertonia is present before the age of one year and evolves into spastic tetraplegia. Other symptoms, reported in a minority of the patients, are congenital cataracts, growth retardation, abnormal hair, inguinal and umbilical hernias, hypogonadism, and megaloblastic anaemia. Magnetic resonance imaging (MRI) of the brain revealed cortical and subcortical hypotrophy and evidence of disturbed myelination.

A *mild, juvenile phenotype* has been described in a brother and sister presenting with intellectual disability and therapy-responsive absence seizures at 5 and 9 years. Brain MRI was normal in both [23].

Finally, a *Charcot-Marie-Tooth phenotype* has been reported in a man who was diagnosed at 31 years with a progressive, severe, axonal sensorimotor polyneuropathy compatible with Charcot-Marie-Tooth disease type 2. Brain MRI showed non-specific T2-weighted hyperintensities [24].

▪ Metabolic Derangement

The deficiency of 3-phosphoglycerate dehydrogenase, the first step of serine biosynthesis (enzyme 1, ◘ Fig. 24.2), causes decreased concentrations of serine and, to a lesser extent, of glycine in CSF and in fasting plasma. Serine thus becomes an essential amino acid in these patients. A significant accumulation of the substrate, 3-phosphoglycerate, is unlikely since it is an intermediate of the glycolytic pathway. Therefore, the deficiency of brain serine seems to be the main determinant of the disease. Serine plays a major role in the synthesis of important brain and myelin constituents, such as proteins, glycine, cysteine, serine phospholipids, sphingomyelins and cerebrosides.

In the two patients with megaloblastic anaemia, decreased methyltetrahydrofolate was found in

CSF. This can be explained by the fact that serine is converted into glycine by a reaction that forms methylenetetrahydrofolate, which is further reduced to methyltetrahydrofolate.

Genetics

This is an autosomal-recessive disease. To date twenty-seven variants in *PHGDH* have been identified. Prenatal diagnosis is only possible by mutation analysis as there is a lack of data on enzyme activity in chorionic villi and amniocytes.

Diagnostic Tests

This disease should be considered in patients with Neu-Laxova syndrome and in encephalopathy comprising congenital microcephaly, epilepsy and intellectual disability. Plasma amino acids must be measured in the fasting state (range of serine in patients: 28–64 μM; normal range: 70–187 μM), since serine and glycine levels can be normal after feeding. In CSF, serine levels are always decreased (6–8 μM; control range 35–80 μM), as are glycine levels, but to a lesser extent. The diagnosis is confirmed by finding a deficient activity of 3-phosphoglycerate dehydrogenase in fibroblasts (reported residual activities from 6–22%) or by mutation analysis. In the patients with the milder, juvenile phenotype, the metabolite and enzymatic findings were indistinguishable from the findings in the patients with the severe phenotype.

Treatment and Prognosis

Treatment with L-serine has a beneficial effect on the convulsions, spasticity, feeding and behaviour of these patients. Oral L-serine treatment (500–700 mg/kg/day in six divided doses) corrected the biochemical abnormalities in all reported patients and abolished the convulsions in most patients, even in those in whom many anti-epileptic treatment regimens had failed previously. During treatment with L-serine, a marked increase in the white matter volume was observed, and in some patients a progression of myelination. In a few patients, convulsions stopped only after adding glycine (200–300 mg/kg/day).

In a girl diagnosed prenatally, because of decelerating head growth, L-serine was given to the mother at 190 mg/kg/day in 3 divided doses from the 27th week of gestation. This normalized foetal head growth and with subsequent postnatal therapy the girl, now aged 22 years, shows a normal development.

The patients with the milder phenotypes responded well to lower L-serine doses (100–150 mg/kg/day), without glycine.

24.2.2 Phosphoserine Aminotransferase Deficiency

This disorder in the second step of the serine biosynthesis (enzyme 2, ◘ Fig. 24.2) has been reported in 6 patients from 5 families [25–28]. The first reported patients were a brother and sister who showed decreased concentrations of serine and glycine in plasma and cerebrospinal fluid [25]. The boy presented with intractable seizures, acquired microcephaly, hypertonia and psychomotor retardation, and died at the age of 7 months despite supplementation with serine (500 mg/kg/day) and glycine (200 mg/kg/day) from the age of 7 weeks. The younger sibling received treatment from birth, which led to a normal outcome at the age of 3 years. Four other reported patients showed a similar neurological syndrome with or without epilepsy. Treatment with oral serine (+/– glycine) failed to improve substantially neurodevelopmental progress. Eight variants in *PSAT1* have been identified. Neu-Laxova syndrome can be caused by mutations in this and in the two other enzymes of the serine biosynthesis (▶ Sects. 24.2.1 and 24.2.3).

24.2.3 3-Phosphoserine Phosphatase Deficiency

Nine patients from 3 families (including 1 adult, a family with 7 affected siblings and a patient with Williams syndrome) have been reported with a deficiency of 3-phosphoserine phosphatase, the third step in the serine biosynthesis (enzyme 3, ◘ Fig. 24.2). The phenotype consisted of intellectual disability, epilepsy, microcephaly, growth deficiency, and, in the adult, contractures and severe axonal neuropathy with a non-healing foot ulcer [29–32]. Fasting plasma and, when tested, CSF showed low serine and glycine levels. Oral serine normalized plasma and CSF serine levels in the patient with Williams syndrome and seemed to have some beneficial clinical effect. After four months of treatment, the adult patient reported decreased neuropathic pain and healing of the foot ulcer, and the serine plasma levels improved. Neu-Laxova syndrome can be caused by mutations in this and in the two other enzymes of the serine biosynthesis pathway (▶ Sects. 24.2.1 and 24.2.2).

24.2.4 Brain Serine Transporter Deficiency

Nineteen patients have been reported with mutations in *SLC4A1*, encoding the ASCT1 brain transporter of

serine and other neutral amino acids [33–38]. They presented with significant psychomotor/intellectual disability, severe progressive microcephaly, epilepsy, spasticity, hypomyelination and thin corpus callosum. This phenotype is strongly reminiscent of the severe presentation of phosphoglycerate dehydrogenase deficiency and suggests that serine deficiency is the main determinant of the disease.

24.2.5 Serine Palmitoyltransferase Defects

These cause the most frequent subtype of hereditary sensory and autonomic neuropathy, HSAN type 1 (HSAN1), an autosomal dominant disease. The disorder is caused by mutations in the *SPTLC* genes, encoding three subunits of serine palmitoyltransferase (SPT), the first step in the de novo synthesis of sphingolipids (see ► Chap. 40).

24.3 Inborn Errors of Asparagine Metabolism

24.3.1 Asparagine Synthetase Deficiency

Asparagine synthase (ASNS) deficiency (enzyme 6 ◘ Fig. 24.1) was first described in 2013 [39] but is being increasingly recognized: thirty-three patients from 22 families from different part of the world are known with this disease [40]. This very recent discovery is due to the fact that the biochemistry is not reliable, and that the diagnosis mostly relies on exome sequencing. However, the clinical phenotype and MRI findings should allow early suspicion indicating a focused exome sequencing. However, therapy is mostly disappointing, mainly because asparagine crosses the blood-brain barrier very poorly, and therefore oral supplementation is mildly or not effective.

▪ Clinical Presentation

All patients showed severe psychomotor/intellectual disability, progressive (mostly congenital) microcephaly, limb hypertonia and hyperreflexia. In most patients there was in addition early-onset intractable epilepsy and axial hypotonia. Six patients died at ages from 9 days to 12 months. The oldest reported patient was 14 years. An apparently broad range of epileptic manifestations (spasms, tonic seizures, generalized tonic-clonic seizures, partial complex seizures) and EEG patterns (hypsarrhythmia, multiple independent spike foci, disorganized background activity, and so-called burst suppression) is on record. However, a striking sequence has been reported, starting with myoclonus and clonic seizures often turning to status epilepticus with focal or multifocal spikes followed by spasms with modified hypsarrhythmia and then discontinuous EEG recordings most often distinct from burst suppression, a sequence similar to what is seen in pyridoxine dependency [41]. A less severe phenotype was reported in two Turkish siblings without symptoms until the first seizures occurred at 6 months of age [42]. Seizures were then asymmetrical tonic and clonic, lasting several minutes, with multifocal interictal spikes. These children could sit at 30 months and walk at 3 years, but then developed major autistic features including lack of visual contact, loss of the few monosyllables they had acquired, hyperactivity and self-injury.

Brain MRI in the early onset phenotype shows a decreased cerebral volume, and in most cases cerebellar atrophy, a decreased size of the pons, a thin corpus callosum, simplified gyri and evidence of deficient myelination. Postmortem findings, available from two siblings, were cortical dysgenesis, periventricular leukomalacia, mesial temporal sclerosis, gliosis, neuronal loss and hydromelia of the spinal cord [43]. In an unrelated child, also with a severe phenotype, there was gliosis of the grey matter with simplified gyration and poor white matter, clearly resulting from loss of cortical neurons [41]. Therefore, ASNS deficiency is both a dysgenetic and a degenerative disease, and damage occurs during fetal life and goes on after birth.

▪ Metabolic Derangement

Plasma levels of asparagine are normal to decreased while plasma glutamine levels are normal to increased. Plasma aspartate (the other substrate of ASNS) is reported to be normal. However, the reports do not mention whether the blood was taken in the fed or in the fasting state, and in disorders of serine synthesis, plasma serine is only decreased in the fasting state. Thus, a normal plasma asparagine level does not rule out ASNS deficiency. Cerebrospinal fluid asparagine levels were measured in only two siblings and found decreased in both (0 and 1 μmol/L; normal range: 1.1–6.9) [43]. The cerebrospinal glutamine level was increased in the first patient and normal in the other. There is no information on aspartate levels in this fluid. As to the pathophysiology, it is assumed that the brain abnormalities are caused by asparagine deficiency during intrauterine development, and maybe by excitotoxicity due to a possible accumulation of aspartate, a substrate of ASNS that is supposed to be an excitatory neurotransmitter, although data are contradictory regarding this issue [44].

▪ Genetics

This disease shows an autosomal recessive inheritance with consanguinity in over half of the reported families. Nevertheless, twice more males than females have been

reported. Over 80% of the variants in *ASNS* were missense changes presumably allowing some residual ASNS activity. It can be assumed that a complete loss of ASNS function would be lethal during embryonic or fetal development. Prenatal diagnosis is feasible by mutation analysis.

Diagnostic Tests

Blood for asparagine measurement must be taken in the fasting state. Finding a decreased plasma asparagine level should be followed by amino acid analysis of the cerebrospinal fluid but there is only a small difference between normal and decreased levels. Therefore, in the absence of a reliable metabolic marker, exome sequencing remains the only diagnostic test. Indeed, the great majority of reported cases were diagnosed based on exome findings. As is true for many other patients, ours had died before we obtained the exome result, so that functional biochemical studies were no longer possible.

Treatment and Prognosis

In the absence of effective substitution, the outcome is very poor, and six patients died within the first year of life. The epilepsy is pharmacoresistant. One patient showed some effect of lamotrigine, an antiepileptic compound that reduces glutamate release. The poor permeability of the blood-brain barrier leaves poor hope for an effect of asparagine substitution: although the condition stabilized in both patients with the milder phenotype [40], one patient with the usual phenotype experienced aggravated epilepsy [45].

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Disorders of Amino Acid Transport at the Cell Membrane

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Contents

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Transepithelial Transport of Amino Acids

Epithelial cells in (for example) renal tubules and intestinal mucosa utilise several different amino acid transport systems (Fig. 25.1), which prefer amino acids with certain physicochemical properties. Cystine and the structurally related dibasic cationic amino acids lysine, arginine and ornithine are transported from the intestinal or renal tubular lumen into epithelial cells by an apical transporter (system $b^{0,+}$) in exchange for neutral amino acids. The dibasic amino acids are then transported from the epithelial cell into the tissues by a basolateral dibasic amino acid transporter (system y^+L) in exchange for neutral amino acids and sodium. Both these transporters are heteromers of a heavy subunit (*N*-glycosylated type 2 membrane glycoprotein) and a light subunit (nonglycosylated polytopic membrane protein) linked by a disulfide bridge. The subunits of an active transporter colocalize in the plasma membrane, but the exact process of dimerization is unclear since direct evidence for the assembly of the transporter in intact human cells has not been available. A third transporter system for neutral amino acids is expressed only at the luminal border of epithelial cells. It transports neutral amino acids i.e. alanine, asparagine, citrulline, glutamine, histidine, isoleucine, leucine, phenylalanine, serine, threonine, tryptophan, tyrosine and valine into epithelial cells. A specific renal transporter for the imino acids glycine, proline and hydroxyproline probably exists. Dicarboxylic amino acids (aspartate, glutamate) have a specific transporter EAAT3 (encoded by *SLC1A1*), located at the luminal border of epithelial cells and also expressed in neurons (not shown on Fig. 25.1).

Cystinuria, lysinuric protein intolerance and Hartnup disorder are caused by defects of the apical cystine/dibasic amino acid transporter (upper left arrow), the antiluminal dibasic amino acid transporter (lower left arrow), and the luminal neutral amino acid transporter (upper right arrow), respectively.

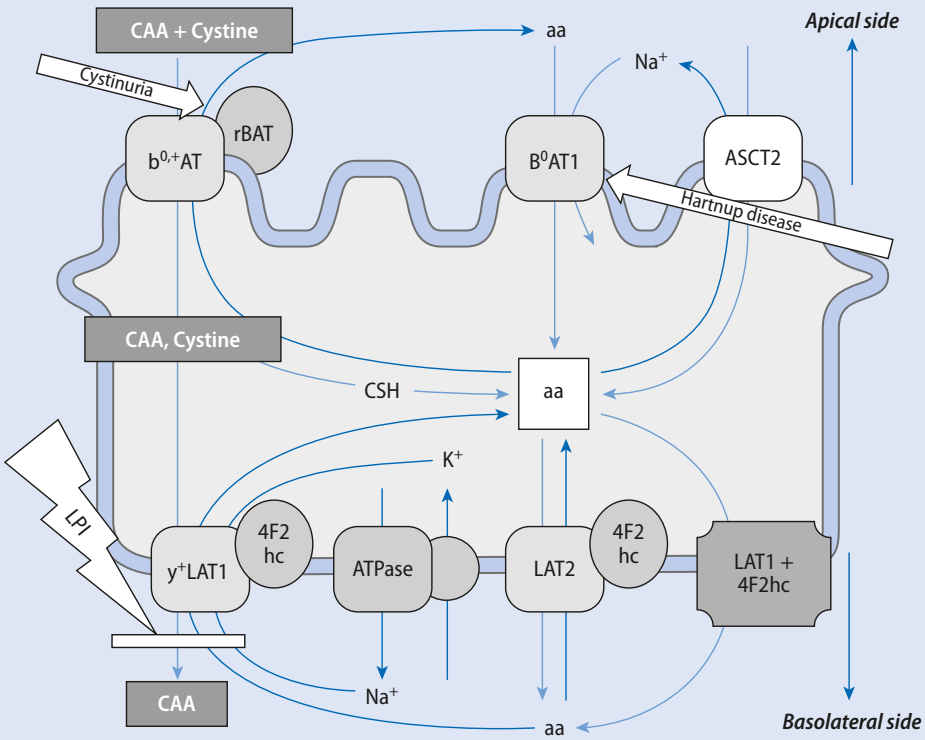

Fig. 25.1 Simplified schematic representation of cationic and neutral amino acid transport in epithelial cells. Courtesy of G. Sebastio. aa, amino acids; CAA, cationic amino acids; rBAT and $b^{0,+}$AT, heavy and light subunits of the high-affinity luminal transporter system $b^{0,+}$; y^+ LAT, system y + L amino acid transporter; 4F2hc, 4F2 cell surface antigen heavy chain; ASCT2, alanine serine cysteine transporter 2; CSH, cysteine

Introduction

Inherited defects in amino acid transport at the cell membrane are usually expressed as selective renal amino aciduria, i.e., the concentration of the affected amino acids is high in the urine while it is normal or low in plasma. Intestinal absorption of the affected amino acids is also almost always impaired. The clinical symptoms thus result from excess amounts of certain amino acids in the urine or lack of them in the tissues. There are systemic and non systemic disorders. Non systemic disorders with normal plasma amino acids (AA) include cystinurias in which renal stones may be formed because of high urinary concentration of poorly soluble cysteine, and iminoglycinuria and dicarboxylic aciduria that are mostly asymptomatic. There are 4 systemic disorders with low plasma AA. In lysinuric protein intolerance (LPI), the transporter defect for the dibasic cationic amino acids leads to poor intestinal absorption and urinary loss of arginine, ornithine and lysine. Subsequently, the patients develop protein intolerance with hyperammonaemia, growth retardation and skeletal and immunological manifestations. SLC6A19 and collectrin deficiency are responsible for Hartnup disease (neutral amino acids with secondary niacin deficiency), and Hartnup like disorder (neutral and acidic amino acids) respectively and may present with postnatal multisystemic manifestations. The pellagra-like dermatitis and ataxia are attributed to deficiency of tryptophan, the precursor of niacin synthesis. An IMD linked to brain carrier defects of essential AA is caused by mutations in *SLC7A5*, resulting in the defective brain transport of branched chain AA (BCAA) responsible for a severe neurodevelopmental disorder (Transepithelial Transport of Amino Acids and ◘ Table 25.1).

25.1 Cystinuria

25.1.1 Clinical Presentation

Cystinuria is linked to a life-long risk of urolithiasis. It is responsible for 1–2% of renal stones in adults and 6–8% in children and for approximately 20% of the metabolic causes of renal stones [2]. Stones typically first manifest during the second decade. Some patients never develop any problems, but others may have recurrent symptoms from early childhood. Acute episodes of abdominal or lower back pain, haematuria, pyuria or spontaneous passing of stones may be the presenting sign. Symptomatic stones often appear in clusters between long asymptomatic periods. Microscopic nephronal obstruction by cystine crystals associated with inflammation may lead to renal injury. Recurrent urinary tract infections, urinary obstruction and, finally, renal failure are possible complications. Transient cystinuria due to an immature transport system may appear during the first years of life [3, 4]. Cystinuria associated with severe neurological findings or Prader-Willi-like syndrome suggests a contiguous gene deletion on chromosome 2p16 or 2p21 (▶ Sect. 25.1.3).

25.1.2 Metabolic Derangement

In cystinuria, the high-affinity luminal transporter (system $b^{0,+}$; consisting of two protein subunits rBAT and $b^{0,+}$AT; ◘ Fig. 25.1) for cystine and the dibasic amino acids at the apical side of the epithelial cells of the proximal renal tubule and in jejunal mucosa is defective. Subsequently, absorption of cystine in the intestine and its reabsorption in the kidney is reduced. Normally, 99% of the filtered cystine is reabsorbed, while homozygotes with cystinuria excrete 600–1400 mg of cystine into the urine per day. Cystine is poorly soluble at neutral or low urinary pH and crystals and stones may be formed [3]. Specific proteins may serve as promoters of cystine precipitation, aggregation or epithelial adherence [5]. No signs of cystine deficiency have been described.

25.1.3 Genetics

The average incidence of cystinuria is 1 in 7000 but varies considerably between different populations. The highest incidence, 1:2500, has been observed in Libyan Jews [6]. In addition, some of the patients may remain undiagnosed as they do not form stones or only form them infrequently. Cystinuria type A (type 1 cystinuria by former classification based on the phenotype of the parents as obligatory carriers) is the autosomal recessive form of the disease and represents over 60% of the cases. It is caused by mutations in *SLC3A1* that encodes rBAT, the heavy subunit of the amino acid transporter. At least 152 mutations have been reported.

Cystinuria type B (non-type 1 cystinuria) is due to mutations in *SLC7A9*, encoding the light subunit of the transporter, $b^{0,+}$AT. At least 104 mutations have been described. Individuals harboring one mutated allele in *SLC7A9* may exhibit an abnormal urinary cystine excretion but generally remain free of clinical symptoms. On the other hand, individuals harboring two mutated alleles are clinically symptomatic. Individuals with the rare type AB cystinuria have homozygous mutation in one gene and additional heterozygous mutation in the other gene (type AAB or ABB). In approximately 5% of the patients with the clinical phenotype, either no mutations have been found in these genes or only one hetero-

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Table 25.1 Inherited defects in amino acid transport at the cell membrane

Disorder	Key clinical characteristics	Excess amino acids in urine	Plasma amino acids	Protein	Solute carrier family	Gene
Cystinuria A	Urolithiasis	Cystine, lysine, arginine, ornithine	Normal	rBAT	Solute carrier family 3 (cystine, dibasic, and neutral amino-acid transporters), member 1	*SLC3A1*
Cystinuria B		Cystine, lysine, arginine, ornithine	Normal	$b^{0,+}$AT	Solute carrier family 7 (cationic amino acid transporter, y^+ system), member 9	*SLC7A9*
Cystinuria AB		Cystine, lysine, arginine, ornithine	Normal			*SLC3A1* and *SLC7A9*: AAB or ABB
Iminoglycinuria	Mostly asymptomatic	Proline, hydroxy-proline, glycine	Normal	PAT-1? SIT-1? $b^{0,+}$AT?		*SLC36A1? SLC6A20? SLC6A19?*
Dicarboxylic aminoaciduria	Asymptom-atic? Neuropsychi-atric symptoms?	Aspartate, glutamate	Normal	SLC1A1 (EAAT3)	Solute carrier family 1, (dicarboxylic amino acid transporter; excitatory amino acid transporter)	*SLC1A1*
Lysinuric protein intolerance	Hyperammo-nemia after protein load, failure to thrive, renal problems, risk of alveolar proteinosis, HLH	Lysine, arginine, ornithine	Low lysine, arginine and ornithine. Increased glutamine, glycine and alanine	y^+LAT1	Solute carrier family 7 (cationic amino-acid transporter, y^+ system), member 7	*SLC7A7*
Hartnup disorder	Mostly asymptomatic; pellagra-like dermatitis, ataxia, neuropsychiat-ric symptoms, intellectual disability in some patients	Neutral amino acids	Low or low normal neutral amino acid	$b^{0,+}$AT	Solute carrier family 6 (neutral amino-acid transporter), member 19	*SLC6A19*
Hartnup like disorder	Pellagra-like dermatitis, ataxia, neuropsychiat-ric symptoms, intellectual disability	Neutral and acidic amino acids	Normal	Transport and activation of $b^{0,+}$AT	Collectrin	*CLTRN*
Brain neutral AA transporter defect	Neurodevel-opment disorder (autism)	No amino aciduria	Normal	Brain neutral AA trans-porter	Solute carrier family 7, member 5	*SLC7A5*

Modified from [62]

zygous mutation has been identified [7]. The mutation spectrum is broad and varies widely between different ethnic groups. No genotype-phenotype correlation has been observed [8].

Homozygous contiguous gene deletions on chromosome 2p21 lead to three syndromes all presenting with cystinuria but otherwise distinct phenotypes. Homozygous deletions involving *SLC3A1* and the adjacent *PREPL* cause the hypotonia-cystinuria syndrome (HCS) that mimics the Prader-Willi syndrome with hypotonia, feeding difficulties and growth hormone deficiency in infancy and hyperphagia and obesity later in childhood [9]. Larger homozygous deletions in this region result in more severe neurological phenotypes, atypical HCS and the 2p21 deletion syndrome [10, 11]. Recessive contiguous gene deletions in chromosome 2p16 lead to a combination of cystinuria and mitochondrial disease [12].

25.1.4 Diagnostic Tests

Cystine stones are usually radio-opaque and also visible on ultrasonography. Hexagonal cystine crystals in urine analysis are pathognomonic for cystinuria. Positive nitroprusside test and analysis of urinary amino acids lead to the diagnosis. Daily urinary cystine excretion is generally more than 400 mg (1.7 mmol)/1.73 m^2/d while the normal amount is less than 50–60 mg (0.26 mmol)/1.73 m^2/d. However, the excretion of cystine varies markedly. Proper alkalinisation of the urine sample after voiding is a prerequisite for correct results. Plasma concentrations of cystine and the dibasic amino acids are normal or slightly decreased [13].

Chemical analysis of the stones alone may be misleading, because mixed stones are not uncommon in cystinuria, and some stones may contain no cystine at all. Interestingly, a hyperechogenic colon, due to accumulated cysteine crystals, is often observed in antenatal echography in babies with cystinuria [14], thus leading to a prenatal diagnosis [15]. The diagnosis may be confirmed by molecular genetic testing. Depending on the ethnic origin, specific mutations may be targeted. Alternatively, NGS sequencing of both causative genes can be applied, keeping in mind that large deletions or duplications will not be identified with this method. In case of atypical phenotypes, chromosomal microarray should therefore be performed to detect contiguous gene deletions and other large genomic inbalances.

25.1.5 Treatment and Prognosis

Increased fluid intake to dilute the urine and alkalinisation to improve cystine solubility are the cornerstones of therapy. Moderate sodium restriction is recommended to reduce cystine excretion. Adults should drink 3000–4000 ml/24 h (1.75–2 l/m^2/24 h), 500 ml of this before bedtime and, if possible, 500 ml during the night to dilute urinary cystine concentration below saturation (less than 243 mg/L or 1 mmol/L). The amount of fluids should be further increased in warmer temperatures or with exercise. Permanent alkalinisation of the urine with a goal of a pH over 7.0 is best achieved by potassium citrate (2–4 mEq/kg/day, adults 30–90 mEq/day, increased if necessary on the basis of urinary pH monitoring). It may be helpful to avoid excessive dietary animal protein [16].

If the standard therapy fails to prevent new or dissolve pre-existing stones, a thiol derivative is added to decrease urinary free cystine concentration. The thiol drugs split the cystine molecule into two cysteines that form highly soluble drug-cysteine disulfide compounds that are then excreted in the urine. D-Penicillamine (30 mg/kg/day up to 3–4 g divided in 3–4 doses) has a large number of side effects including hypersensitivity reactions, bone marrow suppression, liver and kidney problems, trace metal deficiencies and disturbances in taste. In children, an initial dose of 5 mg/kg/day for 1 week and gradual increase up to 20–40 mg/kg/day has been proposed. The better tolerated mercaptopropionylglycine (tiopronin) may be the first option. The dose (10–20 mg/kg/day up to 1000 mg/day in three doses) [17] should be increased gradually and adjusted individually. Captopril, while not as effective, is less toxic, and may have a role as an alternative therapy. Alkaline pH of the urine enhances the efficacy of the thiols. To date, none of the therapeutic agents is preferred over another in efficacy [18], but penicillamine and tiopronin tend to have frequent side effects [19]. Liver enzymes, complete blood count, zinc and copper levels and urinary protein excretion should be monitored. Future therapeutic approaches may include crystal growth inhibition by cystine analogs [3, 4, 7, 13, 20]. Effect of nutritional supplement, alfa-lipoic acid, has been studied in animal models [21].

Percutaneous nephrolithotomy and extracorporeal shock-wave lithotripsy are seldom effective in stone removal, because cystine stones are extremely hard. New, minimally invasive urological techniques minimize the need for open surgery [22, 23]. Surgical procedures should always be combined with preventive therapy.

Regular follow-up is mandatory to support compliance, to monitor renal function and to detect developing stones early. Determination of cystine crystal volume in morning urine in addition to urinary pH and specific gravity [24] or direct assessment of urinary supersaturation [25] may prove helpful. Approx. 50% of adult patients have hypertension [26]. Early detection of the disease by screening the family members of a patient is also essential.

25.2 Asymptomatic Amino Acidurias: Iminoglycinuria and Dicarboxylic Amino Aciduria

Urine screening programmes have detected asymptomatic patients with iminoglycinuria and dicarboxylic amino aciduria. In iminoglycinuria, the excretion of glycine, proline and hydroxyproline is increased. As iminoglycinuria is normal in newborns, probably reflecting renal immaturity, the finding needs to be confirmed later in life.

The incidence of iminoglycinuria is 1 in 10,000. It is inherited in an autosomal recessive mode. Interestingly, the parents of the homozygous individuals (obligate heterozygotes) show glycinuria only. The molecular cause is not known, but candidate genes include *SLC36A1* and *SLC6A20*, encoding a proton-dependent amino acid transporter, PAT-1, and a sodium-dependent iminoacid transporter, SIT-1, respectively, and *SLC6A19* encoding the transporter $b^{0,+}$AT. SIT-1 transporter is not expressed in the small intestine of human newborns [27]. Although iminoglycinuria has occasionally been linked to other diseases in case reports, the present view is that it does not lead to clinical symptoms in spite of constant urinary loss of the three amino acids [28, 29].

Dicarboxylic amino aciduria i.e. excess urinary excretion of the acidic amino acids (aspartate and glutamate), has also been considered to be a largely asymptomatic condition but has recently been linked in the pathogenesis of some neuropsychiatric disorders. The estimated incidence of dicarboxylic amino aciduria is 1 in 36,000. It is caused by loss-of-function mutations in *SLC1A1* (EAAT3) encoding the glutamate transporter, which is also expressed in neurons [30].

25.3 Lysinuric Protein Intolerance

25.3.1 Clinical Presentation

The natural history of LPI still remains to be fully characterized, as only few of the oldest patients have reached the age of 50 years. Breast-fed newborns and infants are usually asymptomatic. Postprandial episodes of hyperammonaemia usually emerge when formula with higher protein content or supplementary high-protein foods are introduced [31]. Hyperammonaemia may present as refusal to eat, vomiting, stupor and drowsiness leading to coma, and can be misdiagnosed as food protein-induced enterocolitis syndrome. In that setting, forced tube feeding may be fatal. Strong aversion to high-protein foods with failure to thrive usually develops around the age of 1 year. The liver and spleen may be moderately enlarged.

In toddlers and school-age children, the presenting signs are most often growth failure and hepatosplenomegaly. The children are usually hypotonic with poor muscular strength. They show easy bruising and may have fractures after minor traumas. Neurological development is normal if severe or prolonged hyperammonaemia has been avoided. Bone maturation is retarded, and there is often pubertal delay.

The clinical heterogeneity of LPI is obvious in adult patients. Some are of moderately short stature, with abundant subcutaneous fat on a square trunk and thin extremities. They may have marked hepatomegaly with or without splenomegaly. Two thirds have exhibit osteopenia [32], but pathological fractures seldom occur in appropriately treated patients. Radiological signs of pulmonary fibrosis are common, but few patients suffer from symptomatic interstitial lung disease [33]. Mental capacity varies from normal to moderate impairment depending on previous history of hyperammonaemia.

Some patients have mild normochromic or hypochromic anaemia, leukopenia and thrombocytopenia, and their reticulocyte count is often slightly elevated. Serum ferritin, zinc and lactate dehydrogenase values are constantly elevated, while serum iron and transferrin concentrations are usually normal. Most adult patients have combined hyperlipidaemia [34]. High serum immunoglobulin-G concentrations and abnormalities in the distribution of lymphocyte subpopulations as well as in humoral immune responses [35] have been reported. Varicella infections are usually severe. Several cases of systemic lupus erythematosus have been reported. Bone marrow involvement with haemophagocytic lymphohistiocytosis (HLH) and interstitial pulmonary disease with alveolar proteinosis are quite frequent complications, and can occasionally be presenting signs. While most if not all LPI patients exhibit biological features of HLH (hypertriglyceridemia, hypofibrinogenemia and other coagulation alterations, hyperferritinemia, hypoalbuminemia, and elevated serum transaminases and LDH), a few develop a true clinical HLH syndrome with fever, hepatosplenomegaly and haemophagocytosis in bone marrow, spleen or lymph nodes [36–38]. Defective pri-

mary hemostasis, coagulopathy and clearly elevated D-dimer and plasmin-α2-antiplasmin complex levels are common in LPI, especially in patients with renal involvement [39].

Disturbed proximal tubular function with mild proteinuria, glucosuria, phosphaturia, tubular acidosis and microscopic haematuria may appear in childhood and often progress to glomerular dysfunction and end-stage renal failure [40]. Systematic screening has revealed renal dysfunction of variable degree in the majority of adult Finnish patients, with rapid deterioration in glomerular filtration in some cases [41]. Renal biopsies show variable histological lesions [42]. Urine β2-microglobulin seems to be a sensitive early marker of renal tubular involvement in LPI [43].

A few children and adults have died after a very uniform course of progressive multiorgan failure, often starting with interstitial lung involvement and alveolar proteinosis, progressive glomerulonephritis that leads to renal insufficiency, and a severe bleeding diathesis [38]. In a French cohort, lung involvement has been observed in as many as 71% of LPI children, often with dismal prognosis [44]. One child with alveolar proteinosis went through an initially successful heart-lung transplantation, but died later after a recurrent disease [45].

Pregnancies of patients with LPI have been complicated by toxaemia, anaemia or bleeding during delivery and variable degrees of intrauterine growth retardation, but many have been completed successfully without any major problems [46].

25.3.2 Metabolic Derangement

In LPI, transport of the dibasic cationic amino acids lysine, arginine and ornithine (system y^+L; ◘ Fig. 25.1) is defective at the basolateral membrane of epithelial cells in the renal tubules and small intestine [47], where y^+LAT1 combines with 4F2hc to generate an active amino acid transporter [48].

Massive amounts of lysine and more moderate amounts of arginine and ornithine are lost in the urine, and their intestinal absorption is limited, resulting in low plasma concentrations. Glutamine, glycine and alanine concentrations are often clearly elevated owing to malfunction of the urea cycle. It is still unclear whether the transport defect is also expressed in non-epithelial cells. Contrary to an earlier report [49], more recent data indicate that fibroblasts and erythrocytes from LPI patients have normal cationic amino acid transport, probably via other transporter isoforms [50, 51]. A transport defect in hepatocytes has been postulated because of normal or even paradoxically elevated cationic amino acid concentrations in liver biopsy in LPI, and abnormal cationic amino acid transport between various intracellular compartments has been suggested [52].

Hyperammonaemia after protein ingestion and diminished protein tolerance in LPI resemble the symptoms of urea cycle enzyme deficiencies (▶ Chap. 19). This is best explained by functional deficiency of the intermediates arginine and ornithine in the hepatocytes [52]. Most patients develop a protective aversion to high-protein foods, which further impairs their amino acid intake, aggravating the amino acid deficiencies. As arginine is the rate-limiting precursor of nitric oxide synthesis, extracellular arginine deficiency may also result in persistently low nitric oxide concentrations that may influence vascular and immunological functions [53]. Reduced availability of lysine, an essential amino acid, probably has a prominent role in the poor growth and skeletal and immunological manifestations in LPI. Occasional patients have exhibited severe carnitine deficiency [54] that may be of dietary origin: the principal dietary source of carnitine is red meat, which is consumed in very small amounts by most patients with LPI. Chronic lysine deficiency may also limit endogenous carnitine biosynthesis.

The pathogenic mechanisms of several clinical manifestations of LPI are still poorly known. Macrophages along with altered regulation of nitric oxide synthesis probably play a central role in the development of alveolar proteinosis and nephropathy: LPI macrophages secrete less nitric oxide than control macrophages while several inflammatory chemokines are elevated [55]. Impaired phagocytic function [56] and abnormal inflammatory and immune responses may contribute to lung injury [44]. Conversely, intracellular nitric oxide accumulation secondary to intracellular arginine trapping might also explain some LPI complications [57]; e.g. intracellular nitric oxide excess in kidney cells might contribute to kidney damage. However, a recent study showed that a significant induction of inflammatory mediators (IL1β, TNFα) was observed in *SLC7A7* silenced lung epithelial cells regardless of intracellular arginine availability [58]. The role of HLH is unclear. High lysine concentration in proximal tubular cells in LPI may induce ROS and NADPH oxidase generation and thus make the tubular cells susceptible to apoptosis [59], potentially contributing to the proximal tubular dysfunction.

25.3.3 Genetics

LPI is a rare autosomal-recessive disease, with only few hundred patients reported worldwide, over 50 of them from Finland. The incidence is highest in Finland (1 in 60,000); clusters of families are also known at least in Italy, Norway and Japan, and sporadic cases have been

reported on all continents. *SLC7A7* encodes the light subunit of the dibasic amino acid transporter y^+LAT-1. Nearly 70 different mutations spread along the entire gene have been reported [60–62]. The majority of them are missense and nonsense variants, but deletions, insertions, splicing variants and large genomic rearrangements have been described as well. One case of a homozygous LPI mutation associated with maternal uniparental isodisomy of chromosome 14 has been reported [63].

All Finnish patients except one are homozygous for the Finnish founder mutation, 1181-2A > T, which causes a frame shift leading to a premature stop codon. However, the phenotypic variability is wide even within this genetically homogeneous patient group and no genotype/phenotype correlation in LPI has been established.

25.3.4 Diagnostic Tests

The diagnosis LPI is based on the combination of increased urinary excretion and low plasma concentrations of the cationic amino acids, especially lysine. The concentrations of plasma lysine, arginine and ornithine are usually less than 80 μmol/l, 40 μmol/l, and 30 μmol/l, respectively. If plasma amino acid concentrations are remarkably low owing to very limited protein intake, urinary cationic amino acid excretion may on rare occasions be within the reference range.

Blood ammonia concentration increases after protein-rich meals. Postprandial orotic aciduria is practically always seen in untreated patients (see hyperammonaemia algorithm in ▶ Chap. 19). Nonspecific but almost constant findings include elevated serum lactate dehydrogenase activity and increased ferritin and triglyceride concentrations due to secondary HLH (see above). Also serum zinc is often elevated.

In the genetically homogenous Finnish population, the diagnosis is easily confirmed by founder mutation analysis. In all other patients molecular analysis of the entire *SLC7A7* gene, possibly as a part of a targeted gene panel or exome / whole-genome sequencing, is necessary to confirm the diagnosis whenever the biochemical data are unclear or if the clinical presentation is that of isolated HLH or alveolar proteinosis that might be erroneously ascribed to other etiologies. It has to be acknowledged that large deletions and duplications cannot be excluded by next generation sequencing, and other methods such as multiplex ligation–dependent probe amplification (MLPA) or chromosomal microarray need to be applied.

25.3.5 Treatment and Prognosis

The principal aims of the treatment are to prevent hyperammonaemia and to provide a sufficient supply of protein and essential amino acids for normal metabolism and growth. Protein tolerance in LPI can be improved with supplementary low-dose citrulline, a neutral amino acid that is also an intermediate of the urea cycle. Citrulline is readily absorbed and partially converted to arginine and ornithine, all of which improve the function of the urea cycle. Approximately 50–100 mg/kg/day of L-citrulline is given in three to five doses in association with protein-containing meals [64], with a target of maintaining plasma citrulline within the high-normal range. On such a regimen, children usually tolerate 1.0–1.5 and adults 0.5–0.8 g/kg/day of natural protein [65]. There is marked interindividual variation in protein tolerance, and infections, pregnancy and lactation may alter it extensively. Frequent monitoring of urinary orotic acid excretion is necessary. In patients with constantly highly elevated glutamine and glycine levels, sodium benzoate or sodium or glycerol phenylbutyrate (both up to 250 mg/kg/day or 13 g/m^2/day) help to reduce the nitrogen load.

A carefully titrated dose of l-lysine-HCl (20–30 mg/kg/day in three doses) is able to elevate the plasma lysine concentrations to low-normal range without side effects.

Carnitine supplementation is indicated for the patients with carnitine deficiency [66]. Owing to their restricted diet, all patients need regular supplementation with calcium, vitamins and trace elements, and the involvement of an experienced nutritionist is essential. Growth hormone therapy has been used in several children with growth retardation, with a good response and no side effects [67]. Hypercholesterolemia has successfully been treated with statins, and high triglyceride levels may also need dietary and/or pharmacological treatment [34].

The rare cases of acute hyperammonaemia in LPI patients should be treated as in other urea cycle defects (▶ Chap. 19).

LPI patients should be immunised against pneumococci and varicella zoster, and non-immunised patients should be treated immediately with acyclovir if they get varicella infection [35]. Immunization against seasonal influenza is also recommended. The treatment of the immunological and bone marrow complications, including clinical HLH, is still experimental. Good responses have been reported in individual cases with immunosuppressive drugs and with immunoglobulin infusion [68]. In alveolar proteinosis, bronchoalveolar lavage and steroid therapy have been effective in some cases [37].

Granulocyte-macrophage colony-stimulating factor therapy in LPI proteinosis has thus far given contradictory results [69–71]. Heart-lung transplantation is probably contraindicated due to the risk of a relapse of proteinosis in the graft.

Although hyperammonaemia and the associated mental retardation can be avoided with citrulline treatment, renal and other complications of LPI develop and progress during current therapy. Too large dosages of citrulline (>100 mg/kg/day) may increase the intracellular synthesis of arginine and may further stimulate the immune cascade in tubular, glomerular and mesangial cells in the kidney, in alveolar macrophages and epithelial cells in the lung, and in reticular endothelial cells, with overt clinical complications [57].

25.4 Hartnup Disease

25.4.1 Clinical Presentation

The classical symptoms of Hartnup disease, pellagra-like dermatitis, intermittent ataxia and neuropsychiatric abnormalities, closely resemble those of nutritional niacin (nicotinic acid and nicotinamide) deficiency. Since the first description of the syndrome in several members of the Hartnup family in 1956 [72], an extensive number of subjects who fulfil the biochemical diagnostic criteria have been reported, mostly detected in newborn screening programmes. However, most of them remain asymptomatic.

In the few patients who develop clinical symptoms, the skin lesions and neurological problems usually appear in early childhood [73] and tend to ameliorate with age. Exposure to sunlight, fever, diarrhoea, inadequate diet or psychological stress may precipitate the symptoms. Pellagra-like skin changes are found on light-exposed areas. Eruptions may mimic those seen in zinc deficiency, and the rare combination of coeliac disease and Hartnup disorder has led to severe skin problems [74], intermittent cerebellar ataxia, attacks of headache, muscle pain and weakness may appear. Occasionally, patients present with mental retardation, seizures or psychosis-like symptoms [75]. Maternal Hartnup disorder seems to be harmless to the foetus [76].

25.4.2 Metabolic Derangement

The molecular defect involves a sodium-dependent and chloride-independent neutral amino acid transporter, $b^{0,+}$AT (*SLC6A19*) in the apical brush border membrane of renal proximal tubule and intestinal epithelium [28, 77]. Mutations in *SLC6A19* impair intestinal uptake and tubular reabsorption of all the neutral amino acids, i.e. alanine, serine, threonine, valine, leucine, isoleucine, phenylalanine, tyrosine, tryptophan, histidine and citrulline and the monoamino-dicarboxylic amides asparagine and glutamine. The transporter is associated with partner proteins that are necessary for its expression, collectrin (Tmem27) in the kidney and angiotensin-converting enzyme 2 (ACE2) in the intestine, both components of the renin angiotensin system [78].

The affected amino acids are readily absorbed in the intestine as short oligopeptides but not as free amino acids. They are excreted in 5- to 20-fold excess into the urine, leading to decreased or low normal plasma concentrations. The stools of patients contain increased amounts of free amino acids, closely reflecting the urinary excretion pattern [28, 77]. The unabsorbed amino acids in the colon are exposed to bacterial degradation. Degradation of tryptophan produces large amounts of indole compounds, which are then excreted in the urine.

Systemic tryptophan deficiency plays a crucial role in the development of clinical symptoms such as neuropsychiatric signs since tryptophan is the precursor of the neurotransmitter serotonin. Most importantly, tryptophan deficiency leads to reduced availability of nicotinic acid, the precursor of NAD(P)H. Subsequent deficiency of nicotinic acid (or niacin) and its amide, nicotinamide, may explain the skin abnormalities resembling pellagra that is due to nutritional niacin deficiency. The wide phenotypic variability of Hartnup disorder may be explained by nutritional factors and genetic differences owing to the high frequency of compound heterozygotes. Tissue specific partner proteins may also play a role.

25.4.3 Genetics

The reported incidence of Hartnup disorder in newborns screened for amino aciduria varies from 1 in 14,000 to 1 in 45,000. Hartnup disease is caused by mutations in *SLC6A19* and follows an autosomal recessive pattern of inheritance. At least 21 mutations have been identified, including missense and splicing as well as small deletions and insertions. The most common allele D173N does not completely inactivate the transport mechanism. Most patients are compound heterozygotes [79–81].

25.4.4 Diagnostic Tests

The characteristic excess of neutral amino acids in the urine and their normal or low-normal concentrations in plasma confirm the diagnosis. Urinary excretion of indole compounds may be within the normal range if

the patient consumes normal or low amounts of dietary protein, but an oral load of L-tryptophan (100 mg/kg) in most cases leads to a supranormal increase in indole excretion. Genetic testing is available.

25.4.5 Treatment and Prognosis

Clinical symptoms may be prevented by sufficient dietary intake of niacin or adequate supply of high-quality protein that allows the necessary amount of tryptophan to be absorbed in oligopeptide form. Dermatitis and neurological symptoms usually but not invariably disappear rapidly with oral nicotinamide (50–300 mg/day). Tryptophan ethyl ester has been successfully used to circumvent the transport defect. Oral neomycin reduces intestinal degradation of tryptophan and decreases indole production; however, the role of the indole compounds in the disease has been poorly characterized [1, 28]. Early recognition of the condition in newborn screening programmes permits adequate follow-up and prevention of symptomatic disease.

25.5 Collectrin Deficiency

The protein collectrin (encoded by *CLTRN*) functions in the transport and activation of $b^{0,+}$AT in the renal apical brush border epithelium. Recently two patients were reported with hemizygous *CLTRN* deletions, both of whom presented with neuropsychiatric phenotypes including autistic features, anxiety, and motor tics. They had normal plasma amino acids but neutral amino aciduria led to a clinical diagnosis of Hartnup disease and treatment with niacin. Collectrin deficiency in humans can be associated with amino aciduria and a clinical picture similar to that seen in Hartnup disease but further studies are needed to explore its role in the neurological phenotypes [82].

25.6 SLC7A5/Brain Neutral Amino Acid Transporter Deficiency

SLC7A5, a neutral amino acid transporter localized at the blood brain barrier, has an essential role in maintaining normal levels of brain BCAAs. In mice, deletion of SLC7A5 from the endothelial cells of the blood brain barrier leads to an atypical brain amino acid profile and neurological abnormalities. Several patients with autistic symptoms and motor delay carrying homozygous mutations in *SLC7A5* have been reported [83]. A recent study reported *SLC7A5* heterozygous variants in a small subset of autistic patients [84]. The response to BCAA supplementation needs further study. Furthermore, overexpression of *SLC7A5* has been observed in a wide range of tumour cells, and its potential as a target for anti-cancer drugs is under active investigation [85].

25.7 SLC7A8/LAT2 Neutral Amino Acid Transporter Deficiency

Recently, a deletion in neutral amino acid transporter LAT2 (SLC7A8) has been linked with increased incidence of cataracts in mice, and also a homozygous single nucleotide deletion has been found in a family with congenital cataracts [86]. The contribution of LAT2 variants to the pathology of cataracts needs to be further investigated.

25.8 SLC6A6/Taurine Transporter Deficiency

Recently, retinal degeneration and cardiomyopathy have been reported in a consanguineous family with SLC6A6 taurine transporter deficiency and almost undetectable plasma taurine levels. Oral taurine supplementation at 100 mg/kg/day led to normal blood taurine levels. After 24-months, the cardiomyopathy was corrected in both affected siblings and the retinal degeneration was arrested in the 6 years old, with clinically improved vision [87].

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Cystinosis

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Contents

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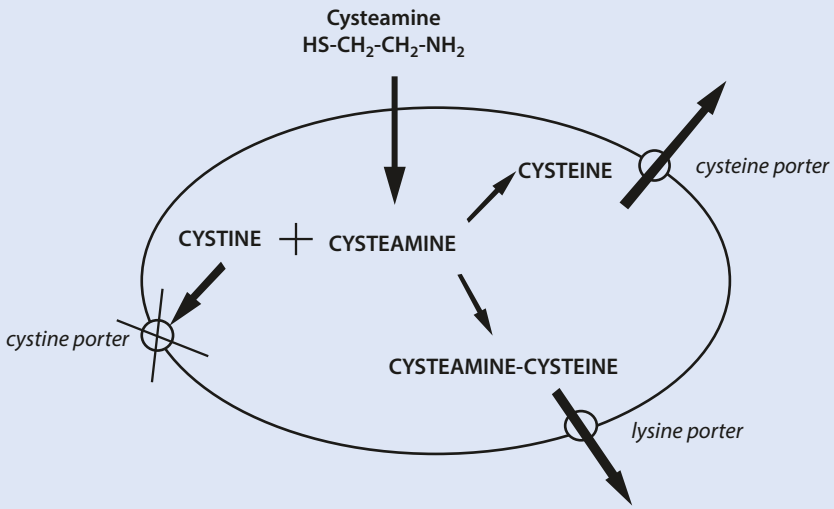

Fig. 26.1 Lysosomal export of cystine and related compounds. The cross represents the defect in cystinosis

Lysosomal Porters for Cystine and Related Compounds

Intralysosomal cystine is formed by protein catabolism in the organelle, and is normally exported by a cystine porter (Fig. 26.1) which contains a membrane protein, *cystinosin*. Defects of this protein cause lysosomal accumulation of cystine. Cysteamine can enter into the lysosome and combine with half-cystine (i.e., cysteine). This results in the formation of cysteine (which can be exported by the cysteine porter), and of mixed disulfide, cysteine-cysteamine (which can be exported by the lysine porter due to its structural analogy).

Introduction

Cystinosis is an autosomal-recessive generalized lysosomal storage disease classified into three clinical phenotypes, of which the nephropathic or infantile form is by far the most frequent. The reported incidence of the disease is about 0.5–1:100,000 live births. The first symptoms start at about 6 months of age with anorexia, polyuria, failure to thrive and are secondary to a proximal renal tubulopathy (renal Fanconi syndrome). In the absence of specific therapy, the renal disease progress to end stage renal failure between 6 and 12 years. Survival beyond this age is associated with the development of extrarenal complications in eyes, thyroid, gonads, endocrine pancreas, muscle and central nervous system. A late-onset or juvenile form, and a benign or adult form limited to the eyes, are caused by pathogenic variants of the same gene. The lysosomal cystine accumulation leads to cellular dysfunction in many organs. The disease is caused by pathogenic variants in *CTNS* coding for cystinosin, a lysosomal cystine/proton symporter. The diagnosis is ascertained by measurement of the cystine content in leukocytes. Treatment is both supportive and specific, the latter based on cysteamine, which effectively decreases intralysosomal cystine accumulation.

26.1 Infantile Cystinosis

26.1.1 Clinical Presentation

First Stage

The first 3–6 months of life are usually symptom-free. The first symptoms develop before 12 months of age [1]. They include feeding difficulties, anorexia, vomiting, polyuria, constipation and failure to thrive. If the diagnosis is delayed, severe rickets develops after 10–18 months in spite of correct vitamin D supplementation. A polyuria of 2–5 L/day develops rapidly due to a severe urinary concentrating defect. Urine from cystinotic patients is characteristic, being pale and cloudy, and the diagnosis can be immediately suspected if both glucose and protein are found. When the disease has become symptomatic, the full expression of the renal Fanconi syndrome is generally present at first examination. It includes normoglycemic glycosuria, generalized

aminoaciduria, low molecular-weight proteinuria (with massive excretion of β2-microglobulin and lysozyme), decreased reabsorption of phosphate with hypophosphatemia, excessive losses of potassium, sodium and bicarbonate leading to hypokalemia, hyponatremia and metabolic acidosis. Hypercalciuria may lead to nephrocalcinosis [2] and hypouricemia is constant. Tubular loss of carnitine may cause carnitine depletion. Kidney biopsy shows tubular abnormalities and some cystine crystals; plurinucleated glomerular podocytes are also characteristic.

The general reabsorptive defect of the proximal tubule explains the severe hydroelectrolyte imbalance, which may be life-threatening. Episodes of fever, probably related to dehydration, are also commonly noted.

Involvement of the eye is an early symptom of cystinosis, starting with photophobia, which usually appears at 2 or 3 years of age [3]. Ophthalmological examination with a slit lamp and a biomicroscope reveals cystine crystal deposits after the first year of life. Confocal microscopy and optical coherence tomography allows a quantitative measurement of the deposits [4].

End Stage Kidney Disease

The natural history of the disease includes severe stunting of growth and a progressive decrease of the glomerular filtration rate, leading to end stage kidney disease (ESKD) between 6 and 12 years of age. Progression to renal failure may be delayed by cysteamine treatment especially when started within the first months of life. This treatment also improves growth velocity. The decrease in glomerular filtration is accompanied by an improvement of urinary losses and a spurious regression of the renal Fanconi syndrome. At this stage, severe renal hypertension may develop. After kidney transplantation, there is no recurrence of the renal Fanconi syndrome even if cystine crystals develop in the recipient mononuclear cells that are present in the graft.

Late Symptoms

The success of renal replacement therapy and renal transplantation has revealed the consequences of long-term cystine accumulation in various organs and has emphasized the multisystemic nature of cystinosis, which may involve the eyes, thyroid, liver, spleen, pancreas muscle and central nervous system (CNS) [5].

Ocular Complications

Crystal deposition in the cornea and the conjunctiva increases with age and gradually leads to photophobia, watering, blepharospasm, superficial punctate keratopathy and recurrent corneal erosions [6]. In older patients, filamentous keratopathy, band keratopathy and peripheral corneal neovascularization are also observed. Vision may be affected by retinopathy and glaucoma [7].

Endocrine Disturbances

Hypothyroidism

Thyroid dysfunction usually appears between 8 and 12 years of age. It is rarely overt with clinical symptoms, but rather discovered by systematic assessment of thyroid function showing elevated TSH but normal T3 and T4 plasma concentrations [8]. Cysteamine was reported to delay or prevent thyroid dysfunction [9].

Gonadal function

Male hypogonadism with delayed and incomplete puberty is observed in most patients with cystinosis [10]. Male patients are generally azoospermic but the presence of spermatogenesis in testicular biopsy may allow in vitro fertilization and a successful conception [11, 12]. Female patients exhibit pubertal delay but seem to have normal gonadal function and there are several reports of successful pregnancies [13].

Endocrine pancreas

Insulin-dependent diabetes has been reported in cystinotic patients in the second or third decade of life, usually after renal transplantation. A progressive loss of insulin production and C-peptide production leads to glucose intolerance in 50% of patients by the age of 18 years [14].

Liver and Spleen Involvement

Hepatomegaly and splenomegaly occur after 15 years of age in one-third to half of the patients who do not receive cysteamine [15]. This enlargement may be the cause of portal hypertension with gastroesophageal varices. Hematological symptoms of hypersplenism may be noted. Cysteamine prevents this type of complications.

Muscle

A myopathy, potentially leading to a severe handicap, has been reported in some patients with generalized muscle atrophy and weakness, mainly of distal muscles of all limbs [16, 17]. Pharyngeal and oral dysfunction, which may also cause voice changes, is often observed [18, 19]. Swallowing dysfunction is inversely related with the duration of cysteamine treatment [20, 21]. Pulmonary dysfunction is correlated with the severity of myopathy [22, 23]. Nocturnal non-invasive positive pressure constitutes an effective treatment [24].

Central Nervous System

Cystinosis does not affect general intellectual performances but may be associated with mild neurocognitive abnormalities [25]. A subtle visuoperceptual defect and lower cognitive performances with impairment of visual memory and tactile recognition have been reported [26]

as well as social difficulties [27]. These anomalies may appear as early as 3–7 years of age favoring the hypothesis of the direct role of the gene defect rather than cystine accumulation [28]. More severe neurological complications may develop in the long-term with pyramidal and cerebellar symptoms, progressive intellectual deterioration leading to a pseudo-bulbar syndrome [29, 30]. This cystinotic encephalopathy has only been observed above 19 years of age. Idiopathic intracranial hypertension has been reported [31, 32]. Acute ischemic episodes may occur with hemiplegia or aphasia. Brain atrophy, calcifications and abnormal features of white matter on magnetic resonance imaging (MRI) examination may be observed.

- **Bone Anomalies**

The renal Fanconi syndrome leads to hypophosphatemia and reduced synthesis of calcitriol resulting in rickets. Later in life, chronic kidney disease results in renal osteodystrophy with bone deformities (genu valgum, scoliosis), osteomalacia, osteoporosis and fractures. Despite correct vitamin D supplements and cysteamine treatment, some patients develop a severe bone disease, called "cystinosis metabolic bone disease". The pathophysiology is not clear and may also involve copper deficiency, iatrogenic effects of cysteamine and a direct effect of cystinosin mutations on osteoblasts and osteoclasts [33, 34].

26.1.2 Metabolic Derangement

Efflux of cystine out of cystinotic lysosomes is significantly decreased in comparison with normal lysosomes [35]. Consequently, cystine accumulates in many tissues including kidney, bone marrow, conjunctiva, thyroid, muscle, choroid plexus, brain parenchyma and lymph nodes. This abnormality is related to a molecular defect of cystinosin, the protein that transports cystine across the lysosomal membrane. The function of this carrier molecule was demonstrated in a cellular model where the lysosomal targeting signal directing cystinosin to the plasma membrane is defective [36]. Cystine transport out of the lysosomes is H^+ driven. Why lysosomal cystine accumulation leads to cellular dysfunction is not clear. It has been shown that cystine accumulation in proximal tubular cells in vitro is associated with ATP depletion and inhibition of Na^+ dependent transporters [37, 38]. Cystinosin knockout mice show decreased expression of megalin, cubilin and sodium transporters at the apical surface of proximal tubular cells [39]. Cystine accumulation favors oxidative stress, apoptosis, mitochondrial dysfunction and inflammation [40, 41]. Alteration of mTOR signaling may contribute to the proximal tubular dysfunction observed early in life [42].

26.1.3 Genetics

Nephropathic cystinosis is an autosomal recessive disorder. The gene, *CTNS*, encodes a protein of 367 amino acids which has the structure of an integral membrane protein with 7 membrane spanning domains and two lysosomal targeting signals [43]. More than 120 pathogenic variants in the first 10 exons and in the promotor of the gene have been identified in association with cystinosis [1]. The most common is a 57 kb deletion found in 76% of patients of European descent. This deletion encompasses *CARKL*, encoding the enzyme sedoheptulokinase (▶ Chap. 7). This explains why patients with homozygous 57 kb deletion have elevated urinary concentrations of sedoheptulose [44]. In the other cases, shorter deletions, point mutations, small insertions, duplications, non-sense or splice-site mutations of *CNTS* are found on both alleles, some of them clustering in certain ethnic and/or geographical areas [45].

Intermediate and adult forms have the same mode of inheritance with variants that do not disrupt the open reading frame and are generally found in the intertransmembrane loops or in the N-terminal region.

26.1.4 Diagnostic Tests

The diagnosis of cystinosis is confirmed by the measurement of leukocyte cystine content, the demonstration of corneal crystals by the slit lamp examination and genetic analysis of *CNTS*. In patients with nephropathic cystinosis, the free cystine content in leukocytes is about 10–50 times normal values as measured by high-performance liquid chromatography (HPLC) or liquid chromatography-tandem mass spectrometry (LC-MS/MS), preferably on granulocytes [46–48]. In cystinosis, the level is usually 5 to 15 nmol of 1/2 cystine/mg protein. The technique enables detection of heterozygous carriers with levels of 0.5 to 1.4 nmol 1/2 cystine/mg protein. In control subjects, cystine is undetectable or <0.4. The results obtained on polymorphonuclear leukocytes are approximately twice those obtained on mixed leukocytes and this must be taken in consideration when comparing data. Prenatal diagnosis can be made by the measurement of cystine by HPLC or LC-MS/MS on chorionic villous biopsy or cultured amniotic cells [49]. The diagnosis can also be made by molecular analysis if both pathogenic variants have been identified in an affected sibling. FISH diagnosis of

the 57 Kb deletion is possible [50]. At birth, diagnosis is possible on placenta or cord blood white cells.

26.1.5 Treatment

The therapy of nephropathic cystinosis is both supportive and specific.

Supportive Treatment of Tubular Losses

Several abnormalities have to be corrected:

Water

The water intake must be adjusted to the level of diuresis, short-term weight variation and, if necessary, plasma protein concentration. Fluid requirement increases with external temperature and with fever. It is also increased by the required mineral supplements.

Acid base equilibrium

Sodium and potassium bicarbonate, which have a better gastric tolerance than citrate, must be given in order to obtain a plasma bicarbonate level between 21 and 24 mmol/l. This is sometimes difficult and may require large amounts of buffer, up to 10–15 mmol/kg/day.

Sodium

Sodium losses sometimes remain uncompensated after achieving acid base equilibrium. This is recognizable by a persistent hyponatremia with failure to thrive.

Potassium

Hypokalemia requires potassium supplements in order to maintain serum potassium above 3 mmol/l. Four to 10 mmol/kg/day are usually necessary to achieve this goal. Prescription of amiloride at a dose of 2–5 mg/day may help in some cases.

Phosphorus

Hypophosphatemia must be corrected with a supplement of sodium/potassium phosphate at a dose of 0.3–1 g/day. The aim is to obtain a plasma phosphate just above 1.0 to 1.2 mmol/l. This poorly tolerated supplement may be gradually withdrawn after some months or years. Excessive phosphorus prescription may lead to nephrocalcinosis.

Vitamin D supplementation

Since tubular 1α-hydroxylation is diminished in this disease, it is justified to give 1α- or 1α-25-OHD_3 (0.10–0.50 µg/day), especially in cases of symptomatic rickets. These prescriptions must be carefully adjusted by regular follow-up of serum calcium.

Carnitine supplementation

A dose of 100 mg/kg per day in four divided doses is proposed in order to correct muscle carnitine depletion [51].

All these supplements need to be given regularly in order to replace the losses, which are permanent. A good way to achieve this goal is to prepare in advance all the supplements, except vitamin D, in a bottle containing the usual amount of water for the day. Feeding problems may require tube or gastric button feeding and, in some cases, continuous or intermittent total parenteral nutrition [52].

- Losses of water, potassium and sodium may be reduced by the prescription of indomethacin at a dose of 1–3 mg/kg/kg in two separate doses [53]. Although there is no consensus on the use of this drug in cystinotic patients, it appears to be relatively safe and well tolerated in young patients. It should be stopped if there is dehydration or if renal function deteriorates.
- It has been shown that the angiotensin converting enzyme (ACE) inhibitors diminish albuminuria and possibly slows down the degradation of renal function [54]. They should not be prescribed in association with indomethacin, which also reduces renal perfusion.
- When the glomerular filtration rate decreases, tubular losses also decrease and the mineral supplements must be adjusted and progressively tapered off in order to avoid overload, especially with sodium and potassium. When dialysis is started, mineral supplements are usually no longer necessary.

Renal Replacement Therapy

Hemodialysis or peritoneal dialysis are both effective and applied according to the circumstances. As for any child with ESKD, kidney transplantation is considered the best approach. Long-term results of kidney transplantation are better than for any other primary renal disease in children [55, 56].

Supportive Treatment of Extrarenal Complications

Hypothyroidism, even if asymptomatic, should be treated with L-thyroxine supplementation. Growth failure, one of the most striking complications of nephropathic cystinosis, is improved by administration of recombinant growth hormone at a dose of 1 U/kg/week [57]. Hypersplenism with permanent leukopenia and/or thrombocytopenia may be an indication for splenectomy. Photophobia and watering may be improved by local symptomatic therapy such as vitamin A eye drops, artificial tears, topical lubricants, and thin bandage soft contact lenses. Corneal graft has been rarely performed, with variable results.

Specific Therapy

Cysteamine, ($HS\text{-}CH_2\text{-}NH_2$), has been shown to reduce cystine leukocyte content and slow the decline in the glomerular filtration rate [55, 58]. The dose is progressively increased from 10 to 50 mg/kg of cysteamine

base per day. Cysteamine, most widely used as cysteamine bitartrate (Cystagon), is rapidly absorbed and its maximum effect, assessed by cystine assay in leukocytes, occurs after 1–2 h, and lasts no longer than 6 h [59]. Consequently, it has to be given in 4 separate doses – one every 6 h – in order to obtain the best prevention of cystine accumulation. The target dose is 1.3 g/m^2/day of free base for children up to 12 years and 2 g/day for older patients or those weighting more than 50 kg. The aim is to keep the cystine content, determined 6 hours after the last dose, under 1 nmol of 1/2 cystine per mg of protein, although there is no definitive evidence that this leads to the optimal depletion at the tissue level. The maximum dose should not exceed 1.95 g/m^2/day. Leucocyte cystine content is the gold standard for therapeutic monitoring but the assay is not widely available. It has recently proposed that the measurement of chitotriosidase enzyme activity may be useful for therapeutic monitoring [60]. A twice daily administration of an enteric release formulation of cysteamine bitartrate has been developed and was shown to be as effective as the current formulation of cysteamine [61].

The drug should be started as soon as the diagnosis is confirmed [62]. Cysteamine has been shown to delay the progression to renal failure, prevent extra-renal manifestations and improve growth [21, 63]. However, it does not prevent the development of the renal Fanconi syndrome and has no effect on corneal deposits.

Side effects of the drug include nausea and vomiting which can be managed with omeprazole [64]. Less commonly, allergic rashes, seizures, neutropenia and angioendotheliomatosis lesions on the elbows are seen [65]. In addition, the metabolite of cysteamine, dimethyl sulfide, is responsible for an unpleasant breath smell so that compliance is difficult to maintain in the long term, especially in adolescents [66].

Eye drops containing 0.55% cysteamine may prevent corneal deposits [6], and decrease and even suppress the deposits already present. However, they have to be given 6–10 times a day, a task difficult to achieve. A 0.55% gel formulation (Cystadrops) allows a reduced number of instillations with a good efficacy [67].

A phase 1/2 open-label clinical trial using gene modified CD34$^+$ enriched autologous hematopoietic stem and progenitor cells has recently started with encouraging results [68].

26.2 Late-Onset Cystinosis

This is a rare, milder form of the disease, with later clinical onset and delayed evolution to ESKD. It represents less than 2 or 3% of cases. The first symptoms usually appear after 6–8 years of age. Proteinuria may be misleading because of its severity, sometimes in the nephrotic range. The renal Fanconi syndrome may be absent or mild and tubular losses are less important than in infantile cystinosis [69]. The same is true for extrarenal symptoms. ESKD may develop during adolescence or adult life.

The diagnosis is ascertained by the measurement of cystine in leukocytes. Genetic analysis shows homozygous or compound heterozygous *CNTS* pathogenic variants with at least one 'mild' variant [69].

26.3 Ocular Cystinosis

Ocular (or adult or benign) cystinosis was first reported by Cogan et al. in 1957 [70]. This exceptional disorder is characterized by the presence of cystine crystals in the eye and the bone marrow [71]. Crystals in the cornea are usually found by chance examination. The level of cystine in leukocytes is intermediate between that of heterozygotes and homozygotes for nephropathic cystinosis. All systemic manifestations of the other forms of cystinosis are lacking. The variants in *CTNS* found in these patients encode a protein that allows sufficient residual cystine transport.

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Biotin-Responsive Disorders

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Contents

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27

The Biotin Cycle and Biotin-Dependent Enzymes

Biotin is a water-soluble vitamin widely present in small amounts in natural foodstuffs, in which it is mostly protein bound. The classic role of biotin is to function as the coenzyme of five important carboxylases involved in gluconeogenesis, fatty acid synthesis and the catabolism of several amino acids (Fig. 27.1). Covalent binding of biotin to the inactive apocarboxylases, catalysed by *holocarboxylase synthetase* (HCS), is required to generate the active holocarboxylases (Fig. 27.2). Recycling of biotin first

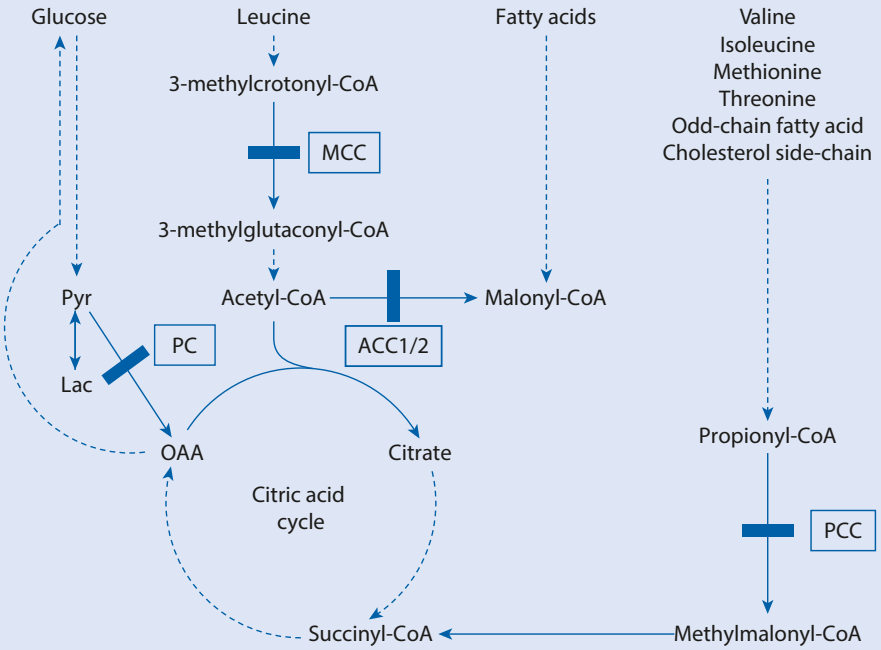

Fig. 27.1 Location of the biotin-dependent carboxylases in intermediary metabolism. ACC acetyl-CoA carboxylases (ACC-1 cytosolic, ACC-2 outer mitochondrial membrane), CoA coenzyme A, HCS holocarboxylase synthetase, LAC lactate, MCC 3-methylcrotonyl-CoA carboxylase, OAA oxaloacetate, PC pyruvate carboxylase, PCC propionyl-CoA carboxylase, PYR pyruvate. *Full lines* indicate one enzyme, and dotted lines indicate that several enzymes are involved. Sites of the enzyme defects are indicated by **solid bars**

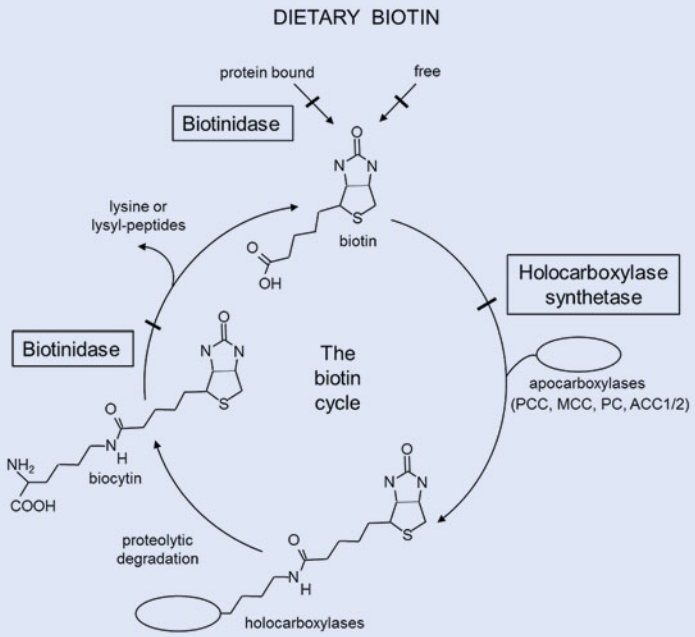

Fig. 27.2 The biotin cycle. Abbreviations as Fig. 27.1 Sites of the enzyme and transport defects are indicated by **solid bars**

involves proteolytic degradation of the holocarboxylases, yielding biotin bound to lysine (biocytin) or to short biotinyl peptides. *Biotinidase* then releases biotin from the latter compounds, which are derived from either endogenous or dietary sources. Outside of these enzymatic roles, HCS has further been implicated in cellular regulation: in the cytosol as part of a guanylate cyclase-dependent pathway to induce transcription of genes involved in biotin transport and utilization, and in the nucleus as part of a transcriptional co-regulatory complex either including or excluding its biotinyltransferase activity [1–3]. The extent to which these alternative functions of HCS may contribute to disease pathomechanisms is not well understood.

Introduction

Two inherited defects affecting the coenzyme function of biotin are known: *holocarboxylase synthetase* (HCS) *deficiency* and *biotinidase deficiency*. Both lead to deficiency of all biotin-dependent carboxylases, i.e. to *multiple carboxylase deficiency* (MCD). In HCS deficiency, the binding of biotin to apocarboxylases is impaired. In biotinidase deficiency, biotin depletion ensues from the inability to recycle endogenous biotin and to utilise protein-bound biotin from the diet. As the carboxylases play an essential role in the catabolism of several amino acids, in gluconeogenesis and in fatty-acid synthesis, their deficiency provokes multiple, life-threatening metabolic derangements, eliciting characteristic organic aciduria and neurological symptoms. The clinical presentation is extremely variable in both disorders. Characteristic symptoms include metabolic acidosis, hypotonia, seizures, ataxia, impaired consciousness and cutaneous symptoms, such as skin rash and alopecia. All patients with biotinidase and a majority of patients with HCS deficiency respond dramatically to oral therapy with pharmacological doses of biotin. Delayed diagnosis and treatment in biotinidase deficiency may result in irreversible neurological damage. A few patients with HCS deficiency show only a partial or even no response to biotin and seem to have an impaired long-term outcome. Four patients with *sodium-dependent multivitamin transporter (SMVT) deficiency*, due to mutation of *SLC5A6*, have been described. They presented in the first 2 years of life with variable symptoms including feeding difficulties and developmental delay that partially resolved upon treatment with biotin and pantothenic acid +/− lipoic acid. A further biotin-regulated gene is *SLC19A3* encoding the thiamine transporter hTHTR2. *SLC19A3* mutations cause biotin-responsive basal ganglia disease [4] (▶ Chap. 29). *Acquired biotin deficiency,* which also causes MCD, is extremely rare. Ultra-high dose biotin therapy has recently been examined as a treatment for disability and progression of multiple sclerosis. However, larger scale clinical trials have not replicated the promise of earlier, smaller, interventions, leaving unclear its potential to inhibit disease progression in this disorder.

27.1 Clinical Presentation

The characteristic manifestation of multiple carboxylase deficiency (MCD) is metabolic acidosis associated with neurological abnormalities and skin disease. The expression of the clinical and biochemical features is variable in both holocarboxylase synthetase (HCS) and biotinidase deficiency [5]. While patients with HCS deficiency commonly present with the typical symptoms of MCD, those with biotinidase deficiency show a less consistent clinical picture, particularly during the early stage of the disease. The onset in biotinidase deficiency may be insidious, and the manifestation is usually very variable, neurological symptoms often being prominent without markedly abnormal organic acid excretion or metabolic acidosis. Later-onset forms of HCS deficiency cannot be clinically distinguished from biotinidase deficiency, necessitating confirmation of the diagnosis by enzyme or molecular genetic analysis.

Four patients have so far presented with deficiency of the sodium-dependent multivitamin transporter (SMVT), with a variable MCD like clinical picture, while one patient has presented with suspected acquired biotin deficiency. Other patients with MCD-like biochemical features have been found to have mutation of *MT-ATP6*, usually associated with NARP (neuropathy, ataxia and retinitis pigmentosa) [6].

27.1.1 Holocarboxylase Synthetase Deficiency

Although HCS deficiency was initially termed early-onset MCD, experience shows that the age of onset varies widely, from a few hours after birth to 8 years of

age [7]. Nevertheless, patients typically present acutely in the first hours to weeks of life with symptoms very similar to those observed in other severe organic aciduria, i.e. lethargy, hypotonia, vomiting, seizures and hypothermia. The most common initial clinical features consist of respiratory difficulties, such as tachypnoea or Kussmaul breathing associated with severe metabolic acidosis, ketosis and hyperammonaemia that – without biotin supplementation – may lead to coma and early death. Patients with a less severe defect and later onset may also present with recurrent life-threatening attacks of metabolic acidosis and typical organic aciduria [8, 9].

Episodes of acute illness are often precipitated by catabolism during intercurrent infections or by a higher protein intake. Early-onset patients who recover without biotin therapy and untreated patients with a less severe defect may additionally develop psychomotor retardation, hair loss and skin lesions. These include an erythematous, scaly skin rash that spreads over the whole body but is particularly prominent in the diaper and intertriginous areas; alternatively, the rash may resemble seborrhoeic dermatitis or ichthyosis [10]. In contrast to biotinidase deficiency, deafness has not been reported in patients with HCS.

27.1.2 Biotinidase Deficiency

Important features are the gradual development of symptoms and episodes of remission, which may be related to increased free biotin in the diet. The full clinical picture has been reported as early as 7 weeks, but discrete neurological symptoms may occur much earlier, even in the neonatal period [11]. Neurological manifestations (lethargy, muscular hypotonia, grand mal and myoclonic seizures, ataxia) are the most frequent initial symptoms. In addition, many children have developmental delay, hearing loss, conjunctivitis and visual problems, including optic atrophy. Biotinidase deficiency should be considered in any child with unexplained developmental delay and particularly in those with sensorineural hearing loss. Skin rash and/or alopecia are hallmarks of the disease; however, these may develop late or not at all [12, 13]. Skin lesions are usually patchy, erythematous/exudative and typically localised periorificially. Eczematoid dermatitis or an erythematous rash covering large parts of the body has also been observed, as has keratoconjunctivitis. Hair loss is usually discrete but may, in severe cases, become complete, including the eyelashes and eyebrows. Immunological dysfunction may occur in acutely ill patients.

Some children with profound biotinidase deficiency (defined as <10% control activity) may not develop symptoms until later in childhood or during adolescence [12, 14]. Their symptoms may include motor limb weakness, spastic paraparesis, spinal cord demyelination and unusual symmetrical findings on brain MRI [12, 14, 15], or eye problems such as myelopathy and vision loss mimicking neuromyelitis optica spectrum disorder, optic neuropathy, loss of visual acuity and scotomata [12, 14].

Asymptomatic adults and siblings with profound biotinidase deficiency were ascertained after identification of their affected children/siblings, often by newborn screening [12, 16]. Therefore, investigation of all family members of patients with biotinidase deficiency is very important for the detection of asymptomatic individuals who are at risk of exhibiting symptoms at any age.

Because of the variability and nonspecificity of clinical manifestations, there is a very high risk of a delay in diagnosis [15, 17–19]. Late-diagnosed patients often have psychomotor retardation and neurological symptoms, such as leukoencephalopathy and delayed myelination, hearing loss and optic atrophy, which may be irreversible [13, 15, 18–21]. Outcome may even be fatal.

Metabolic acidosis and the characteristic organic aciduria of MCD are frequently lacking in the early stages of the disease. Plasma lactate and 3-hydroxyisovalerate may be only slightly elevated, whereas cerebrospinal fluid levels may be significantly higher [22]. This fact and the finding of severely decreased carboxylase activities in brain but moderately deficient activity in liver and kidney in a patient with lethal outcome [17] are in accordance with the predominance of neurological symptoms and show that, in biotinidase deficiency, the brain is affected earlier and more severely than other organs. The threat of irreversible brain damage demands that biotinidase deficiency should be considered in all children with neurological problems including a therapeutic trial with oral biotin (10 mg/day for 5 days), even if obvious organic aciduria and/or cutaneous findings are not present. Thus, *BTD* should be included in genetic panels and WES analysis of all patients with neurological symptoms regardless of the specific clinical manifestation. Sadly, in regions where no neonatal screening for biotinidase deficiency is performed there seems to have been little improvement in the diagnostic delay [18, 20]. Therefore, neonatal screening provides the best chance of improving outcome in biotinidase deficiency. Importantly, treatment should be instituted without delay, since patients may become biotin depleted within a few days after birth [11].

27.1.3 Sodium-Dependent Multivitamin Transporter Deficiency (SLC5A6)

Four patients from three families have thus far been described with deficiency of SLC5A6, a sodium-dependent multivitamin transporter (SMVT) [23–25].

Each patient presented between 12 and 17 months of life with variable symptoms including feeding problems, failure to thrive, neurological features such as developmental delay, epileptic seizures, microcephaly and cerebral palsy as well as immunodeficiency. Single patients also showed severe metabolic acidosis, cyclical vomiting as well as osteoporosis leading to pathologic bone fractures.

Biotin dependency due to a defect in biotin transport was suggested in a 3-year-old boy with normal biotinidase activity and nutritional biotin intake [26], but the genetic defect remains unresolved.

27.1.4 Acquired Biotin Deficiency

Dietary deficiency of biotin was documented in an 11-year-old retarded boy as a consequence of a dietary prescription containing raw eggs rich in avidin that chelates biotin and avoids its intestinal absorption [27].

27.2 Metabolic Derangement

In HCS deficiency, a decreased affinity of the enzyme for biotin and/or a decreased maximal velocity lead to reduced formation of the five holocarboxylases from their corresponding inactive apocarboxylases at physiological biotin concentrations (Fig. 27.2) [28, 29]. In biotinidase deficiency, biotin cannot be released from biocytin and short biotinyl peptides. Thus, patients with biotinidase deficiency are unable to recycle endogenous biotin and use protein-bound dietary biotin (Fig. 27.2) [5]. Consequently, biotin is lost in the urine, mainly in the form of biocytin [11, 30], and progressive biotin depletion occurs. Depending on the amount of free biotin in the diet and the severity of the enzyme defect, the disease becomes clinically manifest during the first months of life or later in infancy or childhood.

Deficient activity of biotin-dependent carboxylases in both HCS and biotinidase deficiencies (Fig. 27.1) results in accumulation of lactic acid and derivatives of 3-methylcrotonyl-CoA carboxylase (MCC; ▶ Chap. 18) and propionyl-CoA carboxylase (PC; ▶ Chap. 11).

Isolated inherited deficiencies of each of the three mitochondrial carboxylases, propionyl-CoA carboxylase (PCC; ▶ Chap. 18), 3-methylcrotonyl-CoA carboxylase (MCC; ▶ Chap. 18), and pyruvate carboxylase (PC; ▶ Chap. 11), are also known. Two patients with a potential defect of acetyl-CoA carboxylase (ACC-1, cytosolic) have also been reported [31, 32]. These isolated deficiencies are due to absence or abnormal structure of the apoenzyme and usually do not respond to biotin therapy.

Acquired biotin deficiency is rare, but may result from excessive consumption of raw egg white [27], malabsorption, long-term parenteral nutrition, haemodialysis and long-term anticonvulsant therapy.

In SMVT deficiency, transport of biotin, pantothenate (the precursor of Coenzyme-A) and α-lipoic acid in the digestive system and across the blood-brain-barrier may be disrupted. Since each vitamin is required for multiple energetic pathways, loss of this transporter may have devastating pleiotropic effects on the tricarboxylic acid cycle, β-oxidation, branched-chain amino acid catabolism and the glycine cleavage system.

27.3 Genetics

Both HCS and biotinidase deficiency are inherited as autosomal recessive traits. HCS deficiency seems to be rarer than biotinidase deficiency with the exception of the isolated small population in Faroe Islands where the incidence was calculated to be 1 in 1200 newborns [33].The incidences of profound (<10% residual activity) and partial (10–30% residual activity) biotinidase deficiencies are estimated to be 1:80,000 and 1:30,000–40,000 in the United States, respectively [34]. However, disease incidence varies between countries, with estimated combined incidences of approximately 1:14,000 in Brazil, 1:8,500 in the Czech Republic and 1:8,200 in the Netherlands, as identified by newborn screening.

27.3.1 Holocarboxylase Synthetase Deficiency

More than 50 different disease-causing mutations have been reported in the *HLCS* gene [35]. About two-thirds of them are within the putative biotin-binding region of HCS and some mutations have been shown to result in decreased affinity of the enzyme for biotin [29, 36, 37]; this is in accordance with elevated K_m values for biotin measured in fibroblasts of several HCS deficient patients [28, 38], and probably accounts for the in vivo responsiveness to biotin therapy of these patients. The degree of abnormality of the K_m values correlates well with the time of onset and severity of illness, i.e. highest K_m with early onset and severe disease [28].

Other mutations, located outside the biotin-binding site in the N-terminal region, are associated with virtually normal K_m for biotin but decreased V_{max} [29]. Most patients with this type of mutation also respond to biotin, although higher doses are usually required and residual biochemical and clinical abnormalities mostly persist. Biotin responsiveness in such

patients most probably derives from a positive effect of biotin on *HLCS* mRNA transcription and thus on the level of HCS protein [39]. However, since this mechanism involves HCS protein itself, it requires the presence of residual HCS activity. One mutant allele, c.647T>G (p.Leu216Arg), when present in the homozygous state, has been associated with a virtually biotin-unresponsive, severe clinical phenotype [40]. Nevertheless, favourable outcome in two siblings with very high-dose (1.2 g oral/day) biotin has been reported [41].

27.3.2 Biotinidase Deficiency

Over 200 mutations in *BTD* have been reported [34]. The most commonly identified variant is p.Asp444His, which reduces protein activity by 50%, and is found in almost all patients with partial biotinidase deficiency, with a severe mutation in the trans allele. Alternatively, the double mutant p.[(Ala171Thr);(Asp444His)] in trans with a severe mutation is a common finding associated with profound biotinidase deficiency. Other common variants associated with profound deficiency include c.98_104delinsTCC, found in up to 50% of individuals, and p.Arg538Cys, identified in up to 30% of patients in the USA. Alternatively, p.Arg157His, p.Gln456His and p.Asp252Gly have been frequently identified by newborn screening and are associated with profound deficiency. Since most patients identified by newborn screening are treated with biotin, including those with partial deficiency, genotype-phenotype relationships are difficult to discern.

27.3.3 SLC5A6 Deficiency

Only four patients, from three families, are known [23–25]. Inheritance was autosomal recessive with mutation of *SLC5A6*.

27.4 Diagnostic Tests

A characteristic organic aciduria due to systemic deficiency of the carboxylases is the key feature of MCD. In severe cases, an unpleasant urine odour (cat's urine) may even be suggestive of the defect. MCD is reflected in elevated urinary and plasma concentrations of organic acids as follows:

- Deficiency of MCC: 3-hydroxyisovaleric acid and 3-hydroxyisovalerylcarnitine (C5-OH) in high concentrations, 3-methylcrotonylglycine and tiglylcarnitine (C5:1) in smaller amounts.
- Deficiency of PCC: methylcitrate, 3-hydroxypropionate, propionylglycine, tiglylglycine, propionic acid and propionylcarnitine (C3) in small to moderate amounts.
- Deficiency of PC: lactate in high concentrations, pyruvate in smaller amounts.
- It should be noted that a similar organic acid profile can occur in patients with hyperammonemia due to carbonic anhydrase VA deficiency (▶ Chap. 19) and in MT-ATP6 mutations (▶ Chap. 10). Cases also need to be distinguished from other causes of increased C5-hydroxyacylcarnitines (see ▶ Chap. 13).

The majority of HCS-deficient patients excrete all of the typical organic acids in elevated concentrations, provided that the urine sample has been taken during an episode of acute illness. In contrast, in biotinidase deficiency elevated excretion of only 3-hydroxyisovalerate may be found, especially in early stages of the disease. In 20% of untreated biotinidase-deficient children urinary organic acid excretion was normal when they were symptomatic [12].

The measurement of carboxylase activities in lymphocytes provides direct evidence of MCD. These activities are low in HCS deficiency but may be normal in biotinidase deficiency, depending on the degree of biotin deficiency [8, 42].

Determination of biotin concentrations in plasma and/or urine has little diagnostic value but can be used in evaluation of therapeutic compliance. Untreated, biotin concentrations are normal in HCS deficiency and usually decreased in symptomatic patients with biotinidase deficiency [11, 42], provided that an assay method that does not detect biocytin is used [43].

The two inherited disorders can easily be distinguished by the assay of biotinidase activity in serum. Today, this assay is included in the neonatal screening programmes in many countries worldwide.

27.4.1 Holocarboxylase Synthetase Deficiency

The diagnosis can be confirmed by molecular genetic analysis of the *HLCS* gene or indirect assay of HCS activity by demonstrating severely decreased activity of at least 2 mitochondrial biotin-dependent carboxylases in skin fibroblasts cultured in a medium with low biotin concentration (0.1 nM) and normalization (or at least an increase) of the activities in cells cultured in a medium

supplemented with a high biotin concentration (0.2–10 μM) [8, 28].

Carboxylase activities in lymphocytes are deficient and may remain reduced even during prolonged biotin supplementation [5]. Direct measurement of HCS activity requires a protein substrate, e.g. an apocarboxylase or a subunit or fragment of an apocarboxylase that contains the biotin attachment site [28, 44]; therefore, it is not routinely performed.

27.4.2 Biotinidase Deficiency

Biotinidase activity in serum is absent or decreased [12, 42]. Many patients have measurable residual activity. The level of residual activity should be confirmed in a second serum sample obtained at the age of 4 months or later. Mutation analysis of the entire *BTD* gene is rarely necessary, because there are no therapeutic consequences.

Carboxylase activities in lymphocytes are decreased in those patients that have biotin deficiency, but are normalised within hours after a single dose of oral biotin [11]. Carboxylase activities in fibroblasts cultured in low-biotin medium are similar to those in control fibroblasts, and are always normal in fibroblasts cultured in standard FCS based medium.

27.4.3 SLC5A6 Deficiency

So far, diagnosis has only been confirmed following mutation analysis of *SLC5A6*.

27.4.4 Prenatal Diagnosis

Prenatal diagnosis of HCS deficiency is possible by mutation analysis if mutations of an index patient are known, by enzymatic studies in cultured chorionic villi or amniotic fluid cells, or by demonstration of elevated concentrations of metabolites by stable isotope dilution techniques in amniotic fluid. In milder forms of HCS deficiency organic acid analysis may fail to show an affected fetus, necessitating molecular genetic or enzymatic investigation [45]. Prenatal diagnosis allows rational prenatal therapy, preventing severe metabolic derangement in the early neonatal period [45, 46]. Prenatal diagnosis of biotinidase deficiency is possible by mutation analysis but, in our opinion, not warranted, because prenatal treatment is not necessary.

27.5 Treatment and Prognosis

With the exception of some cases of HCS deficiency, both biotinidase and HCS deficiency can be treated effectively with oral biotin in pharmacological doses as long as treatment is started before irreversible neurological damage has occurred, e.g. deafness in biotinidase deficiency. No adverse effects have been observed from such therapy over a more than 30-year experience of treating biotinidase deficiency [47, 48] and, importantly, there is no accumulation of biocytin in body fluids [30], which was previously suspected to be a possible risk. In SLC5A6 deficiency, three patients responded positively to either oral or i.m. supplementation of biotin, pantothenic and lipoic acid.

Restriction of protein intake is not necessary except in very severe cases of HCS deficiency. Raw eggs should be avoided because they contain avidin, an egg-white protein that binds biotin, thereby decreasing the vitamins bioavailability [12]. Acutely ill patients with metabolic decompensation require general emergency treatment in addition to biotin therapy (► Chap. 4).

27.5.1 Holocarboxylase Synthetase Deficiency

The required dose of biotin is dependent on the severity of the enzyme defect and has to be assessed individually [5]. Most patients have shown a good clinical response to 10–20 mg/day, although some may require higher doses, i.e. 40–200 mg/day or even higher doses [5, 8, 40, 41, 46, 48, 49]. In spite of apparently complete clinical recovery, some patients continue to excrete abnormal metabolites (particularly 3-hydroxyisovalerate), a finding that correlates inversely with the actual level of carboxylase activities in lymphocytes. Exceptionally, persistent clinical and biochemical abnormalities have been observed despite treatment with very high doses of biotin [5, 40, 41, 46, 49]. All patients with HCS deficiency have at least partially responded to pharmacological doses of biotin with the exception of the majority of those homozygous for the missense mutation p.Leu216Arg [40, 41]. Patients homozygous for the c.1519+5G>A (IVS10+5G>A) mutation, a founder mutation in Scandinavia originating from Faroe Islands, show a unique clinical character with onset between 2 months and 8 years, and a slow and in some patients only partial clinical response to biotin treatment [33, 49].

To date, the prognosis for most surviving, well-treated patients with HCS deficiency seems to be good,

with the exception of those who show only a partial or no response to biotin [5, 40, 41, 46, 49]. Careful follow-up studies are needed to judge the long-term outcome. Irreversible neurological auditory-visual deficits, as described for biotinidase deficiency, have not been reported. Prenatal biotin treatment (10 mg/day) has been reported in a few pregnancies [46]. It is unclear whether prenatal treatment is essential; treatment of at-risk children immediately after birth may be sufficient.

27.5.2 Biotinidase Deficiency

The introduction of neonatal screening programmes has resulted in the detection of asymptomatic patients with residual biotinidase activity [48]. Based on measurement of serum biotinidase activity, the patients are classified into those with profound biotinidase deficiency, with less than 10% of mean normal biotinidase activity, and those with partial biotinidase deficiency, with 10–30% residual activity.

■ Profound Biotinidase Deficiency

In early-diagnosed children with complete biotinidase deficiency, 5–10 mg of oral biotin per day promptly reverses or prevents all clinical and biochemical abnormalities. For chronic treatment, the same dose is recommended. Under careful clinical and biochemical control, it may be possible to reduce the daily dose of biotin to 2.5 mg. However, biotin has to be given throughout life and regularly each day, since biotin depletion develops rapidly [11]. Some patients with profound deficiency have been reported to develop symptoms, e.g. hair loss, during puberty and adulthood that could be resolved when biotin dosage was increased to 15 or 20 mg [12].

Neonatal screening for biotinidase deficiency [50] allows early diagnosis and effective treatment. In such patients, the diagnosis must be confirmed by quantitative measurement of biotinidase activity. Treatment should be instituted without delay, since patients may become biotin deficient within a few days after birth [11].

In patients who are diagnosed late, irreversible brain damage may have occurred before the commencement of treatment. In particular, auditory and visual deficits often persist in spite of biotin therapy [13, 20–22], and intellectual impairment and ataxia have been observed as long-term complications [13, 18, 20, 21].

Patients with residual activity up to 10%, usually detected by neonatal screening or family studies, may remain asymptomatic for several years or even until adulthood [16, 42]. According to our experience with 61 such patients (52 families), however, they show a very high risk of becoming biotin deficient and should be treated [12, 42, 50].

■ Partial Biotinidase Deficiency

Patients with partial biotinidase deficiency (10–30% residual activity) are mostly detected by neonatal screening or in family studies and usually remain asymptomatic. However, over 20 children with partial deficiency who were identified by newborn screening but were not treated with biotin eventually did develop symptoms typical of profound biotinidase deficiency, such as hypotonia, skin rashes and loss of hair, particularly when they were stressed by an infectious disease or moderate gastroenteritis. In the vast majority all symptoms were readily reversed upon biotin treatment [47, 48]. Thus, because some untreated children will develop symptoms and conclusive evidence is lacking, it seems prudent to supplement patients with 10–30% of residual activity with biotin, e.g. 2.5–5 mg/day [47, 48].

27.5.3 SLC5A6 Deficiency

One patient was treated with large doses of biotin (10 mg/day, then 30 mg/ day), pantothenic acid (250 mg/day, then 500 mg/day), and lipoic acid (150 mg/day, then 300 mg/day) and exhibited limited improved motor and verbal skill development, but normalized height and weight, and improved head growth [23]. Another patient made some recovery on biotin alone (5 mg/day) however, escalation of biotin (to 10 mg/ 2× day) and introduction of pantothenic acid (250 mg/day), was required for improved appetite and growth, and resolution of diarrhoea. Nevertheless, delayed gross motor development remained [24]. The final treated patient was given weekly biotin (10 mg, intramuscular), dexpanthenol (250 mg, intramuscular) and α-lipoic acid (300 mg, intravenous) at the age of 7 years of age. The patients overall condition improved, including attenuation of cyclical vomiting, good seizure control, improved attention and recovery of a limited vocabulary and ability to walk with a frame [25].

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Disorders of Cobalamin and Folate Transport and Metabolism

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Contents

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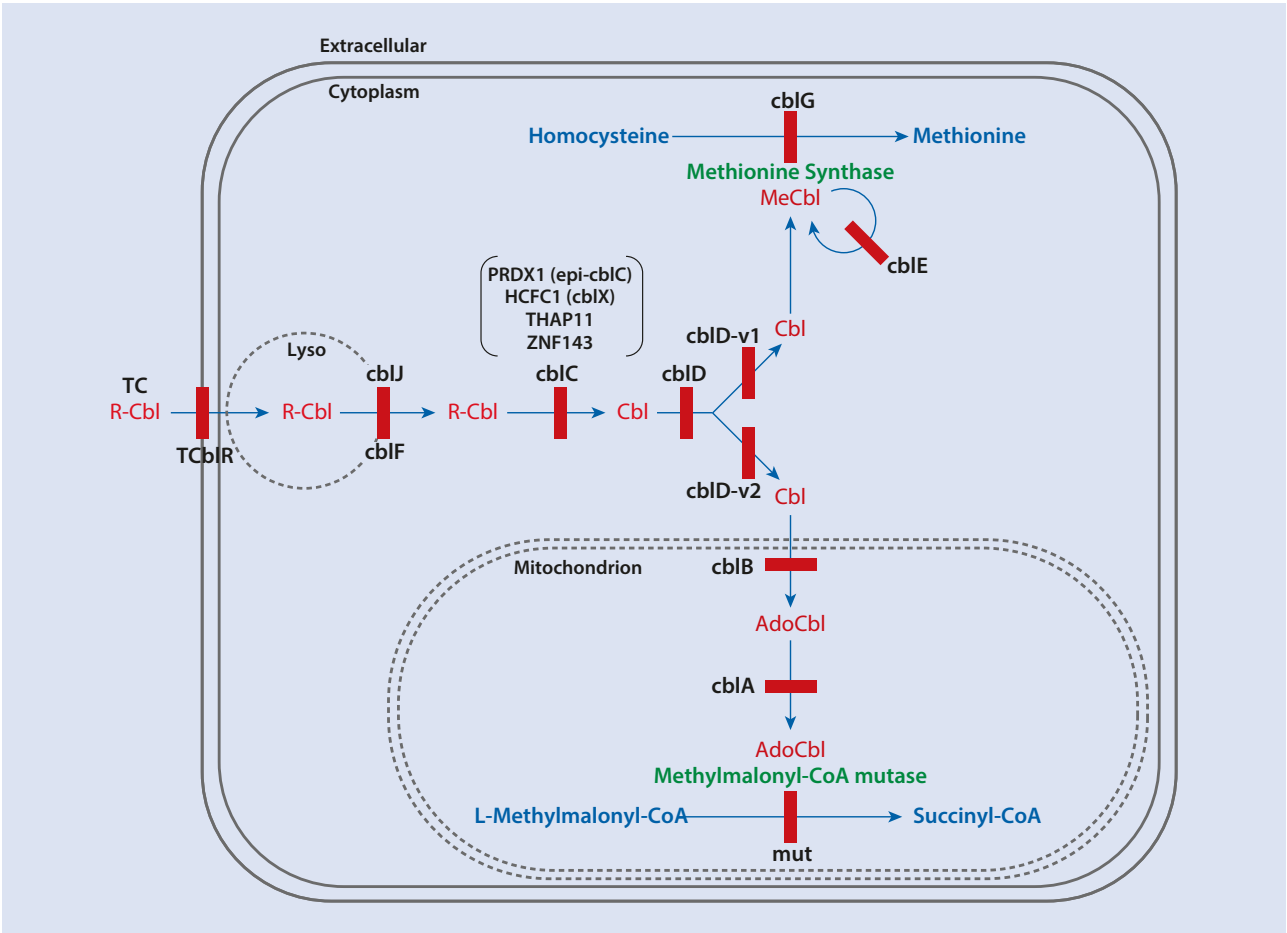

Fig. 28.1 Cobalamin (Cbl) endocytosis and intracellular metabolism. The cytoplasmic, lysosomal, and mitochondrial compartments are indicated: AdoCbl adenosylcobalamin, CoA coenzyme A, MeCbl methylcobalamin, OHCbl hydroxocobalamin, TC transcobalamin (previously TCII), V1 variant 1, V2 variant 2, cblA-cblG, and cbIJ, refer to the sites of blocks. PRDX1 (epi-cblC), HCFC1 (cblX), THAP11 and ZNF143 refer to disorders affecting expression of the cblC protein (MMACHC). Inborn errors are indicated by *solid bars*

Cobalamin Transport and Metabolism

Cobalamin (Cbl or vitamin B_{12}) is a cobalt-containing water-soluble vitamin that is synthesised by lower organisms but not by higher plants and animals (Fig. 28.1). The only source of Cbl in the human diet is animal products. Cbl is needed for only two reactions in man, but its metabolism involves complex absorption and transport systems and multiple intracellular conversions. As methylcobalamin (MeCbl), it is a cofactor of the cytoplasmic enzyme methionine synthase, which converts homocysteine to methionine. As adenosylcobalamin (AdoCbl), it is a cofactor of the mitochondrial enzyme methylmalonyl-coenzyme A mutase, which is involved in the catabolism of valine, threonine and odd-chain fatty acids into succinyl-CoA, an intermediate of the Krebs cycle:

Absorption of dietary Cbl first involves binding to a glycoprotein (haptocorrin, R binder) in the saliva. In the intestine, haptocorrin is digested by proteases, allowing the Cbl to bind to intrinsic factor (IF), which is produced in the stomach by parietal cells. Using the specific receptor cubam, the IF-Cbl complex enters the enterocyte. Following release from this complex Cbl enters the portal circulation bound to transcobalamin (TC), the physiologically important circulating Cbl-binding protein. Inherited defects of several of these steps are known.

28.1 Disorders of Absorption and Transport of Cobalamin

The serum cobalamin (Cbl) level is usually low in patients with disorders affecting absorption and transport of Cbl, with the exception of transcobalamin (TC) deficiency. Patients with disorders of intracellular Cbl metabolism typically have serum Cbl levels within the reference range, although levels may be reduced in the cblF and cblJ disorders. Homocystinuria (Hcy) and hyperhomocysteinaemia, as well as megaloblastic anaemia and neurological disorders, are major clinical findings in patients with disorders of Cbl absorption and transport, as well as those with defects of cellular metabolism that affect synthesis of MeCbl. Methylmalonic aciduria and acidaemia (MMA), resulting in metabolic acidosis, are seen in disorders that result in decreased synthesis of AdoCbl.

Increased urine MMA and plasma Hcy are also found in nutritional vitamin B_{12} deficiency. Severe vitamin B_{12} deficiency in newborn infants, which may occur in breast fed infants born to vegan mothers or those with subclinical pernicious anaemia, can result in a disorder that ranges from an elevation in serum concentration of propionylcarnitine detected by newborn screening, to one presenting with severe neonatal encephalopathy. The mother does not necessarily have a very low serum concentration of vitamin B_{12}. IM vitamin B_{12} replacement therapy to normalize the vitamin B_{12} serum concentration reverses the metabolic abnormality [1].

Inherited disorders of Cbl metabolism are divided into those involving absorption and transport and those involving intracellular utilisation [2–4]. New disorders continue to be discovered and the spectrum of clinical abnormalities widens. Developing guidelines [5] lead to harmonised treatment strategies, although doses given need to be individually tested.

28.1.1 Hereditary Intrinsic Factor Deficiency

Clinical Presentation

Presentation is usually from 1 to 5 years of age, but in cases of partial deficiency can be delayed until adolescence or adulthood. Patients present with megaloblastic anaemia as the main finding, together with failure to thrive, often with vomiting, alternating diarrhoea and constipation, anorexia and irritability. Hepatosplenomegaly, neutropenia/thrombocytopenia, stomatitis or atrophic glossitis, developmental delay and myelopathy or peripheral neuropathy may also be found [6].

Metabolic Derangement

IF is either absent or immunologically detectable but non-functional. There have been reports of IF with reduced affinity for Cbl or cubam, or with increased susceptibility to proteolysis.

Genetics

Approximately 100 patients of both sexes have been reported. Inheritance is autosomal recessive. Mutations in the gene for IF (*CBLIF*) have been identified in several patients with IF deficiency [7, 8].

Diagnostic Tests

The haematological abnormalities in the defects of Cbl absorption and transport should be detected by measurement of red blood cell indices, complete blood count and bone marrow examination. Low serum Cbl levels are present. In contrast to acquired forms of pernicious anaemia, there is normal gastric acidity and cytology, and anti-IF antibodies are absent. Cbl absorption, as measured by the Schilling test, is abnormal and normalised by exogenous IF. Because the Schilling test is rarely available, and because differentiation between hereditary IF deficiency and Imerslund-Gräsbeck syndrome on the basis of other clinical findings has proven difficult in some cases, sequencing of *CBLIF*, *AMN* and *CUBN* may represent an appropriate first-line means of correctly diagnosing these disorders [7].

Treatment and Prognosis

IF deficiency can be treated initially with hydroxocobalamin (OHCbl), 1 mg/day i.m., to replenish body stores until biochemical and haematological values become normal. The subsequent dose of OHCbl required to maintain values within or above the reference range may be as low as 0.25 mg every 3 months.

28.1.2 Defective Transport of Cobalamin by Enterocytes (Imerslund-Gräsbeck Syndrome)

Clinical Presentation

Defective transport of Cbl by enterocytes, also known as Imerslund-Gräsbeck syndrome or megaloblastic anaemia 1 (MGA1), is characterised by prominent megaloblastic anaemia with pallor, fatigue and failure to thrive manifesting once foetal hepatic Cbl stores have been depleted. The disease usually appears between the ages of 1 year and 5 years, but onset may be even later [9]. Many patients have

proteinuria that is not of the classic glomerular or tubular types, does not respond to therapy with Cbl and is not progressive [10]. A benign form of albuminuria has been associated with reduced cubilin function [11]. Neurological abnormalities, such as spasticity, truncal ataxia and cerebral atrophy, may be present as a consequence of the Cbl deficiency. A loss of interaction between cubilin and the vitamin D binding protein in the kidney may lead to hypovitaminosis D due to excess vitamin D excretion in patients with null mutations of cubulin [12, 13].

Metabolic Derangement

This disorder is caused by defects of the IF-Cbl receptor, cubam, which comprises two components. Cubilin was first purified as the IF-Cbl receptor from the proximal renal tubule. A second component, amnionless, colocalises with cubilin in the endocytic apparatus of polarised epithelial cells, forming a tightly bound complex that is essential for endocytosis of IF-Cbl and other molecules, including vitamin D-binding protein, albumin, transferrin and apolipoprotein A [2, 14]. Thus, defective function of either protein may cause this disorder.

Genetics

Around 300 cases have been reported. Inheritance is autosomal recessive, with environmental factors affecting expression [15]. Most patients are found in Finland, Norway, Saudi Arabia and Turkey, and among Sephardic Jews. A p.Pro1297Leu mutation in the cubilin gene (*CUBN*) was the most common causal variant in Finnish families, while mutations in the amnionless gene (*AMN*) were identified in Norwegian patients. Mutations of both *CUBN* and *AMN* have been identified in patients of Eastern Mediterranean origin [8].

Diagnostic Tests

The diagnosis is aided by finding low serum Cbl levels, megaloblastic anaemia and proteinuria. Most of the reports in the literature do not comment on the levels of homocysteine and methylmalonic acid. Gastric morphology and pancreatic function are normal; there are no IF autoantibodies and IF levels are normal. As previously noted, in the absence of the Schilling test, molecular analysis of *CBLIF*, *CUBN* and *AMN* may be the best means of differentiating between hereditary IF deficiency and Imerslund-Gräsbeck syndrome [7].

Treatment and Prognosis

Treatment with parenteral OHCbl corrects the anaemia and the neurological findings, but not the proteinuria. As with hereditary IF deficiency, once Cbl stores are replete, low doses of parenteral OHCbl may be sufficient to maintain normal haematological and biochemical values.

28.1.3 Haptocorrin (R Binder) Deficiency

Clinical Presentation

Very few cases have been described, and it is not clear whether this entity has a distinct phenotype. Haematological findings are absent and neurological findings such as subacute combined degeneration of the spinal cord in one man in the fifth decade of life and optic atrophy, ataxia, long-tract signs and dementia in another may be coincidental. It has been suggested that a deficiency of haptocorrin may be responsible for a number of patients with unexplained low serum Cbl levels. Haptocorrin deficiency has also been identified in individuals with serum Cbl levels within the reference range [16, 17].

Metabolic Derangement

The role of haptocorrin is uncertain, but it could be involved in the scavenging of toxic Cbl analogues or in protecting circulating MeCbl from photolysis. Deficiency of haptocorrin has been described in isolation and in association with deficiency of other specific granule proteins such as lactoferrin [18].

Genetics

A patient with severe deficiency of haptocorrin was shown to be compound heterozygous for two nonsense mutations (c.270delG and c.315C > T) in *TCN1*, which encodes haptocorrin. Members of this patient's family with moderate haptocorrin deficiency, as well as unrelated individuals with moderate deficiency, were found to be heterozygous for one of the mutations [17].

Diagnostic Tests

Serum Cbl levels are low because most circulating Cbl is bound to haptocorrin. TC-Cbl levels are normal, and there are no haematological findings of Cbl deficiency. A deficiency or absence of haptocorrin is found in plasma, saliva and leukocytes.

Treatment and Prognosis

It is likely that no treatment is needed because of the lack of a clearly defined phenotype.

28.1.4 Transcobalamin Deficiency

Clinical Presentation

In transcobalamin (TC) deficiency, symptoms usually develop much earlier than in other disorders of Cbl absorption, typically within the first few months of life. Even though the only TC in cord blood is of foetal ori-

gin, patients are not sick at birth. Presenting findings include pallor, failure to thrive, mouth ulcerations, weakness and diarrhoea. Although the anaemia is usually megaloblastic, patients with pancytopenia or isolated erythroid hypoplasia have been described [19]. Leukaemia may be mistakenly diagnosed because of the presence of immature white cell precursors in an otherwise hypocellular marrow [20]. Neurological disease is not an initial finding but may develop with delayed treatment, with administration of folate in the absence of Cbl, or with inadequate Cbl treatment. Neurological features include developmental delay, weakness, ataxia, hypotonia, neuropathy, myelopathy and encephalopathy and, rarely, retinal degeneration. Immunologic abnormalities including agammaglobulinaemia, low IgG and low T and B cell counts may be present; some patients have had recurrent infections.

Metabolic Derangement

The majority of patients have no immunologically detectable TC, although others have some detectable TC that is able to bind Cbl but cannot support cellular Cbl uptake.

Genetics

Inheritance is autosomal recessive. There have been at least 50 cases, including both twins and siblings. Disease-causing deletions, nonsense mutations and activation of an intra exonic cryptic splice site have been described in *TCN2*, which encodes transcobalamin [19].

Diagnostic Tests

Serum Cbl levels are not usually low, because the majority of serum Cbl is bound to haptocorrin and not to TC. Cbl bound to TC, as reflected by the unsaturated vitamin B_{12}-binding capacity, is low provided that the test is performed before Cbl treatment is started. Reports of levels of Cbl-related metabolites are inconsistent. Patients with plasma total homocysteine within the reference range and moderately increased urine methylmalonic acid have been reported, as well as patients with methylmalonic aciduria and homocystinuria. DNA testing is possible for both diagnosis and heterozygote detection in families in which the molecular defect has been identified. Assays using antibodies generated against recombinant human TC allow reliable measurement of serum TC even in patients who have been treated with Cbl [21]. In atypical cases, study of TC synthesis in cultured fibroblasts or amniocytes allows both pre- and postnatal diagnosis in patients [22].

Treatment and Prognosis

Adequate treatment requires administration of oral or parenteral OHCbl or cyanocobalamin (CNCbl) at a dose of 0.5–1 mg, initially daily then twice weekly, to maintain serum Cbl levels in the range of 1000–10,000 pg/ml. Intravenous Cbl is not recommended because of its rapid loss in the urine. Folic acid or folinic acid can reverse the megaloblastic anaemia and has been used in doses up to 15 mg p.o. four times daily. However, folates must never be given as the only therapy in TC deficiency, because of the danger of neurological deterioration. Treatment with Cbl, particularly when instituted during the first months of life, has been associated with favourable patient outcomes. A review of TC-deficient patients found a single patient, among 19 older than 6 years old at the latest follow-up, with significant intellectual deficits, possibly due to sub-optimal therapy. A second patient had neurological findings that responded to treatment optimization [19].

28.1.5 Transcobalamin Receptor Deficiency

Clinical Presentation

Several subjects with a defect affecting the cell surface receptor that recognises the TC-Cbl complex and modulates its uptake by carrier-mediated endocytosis have been identified on newborn screening. They had moderate elevations of serum methylmalonic acid and, in most cases, also of homocysteine, but most did not show clinical signs of Cbl deficiency [23]. Attributing bilateral central artery occlusions to hyperhomocysteinemia [24] in a single patient is questionable.

Metabolic Derangement

Cellular uptake of Cbl bound to TC is markedly decreased in patient fibroblasts [23], apparently without prejudicing synthesis of MeCbl and AdoCbl.

Genetics

Inheritance is autosomal recessive. Eight individuals homozygous for a 3-bp deletion (c.262_264delGAG) in *CD320* that encodes the TC receptor, have been described, in addition to one patient heterozygous for this variant together with c.297delA [25]. The c.262_264delGAG variant has been shown to cause diminished Cbl uptake in an in vitro system [23]. It was present at a frequency of 3% in an Irish control population [26].

Treatment and Prognosis

Since most of the affected individuals lack clinical signs of Cbl deficiency, it is likely that treatment is not necessary. Mild or moderate elevations of homocysteine or methylmalonic acid experienced by these individuals appear to be responsive to oral supplementation with cobalamin.

28

28.2 Disorders of Intracellular Utilisation of Cobalamin

Disorders of intracellular Cbl metabolism have been classified as *cbl* mutants (*A-G, J*), based on the biochemical phenotype and on somatic cell analysis (Fig. 28.1). They encompass disruption of proteins required for transport or metabolism of Cbl to its cofactor forms. Recently, a new group of disorders that disrupt transcription of one of these enzymes, MMACHC, has been described. These include variants of *HCFC1* (cblX), *THAP11*, *ZNF143* and *PRDX1*. Precise diagnosis of the inborn errors of Cbl metabolism requires either identification of causal mutations or tests in cultured fibroblasts, including complementation analysis.

28.2.1 Combined Deficiencies of Adenosylcobalamin and Methylcobalamin

Five distinct disorders are associated with functional defects in both methylmalonyl-CoA mutase and methionine synthase. They are characterised by both methylmalonic aciduria and homocystinuria.

28.2.1.1 CblF (*LMBRD1*)

- **Clinical Presentation**

Most patients with cblF disease have presented in the first year of life. Frequent findings have included intrauterine growth retardation (2 patients), feeding difficulties (4), failure to thrive (8), developmental delay (8) and persistent stomatitis (5). Other findings are haematological features (6), congenital cardiac anomalies (6), and small for gestational age (6) [27]. A complete blood count and bone marrow examination may reveal megaloblastic anaemia, neutropenia and thrombocytopenia. Two patients have had minor facial anomalies including pegged teeth and bifid incisors; four have had structural heart defects. One patient died suddenly at home in the first year of life; two others died after cardiac surgery [28].

- **Metabolic Derangement**

The defect in cblF appears to be a failure of Cbl transport across the lysosomal membrane following its release from TC in the lysosome. As a result, Cbl accumulates in lysosomes and cannot be converted to either AdoCbl or MeCbl. The inability of cblF patients to absorb oral Cbl suggests that IF-Cbl also has to pass through a lysosomal stage in the enterocyte before Cbl is released into the portal circulation.

- **Genetics**

Sixteen patients with the cblF disorder have been reported. Mutations in *LMBRD1* have been identified in all reported patients [28–30], inherited in an autosomal recessive manner. This gene encodes a lysosomal membrane protein. A deletion mutation (c.1056delG), which is found on a common haplotype [31], occurs in patients from different ethnic groups and represents two-thirds of disease-causing alleles that have been identified.

- **Diagnostic Tests**

The serum Cbl level may be low, and the Schilling test has been abnormal when tested. Usually, increased plasma total homocysteine, methylmalonic acid and C3 acylcarnitine, low to normal plasma methionine, homocystinuria and methylmalonic aciduria are found, although urine and plasma elevations of homocysteine were not reported in the original patient. In fibroblasts from cblF patients, total incorporation of labelled CNCbl is elevated, but CNCbl is not converted to either AdoCbl or MeCbl. Most of the label is found as free CNCbl in lysosomes. There is decreased function of both Cbl-dependent enzymes.

- **Treatment and Prognosis**

Treatment with parenteral OHCbl (first daily and then biweekly, or even less frequently) at a dose of 1 mg/day seems to be effective in correcting the metabolic and clinical findings. The original patient responded to oral Cbl before being switched to parenteral Cbl, despite the fact that the Schilling test performed on two occasions showed an inability to absorb Cbl with or without IF.

28.2.1.2 CblJ (*ABCD4*)

- **Clinical Presentation**

Six patients have been reported with the cblJ disorder. The first two patients presented in the newborn period. One had feeding difficulties, hypotonia, lethargy and bone marrow suppression; the second had feeding difficulties, macrocytic anaemia and congenital heart defects [32]. Subsequently, three patients of Chinese descent were reported with later onset (4 to 6 years of age), hyperpigmentation and prematurely grey hair; one additionally reported dizziness and headaches [33–35]. Macrocytic anaemia, methylmalonic aciduria and hyperhomocysteinemia were present in all cases.

- **Metabolic Derangement**

As in the cblF disorder, there is decreased ability to transfer Cbl across the lysosomal membrane into the cytoplasm, resulting in accumulation of free Cbl in lyso-

somes. Although the exact role of the two proteins involved in lysosomal transport of Cbl remains to be elucidated, current evidence suggests that LMBRD1 (responsible for cblF) may be involved in lysosomal targeting and subsequent protection of ABCD4 (responsible for cblJ), which mediates Cbl transport across the lysosomal membrane.

Genetics

Mutations in *ABCD4*, which encodes an ATP-binding cassette transporter, have been identified in all six cases. Inheritance is autosomal recessive. Three patients, from Taiwan (2) and China (1), were homozygous for c.423C > G (p.Asn141Lys).

Diagnostic Tests

Patients have had elevated urine methylmalonic acid and hyperhomocysteinemia. Serum Cbl was low in several patients; intestinal absorption has not been investigated. Results of studies of cultured fibroblasts in the early-onset patients were identical to those of cblF fibroblasts; studies of the first Taiwanese patient showed a milder cellular phenotype, with moderately reduced MeCbl synthesis and apparently normal AdoCbl synthesis.

Treatment and Prognosis

As for cblF, biochemical abnormalities respond dramatically to parenteral B_{12}.

28.2.1.3 CblC (*MMACHC*)

Clinical Presentation

This is the most frequent inborn error of Cbl metabolism, and over 750 patients are known [36–38]. Many were acutely ill in the first month of life, and most were diagnosed within the first year. This early-onset group shows feeding difficulties and lethargy, followed by progressive neurological deterioration. This may include hypotonia, hypertonia or both, abnormal movements or seizures and coma. Severe pancytopenia or a non-regenerative anaemia, which is not always associated with macrocytosis and hypersegmented neutrophils, but which is megaloblastic on bone marrow examination, may be present. Patients may develop multisystem pathology, such as renal failure, hepatic dysfunction, cardiomyopathy, interstitial pneumonia or the haemolytic uraemic syndrome characterised by widespread microangiopathy. Additional features include an unusual retinopathy consisting of perimacular hypopigmentation surrounded by a hyperpigmented ring and a more peripheral salt-and-pepper retinopathy sometimes accompanied by nystagmus, microcephaly and hydrocephalus [38, 39]. Congenital structural heart defects and dysmorphic features may be present [40]. A small number of cblC patients were not diagnosed until after the first year of life and some as late as the end of the fourth decade of life. The patients in this group who were diagnosed earlier had findings overlapping those found in the younger onset group. Major clinical findings in the late-onset cblC group included confusion, disorientation and gait abnormalities and incontinence. Macrocytic anaemia was seen in only about a third of the oldest patients [38, 39]. Therefore, it is important to search for the cblC disorder by determination of metabolite levels in the presence of neurological findings alone.

Metabolic Derangement

In the cblC disorder, there is disruption of a protein (MMACHC) that plays a role in the early steps of cellular Cbl metabolism [2]. MMACHC binds Cbl and catalyses removal of upper axial ligands from alkylcobalamins (including the methyl group from MeCbl and the adenosyl group from AdoCbl) and from CNCbl.

Genetics

In most cases, the cblC disorder is caused by biallelic mutations in *MMACHC*. A common mutation, c.271dupA, accounts for 40% or more of all disease alleles in patient populations of European origin [37]. In the homozygous state, or in combination with other truncating variants, this mutation is generally associated with early, severe disease [36, 38]. Alternatively, c.394C > T has been associated with a later, milder presentation. A different mutation, c.609G > A (p.Trp203*), represents over 50% of disease-causing alleles in Chinese cblC patients [41, 42], and is predominantly associated with early onset disease, although there is considerable phenotypic variability.

Mutation of *PRDX1*, an overlapping proximal gene of *MMACHC*, results in epigenetic silencing of *MMACHC* transcription (called: epi-cblC). Mutation of *PRDX1* leading to silencing of MMACHC has been described in the heterozygous state combined with genetic mutation of *MMACHC* in three patients with cblC disease [43].

Diagnostic Tests

Increased plasma total homocysteine, low to normal plasma methionine, homocystinuria and methylmalonic acidaemia and aciduria are the biochemical hallmarks of this disease. In general, the methylmalonic acid levels seen are lower than those found in patients with methylmalonyl-CoA mutase deficiency but higher than those seen in the Cbl transport defects. A complete

blood count and bone marrow examination allow detection of the haematological abnormalities.

Fibroblast studies show decreased accumulation of CNCbl, decreased synthesis of both AdoCbl and MeCbl, and decreased function of both methylmalonyl-CoA mutase and methionine synthase. Cells fail to complement those of other cblC patients and patients with mutations in *HCFC1*. Differentiation between cblC and cblX-like disorders (next section) requires DNA sequencing. Prenatal diagnosis can be performed by mutation analysis, by in vitro studies in cultured chorionic villus cells (interpreted with caution, chorionic villus biopsies should not be used) and amniocytes, and by measuring methylmalonic acid and total homocysteine levels in amniotic fluid. Except for mutation analysis, these techniques cannot detect heterozygotes.

■ Treatment and Prognosis

Treatment is usually with 1 mg/day OHCbl (parenteral) in combination with oral betaine. Elevated metabolite levels improve but are not usually completely normalised. Oral OHCbl has been found to be insufficient, and neither folinic acid nor carnitine was effective. Doses as high as 20 mg OHCbl a day have been used, emphasizing the need to titrate doses in individual patients, while blood vitamin B_{12} levels has been proposed as a prognostic marker [44]. Both in vitro studies and studies of patients indicate that CNCbl is ineffective in treatment of this disease, possibly reflecting the role of the MMACHC protein in decyanation of CNCbl.

12–30% of early-onset cblC patients have previously been reported to have died, and most survivors have had moderate or severe neurological impairment despite treatment [38, 45]. However, a more recently studied cohort indicated that prognosis is likely improving [46]. Patients with later onset tend to have better outcomes. Treatment starting early in life, before neurologic impairment becomes established, is important for optimal patient response, but long-term outcome remains uncertain. Thus, newborn screening plays an increasing role in early detection and treatment [47].

28.2.1.4 Disorders of *MMACHC* Transcription: CblX (*HCFC1*) and Related Disorders (*HAP11*; *ZNF143*)

■ Clinical Presentation

Exome sequencing of a male patient with a diagnosis of cblC disease based on complementation analysis, in whom no *MMACHC* mutations could be detected, identified a hemizygous mutation in *HCFC1* on the X chromosome. Subsequently, *HCFC1* mutations were identified in an additional 14 male patients [48, 49]. Patients have presented in the first months of life with a similar clinical presentation to cblC patients, although the metabolic abnormalities are milder and the neurologic presentation is more severe, with choreoathetosis, intractable epilepsy and severe developmental delay and sometimes with manifestations before birth (such as microcephaly and cortical malformations).

■ Metabolic Derangement

The cblX disorder is caused by mutations at *HCFC1*, which functions as a transcriptional co-regulator in association with other transcription factors, including THAP11 and ZNF143. This trio affects expression of a number of genes, including *MMACHC*. The metabolic consequences relating to cobalamin metabolism stem from decreased *MMACHC* expression leading to decreased synthesis of both AdoCbl and MeCbl. This is also a vesicular trafficking disorder (between the nucleus and the endoplasmic reticulum) (► Chap. 43).

■ Genetics

HCFC1 is X-linked. All cblX patients have been male, with hemizygous *HCFC1* mutations affecting the kelch domain near the N-terminus of the protein [48]. The most common mutation is c.344C > T (p.Ala115Val). Mutation of *ZNF143* [50] and *THAP11* [51] have each been identified in single patients with autosomal recessive inheritance.

■ Diagnostic Tests

Patients have moderately elevated serum and urine levels of methylmalonic acid that are usually lower than those seen in other inborn errors of Cbl metabolism. Serum total homocysteine has been elevated in some patients, but others had values within the reference range. Fibroblasts studies place these patients within the cblC complementation group and diagnosis therefore depends on identification of mutations.

■ Treatment and Prognosis

Since MMACHC deficiency alone appears not to be responsible for all clinical abnormalities, correction of methylmalonic acid and homocysteine levels with OHCbl is unlikely to prevent all manifestations of this disorder.

28.2.1.5 CblD (*MMADHC*)

■ Clinical Presentation

This defect was first described in two brothers with combined methylmalonic aciduria and homocystinuria. The elder sibling had behavioural problems and mild mental retardation at the age of 14 years, and also ataxia and nystagmus. Heterogeneity of the cblD defect was established by discovery of one patient with isolated methylmalonic aciduria who presented prematurely with respiratory distress, cranial haemorrhage, necrotising enterocolitis and convulsions but without anaemia, and

two unrelated patients with isolated homocystinuria, megaloblastic anaemia and neurological changes but without metabolic decompensation [52]. Following the discovery of *MMADHC*, the gene responsible for cblD [53, 54], further patients were described whose clinical presentation broadly resembled that of the cblC, cblA/B and cblE/G defects, respectively.

Metabolic Derangement

The cblD defect is caused by mutations in *MMADHC* and can cause deficient synthesis of both AdoCbl and MeCbl together, or of either in isolation. This suggests that the product of *MMADHC* plays a role in directing Cbl from the MMACHC protein to the two Cbl-dependent enzymes.

Genetics

Biallelic *MMADHC* mutations have been found in all patients belonging to the cblD complementation group regardless of the phenotype. The nature and location of mutations within the gene seem to determine the phenotype. Thus the combined-defect patients have crippling mutations towards the C-terminus; isolated homocystinuria patients have missense mutations towards the C-terminus; and isolated methylmalonic aciduria patients have mutations leading to a stop codon toward the N-terminus, in which case re-initiation of translation occurs at one of two downstream start codons.

Diagnostic Tests

Methylmalonic aciduria with or without increased plasma total homocysteine and homocystinuria, or isolated homocystinuria may be found. In fibroblast studies, findings can be similar to those of the cblC, cblA/B or cblE/G defects, although differences in the severity and responsiveness to addition of OHCbl to the culture medium may be seen. This heterogeneity emphasises the necessity of complementation or genetic analysis to make a specific diagnosis.

Treatment and Prognosis

This depends on the sub-type and is similar to that described for the cblC, cblA/B and cblE/G defects, respectively. Too few patients have so far been identified to allow clear conclusions on outcome to be made.

28.2.2 Adenosylcobalamin Deficiency: CblA (*MMAA*) & CblB (*MMAB*)

Clinical Presentation

Adenosylcobalamin (AdoCbl) deficiency comprises cblA and cblB, two disorders characterised by methylmalonic aciduria (MMA) which is often Cbl-responsive. The phenotype resembles methylmalonyl-CoA mutase deficiency (► Chap. 19), although often less severe.

Metabolic Derangement

The defect in cblB is deficiency of Cbl adenosyltransferase, which catalyses the final step in intramitochondrial synthesis of AdoCbl, the cofactor for methylmalonyl-CoA mutase [55]. The defect in cblA results from mutations in *MMAA* [56]. Enzymatic studies of the *MMAA* gene product suggest that this protein is involved in transfer of AdoCbl from adenosyltransferase to methylmalonyl-CoA mutase and in maintaining mutase-bound AdoCbl in its active form.

Genetics

MMAA encodes a polypeptide belonging to the G3E family of GTP-binding proteins. Over 60 mutations in *MMAA* have now been described among approximately 200 *cblA* patients [56–58]. The most common of these is a c.433C > T (p.Arg145*) nonsense mutation. Inheritance is autosomal recessive.

MMAB encodes cobalamin adenosyltransferase. Over 30 mutations in *MMAB* have been identified in cblB patients [55, 59]. Virtually all of these mutations are clustered in the regions of the protein identified as the active site of adenosyltransferase. Inheritance is autosomal recessive.

Diagnostic Tests

Total serum Cbl is usually normal. Urinary methylmalonic acid levels are elevated above reference values (typically <5 μmol/mmol creatinine), sometimes to greater than 20,000 μmol/mmol creatinine [60], but there is no increase of plasma total homocysteine or homocystinuria.

The differentiation of cblA and cblB from methylmalonyl-CoA mutase deficiency depends on fibroblast studies, including complementation analysis, or sequencing. In cell lysates from patients with cblA and cblB, activity of methylmalonyl-CoA mutase is within the reference range in the presence of added AdoCbl. Incorporation of radio-labelled propionate into TCA-precipitable protein, an indirect measure of methylmalonyl-CoA pathway function, is decreased in fibroblasts from both cblA and cblB patients; this is usually responsive to the addition of OHCbl to the culture medium in cblA, while many cblB patients do not show such a response. Uptake of labelled CNCbl is within the reference range, but there is decreased synthesis of AdoCbl. Adenosyltransferase specific activity is clearly deficient in cblB, but normal in cblA fibroblast extracts.

Treatment and Prognosis

Most of these patients respond to protein restriction and methylmalonic acid excretion may decrease in

response to Cbl therapy (10 mg p.o. daily or 1 mg i.m. once or twice weekly) in some patients although many cblB patients do not respond. There has been marked variation in the form and dosage of Cbl used and its mode of administration, as well as in the parameters used to assess response. A standardised protocol involving administration of 1 mg OHCbl intramuscularly on 3 consecutive days has been suggested, with a decrease in plasma or urine methylmalonic acid of 50% or more over 10 days considered a positive result [60]. Carnitine (50–100 mg/kg/day) is also required. For details of the planning of a protein-restricted diet, see ▶ Chap. 18. Some patients appear to become resistant to Cbl treatment. Therapy with AdoCbl has been attempted in cblB with and without success, possibly reflecting removal of the adenosyl group by the MMACHC protein after entry into cells. There have been reports of prenatal therapy with Cbl in AdoCbl deficiency. Most (90%) cblA patients improve on Cbl therapy, with 70% doing well long term. However, late severe renal and neurological complications, including optic atrophy, have been observed. Only 40% of cblB patients respond to Cbl, and the long-term survival of cblB patients is poorer than that of cblA patients [61].

Renal transplantation may be required in patients with end-stage renal disease. Liver transplantation has resulted in prevention of metabolic decompensation, although serum methylmalonic acid levels remain markedly elevated and neurological and renal deterioration may continue even in the absence of decompensation [62].

28.2.3 Methylcobalamin Deficiency: CblE (*MTRR*) & CblG (*MTR*)

Clinical Presentation

Formation of MeCbl is disturbed in the cblE and cblG disorders. The most common clinical findings are megaloblastic anaemia and neurological disease [63]. The latter includes poor feeding, vomiting, failure to thrive, developmental delay, nystagmus, hypotonia or hypertonia, ataxia, seizures and blindness. Cerebral atrophy may be seen on imaging studies of the central nervous system, and at least one cblE patient showed a spinal cord cystic lesion on autopsy. Most patients are symptomatic in the first year of life (peak at 3 months), but one cblG patient was not diagnosed until the age of 21 years and carried a misdiagnosis of multiple sclerosis. Another cblG patient, who was diagnosed during his fourth decade of life, had mainly psychiatric symptoms. Patients with minimal findings and without clear neurological features have also been reported.

Metabolic Derangement

The defect in cblE is deficiency of the enzyme methionine synthase reductase, which is required for the activation by reductive methylation of the methionine synthase apoenzyme. The cblG defect is caused by deficient activity of the methionine synthase apoenzyme itself.

Genetics

Both the cblE and cblG disorders are inherited in an autosomal recessive manner. Over 30 patients are known with each [63]. Approximately 20 mutations have been identified in the methionine synthase reductase gene, *MTRR*, in cblE patients [64]. The most common of these mutations is c.903 + 469 T > C, which may represent up to 25% of mutant alleles. Mutations in the methionine synthase gene, *MTR*, have been found in cblG patients [65]. The most common of these mutations is c.3518C > T (p.Pro1173Leu).

Diagnostic Tests

Homocystinuria and hyperhomocysteinaemia are almost always found in the absence of methylmalonic acidaemia, although one cblE patient had transient unexplained methylmalonic aciduria. Hypomethioninaemia and cystathioninaemia may be present, and there may be increased serine in the urine. Methionine synthase function is decreased in cultured fibroblasts from both cblE and cblG patients. Uptake of CNCbl is normal but synthesis of MeCbl is decreased in both disorders. Complementation analysis and gene sequencing distinguish cblE from cblG and cblD-HC patients.

Treatment and Prognosis

Both disorders are treated with OHCbl or MeCbl, 1 mg i.m., first daily and then once or twice weekly. Although the metabolic abnormalities are nearly always corrected, existing neurological findings and eye abnormalities such as nystagmus and impaired visual acuity tend to persist. Treatment with betaine (250 mg/kg/day) has been used, and one cblG patient was treated with L-methionine (40 mg/kg/day) and showed neurological improvement. In one family with cblE, a boy developed normally to the age of 14 years following maternal treatment with OHCbl during the second trimester and from birth, in contrast to his older brother treated with delay who showed significant developmental delay at 18 years. Some patients may benefit from high-dose folic or folinic acid treatment.

Folate Metabolism

Folic acid (pteroylglutamic acid) is plentiful in foods such as liver, leafy vegetables, legumes and some fruits. Its metabolism involves reduction to dihydrofolate (DHF) and tetrahydrofolate (THF), followed by addition of a single-carbon unit, which is provided by serine, glycine or histidine; this carbon unit occurs in various redox states (methyl, methylene, methenyl or formyl). Transfer of this single-carbon unit is essential for the endogenous formation of methionine, thymidylate (dTMP) and formylglycineamide ribotide (FGAR) and formylaminoimidazolecarboxamide ribotide (FAICAR), two intermediates of purine synthesis (Fig. 28.2). These reactions also allow regeneration of DHF and THF. The predominant folate derivative in blood and in cerebrospinal fluid is 5-methyltetrahydrofolate (the product of the methylenetetrahydrofolate reductase reaction).

Folate can be measured by folate binding protein assay in serum, red blood cells, or cerebrospinal fluid. Serum folate level is the most common test; red cell folate is thought to reflect body stores more accurately than serum folate, but on the whole adds little diagnostic value and is usually unavailable in the clinical setting. Differentiation of folate derivatives present in clinical samples requires mass spectrometric analysis and is not available in most clinical laboratories.

Several proteins have been shown to play a role in transport of folates across cellular membranes [66, 67]. The reduced folate carrier (RFC) supports a low-affinity high-capacity system for uptake of reduced folates at micromolar concentrations. It appears to play an important role in folate uptake by many types of cells, including haematopoietic cells. The folate receptors (FRα and FRβ) are a family of folate-binding proteins that are attached to the cell surface by a glycosylphosphatidylinositol anchor; they support a high-affinity low-capacity uptake system for 5-methyltetrahydrofolate and folic acid that is active at nanomolar concentrations of folate. The protein-coupled folate transporter (PCFT) supports uptake of reduced and oxidised folates at acid pH [68]. Uptake of folate in the intestine appears to depend on function of the PCFT and not RFC whereas transport of folate across the blood-brain barrier at the choroid plexus requires both PCFT and FRα [69].

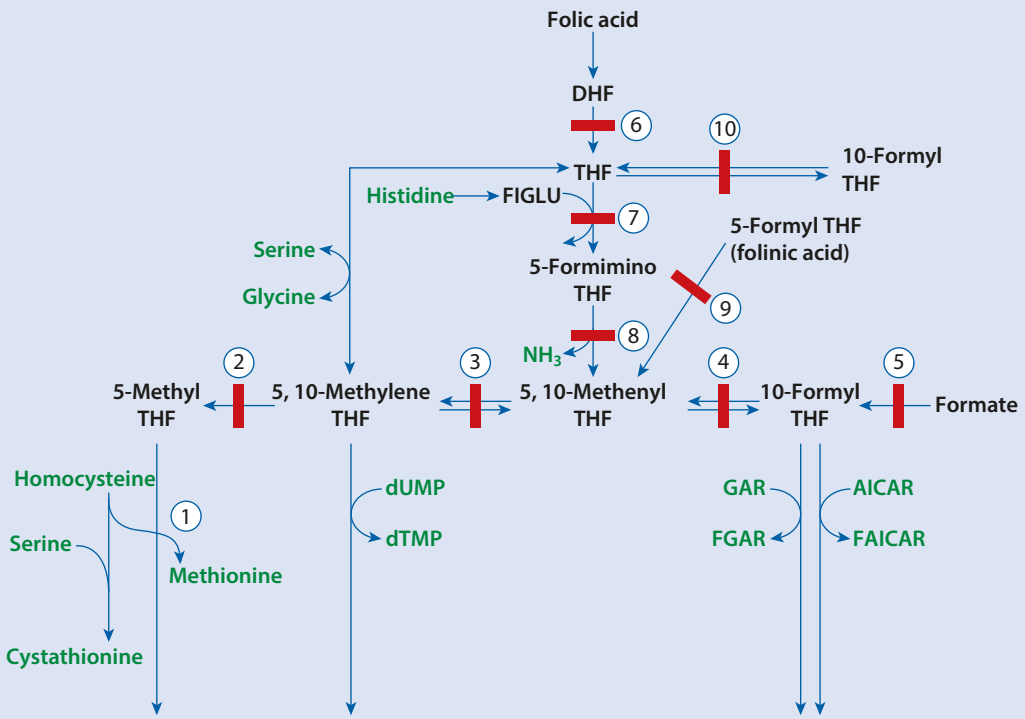

Fig. 28.2 Folic acid metabolism (genes in *italics*): **1**, methionine synthase; **2**, methylenetetrahydrofolate reductase (*MTHFR*); **3**, methylenetetrahydrofolate dehydrogenase (*MTHFD1*); **4**, methenyltetrahydrofolate cyclohydrolase (*MTHFD1*) **5**, formyltetrahydrofolate synthetase (*MTHFD1*); **6**, dihydrofolate reductase (*DHFR*); **7**, glutamate formiminotransferase (*FTCD*); **8**, formiminotetrahydrofolate cyclodeaminase (*FTCD*); **9**, methenyltetrahydrofolate synthetase (*MTHFS*); **10**, formyltetrahydrofolate dehydrogenase (*ALDH1L2*); AICAR aminoimidazole carboxamide ribotide, DHF dihydrofolate, dTMP, deoxythymidine monophosphate, dUMP deoxyuridine monophosphate, FAICAR formylaminoimidazole carboxamide ribotide, FGAR formylglycinamide ribotide, FIGLU formiminoglutamate, GAR glycinamide ribotide, THF tetrahydrofolate. Enzyme defects are indicated by *solid bars*

28.3 Disorders of Absorption and Metabolism of Folate

28.3.1 Hereditary Folate Malabsorption (Proton-Coupled Folate Transporter Deficiency, *SLC46A1*)

Clinical Presentation

This rare condition presents in the first months of life with severe megaloblastic anaemia, diarrhoea, stomatitis, failure to thrive and usually progressive neurological deterioration with seizures and sometimes with intracranial calcifications. Peripheral neuropathy has been seen, as have partial defects in humoral and cellular immunity [70, 71].

Metabolic Derangement

All patients have severely decreased intestinal absorption of oral folic acid or reduced folates, such as 5-formyltetrahydrofolic acid (formyl-THF, folinic acid) or 5-methyl-THF. There is also decreased transport of folate across the blood-brain barrier. Transport of folates across other cell membranes is not affected in this disorder. The disorder is the result of decreased function of the proton-coupled folate transporter (PCFT) [68]. Folate metabolism in cultured fibroblasts is normal.

Genetics

Approximately 30 patients with this disorder have been reported. It is caused by mutations affecting *SLC46A1*, which encodes the PCFT. It is an autosomal recessive trait.

Diagnostic Tests

Measurement of serum, red blood cell and CSF folate levels and a complete blood count and bone marrow analysis should be performed. The most important diagnostic features are the severe megaloblastic anaemia in the first few months of life, together with low serum folate levels. Measurements of related metabolite levels have been sporadically reported and inconsistently found abnormalities include increased excretion of formiminoglutamate, orotic aciduria, increased plasma sarcosine and cystathionine and low plasma methionine. Folate levels in CSF remain low even when blood levels are high enough to correct the megaloblastic anaemia [70]. Folate absorption can be investigated by measuring serum folate levels following an oral dose of between 5 and 100 mg of folic acid.

Treatment and Prognosis

High-dose oral folic acid (up to 60 mg daily) or lower parenteral doses in the physiological range correct the haematological and gastrointestinal abnormalities but are less effective in correcting the neurological findings and in raising the level of folate in the CSF. Folinic acid (5-formyl-THF) [71] is more effective in raising CSF levels and has been given in combination with high-dose oral folic acid. The clinical response to folates has varied with worsening seizures in some cases. It is important to maintain both blood and CSF folate in the normal range using parenteral or even intrathecal routes if necessary, although the optimal dose of folate is unknown. In some cases high oral doses of folinic acid (up to 400 mg orally daily) may eliminate the need for parenteral therapy.

28.3.2 Cerebral Folate Deficiency (Folate Receptor α Deficiency, *FOLR1*)

Clinical Presentation

This disorder usually presents in the first year of life, with psychomotor retardation, spastic paraplegia, cerebellar ataxia and dyskinesia and refractory myoclonic epilepsy, associated with normal blood folate levels and low folate levels only in the cerebrospinal fluid (CSF) [72]. Several affected children have developed autistic features. It is important to distinguish between the primary defect and secondary causes such as acquired (perinatal asphyxia, CNS infection) and genetic (Rett syndrome, Kearn Sayre disease, MTHFR deficiency, white matter disease) disorders which also have decreased cerebral folate levels (although, in general, these secondary defects show less depleted levels than folate receptor deficiency) [73].

Metabolic Derangement

There is a decreased level of 5-methyl-THF, the major circulating form of folate in the CSF, with normal blood levels of the vitamin. This is the result of decreased FRα (folate receptor α) function at the choroid plexus.

Genetics

Mutations in *FOLR1*, which encodes FRα, have been identified in a small number of families with cerebral folate deficiency [74]. The disorder segregates as an autosomal recessive trait in these families. Cerebral folate deficiency without *FOLR1* mutations has been attributed to antibodies directed against FRα.

Diagnostic Tests

Patients are characterised by decreased CSF levels of folate in the presence of normal serum folate levels.

Treatment and Prognosis

The cerebral folate deficiency syndrome responds exclusively to folinic acid (10–20 mg/day) and not to folic acid. Folinic acid therapy can restore CSF folate concentrations, reverse white matter choline and inositol depletion and improve clinical symptoms [74, 75].

28.3.3 Reduced Folate Carrier Deficiency (*SLC19A1*)

Clinical Presentation

A single patient in his teens has been reported with a homozygous mutation in the gene encoding the reduced folate carrier and recurrent severe megaloblastic anaemia, in the context of dietary insufficiency [76]. Diagnosis was complicated by the presence of low serum vitamin B_{12} levels, but his haematological symptoms did not respond to therapy with cyanocobalamin.

Metabolic Derangement

There is decreased uptake of folate by haematopoietic cell precursors due to decreased function of the reduced folate carrier.

Genetics

The patient was homozygous for a 3-bp deletion in *SLC19A1*, the gene encoding the reduced folate carrier, resulting in deletion of a highly conserved phenylalanine residue. In vitro analyses demonstrated that this sequence variant adversely affected reduced folate carrier function.

Diagnostic Tests

The patient had serum folate levels within the reference interval but decreased red blood cell folate. Levels of homocysteine and AICAR were elevated.

Treatment and Prognosis

The patient responded to therapy with folic acid.

28.3.4 Methylenetetrahydrofolate Dehydrogenase Deficiency (*MTHFD1*)

Clinical Presentation

Nine individuals from seven families have been reported. Affected individuals have had megaloblastic anaemia, severe combined immunodeficiency and atypical haemolytic uraemic syndrome [77–79]. Seizures, developmental delay and neuroimaging abnormalities have also been reported [72]. Two untreated patients died at 9 weeks of age.

Metabolic Derangement

Serum folate levels are within the reference range, while cerebrospinal fluid folate levels are reduced. The product of *MTHFD1* is a trifunctional cytoplasmic enzyme that catalyzes synthesis of 10-formyl-THF from THF and formate and its conversion to 5,10-methylene-THF. Biochemical studies demonstrated deficient 5,10-methylene-THF dehydrogenase specific activity in fibroblasts from four patients [80]. Studies in fibroblasts from the first identified patient showed adequate function of 10-formyl-THF-dependent purine biosynthesis with impairment of methylene-THF-dependent thymidylate synthesis and methyl-THF-dependent conversion of homocysteine to methionine [81]. Synthesis of MeCbl from exogenous CNCbl was somewhat reduced due to deficiency of methyl-THF [80].

Genetics

Mutations in *MTHFD1* have been identified in affected individuals in all three families, consistent with autosomal recessive inheritance [77–79].

Diagnostic Tests

Patients have normal serum folate levels and decreased cerebral folate levels. Serum total homocysteine is elevated, with normal or low-normal methionine levels. Diagnosis in all cases has depended on identification of mutations in *MTHFD1*.

Treatment and Prognosis

Treatment with oral folinic acid has been associated with reduction of total homocysteine to within the reference range and correction of megaloblastic marrow morphology, and with improved neurological function, although seizures in the initial patient were not corrected and maculopathy with retinal atrophy in a second patient was resistant to therapy. Two patients that had been treated long-term with folinic acid had normal neurological development at 8 and 22 years.

28.3.5 Dihydrofolate Reductase Deficiency (*DHFR*)

Clinical Presentation

Three families with apparent dihydrofolate reductase deficiency have been described [82, 83]. Findings included megaloblastic anaemia, cerebral folate deficiency and seizures, and in severe cases, pancytopenia, cerebral atrophy and severe developmental delay,

Metabolic Derangement

Plasma and red cell folate levels are within the normal range. However, there are relatively high levels of the oxidised forms of folates (dihydrofolate and folic acid), reflecting the deficiency in dihydrofolate reductase, which catalyses reduction of dihydrofolate to tetrahydrofolate, and (at a slower rate) folic acid to dihydrofolate.

Genetics

Homozygous mutations in *DHFR* have been identified in affected individuals in all three families, consistent with autosomal recessive inheritance. The mutations

affect well-conserved amino acid residues, and decreased dihydrofolate reductase function has been shown in affected individuals.

Diagnostic Tests

Patients have decreased cerebral folate levels. Serum and red cell folate levels are normal, but the proportion of tetrahydrofolate derivatives is decreased. This disorder can be differentiated from cerebral folate deficiency due to mutations in *FOLR1* by the presence of megaloblastic anaemia.

Treatment and Prognosis

Treatment with oral folinic acid has been associated with normalisation of red cell volume and of megaloblastic marrow morphology, and with improved neurological function. There may be transient improvement of seizures, but ultimately folinic acid therapy has not proved effective in seizure control. In severely affected individuals, neurological dysfunction and developmental delay persist despite therapy.

28.3.6 Glutamate Formiminotransferase Deficiency (*FTCD*)

Clinical Presentation

Patients have been identified on the basis of elevated blood levels of formiminoglutamate. Approximately 50 patients have been described but the clinical significance of this disorder has been unclear. Identification of multiple patients by newborn screening has led to the conclusion that the disorder is generally asymptomatic [84, 85].

Metabolic Derangement

Histidine catabolism is associated with a formimino group transfer to THF, with the subsequent release of ammonia and the formation of 5,10-methenyl-THF. A single octameric enzyme catalyses two different activities: glutamate formiminotransferase and formiminotetrahydrofolate cyclodeaminase. These activities are found only in the liver and kidney, and defects in either of them will result in formiminoglutamate excretion.

Genetics

The condition is caused by mutations in *FTCD* and is inherited as an autosomal recessive trait [84, 85]. When tested, expressed enzyme activity was 60% of controls.

Diagnostic Tests

Elevated formiminoglutamate and hydantoin propionate excretion and elevated levels of formiminoglutamate in the blood following a histidine load have been used traditionally to help to establish the diagnosis. Normal to high serum folate levels are found. Hyperhistidinaemia and histidinuria have been reported. Formiminoglutamate overlaps with C4 acylcarnitine on tandem mass spectrometry, which has allowed its detection on newborn screening.

Treatment and Prognosis

Formiminoglutamate excretion has responded in some cases to folate therapy. Since most cases have been asymptomatic, clinical benefit of treatment of this condition is unclear.

28.3.7 Methylenetetrahydrofolate Reductase Deficiency (*MTHFR*)

This section is restricted to the severe form of this deficiency. The role of polymorphisms in methylenetetrahydrofolate reductase (MTHFR) with respect to the risk for common disease, such as neural tube defects or cardiovascular disease, is beyond the scope of this chapter [86].

Clinical Presentation

Over 200 patients with severe MTHFR deficiency have been described [87–90]. Most were diagnosed in infancy, and more than half presented in the first year of life. The most common early manifestation was progressive encephalopathy with apnoea, seizures, microcephaly, hypotonia, feeding problems and failure to thrive. However, patients became symptomatic at any time from infancy to adulthood, and in the older patients ataxic gait, psychiatric disorders (schizophrenia) and symptoms related to cerebrovascular events have been reported. At least one adult with severe enzyme deficiency was completely asymptomatic. Autopsy findings have included dilated cerebral vessels, microgyria, hydrocephalus, perivascular changes, demyelination, gliosis, astrocytosis and macrophage infiltration. In some patients, thrombosis of both cerebral arteries and veins was the major cause of death. There have been reports of patients with findings similar to those seen in subacute degeneration of the spinal cord due to Cbl deficiency. It is important to note that MTHFR deficiency is not associated with megaloblastic anaemia.

Metabolic Derangement

Methyl-THF is the methyl donor for the conversion of homocysteine to methionine, and in MTHFR deficiency its lack results in an elevation of total plasma homocysteine levels and decreased levels of methionine. Total CSF folate levels are also severely reduced. The block in the conversion of methylene-THF to methyl-THF does not result in the trapping of folates as methyl-THF and

does not interfere with the availability of reduced folates for purine and pyrimidine synthesis in contrast to disorders at the level of methionine synthase. This explains why patients do not have megaloblastic anaemia. It is not clear whether the neuropathology in this disease results from the elevated homocysteine levels, from decreased methionine and resulting interference with methylation reactions or from some other metabolic effect. It has been reported that individuals with a severe deficiency in MTHFR may be at increased risk following exposure to nitrous oxide anaesthesia [91].

Genetics

MTHFR deficiency is inherited as an autosomal recessive disorder. Over 100 mutations causing severe deficiency have been described in *MTHFR*, in addition to polymorphisms that result in intermediate enzyme activity and that may contribute to disease in the general population [46, 88]. Most of these mutations are restricted to one or two families. Exceptions are a c.1542G > A mutation that results in a splicing mutation, that was seen in 21 of 152 mutant alleles in a large study [88]; and a c.1141C > T mutation that is present at high frequency in the Old Order Amish [92]. A recent review has summarized all known mutations [89]; mutations in this publication are listed using the original as well as the HGVS numbering system.

Diagnostic Tests

Because methyl-THF is the major circulating form of folate, serum folate levels may sometimes be low. There is a severe increase of plasma total homocysteine (60–320 μmol/l, with controls less than 14 μmol/l), together with low plasma methionine levels ranging from zero to 18 μmol/l (mean: 12 μmol/l, range of control means from different laboratories: 23–35 μmol/l). Although neurotransmitter levels have been measured in only a few patients, they are usually low. Direct measurement of MTHFR specific activity can be performed in liver, leukocytes, lymphocytes and cultured fibroblasts. Severe MTHFR deficiency has been associated with complete lack of enzyme activity, and with mutations causing reduced affinity for NADPH, decreased FAD responsiveness, abnormal inhibition of enzyme activity by S-adenosylmethionine and reduced affinity for methylene-THF [88]. The level of residual enzymatic activity is associated with disease severity, whereby those with strongly reduced or completely absent activity are more likely to present with earlier, more severe disease [89].

Treatment and Prognosis

It is important to diagnose MTHFR deficiency early because, in the infantile forms, the only patients who have done well are those who were treated from birth. This underpins the need for effective newborn screening that currently needs to be based on a critical selection of the window for low methionine supported by second tier testing for total homocysteine [5]. Early treatment with betaine following prenatal diagnosis has resulted in the best outcome [93]. Suggested doses have been in the range of 2–3 g/day (divided twice daily) in young infants and 6–9 g/day in children and adults, good results were claimed with a dosage of 100/mg/kg/day [94]. Betaine is a substrate for betaine-homocysteine S-methyltransferase (BHMT), which converts homocysteine to methionine but is mainly active in the liver. Therefore, betaine may be expected to have the doubly beneficial effect of lowering homocysteine levels and raising methionine levels. Because BHMT is not present in the brain, the central nervous system effects must be mediated through the effects of the circulating levels of metabolites. The dose of betaine should be modified according to plasma levels of homocysteine and methionine. Other therapeutic agents with unproven efficacy but possibly helpful in isolated cases include folic acid or reduced folates, methionine, pyridoxine, Cbl and carnitine. In 3 patients with severe MTHFR deficiency measurable 5-methyltetrahydrofolate in cerebrospinal fluid was only achieved with mefolinate (5-methyltetrahydrofolate) supplements and not with either folic acid or folinic acid [95]. Most of the treatment protocols omitting betaine have not been effective. Dramatic improvement was reported in a patient with severe enzyme deficiency following early introduction of methionine supplements.

28.3.8 Methenyltetrahydrofolate Synthetase Deficiency (*MTHFS*)

Clinical Presentation

Three patients have been reported with deficiency in 5,10-methenyltetrahydrofolate synthetase (MTHFS) [96, 97]. They have presented with global developmental delay, microcephaly, hypotonia, seizures, spasticity and cerebral hypomyelination. Cerebral 5-methyltetrahydrofolate levels were low or low normal. One patient had macrocytic anaemia; another had unexplained episodes of hyperthermia.

Metabolic Derangement

MTHFS catalyzes the ATP-dependent conversion of 5-formyltetrahydrofolate (folinic acid) to methenyltetrahydrofolate. Folinic acid plays no known role in folate-dependent one-carbon metabolism; it is generated from methenyltetrahydrofolate in a secondary reaction catalyzed by serine hydroxymethyltransferase. It has been suggested the folinic acid functions as a storage form of

tetrahydrofolate. Deficiency of MTHFS results in trapping of folinic acid generated by the activity of serine hydroxymethyltransferase and accumulation of folinic acid in cells.

Genetics

This is an autosomal recessive disorder caused by mutations in *MTHFS*.

Diagnostic Tests

Patients have been characterized by low or low normal cerebral folate levels that did not respond to therapy with folinic acid.

Treatment and Prognosis

Treatment with 5-methyltetrahydrofolate resulted in increase of cerebral folate levels and increased alertness and vocalizations in one patient; no change in EEG or MRI findings were detected. Long-term response to this therapy is not known.

28.3.9 10-Formyltetrahydrofolate Dehydrogenase Deficiency (*ALDH1L2*)

Clinical Presentation

A single patient with ichthyosis, significant developmental delay and hypotonia has been reported [98]. There was increasing hyperactivity and attention deficit in his teens. Diagnosis was complicated by previous identification of a deleterious RPS6KA3 mutation, which causes Coffin-Lowry syndrome, but did not explain all clinical features.

Metabolic Derangement

The patient had deficiency of the mitochondrial form of 10-formyl-THF dehydrogenase, which catalyses breakdown of 10-formyl-THF to CO2 and THF with generation of NADPH from NADP+. This results in increased cellular levels of 10-formyl-THF and possibly decreased NADPH levels.

Genetics

The disorder is apparently the result of compound heterozygous mutations in *ALDH1L2*, which encodes the mitochondrial form of formyl-THF dehydrogenase [98].

Diagnostic Tests

Abnormalities on MRI and MR (^{1}H-MRS) spectroscopy were observed and metabolic changes were reported in cultured fibroblasts.

Treatment and Prognosis

Not known.

28.3.10 Serine Hydroxymethyltransferase 2 Deficiency (*SHMT2*)

Clinical presentation

Five patients from four families have been recently described with SHMT2 deficiency [99]. Each presented with a similar phenotype, characterized by congenital microcephaly, dysmorphic features, intellectual disability, and motor dysfunction, in the form of spastic paraparesis, ataxia, and/or peripheral neuropathy. Also, four out of five patients showed hypertrophic cardiomyopathy or atrial-septal defects, which tended to progress over time. MRI revealed corpus callosum abnormalities in all patients and perisylvian polymicrogyria-like pattern in four patients.

Metabolic Derangement

In plasma all metabolites were in the normal range whereas in fibroblasts affected individuals showed a significant decrease in glycine/serine ratios compared to controls. Folate metabolism was also impaired in that 5-methyltetrahydrofolate levels were increased in relation to total folate. The substrate of SHMT2, tetrahydrofolate (THF), was undetectable in mitochondria-enriched control fibroblast samples, but low levels were measurable in patient fibroblasts. ATP and ROS production were also impaired in fibroblasts.

Genetics

Biallelic SHMT2 variants were identified in all individuals. They were missense/in-frame deletion and homozygous in one family.

Diagnostic Tests

Consistent clinical manifestations in all individuals, albeit of variable severity, seem to constitute a well-defined and recognizable clinical syndrome. Studies of folate forms and amino acids in fibroblasts, together with genetic analysis confirm the diagnosis.

Treatment and Prognosis

Not known.

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Disorders of Thiamine and Pyridoxine Metabolism

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Contents

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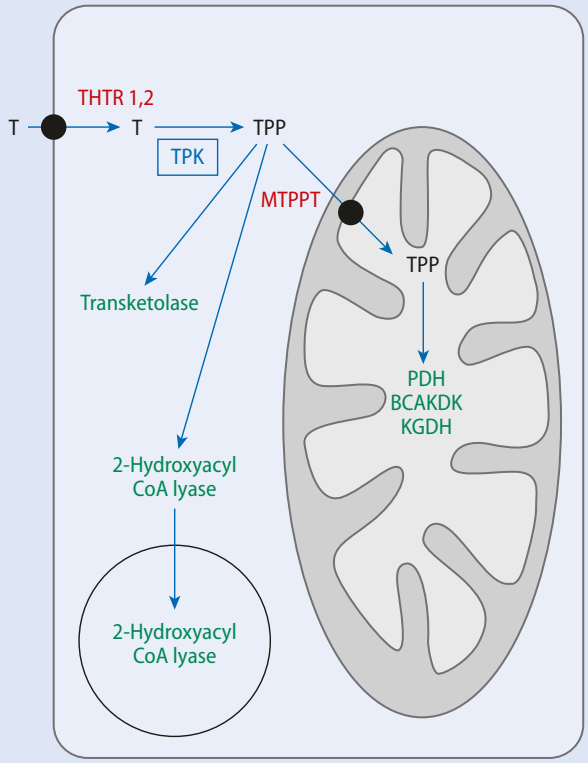

Fig. 29.1 Thiamine transport. THTR1 and THTR2 thiamine transporter 1 and 2, TPP thiamine pyrophosphate, MTPPT mitochondrial, TPP transporter, TPK thiamine pyrophosphate kinase, PDH pyruvate dehydrogenase, BCAKDK branched chain aminoacid dehydrogenase kinase, KGDH ketoglutarate dehydrogenase

Thiamine Metabolism

Thiamine is transported across cell membranes by two closely related transporters, THTR1 and THTR2, encoded by *SLC19A2* and *SLC19A3*, respectively (Fig. 29.1). Both transporters are widely expressed in the body, but they differ in kinetic properties and in the level of expression in different tissues. In the upper small intestine, where dietary thiamine is absorbed, THTR2 is the major transporter at the luminal surface whereas THTR1 predominates at the basal surface. The active cofactor of thiamine, thiamine pyrophosphate (TPP), is formed in the cytoplasm by the enzyme thiamine pyrophosphokinase (TPK). There the cofactor is attached directly to the transketolase and 2-hydroxyacyl CoA lyase apoproteins, while a TPP transporter (MTPPT) in the inner mitochondrial membrane delivers the cofactor to the α-ketoacid dehydrogenases in the mitochondrial matrix.

Introduction

Thiamine (vitamin B_1) is a water-soluble vitamin transported across cell membranes by two closely related transporters, THTR1 and THTR2. The active cofactor of thiamine, thiamine pyrophosphate (TPP), is formed in the cytoplasm by the enzyme thiamine pyrophosphokinase. TPP enters mitochondria with a specific TPP transporter.

Pyridoxine (vitamin B_6) is a water-soluble vitamin with broad availability from various food sources, including dairy products, meat, cereals and vegetables. The three vitamers, pyridoxal, pyridoxamine and pyridoxine and their phosphorylated esters are absorbed in the small intestine. Within the cells vitamers are re-phosphorylated by kinases and further oxidised to the active cofactor pyridoxal 5′-phosphate (PLP) by pyridox(am)ine 5′- phosphate oxidase (PNPO). Within the cell PLP homeostasis is regulated by the PLP-binding protein (PLPBP, formerly called PROSC).

29.1 Disorders of Thiamine (vitamin B_1) Metabolism

Thiamine (vitamin B_1) has long been recognised as an essential dietary component. The minimal daily requirement is about 0.5 mg/1000 Kcal and this is normally provided by a well-balanced diet. Requirements do vary,

however, and are increased in parallel with carbohydrate intake, during pregnancy and lactation, in hypermetabolic states and in infants. The active form of the vitamin is thiamine pyrophosphate (TPP) and this is a coenzyme for a number of important metabolic enzymes: pyruvate dehydrogenase, branched chain α-ketoacid dehydrogenase, α-ketoglutarate dehydrogenase, transketolase and the peroxisomal enzyme, 2-hydroxyacyl CoA lyase. As an essential component of these highly regulated enzymatic reactions, thiamine plays a crucial role in carbohydrate metabolism and the metabolic switch from the fed to the fasting state. Acute thiamine deficiency states (such as total parenteral nutrition without thiamine supplement) are life threatening emergencies and present as cardiac failure, Gayet Wernicke encephalopathy, or lactic acidosis [1, 2]. Metabolic markers are hyperlactatemia with hyperpyruvic acidaemia, a normal lactate to pyruvate ratio, slight elevation of branched chain amino acids in plasma, presence of α-ketoglutarate, pyruvate and branched chain α- ketoacids in urine, with a positive DNPH reaction, and low transketolase activity in red blood cells. However, these markers are rarely available under emergency conditions and diagnosis relies on primary care physicians in the emergency room and the life-saving therapeutic test of administration of thiamine intravenously at a dose of 5 mg/kg/day. This dose may be given without risk of adverse effects.

Thiamine-dependent inborn errors of metabolism are rare and can arise from defects in thiamine transport or the biosynthesis and intracellular transport of thiamine pyrophosphate. They can also be due to intrinsic structural defects in thiamine-dependent enzymes which alter the affinity of the enzyme for the cofactor (◘ Table 29.1). Patients with these different conditions present with a wide range of clinical and biochemical manifestations, reflecting different patterns and degrees of involvement of the thiamine-dependent enzymes. Many details remain to be elucidated about the natural history and response to treatment of these conditions. In many cases, patients only respond to high doses of thiamine, however, these are readily tolerated and can be used safely.

29.1.1 Thiamine Metabolism Dysfunction Syndrome 1 (*SLC19A2*, THTR1 Deficiency)

Clinical Presentation

THTR1 deficiency results in thiamine-responsive megaloblastic anaemia [3, 4]. The hallmarks of this condition are megaloblastic anaemia, diabetes mellitus and sensorineural deafness. The anaemia is often the first manifestation and develops during infancy or early childhood. Although the anaemia is megaloblastic in character, ringed sideroblasts may be present in the marrow and some patients develop thrombocytopaenia. Diabetes usually develops later in childhood, although patients have been reported with neonatal diabetes. Other manifestations of the condition include cardiac abnormalities, short stature, retinal abnormalities, optic atrophy and stroke-like episodes. Cardiac involvement includes arrhythmias, congenital malformations and cardiomyopathy [5].

Metabolic Derangement

There have been few biochemical studies in patients with THTR1 deficiency. It is likely that the megaloblastic erythropoiesis is related to deficiency of transketolase in the pentose phosphate shunt, with impaired synthesis of ribose-5-phosphate. The blood thiamine and TMP concentrations have been reduced in some patients, but are often normal. This is consistent with experimental evidence that intestinal absorption of thiamine does not depend on this transporter. Apart from the anaemia, many of the features of THTR1 deficiency are shared with various mitochondrial diseases, however, biochemical defects in energy metabolism have not been widely documented. The blood and cerebrospinal fluid lactate concentrations are usually normal. The diabetes is non-autoimmune and insulin secretion is not impaired, at least initially. Diabetic ketoacidosis has developed in a small number of patients.

Genetics

Many mutations in *SLC19A2* have now been described, with small deletions, duplications, frameshift and nonsense mutations more common than missense mutations. Many are clustered in exon 2 of the gene. Most have been found in single individuals and there is no clear genotype/phenotype correlations [6].

Treatment and Prognosis

Patients with THTR1 deficiency generally respond well to thiamine supplementation, but not all clinical manifestations respond to the same extent. Treatment with doses of thiamine between 25-50 mg/day usually produces a good response in the anaemia, diabetes and cardiac arrhythmias. Deafness and other neurological features do not usually respond as well, however this may be improved with early diagnosis and treatment. With long term treatment, thiamine-responsiveness may decrease and previously well-controlled patients may become transfusion- and insulin-dependent [4].

Table 29.1 Disorders of Thiamine Metabolism

Defect (mechanism)	Disorder	Diagnostic tests	Effective dose of thiamine
Defective intake	Total parenteral nutrition without B_1 supplementation Breast-fed babies of B_1 deficient mothers Berri-Berri, Wernicke encephalopathy	Raised blood lactate Excretion of α-ketoacids in urine Low erythrocyte transketolase	2-4 mg/day (20 mg in emergency)
Defective transport	Thiamine metabolism dysfunction syndrome 1: Thiamine transporter 1 (THTR1, *SLC19A2*) deficiency: Thiamine-responsive megaloblastic anaemia with diabetes and deafness	Megaloblastic anaemia Hyperglycemia No specific abnormalities relating to thiamine or TPP DNA testing	25-50 mg/day
Defective transport	Thiamine metabolism dysfunction syndrome 2: Thiamine transporter 2 (THTR2, *SLC19A3*) deficiency: Biotin/thiamine-responsive basal ganglia disease	Reduced free thiamine in CSF Raised blood and cerebrospinal fluid lactate (only in some patients) DNA testing	50–100 mg/day (Biotin 2–10 mg/day)
Defective mitochondrial TPP transport	Thiamine metabolism dysfunction syndrome 3: microcephaly, Amish type Thiamine metabolism dysfunction syndrome 4: bilateral striatal degeneration and progressive polyneuropathy type Mitochondrial TPP transporter (*SLC25A19*) deficiency	Raised blood and CSF lactate Urinary excretion of α-ketoglutarate (in Amish microcephaly type) DNA testing	400-600 mg/day Some effect in patients with striatal degeneration and polyneuropathy if diagnosed early
Defective cofactor biosynthesis	Thiamine metabolism dysfunction syndrome 5: Episodic encephalopathy type: TPK1 deficiency	Low blood TPP Raised blood and CSF lactate Urinary excretion of α-ketoglutarate DNA testing	100-500 mg/day (more effective with early diagnosis)
Defective binding of TPP to apoenzyme	Thiamine-responsive pyruvate dehydrogenase complex deficiency	Raised blood lactate Normal L/P ratio DNA testing	50-1000 mg/day
Defective binding of TPP to apoenzyme	Thiamine-responsive maple syrup urine disease	Raised plasma leucine, isoleucine, valine and alloisoleucine DNA testing	50-1000 mg/day

29.1.2 Thiamine Metabolism Dysfunction Syndrome 2 (*SLC19A3*, THTR2 Deficiency)

Clinical Presentation

Deficiency of this transporter most commonly results in biotin-responsive basal ganglia disease [7]. Onset is usually during childhood when patients develop a subacute encephalopathy characterised by speech and swallowing difficulty, confusion, dystonia and rigidity. This is associated with symmetric lesions in the caudate nucleus and putamen. However, patients with different clinical presentations have also been identified and the clinical spectrum continues to evolve.

The earliest, and most severe presentation of this condition is infantile Leigh syndrome. Patients present soon after birth with seizures, feeding difficulty and respiratory distress. Typical MRI findings in the brain are progressive cerebral atrophy and bilateral lesions in the thalami and basal ganglia [7]. Later presentations, in adolescence or adulthood, include Leigh-like [8], or Wernicke-like encephalopathy [9] and generalised dystonia and seizures [10].

Metabolic Derangement

The most significant biochemical finding is a marked reduction in the concentration of free thiamine in CSF [11]. Blood and CSF lactate concentration is elevated in

only minority of patients, and there is increased, but variable urinary excretion of organic acids.

Genetics

In the original cohort of patients from Saudi Arabia, there was a high degree of parental consanguinity and a common missense mutation, p.Thr422Ala, in *SLC19A3* [12]. In the remaining patients, different missense, nonsense, splicing and frameshift mutations have been identified. There is no clear genotype-phenotype correlation in relation to course, outcome or response to treatment.

Treatment and Prognosis

The first patients to be described all had a rapid response to 5-10 mg/day biotin and remained symptom-free provided the diagnosis was established promptly and treatment was continued. Other patients have been treated effectively with a combination of biotin and thiamine (2-10 mg/day of biotin and 50-100 mg/day of thiamine), or thiamine alone. The failure of some patients to respond to biotin alone, and the effectiveness of thiamine supplementation means that this is now the usually recommended treatment. In a controlled study of combined biotin and thiamine versus thiamine alone, all patients diagnosed and treated early had a favourable outcome. There was no long term difference between the two treatments in terms of sequelae, but recovery from acute episodes was slightly faster in patients treated with both biotin and thiamine [13]. Patients with severe infantile Leigh syndrome do not respond as well to thiamine treatment and generally have a much poorer prognosis.

29.1.3 Thiamine Metabolism Dysfunction Syndrome 3 (Microcephaly, Amish Type) and Thiamine Metabolism Dysfunction Syndrome 4 (Bilateral Striatal Degeneration and Progressive Polyneuropathy Type): Mitochondrial TPP Transporter deficiency *(SLC25A19)*

Clinical Presentation

Deficiency of the mitochondrial TPP carrier (*SLC25A19*) was first described in patients with Amish lethal microcephaly. These patients have a distinctive facial appearance and a characteristic pattern of brain abnormalities [14]. Death usually occurs within the first six months.

A different presentation of TPP transporter deficiency has been identified in five families from outside of the Amish community. These patients experienced acute episodes of flaccid paralysis and encephalopathy, with motor and sensory neuropathy, precipitated by intercurrent illness. Age at presentation ranged from early childhood to teenage and patients developed a progressive axonal neuropathy, dystonia and dysarthia [15]. MRI changes were present in the caudate nucleus and putamen, but not the globus pallidus.

Metabolic Derangement

In patients with Amish lethal microcephaly, increased urinary excretion of α-ketoglutarate is a consistent finding, however this was absent in the patients from the unrelated families described subsequently. In these patients, the lactate concentration in cerebrospinal fluid was raised during acute episodes.

Genetics

Patients from the Amish community with lethal microcephaly have a common missense mutation, p.Gly177Ala in *SLC25A19* [16]. Different missense mutations have been identified in the reported patients from outside the Amish community.

Treatment and Prognosis

Most patents with Amish type microcephaly die during infancy. Some of the patients with striatal degeneration and polyneuropathy with early diagnosis have improved with thiamine treatment, although one patient with chronic symptoms, diagnosed at age 18, did not respond.

29.1.4 Thiamine Metabolism Dysfunction Syndrome 5 (Episodic Encephalopathy Type, TPK1 Deficiency)

Clinical Presentation

This is a rare condition, with only twenty patients reported to date (reviewed in [17]). Patients usually present during early childhood with episodic encephalopathy, ataxia, psychomotor retardation, dystonia and dysarthria and seizures. In one family, two siblings developed generalised dystonia without any episodes of encephalopathy or ataxia. Brain MRI changes include global atrophy and abnormal signal in the cerebellum, dentate nuclei, basal ganglia, brain stem and spinal cord.

Metabolic Derangement

Elevated blood and CSF lactate concentrations during episodes of ataxia, and enhanced urinary excretion of α-ketoglutarate are consistent findings. Blood and muscle TPP concentrations are significantly reduced and

measurement of the blood concentration is an effective screening test. The major biochemical consequence of the enzyme defect appears to be deficiency of pyruvate and α- ketoglutarate dehydrogenases, however the activity of these enzymes in vitro is normal in the presence of TPP.

Genetics

A variety of mainly missense mutations in *TPK1* have been identified, with no common mutation in unrelated families.

Treatment and Prognosis

Of the twenty reported patients, five died in childhood. Fourteen of the patients received thiamine supplementation (range (100-500 mg/day). Eight improved significantly, while the remainder (in whom neurological abnormalities were already established at diagnosis), showed no improvement.

29.1.5 Thiamine-Responsive α-ketoacid Dehydrogenase Deficiencies

In some individuals, the normal dietary thiamine intake is not sufficient to sustain function of some TPP-dependent enzymes. This is the case in a small number of patients with pyruvate dehydrogenase deficiency and maple syrup urine disease, in whom high doses of thiamine have been reported to improve the clinical and/or biochemical features. The impaired enzyme activity in these patients is proposed to result from a structural defect which reduces the affinity of the enzyme for the cofactor, but which can be overcome if the cofactor concentration is increased by pharmacological doses of the vitamin precursor.

29.1.6 Thiamine-Responsive Pyruvate Dehydrogenase Deficiency

Clinical Presentation

Over 20 patients with pyruvate dehydrogenase deficiency have been claimed to be thiamine- responsive [18]. They usually present later, and are less severely affected, than is usual with this condition. Most common features are delayed development and hypotonia from late infancy, sometimes with episodes of ataxia in association with intercurrent illness. The great majority have lesions in the brain characteristic of Leigh Syndrome. A small number of these patients have normal development and cognition, but they may develop problems due to peripheral neuropathy. The rare adult patient with unusual presentation of pyruvate dehydrogenase deficiency may also respond to thiamine supplementation.

Biochemical Derangement

Blood and cerebrospinal fluid lactate concentrations are often normal or elevated only during acute episodes. In a number of patients, in vitro studies with cultured fibroblasts have been performed to correlate the clinical response with correction of the enzyme defect in the presence of excess TPP, however, these do not always yield unequivocal results.

Genetics

The TPP binding site is shared between the α and β subunits of the E1 component of the pyruvate dehydrogenase complex, however all thiamine-responsive patients identified to date have had mutations in *PDHA1,* the gene for the E1α subunit. The mutations are missense changes, mostly involving amino acid residues adjacent to the TPP binding site.

Treatment and Prognosis

It is difficult to establish definitively if any of the reported cases of thiamine-responsive pyruvate dehydrogenase deficiency are truly responsive. Almost all patients have received other treatments, as thiamine alone has not controlled symptoms. Doses of thiamine have varied widely from 50–1200 mg/day, and while this has led to clinical improvement in some cases, more often only the biochemical abnormalities have normalised and the clinical course has remained unaltered. Reports of initial improvement are rarely followed up with documentation of later outcome, and only a small number of patients have survived to adolescence with normal cognitive development.

29.1.7 Thiamine-Responsive Maple Syrup Urine Disease

Clinical Presentation

This has been described in over 10 patients [19]. Presentation is usually similar to the intermediate form of maple syrup urine disease, with episodes of ketoacidosis or ataxia in late infancy, and delayed development.

Metabolic Derangement

These patients have elevated branched chain amino acid and α-ketoacid concentrations in blood and

urine at diagnosis which reduce, but do not necessarily normalise, with thiamine supplementation. Episodes of acute decompensation with severe ketoacidosis are less common than in classic maple syrup urine disease and are usually suppressed with thiamine treatment.

Genetics

Almost all thiamine-responsive maple syrup urine disease patients have mutations in *DBT* the gene for the E2 component of the complex [20] and this may reflect the fact that binding of the E1 enzyme to the E2 core of the complex influences its affinity for TPP. A single thiamine-responsive patient with a mutation in *BCKDHB*, encoding the E1β subunit, has been reported.

Treatment and Prognosis

It is again difficult to assess the status of thiamine-responsiveness in patients with maple syrup urine disease. Patients have been given a wide range of thiamine dosage, up to 1000 mg/day, and most have also received dietary branched chain amino acid restriction. There are few long term follow up studies, although several patients remain healthy as adults, with normal cognitive function and no episodes of metabolic decompensation.

29.2 Disorders of Pyridoxine Metabolism

Vitamin B_6 Metabolism

Pyridoxine (vitamin B_6) is a water-soluble vitamin with broad availability from various food sources, including dairy products, meat, cereals and vegetables. The three vitamers, pyridoxal, pyridoxamine and pyridoxine and their phosphorylated esters are absorbed in the small intestine. For cellular uptake and transport across the blood-brain barrier, phosphorylated forms undergo dephosphorylation by intestinal phosphatases and tissue non-specific alkaline phosphatase (TNSAP) respectively. The transport mechanism of B_6 vitamers across cell membranes has not yet been fully elucidated, but it is assumed, that there are two different transporters, one across the endothelium and one across the mitochondrial membrane [21]. Within the cells vitamers are rephosphorylated by kinases and further oxidised to the active cofactor pyridoxal 5'-phosphate (PLP) by pyridox(am)ine 5'-phosphate oxidase (PNPO). As free PLP is highly reactive by its aldehyde group, its intracellular concentration is tightly regulated by negative feedback mechanisms as well as by the action of PLPBP (Fig. 29.2).

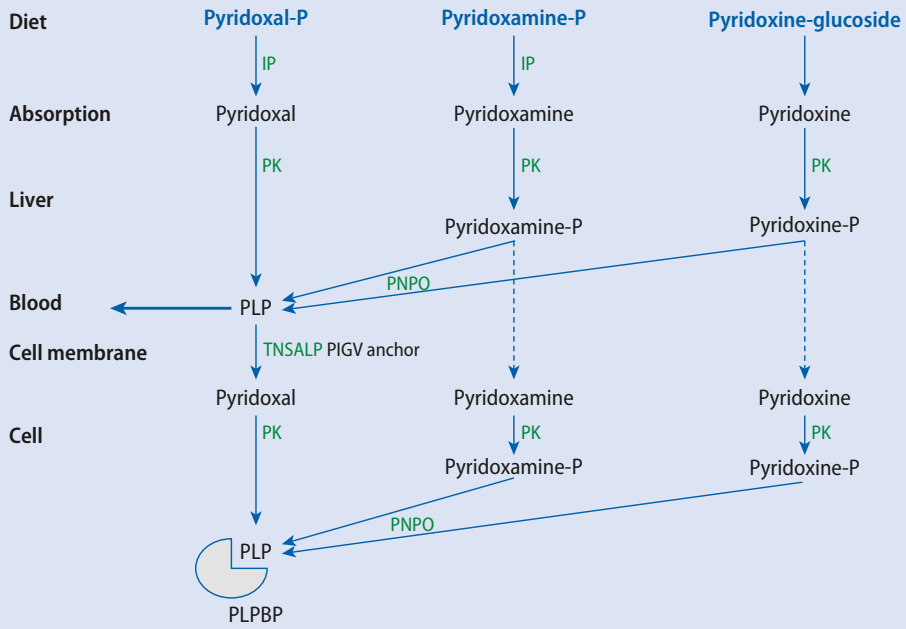

Fig. 29.2 Pyridoxine metabolism. IP intestinal phosphatases, PK pyridoxine kinase, PNPO pyridox(am)ine 5′-phosphate oxidase, TNSAP tissue non-specific alkaline phosphatase, PLPBP pyridoxal 5′-phosphate binding protein

Introduction

While the liver seems to be the most important organ of pyridoxal 5'-phosphate (PLP) formation, pyridox(am)ine 5'-phosphate oxidase (PNPO) is expressed in various cell types including neurons. PLP is one of the most abundant cofactors and, in humans participates in about 70 reactions mainly in amino acid and neurotransmitter metabolism. The daily requirement is 0.1 to 0.3 mg/day in infants and 1.2-1.4 mg/day in adults.

Systemic vitamin B_6 deficiency causes seizures, failure to thrive and anemia in a variety of species, and also human infants fed a formula with low vitamin B_6 content due to overheating during sterilisation. Nutritional vitamin B_6 deficiency is rarely seen nowadays and usually occurs together with other vitamin deficiencies in malnutrition or in association with severe chronic disease.

There are several mechanisms that lead to an increased requirement for pyridoxine and/or PLP [21] (Table 29.2): (i) inborn errors affecting the pathways of B_6 vitamer metabolism: PNPO deficiency, alkaline phosphatase defects and congenital hyperphosphatasia;

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Table 29.2 Disorders of Pyridoxine Metabolism

PLP related mechanism	Biochemical abnormalities			Response to vitamin B_6
	Urine	Plasma	CSF	
Coeliac disease Chronic dialysis Malabsorption, depletion	↑ xanthurenic acid	↑ threonine, glycine and serine		To very low doses of pyridoxine
Drug interaction (eg. hydrazines, D- penicillamine, enzyme inducing anticonvulsants)		↑ homocysteine		Preventive B_6 supplementation
PNPO Deficiency Reduced PLP formation	Vanillactate[a]	↑ PM and PM/PA	↓ to normal PLP° sec. AA and NT changes NTchanges	Mainly to PLP, in certain mutations also pyridoxine
Congenital Hypophosphatasia Reduced PLP uptake		↓ AP, ↓Ph, ↑ Ca ↑PLP		To pyridoxine (or PLP)
Congenital Hyperphosphatasia Reduced PLP uptake		↑ AP		unknown
PLPBP Deficiency (formerly PROSC)			↓ PLP, secondary AA and NT changes°	To pyridoxine or PLP
Antiquitin Deficiency (PDE) PLP inactivation	AASA, P6C ↑6-oxo PIP	↑ Pipecolic acid[a] ↑6-oxo PIP	↑ AASA, ↑6-oxo PIP ↓ PLP° secondary AA and NT changes	To pyridoxine (or PLP)
Hyperprolinemia II PLP inactivation	↑ Prolin, P5C	↑ Prolin, P5C		To pyridoxine (or PLP)
Classical Homocystinuria Chaperone	↑ homocyst(e)ine	↑↑ homocysteine, methionine		To pyridoxine in about 50% of patients
Gyrate atrophy, OAT Chaperone		↑ ornithine		To pyridoxine in some patients
X-linked sideroblastic anaemia		Enzyme assay in RBC and DNA		To pyridoxine in about 90% of patients

AA aminoacid, AP alkaline phosphatase, AASA alpha aminoadipic acid, Ca calcium, PA pyridoxic acid, 6-oxo PIP 6-oxo pipecolate, PDE pyridoxine dependent epilepsy, P6C piperideine-6-carboxylate, P5C pyrrolin-5-carboxylate, Ph phosphate, PLP pyridoxal 5'-phosphate, PM pyridoxamine, PLPBPD pyridoxal 5′-phosphate binding protein deficiency, PNPO pyridox(am)ine 5'-phosphate oxidase, NT neurotransmitter, OAT ornithine delta-aminotransferase

[a]Inconsistent findings, °before specific treatment with vitamin B_6

(ii) inborn errors that lead to accumulation of small molecules that react with PLP and inactivate it: hyperprolinemia type II and antiquitin deficiency (pyridoxine dependent epilepsy); (iii) inborn errors affecting intracellular PLP homeostasis: PLP-binding protein (PLPBP) deficiency (formerly named PROSC deficiency) and (iv) specific PLP dependent enzymes: X-linked sideroblastic anemia, classical homocystinuria, gyrate atrophy of the choroid; (v) drugs as D-penicillamine or isozianid that affect the metabolism of B_6 vitamers or react with PLP; (vi) coeliac disease, which is thought to lead to malabsorption of B_6 vitamers or renal dialysis, which leads to increased losses of B_6 vitamers from the circulation;

There are currently six known inborn errors of metabolism, all of autosomal recessive inheritance, that lead to vitamin B_6 dependent epilepsy, either by inactivation, reduced formation, reduced cellular uptake of PLP or disturbed intracellular PLP homeostasis, that can be recognized by respective biomarkers, except for PLPBPD (◘ Table 29.2). In each of these entities, seizures are a hallmark of the disease, with no or incomplete response to common anticonvulsants, but a good response to pyridoxine or PLP. As PLP is an abundant cofactor in several metabolic pathways, secondary metabolic changes as hypoglycaemia, hyperglycinaemia and elevated lactate are frequent confounders and may mislead the clinician towards other (untreatable) IEM. In the group of vitamin B_6 dependent epilepsies, seizures typically recur upon withdrawal of vitamin B_6 and illustrate B_6 dependency in contrast to mere responsiveness, as seen in nutritional deficiencies and also as a non-specific phenomenon due to GABA-ergic effects of vitamin B_6 supplementation. As biomarkers and/or genetic analysis are both available to test for IEM associated with vitamin B_6 responsive seizures (◘ Table 29.2), withdrawal in responders is no longer relevant.

29.2.1 Antiquitin Deficiency (*ALDH7A1*)

Clinical Presentation

Antiquitin deficiency is the most common form of pyridoxine dependent epilepsy (PDE). Typically, patients present in the neonatal period with myoclonic and tonic seizures or status epilepticus, but later onset from childhood to even adolescence has been observed [22, 23, 24]. About one third of affected neonates have a history of asphyxia and some may also present with encephalopathy (inconsolable crying, sleeplessness) or systemic features including hypoglycemia, lactic acidosis or acute abdomen. The EEG changes can range from non-specific slowing and discontinuity to focal discharges, or rarely, burst suppression patterns. Seizures are typically resistant to common anticonvulsants aside from a possible partial or transient response to phenobarbitone. Imaging is non-diagnostic, but may show thinning of the corpus callosum, cysts of the posterior fossa, white matter anomalies or cortical dysplasia [24, 25].

Metabolic Derangement

Antiquitin (*ALDH7A1*) encodes for α-aminoadipic semialdehyde dehydrogenase, an enzyme involved in lysine degradation (◘ Fig. 29.3). The accumulating compound, α-aminoadipic acid semialdehyde (AASA), is in equilibrium with L-Δ^1-piperideine-6-carboxylate (PC6) δ, which inactivates PLP by a so called Knoevenagel condensation [26]. The Knoevenagel product has not been shown in vivo to date. AASA (and P6C) in urine (plasma or CSF) can be determined semiquantitatively by LC-MS-MS and serve as reliable biomarkers, even when patients are on treatment with pyridoxine. Simultaneous determination of sulfocysteine is crucial to exclude molybdenum cofactor and sulfite oxidase deficiency causing secondary inhibition of antiquitin [27] (▶ Sect. 20.11). Pipecolic acid in plasma, the first described biomarker of PDE, is less specific as it can also be found in peroxisomal disease and has been found normal in older patients while on pyridoxine [28, 29]. (see also ▶ Fig. 30.1) Recently 6-oxo pipecolate (PIP) has been described as a novel biomarker of *ALDH7A1* deficiency, which is stable at room temperature and would be a suitable biomarker for newborn screening [30]. A number of secondary phenomena have been described in CSF of affected patients: low GABA, homovanillic acid (HVA) and hydroxyindole acetic acid (HIAA) concentrations. PLP levels in CSF, if measured pre-treatment, are markedly decreased, while PLP in plasma is low-normal. Enzymatic testing of antiquitin activity in fibroblasts, though feasible, has not been established as a routine investigation.

Genetics

Diagnosis is confirmed by Sanger sequencing of *ALDH7A1*. Over 165 different mutations have been described to date with no clear genotype to phenotype correlation. The carrier frequency is estimated to be as high as 1:127 with an estimated incidence of antiquitin deficiency of about 1:64.300 pregnancies [31]. The E427Q mutation, which results in complete enzyme deficiency, accounts for about 30% of all mutant alleles within Europe. Deletions of *ALDH7A1* have been reported and can only be detected by MLPA techniques [32]. In 2009 it was shown, that antiquitin deficiency is allelic to folinic acid responsive seizures [33].

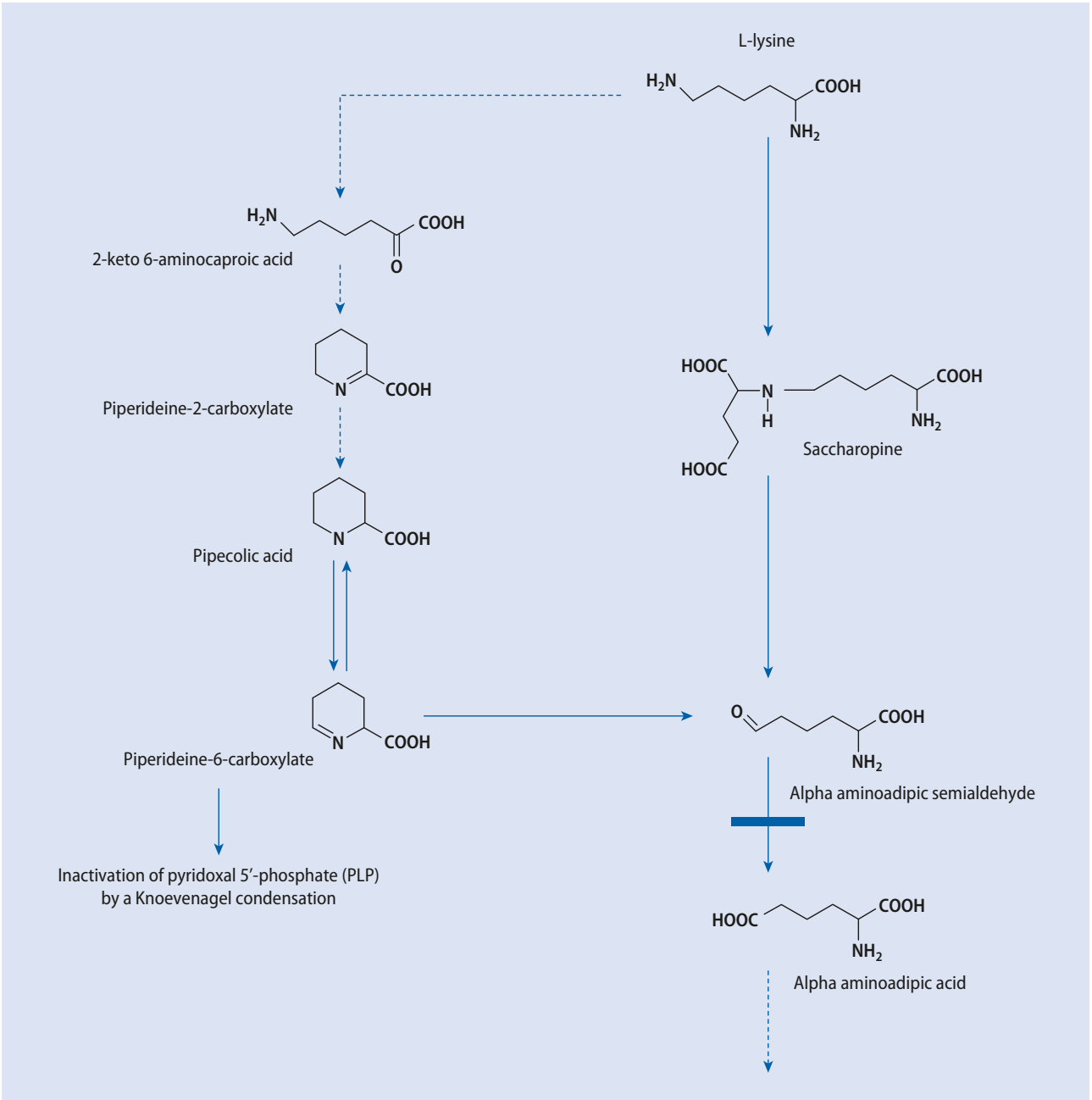

Fig. 29.3 Adapted Lysine degradation and antiquitin deficiency *(blue bar)*

Treatment and Prognosis

In most cases the administration of pyridoxine, 100 mg iv. or po., leads to prompt cessation of seizures. In about 14% the response to pyridoxine has been ambiguous and may be missed by a single dose administration [34]. Therefore, the administration of 30 mg/kg in 2 to 3 SD over three consecutive days has been recommended to identify patients with delayed response [22]. As first administration of pyridoxine may lead to severe apnea, resuscitation equipment should be at hand.

About 90% of patients have complete seizure control on pyridoxine monotherapy. To prevent side effects of pyridoxine, such as peripheral neuropathy, dosages should not exceed 300 mg/day. In those with breakthrough seizures during febrile illness, doubling of the pyridoxine dose over a few days is effective. Despite complete seizure control only 25% of PDE patients show normal development, irrespective of treatment delay. This may be due to the accumulation of potentially toxic metabolites within the L-lysine pathway that

do not normalise upon pyridoxine treatment. Therefore add-on therapies, such as a lysine restricted diet (LRD) and arginine supplementation for competitive inhibition of cellular lysine uptake have been applied in a still limited number of patients [35, 36, 37]. Patients with early initiation of add-on LRD with or without additional arginine supplementation have shown a variable decrease of intermediary lysine metabolites, in some cases associated with cognitive improvement as well as better seizure control. Prenatal treatment with 100 mg of pyridoxine starting from 3 months in pregnancies at risk may lead to better outcome [38], but warrants rapid confirmation testing after birth, as an unaffected offspring had proconvulsive effects on high dose pyridoxine therapy [39]. As some patients with antiquitin deficiency have been shown to have additional benefit from folinic acid, mainly during the neonatal period or infancy, folinic acid (3–5 mg/kg/day) is recommended for patients who fail to respond to pyridoxine alone. The role of folinic acid is not completely understood, but might be due to partially overlapping cofactor function and a 'B_6 sparing effect' (P. Clayton, personal communication, 2014).

29.2.2 Hyperprolinemia Type II

Among the six IEM with vitamin B_6 dependent seizures, this has probably the most attenuated phenotype [40]. It is in this IEM that the inactivating mechanism of PLP by a Knoevenagel condensation was first described [41]. The accumulated inactivating compound is Δ^1-pyrroline-5-carboxylate (P5C) due to deficiency of Δ^1-pyrroline-5-carboxylate dehydrogenase. The diagnosis can be made by marked elevation of plasma proline concentration and the presence of P5C in urine. The disorder is described in ► Chap. 21, ► Sect. 21.7.

29.2.3 Pyridox(am)ine 5'-phosphate Oxidase (PNPO) Deficiency

Clinical Presentation

The clinical presentation of PNPO deficiency is indistinguishable from antiquitin deficiency except for a higher rate of prematurity which is found in 61% of all published cases [42]. The disease was first described in Taiwan, where PLP is the first line drug to test for vitamin B_6 responsiveness [43]. Seizures recurred when patients were switched to pyridoxine and they showed a neurotransmitter profile that mimicked aromatic L-amino acid decarboxylase deficiency [44]. In contrast to antiquitin deficiency, patients with PNPO deficiency show signs of systemic PLP deficiency beyond the neonatal period, as failure to thrive and anaemia. In the neonatal period, the EEG is usually severely abnormal and a burst suppression pattern has been described in two thirds of published cases. Brain MRI imaging can be normal, but shows white matter changes and atrophy if diagnosis and specific treatment are significantly delayed. The function of PNPO might in fact be much broader than previously thought as some mutations were shown to be associated with infertility and miscarriage [45].

Metabolic Derangement

PNPO deficiency leads to severe (systemic) PLP deficiency and impaired function of PLP dependent enzymes [46]. A plasma profile of vitamin B_6 vitamers reveals a high pyridoxamine/pyridoxic acid ratio and is indicative of PNPO deficiency even when on vitamin B_6 supplementation [47]. PNPO activity can now be measured in dried blood spots and allows a quick and accurate diagnosis [48]. Vanillactate in urine reflects the buildup of dopamine metabolites, but is an inconstant and unspecific finding. While the original patients with PNPO deficiency were found to have decreased urinary HVA and HIAA, there are now two reports of elevated levels of these metabolites prior to treatment, an observation which remains unexplained. PLP concentrations in CSF prior to treatment have been found to be low but this may also be an inconsistent finding [48].

Genetics

In 2005 PLP dependent seizures were shown to be caused by PNPO deficiency [46]. To date a total of 27 different mutations in *PNPO* have been reported [21]. The R116Q variant is of special interest, as it shows a high carrier frequency in the general population but incomplete penetrance of epilepsy in the homozygous state. In 2014, two reports documented a significant proportion of patients with novel pyridoxine-responsive *PNPO* mutations, with residual enzyme activity of 8% or above [45, 49]. R225H seems to be a prevalent pyridoxine-responsive founder mutation in Kosovo and neighbouring countries [52].

Treatment and Outcome

Patients with PNPO deficiency have a short time window for specific treatment in order to prevent irreversible brain damage. Outside of Asia, PLP is an unlicensed chemical and can be purchased from naturopathic stores with uncertainty about the exact PLP content [50]. Effective dosages vary from 30 to 60 mg/kg/day. Patients often require frequent dosing of 4–6 single dosages/day. To avoid oxidation PLP should be dissolved immediately before oral administration. According to recent

reports on liver toxicity and cirrhosis, transaminases should be monitored and the lowest effective dose used [51, 52]. PNPO patients who are seizure free on pyridoxine monotherapy should not necessarily be switched to PLP, as in addition to the risk of liver toxicity of PLP, status epilepticus has been observed in single patients when switched to PLP quickly [52].

29.2.4 Congenital Hypophosphatasia (Tissue Non Specific Alkaline Phosphatase)

Clinical Presentation

Only patients affected by the severe form of perinatal or infantile congenital hypophosphatasia (CHP) present with neonatal seizures, sometimes before the skeletal manifestation of osteomalacia due to poor bone mineralisation becomes apparent [53]. The EEG is usually severely abnormal and can show a burst suppression pattern. Prior to the availability of enzyme replacement therapy, this condition was fatal as a result of respiratory insufficiency.

Metabolic Derangement

CHP is caused by a deficiency of Tissue Non Specific Alkaline Phosphatase (TNSAP). This enzyme has three substrates, namely inorganic pyrophosphate, phosphoethanolamine and PLP for cellular uptake. Cerebral PLP deficiency is supported by secondary changes of neurotransmitters reported in single patients [54], but expected phenomena of ubiquitous intracellular PLP deficiency, such as severe anemia, are absent. The diagnosis of CHP is straight forward with markedly reduced levels of alkaline phosphatase (AP) in routine clinical chemistry, elevated serum calcium and reduced serum phosphate, and elevated phosphoethanolamine upon amino acid analysis. PLP in plasma prior to treatment is markedly elevated.

Genetics

CHP is caused by mutations in *ALPL* and there is some genotype-phenotype correlation and an increased frequency of specific mutations in some ethnic populations.

Treatment and Prognosis

There is a variable and inconsistent response to treatment with pyridoxine and worsening of seizures upon pyridoxine has been observed [55]. It is questionable if the availability of enzyme replacement therapy will alter the CNS manifestation of infantile CHP, as enzyme replacement therapy cannot cross the blood brain barrier [56, 57].

29.2.5 Hyperphosphatasia-Mental Retardation Syndrome (HPMRS)

HPMRS or Mabry syndrome, is a clinically recognizable syndrome with facial dysmorphism, brachytelephalangy and seizures of neonatal or childhood onset. The majority of cases are caused by mutations in *PIGV*, or less frequently in *PIGO* or *PGAP2*, that encode the synthesis of phosphatidylinositol (GPI) –anchors of various membrane-bound proteins such as TNSAP [58] (▶ Chap. 35). Mildly to moderately elevated AP in serum is a hallmark of the disease. To date it remains unclear, if seizures of these patients respond to pyridoxine.

29.2.6 PLP Binding protein (PLPBP, Formerly PROSC) Deficiency

In 2016 Darin et al. reported seven patients with a new genetic background of vitamin B_6 dependent epilepsy - at that time called PROSC deficiency [59]. Since then a total of 31 patients have been described, most of them presenting with neonatal onset of therapy-resistant, clonic, tonic clonic or myoclonic seizures [60–64]. Seizure onset beyond the neonatal period may occur and one patient has been identified with an isolated dystonic movement disorder presenting from age 3 months [63]. Primary or acquired microcephaly and intellectual disability are frequently observed and may correlate with the underlying genotype rather than with therapeutic delay. Cranial MRI may be normal or show structural anomalies as a simplified gyral pattern, anterior cysts or swelling of white matter as well as hyperdensities of dentate nuclei. MRI changes and the presence of lactic acidosis in 50% of cases reported so far may mislead towards primary mitochondrial disorders and prevent a therapeutic trial with pyridoxine. EEG changes vary from normal to diffuse slowing or burst suppression patterns.

Metabolic Derangement

PLPBP is a cytosolic as well as mitochondrial protein involved in intracellular PLP homeostasis and suggested to act as a carrier of PLP towards PLP-dependent enzymes [21, 64]. There is no specific biomarker to indicate PLPBP deficiency. Pre-treatment concentration of PLP in CSF and plasma is markedly reduced. Dysfunction of PLP-dependent enzymes may be indicated by elevated lactate (about 50% of cases), glycine, methionine, threonine or vanillactate in urine.

Genetics

PLPBP deficiency is a panethnic condition. Functional and structural studies have been performed in a limited number of missense mutations [65] and have shown a

dimeric protein structure as well as an additonal role of PLPBP in the regulation of cell division and proper muscle function [66].

Treatment and Prognosis

A considerable proportion of patients with PLPBP deficiency shows a favorable response to pyridoxine monotherapy, 100–200 mg/day [60–64], while some patients needed a switch from pyridoxine to PLP [59, 63] or even addition of folinic acid to become seizure free [63]. Emerging genotype phenotype correlation suggests that loss of function mutations cause a more severe phenotype associated with intellectual disability, whereas missense variants that do not affect the PLP binding site seem to be associated with a better outcome [63].

29.2.7 Other B_6 Responsive Disorders

Some IEM caused by defects of PLP dependent enzymes benefit from cofactor supplementation [21]. This is true for about 50% of all cases with classical homocystinuria and warrants a pyridoxine challenge prior to the initiation of a methionine restricted diet and/or medication (► Chap. 20). Pyridoxine responsiveness is also seen in some cases of gyrate atrophy, caused by deficiency of ornithine aminotransferase (► Chap. 21). The pyridoxine-responsive anaemia (or X-linked sideroblastic anaemia) is caused by a defect in the erythroid-specific form of 5-aminolevulinate synthase (► Chap. 33). In all these B_6 responsive disorders vitamin B_6 vitamers may act as a chaperone on the mutated protein [67]. In aromatic acid decarboxylase (AADC) deficiency a trial with pyridoxine is recommended, as PLP can optimize residual AADC activity with positive response reported in some patients (► Chap. 30). A stepwise increase of pyridoxine is advised to identify the lowest effective dose. For the risk of (reversible) neuropathy pyridoxine doses should be kept below 300 mg/day wherever possible.

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Disorders of Neurotransmission

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Contents

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Neurotransmitters

Chemical transmission in the nervous system is characterized by amazing complexity. Classical neurotransmitter systems involve inhibitory aminoacidergic [γ-aminobutyric acid (GABA) and glycine], excitatory aminoacidergic (aspartate and glutamate), cholinergic (acetylcholine), monoaminergic (mainly adrenaline, noradrenaline, dopamine, and serotonin), and purinergic (adenosine and adenosine mono-, di-, and triphosphate). New approaches based on synaptic physiology include synaptic vesicle defects as a new category of inborn errors of neurotransmission and cell trafficking disorders. Defects of neuropeptides, channels (as neurotransmitter modulators), other signalling molecules and cellular processes will be gradually integrated in the complexity of neurotransmission diseases in the future.

GABA is formed from glutamic acid by glutamic acid decarboxylase (Fig. 30.1). It is catabolized into succinic acid through the sequential action of two mitochondrial enzymes, GABA transaminase and succinic semialdehyde dehydrogenase. Glutamic acid decarboxylase and GABA transaminase require pyridoxal phosphate as a coenzyme. Pyridoxal phosphate also participates in the synthesis of dopamine and serotonin, and in many other pathways including the glycine cleavage system. A major inhibitory neurotransmitter, GABA is present in high concentration in the central nervous system, predominantly in the grey matter. GABA modulates brain activity by binding to sodium-independent, high-affinity, mostly $GABA_A$ receptors (Fig. 30.2). Glutamate is the major excitatory neurotransmitter in the brain. Its function requires rapid uptake to replenish intracellular neuronal pools following extracellular release. Glutamatergic receptors (Fig. 30.2) mediate neuronal plasticity, learning and behaviour.

Glycine, a non-essential amino acid, is an intermediate in many metabolic processes but also one of the major inhibitory neurotransmitters in the central nervous system. The inhibitory glycine receptors and transporters (Fig. 30.3) are mostly found in the brain stem and spinal cord. Choline is the major neurotransmitter at the neuromuscular junction (Fig. 30.4).

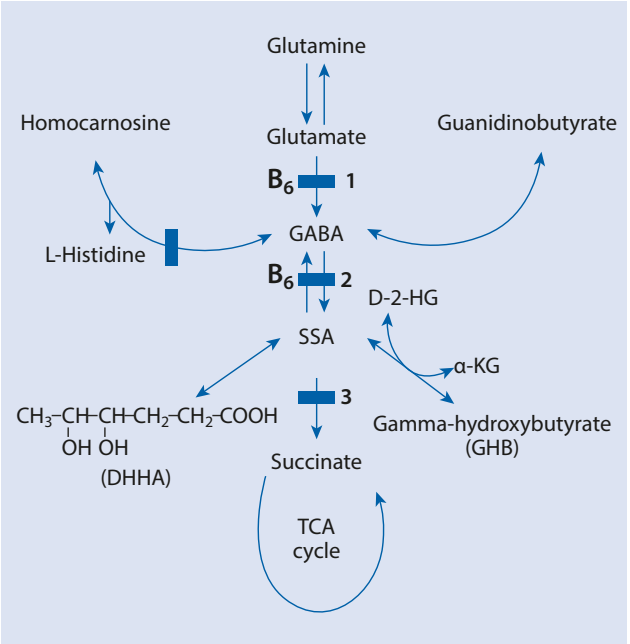


Fig. 30.1 Brain metabolism of γ-aminobutyric acid (GABA). B_6 pyridoxal phosphate, 1 glutamic acid decarboxylase, 2 GABA transaminase, 3 succinic semialdehyde dehydrogenase, D-2-HG D-2-hydroxybutyrate, α-KG alpha ketoglutarate, TCA tricarboxylic c-ycle, DHHA 4,5-dihydroxyhexanoic acid, SSA succinic semialdehyde. Enzyme defects are depicted by **solid bars**. The block at conversion of homocarnosine to GABA remains to be clarified

Introduction

This Chapter deals with inborn errors of neurotransmitter biosynthesis, catabolism, and defects of their transporters, receptors and synaptic vesicle trafficking disorders at the pre-synaptic terminal. Defects of GABA catabolism include GABA transaminase deficiency and succinic semialdehyde dehydrogenase (SSADH) deficiency. Glutamic acid decarboxylase (GAD) deficiency is a new defect of GABA synthesis that presents as neonatal seizures. Mutations in GABA receptors and GABA transporter cause dominantly inherited epilepsies while mutations in glutamate receptors associate with complex neurodevelopmental and psychiatric disorders. Mitochondrial glutamate transporter is a cause of severe epileptic encephalopathy. Hyperekplexia is usually due to a dominantly inherited defect of the α1 subunit of the glycine receptor which causes excessive startle responses and is treatable with clonazepam. Defects of choline synthesis, transporter and their receptors lead to congenital myasthenia. Monoamine metabolism synthesis defects are: Tyrosine hydroxylase (TH) deficiency, which impairs synthesis of L-dopa and causes a neurological disease with prominent extrapyramidal signs, and a variable response to L-dopa; Aromatic L-amino acid decarboxylase (AADC) located upstream of the neurotransmitter amines with a challenging treatment; Dopamine β-hydroxylase deficiency which presents with severe orthostatic hypotension and sympathetic failure. Monoamine-oxidase A (MAO-A) is a catabolism deficiency, located downstream that causes behavioral disturbances with no effective treatment. Monoamine

"transportopathies" produce early parkinsonism-dystonia and include dopamine transporter defect and vesicular monoamine transporter type 2 defect. Guanosine triphosphate cyclohydrolase-I (GTPCH-I) and sepiapterin reductase (SR) deficiencies are pterin disorders upstream of L-dopa and 5-hydroxytryptophan (5-HTP) with normal baseline phenylalaninaemia and effective treatment (especially GTPCH-I deficiency). Synaptic vesicle trafficking defects at the pre-synaptic terminal include 14 disorders of exocytosis, which frequently cause severe neurodevelopmental encephalopathies, and 20 endocytosis defects, that mostly present as early-onset parkinsonism, but also as classical Parkinson disease.

30.1 Gamma Amino Butyric Acid (GABA) Neurotransmitter Disorders

30.1.1 Gamma Amino Butyric Acid Transaminase Deficiency

GABA-Transaminase deficiency is an extremely rare autosomal recessive (AR) disease, with reports published thus far for only 17 individuals from 11 families. The oldest identified patient is currently 32 years old [1, 2].

Clinical Presentation

Clinical findings in the index family were neonatal seizures, lethargy, hypotonia, hyperreflexia, poor feeding, severe developmental impairment, and a high-pitched cry [3]. Linear growth was accelerated, attributable to a pro-growth hormone secreting effect by GABA. In this Flemish sibship, there was early mortality and spongiform leukodystrophy. Patients have uniformly presented with neonatal or early infantile encephalopathy, often accompanied by hypotonia, lethargy, seizures, and extrapyramidal manifestations. Choreoathetosis, subcortical myoclonus, and accelerated growth are common clinical manifestations. EEGs show burst suppression, modified hypsarrhythmia, multifocal spikes, generalized spike-wave, or diffuse background slowing. MRIs show delayed myelination and progressive cerebral atrophy, specifically at the thalami, and thin corpus callosum and brainstem. Milder phenotypes related to higher residual enzymatic activity are emerging with survival into adulthood [2, 4].

Metabolic Derangement

Cerebrospinal fluid (CSF) GABA conjugates and β-alanine are increased. CSF free GABA was elevated up to 60-fold for all patients where CSF was studied (reference <0.12 μM). Diagnosis via proton magnetic resonance spectroscopy with spectral editing for GABA in the basal ganglia at 8 months of age was implemented in one patient (patient GABA, 2.9 mmol/L; control, 0.8 mmol/L). GABA-transaminase activity was deficient in white cells derived from patients (1.2–2 nmol/hr./mg protein) (normal range: 20–64), with intermediate enzyme activities in parents [5]. Growth hormone was elevated in two patients consistent with the growth hormone releasing effect of GABA. An isotope-dilution enzyme assay for GABA-transaminase suggests that GABA- and β-alanine transaminases are identical, thereby explaining the increase of β-alanine [6].

Genetics and Diagnostic Tests

GABA-T deficiency is an AR condition caused by pathological variants in *ABAT*. Due to enzymatic degradation of homocarnosine, free GABA levels in the CSF show an artefactual increase unless samples are rapidly deep-frozen within a few minutes, at −20 °C if analyzed within a few weeks, or at −70 °C if analysis is to be delayed. Control CSF free GABA levels are quite low (<175 nmol/L) and thus sensitive techniques, such as stable-isotope-dilution analysis, must be employed. Molecular confirmation is now the gold-standard for diagnosis (WES). Pop and colleagues [7] recently reported a high-throughput expression system for GABA-T alleles. Enzymatic confirmation remains possible in lymphocytes, lymphoblasts, and liver in specialized laboratories [7].

Treatment and Prognosis

No clinical or biochemical response was observed in the first three patients using pyridoxine or first-line antiseizure medicines (phenytoin, clonazepam, valproate, midazolam). The 7-year old patient is currently on a ketogenic diet and follow-up is in progress. In a 21-month old patient flumazenil ($GABA_A$ receptor antagonist) infusion was undertaken and the patient tolerated 0.5 mg/kg/hr. with clinical and EEG improvement, raising the potential for this agent to be further (4-hydroxybutyric aciduria [1].

30.1.2 Succinic Semialdehyde Dehydrogenase Deficiency

Succinic semialdehyde dehydrogenase (aldhehyde dehydrogenase 5a1, ALDH5A1) deficiency (SSADHD) first reported as γ-hydroxybutyric aciduria [8]. It is the most prevalent of the disorders of GABA metabolism, with at least 500 cases estimated worldwide and 182 confirmed individual cases reported from 40 countries in the literature [9].

Clinical Presentation

SSADHD is a neurometabolic disorder with relatively nonspecific clinical manifestations including developmental delay and early-onset hypotonia, profound expressive language impairment, obsessive–compulsive symptoms, non-progressive ataxia, and epilepsy [4]. Neuropsychiatric symptoms and seizures are common and most prevalent among adolescents and adults [10]. Imaging abnormalities include T2-signal hyperintensity in the globus pallidi, subthalamic nuclei, and cerebellar dentate nuclei, bilaterally and indicative of cytotoxic edema. Abnormalities of myelination and cerebellar atrophy have been noted.

Metabolic Derangement

The key feature is an accumulation of 4-hydroxybutyrate (γ-hydroxybutyrate GHB) in urine, plasma, and CSF (► Fig. 37.1). GHB and GABA (elevated threefold in CSF) are neuropharmacologically active compounds. Additional biochemical abnormalities relate to GABA, succinic semialdehyde, and GHB. These include increased homocarnosine and guanidinobutyrate, D-2-hydroxyglutarate, succinic semialdehyde, and 4,5-dihydroxyhexanoic acid in physiologic fluids (► Fig. 39.1). Levels of glutathione, the major intracellular antioxidant, are low in both the animal model and patients [11].

Genetics and Diagnostic Tests

SSADHD is an AR disorder caused by variants in *ALDH5A1*. Over 60 disease-associated alleles have been identified, but a mutation hotspot has not been detected [4]. The ALDH5A1 protein is a member of the aldehyde dehydrogenase protein superfamily, which has a number of highly conserved glycine residues. Not surprisingly, mutations that alter these glycine residues are frequently pathogenic.

Diagnosis is primarily achieved by determination of γ-hydroxybutyric acid in urine, followed by molecular diagnosis via *ALDH5A1* sequencing. Concomitant identification of 4,5-dihydroxyhexanoic acid, both the free and lactone forms (*threo-, erythro-*) in the organic acid profile, is highly suggestive. Issues with false-positives are generally not problematic, although GHB is also employed clinically for cataplexy and illicitly (for induction of euphoria). Enzyme analysis in white cells is possible, but molecular diagnosis has become the gold-standard for confirmation [4].

Treatment and Prognosis

There is no standard therapy; most strategies are directed toward symptomatic treatment. Epilepsy tends to be more prevalent and severe in the adolescent and adult populations, and a relatively high rate of SUDEP (sudden unexpected death in epilepsy patients) has been reported, approximately 15% [10]. The nonspecific clinical picture of SSADHD can lead to a diagnostic odyssey, as is evident by a recently reported patient diagnosed postmortem at age 63 [12]. Therapeutic intervention has historically employed vigabatrin (gamma-vinyl GABA), an irreversible inhibitor of GABA-transaminase [4], which has been beneficial in some but without efficacy in others. It remains to be determined whether enhancing GABA levels in SSADHD (which are already elevated) is prudent despite vigabatrin's ability to lower GHB, and the visual field disturbances associated with vigabatrin are treatment limiting. An open-label trial with taurine revealed neither clinical nor metabolic benefit [13] and a double-blind, placebo-controlled trial with the GABAB receptor antagonist SGS-742 has concluded, with results pending at the time of this writing (► www.clinicaltrials.gov; NCT02019667). The recent identification of a role for GABA in autophagy/mitophagy has raised the potential of rapalogue intervention to treat SSADHD [14]. Preliminary evidence of enzyme replacement therapy in the murine model demonstrates improved survival and correction of several GABA(A) receptor subunits [15].

30.1.3 Glutamic Acid Decarboxylase (GAD) Deficiency

GAD1, encoding the enzyme glutamic acid decarboxylase, which converts glutamic acid to GABA, has been suggested as a candidate gene for AR spastic cerebral palsy and a susceptibility gene in schizophrenia, although functional studies have been lacking. Bi-allelic variants in *GAD1*, encoding isoform GAD67, have been recently reported in an early infantile epileptic encephalopathy in 11 patients from six independent consanguineous families, with seizure onset in the first 2 months of life [16].

Clinical Presentation

The phenotype is early onset epileptic encephalopathy with associated myoclonic seizures or epileptic spasms, suppression-burst discontinuity on EEG or hypsarrhythmia. A variable association of dysmorphic facial features including cleft palate, joint contractures, scoliosis, and omphalocele was noted. Other clinical features are axial hypotonia, appendicular quadriparesis, spasticity, abnormal eye movements, and extrapyramidal manifestations including dystonia and hyperkinetic movements. Imaging findings include cerebral and cerebellar atrophy and hypoplasia of the corpus callosum.

Metabolic Derangement, Genetics and Diagnostic Tests

Metabolic studies are limited; no abnormalities in blood, urine, or CSF were noted. GAD67 deficiency is an AR condition caused by pathological variants in *GAD1*. The 11 reported patients have homozygous variants including missense, nonsense, intron variants and deletions [16].

Treatment and Prognosis

As this is a novel syndrome, treatment and prognosis information is limited. In the single case series, mortality occurred prior to the fourth birthday in 4 of the 11 patients. The oldest reported patient was 11 years old. Patients require supportive treatment for seizure control, often with multiple antiseizure drugs. A good response to vigabatrin was noted in five of seven patients, particularly those with epileptic spasms.

30.1.4 GABA Receptor Mutations

Zinc ions play a critical role in regulating GABA-A receptors via an allosteric mechanism dependent on the subunit receptor composition. There are distinct binding sites for benzodiazepines and neurosteroids (◘ Fig. 30.2). In contrast to the ionotropic GABA-A receptors, metabotropic GABA- B receptors' inhibitory properties are mediated through G protein-coupled second messenger systems which regulate potassium conductance. Multiple pathogenic variants in GABA(A) receptor subunits have been reported in genetic epilepsies : childhood absence epilepsy, genetic epilepsy with febrile seizures plus (GEFS+), juvenile myoclonic epilepsy and cases of Dravet syndrome, i.e. severe myoclonic epilepsy of infancy (GABRG2 and GABRA1) [17]. Recently, pathogenic variants in GABRA1, GABRB2, and GABRB3 have been associated with infantile spasms and Lennox-Gastaut syndrome [18]. Patients with GABRD and GABBR2 mutations have been reported to have Rett-like features [19]. (◘ Table 30.1).

Mutations in the GABA(A) receptor alter fast inhibitory neurotransmission facilitated by GABA and chloride flux. Mutations associated with epilepsy have been located at genes encoding α1, α6, β2, β3, γ2, or δ subunits (*GABRA1, GABRA6, GABRB2, GABRB3, GABRG2,* and *GABRD*, respectively) [18] (◘ Table 30.1). Interestingly, other than impaired neurotransmission, mutant GABA(A) receptor γ2(Q390X) subunits, related to Dravet syndrome, accumulate and aggregate intracellularly, activate caspase 3, and cause age-dependent neurodegeneration.

The diagnosis is based on molecular genetic analysis of different GABA(A) receptor subunits. Treatment is generally tailored to the symptoms of the particular epilepsy but may also address febrile episodes in patients with GEFS+. The prognosis also is dependent upon the epilepsy syndrome involved.

30.1.5 GABA Transporter Deficiency

The solute carrier transporter SLC6A1 removes GABA from the synaptic cleft. A large cohort of 24 patients with pathogenic *SLC6A1* variants were reported to have the phenotype of myoclonic-atonic epilepsy (or Doose syndrome), along with language delay and mild or moderate cognitive deficiency before epilepsy onset [20]. Clinical responsiveness to the ketogenic diet has been reported [21], which is furthermore well known to occur in a subset of patients with this epilepsy syndrome, and also showing overlap with glucose transporter 1 (GLUT1) deficiency (▶ Chap. 8).

30.2 Glutamate Neurotransmitter Disorders

30.2.1 Glutamate Receptor Mutations

Glutamate is the main excitatory amino acid neurotransmitter in the brain, with about 90% of excitatory synapses using glutamate for neuronal communication. Upon release in the synaptic cleft, glutamate acts on glutamate receptors, functionally divided into metabotropic (mGluRs) and ionotropic (iGluRs). The latter are divided into three main families, *N*-methyl D-aspartate (NMDA), AMPA, and kainate receptors (◘ Table 30.2). NMDA receptors (NMDARs) (◘ Fig. 30.2) are critical for neuronal survival, circuit formation and synaptic plasticity processes, among others [22].

Next-generation sequencing has diagnosed a growing number of patients with genetic variants in *GRIN* genes (encoding for GluN subunits of the NMDAR), defining GRIN-related disorders or "grinopathies" [23]. To date, about 500 individuals harbouring likely-pathogenic *GRIN* variants have been reported worldwide (▶ www.grin-database.de). The great majority of these diseases are autosomal dominant (AD) de novo variants. Symptoms may vary depending on the affected subunit and include mild-profound developmental delay/severe intellectual disability (ID), infantile-onset seizures, oculogyric crises, bilateral polymicrogyria,

Table 30.1 GABA receptor disorders and clinical manifestations

Clinical manifestations	Disorder name								
	GABA type A receptor α1 subunit deficiency	**GABA type A receptor α6 subunit deficiency**	**GABA type A receptor β1 subunit deficiency**	**GABA type A receptor β2 subunit deficiency**	**GABA type A receptor β3 subunit deficiency**	**GABA type A receptor γ2 subunit deficiency**	**GABA type A receptor δ subunit deficiency**	**GABA type B receptor subunit 2 deficiency**	**GABA Transporter Deficiency**
EIEE	Type 19		Type 45	Type 2	Type 43	Type 74		Type 59	
Genetic epilepsy with febrile seizures plus						Type 3	Type 5		
Seizures	x	x	x	x	x	x	x	x	x
Hypotonia			x					x	
Developmental delay			x	x				x	
Intellectual disability				x				x	x
Speech impairment								x	x
Loss of purposeful hand movements								x	
Rett like phenotype							x	x	

All autosomal dominant
EIEE early infantile epileptic encephalopathy

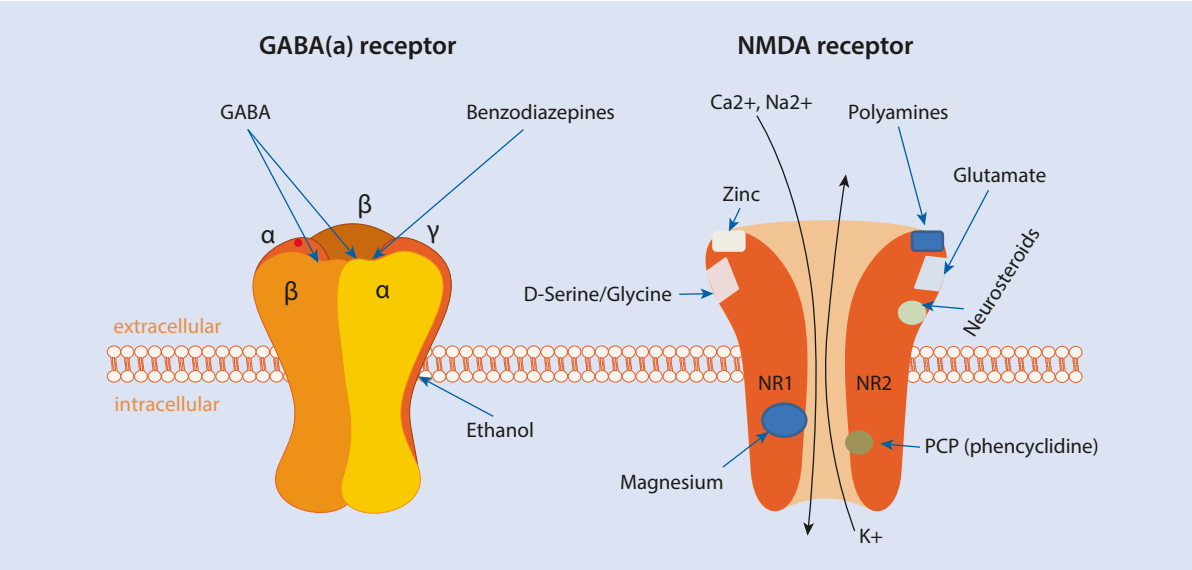


Fig. 30.2 Structure of the GABA(a) and the NMDA receptors and their agonist modulators. GABA-A receptors are composed of pentomeric protein subunits with a central channel that functions as a chloride ion pore. The NMDAR receptor is a heterotetramer resulting from the oligomerization of two GluN1 subunits and a combination of two additional subunits (GluN2A-D, GluN3A, GluN3B)

Landau-Kleffner syndrome and autism/autistic-like behaviours (Table 30.2) [24].

De novo AMPA receptor mutations in *GRIA2* gene have been reported in 28 patients presenting with ID, autism symptoms and Rett-like phenotype [25]. Other AMPA disorders include mutations in *GRIA3*, *GRIA4 GRIK2* and metabotropic receptors type 1 and 6 (Table 30.2). *ATAD1* mutations impair post-synaptic AMPA receptor trafficking and cause a neonatal encephalopathy characterised by hypertonia, absence of spontaneous movements and death within the first months of life; seizures may also be present [26].

The diagnosis of glutamate receptor disorders is based on molecular genetic analysis. Treatment is generally tailored to the symptoms. L-serine was reported to improve a patient with *GRIN2B* loss-of-function variant [27]. *GRIN2D* patients may have reduced seizures with the receptor antagonists memantine and ketamine [28].

30.2.2 Mitochondrial Glutamate Transporter Defect

This disorder, first described in 2005 [29], has been reported in about 15 patients so far. This is characterized by severe, neonatal onset epileptic spasms and migrating focal seizures with a burst-suppression EEG pattern, microcephaly, hypotonia, abnormal electroretinogram, and severe psychomotor delay. MRI imaging in childhood shows cerebellar hypoplasia, an abnormal corpus callosum, abnormal gyration of temporo-parietal regions and abnormal myelination of temporal poles. Mutations in this transporter have been also related to migrating focal seizures in infancy [30]. One patient was diagnosed after a history of ID and absence seizures at 7 years of age [31]. This is an AR disorder caused by missense mutations in *SLC25A22* which encodes a mitochondrial glutamate transporter specifically expressed in the brain during development. The defect impairs oxidation of glutamate. Diagnosis is mostly based on genetic studies although measurement of glutamate oxidation in cultured skin fibroblasts could be used as a functional test. Biochemical abnormalities such as hyperprolinemia and lipid vacuolated fibroblasts have been reported, indicating impairment of the proline/pyrroline-5-carboxylate (P5C) shuttle [30]. There is no specific treatment.

30.3 Glycine Neurotransmitter Disorders

Clinical Presentation

All these diseases present with hyperekplexia, often associated with other symptoms. The diagnosis of hyperekplexia is based on: (1) generalized stiffness immediately after birth that increases with handling and disappears during sleep; (2) excessive startle reflex to unexpected stimuli; (3) a short period of generalized stiffness following the startle response. Associated features include exaggerated head retraction or startle reflex elicited by tapping the tip of the nose, inguinal, umbilical, or epigastric herniations, congenital hip dis-

Table 30.2 GLUTAMATE receptor disorders and clinical manifestations

Receptor type	Subtype	Gene	Inheritance	Phenotype
Iono-tropic	AMPA	GRIA2	AD	Intellectual disability, Rett-like phenotype, EIEE, autism
		GRIA3	X-linked	X-linked Intellectual disability
		GRIA4	AD	Psychomotor delay, Intellectual disability, epilepsy is sometimes present, gait dyspraxia.
		ATAD1	AR	Neonatal Hypertonia, +/– seizures, early lethal encephalopathy. AMPA traffic disturbance
	Kainate	GRIK2	AR	Intellectual disability, behaviour disturbances, epilepsy and dystonia
	NMDA	GRIN1	AD/AR	Severe global psychomotor delay and intellectual disability, hipotonia starting at the first months of life,absence of expressive language, abnormal movments, hyperkinetic moviments including dyskinesia and stereotypies, oculogyric crises, cortical blindness, generalized brain atrophy, epilepsy, EIEE
		GRIN2A	AD	Intellectual disability and/or epilepsy (Landau-Kleffner syndrome)
		GRIN2B	AD	Psychomotor delay with wide spectrum of severity from global early neurodevelopmental encephalopathy to isolated intellectual disability. Epilepsy, hypotonia, autism. Brain malformations including polymicrogyria brain atrophy and visual cortex atrophy.
		GRIN2D	AD	EIEE. Seizues may improve with antagonist receptors such as memantine and ketamine
Metabo-tropic	GRM1		AD/AR	Spinocerebellar ataxia: congenital form, juvenile and adult onset. Intellectual disability is associated
	GRM6		AR	Congenital night blindness with cone dysfunction (cone-rod dystrophy)

AD autosomal dominant, *AR* autosomal recessive, *EIEE* early infantile epileptic encephalopathy

location, and epilepsy. Sudden infant death has been reported. Psychomotor development is usually normal or mildly delayed.

Metabolic Derangement

Hyperekplexia is caused by defective inhibitory glycinergic neurotransmission (Fig. 30.3) due to mutations in genes encoding the α1 subunit of the glycine receptor (GLRA1) [32], the β subunit of the glycine receptor (GLRB) [33], the gene encoding the presynaptic sodium- and chloride- dependent glycine transporter, GlyT2 (SLC6A5) [34], the astrocytic GlyT1 transporter (SLC6A) [35], and the gene encoding the glycinergic clustering molecule, gephyrin (GPHN) [36]. Gephyrin is also involved in molybdenum cofactor (MoCo) synthesis and mutations in *GPHN* can also lead to one form of MoCo deficiency (► Chap. 20). In one individual with hyperekplexia plus severe epilepsy and severe developmental delay, mutations were found in the X-linked *ARHGEF9* encoding collybistin which is also involved in glycinergic receptor clustering [37]. *GLRB* mutations are strongly associated with delays in gross motor development and speech acquisition since β subunits are expressed at a much earlier developmental stage than α1 subunits. GlyT1 deficiency presents with hyperekplexia, severe neonatal encephalopathy, arthrogryposis, dysmorphic features and abnormal brain imaging (thin corpus callosum, ventricumegaly and cysts). It has been reported in six patients since its first description [38].

Genetics and Diagnostic Tests

Hyperekplexia usually has an AD inheritance with nearly complete penetrance and variable expression in most pedigrees. *GLRA1* gene study for point mutations and deletion of exons 1–6 will detect the mutation in approximately 80% of cases. In cases without a family history suggesting dominant inheritance, screening all the genes listed above will detect mutations in approximately 20% of cases (mostly recessive).

Clinical diagnosis is based on the neurological features and the response to medication: clonazepam reduces the startle responses and diminishes the fre-

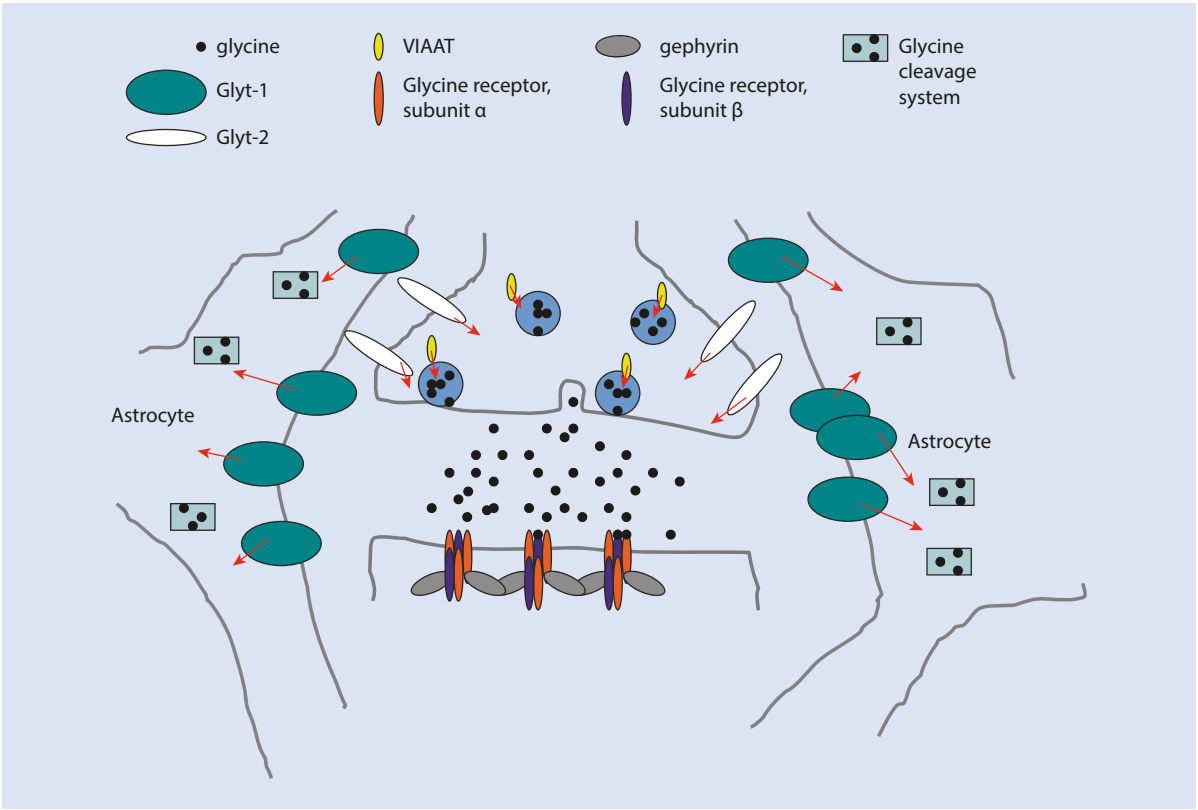


Fig. 30.3 Inhibitory glycinergic synapse. Glycine is stored in vesicles in the presynaptic neuron. Vesicle inhibitory amino acid transporter (VIAAT) transports these vesicles to the presynatic membrane, where glycine is released to the synaptic cleft. Glycine transporters (GlyT1 and 2) are members of the Na+/Cl--dependent neurotransmitter transporter superfamily. The neuronal GlyT2 is essential for glycine uptake into the presynaptic neuron and thereby provides substrate for VIAAT mediated refilling of re-endocytosed vesicles. The glial isoform GlyT1 removes released glycine from post-synaptic receptors and allows for its degradation by the glial glycine cleavage system. Glycine receptors are ligand-gated chloride channels assembled into pentameric complexes consisting of a combination of α (GLRA1) and β (GLRB) subunits. *GLRA1* mutations are the most important cause of hyperekplexia

quency of falls. GlyT1 is the only disease with a biomarker: high levels of glycine in the CSF but normal concentration in plasma [35]. Confirmation of the clinical diagnosis requires DNA sequencing for all the known hyperekplexia genes.

Treatment and Prognosis

The stiffness decreases during the first years of life, but the excessive startle responses remain. Clonazepam, that binds to the benzodiazepine site of the GABA(A) receptor, significantly reduces the startle responses but has less effect on the stiffness. GlyT1 is a severe disease with high lethality in the neonatal period. Benzoate and ketamine have been tried without improvement.

30.4 Choline Neurotransmitter Disorders

Choline is a water-soluble, vitamin-like nutrient involved in structural integrity and signaling for cell membranes, cholinergic neurotransmission, and methylation [39]. Neurotransmission diseases include genetic defects involved in acetylcholine metabolism, release, transport, and signaling. All cause congenital myasthenic syndromes (CMS), characterized by abnormal fatigability or transient or permanent weakness of extra-ocular, facial, bulbar, truncal, respiratory or limb muscles. Onset is neonatal, in infancy or childhood and rarely in adolescence [40].

Pre-synaptic CMS (Fig. 30.4) include defects of biosynthesis, transport and synaptic vesicle trafficking.

Regarding biosynthesis, choline acetyltransferase (CHAT) is necessary for the synthesis of acetylcholine from choline. Cholinergic neurons take up choline by the high-affinity choline transporter CHT1 encoded by *SLC5A7*. Biallelic mutations in both *CHAT* and *SLC5A7* underlie a congenital myasthenic syndrome characterized by neonatal hypotonia, weakness, ptosis, poor sucking and episodic apnea [41, 42], with responsiveness to acetylcholine esterase inhibitors in some

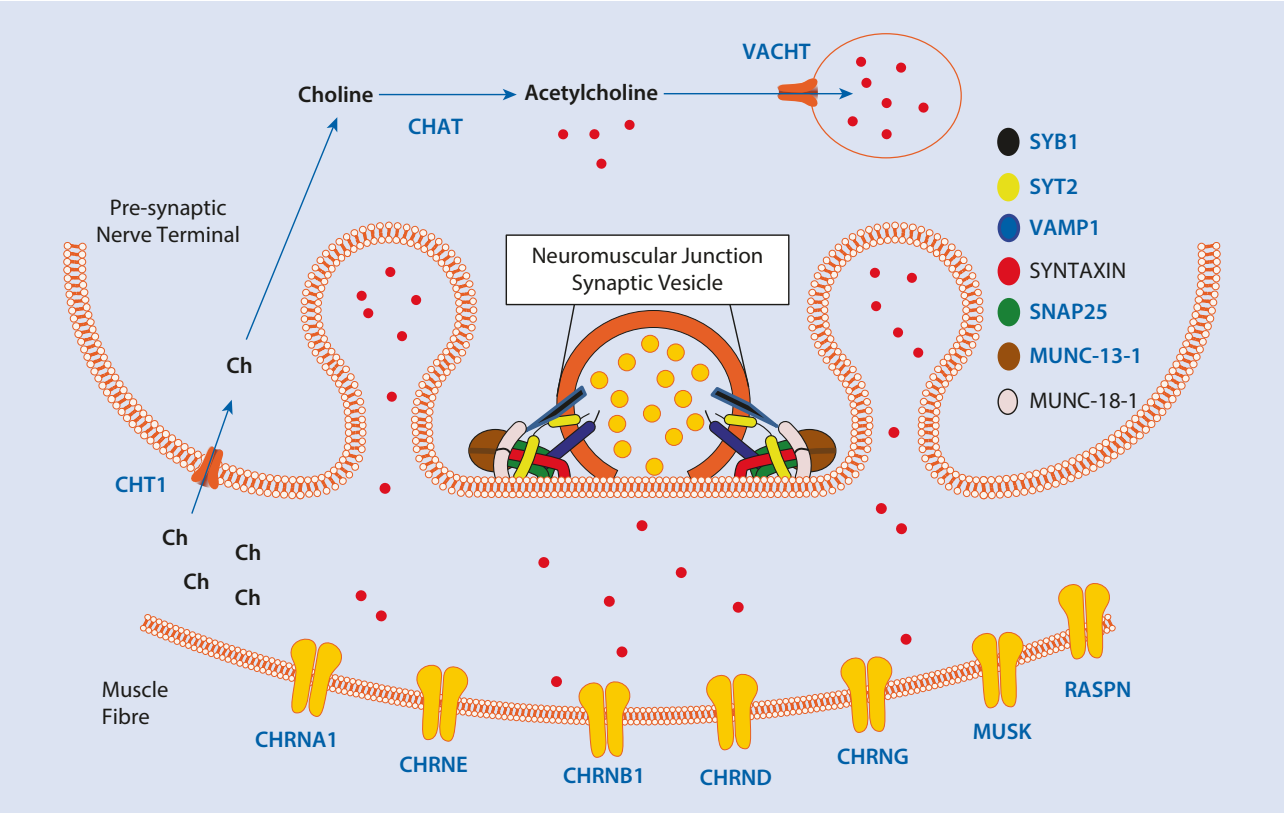


Fig. 30.4 Cholinergic synapse. Pathophysiological mechanisms involved in congenital myasthenic syndromes (CMS) at the neuromuscular junction. Enzymes, proteins and transporters at the presynaptic nerve terminal, and receptors at the muscle fiber that cause CMS are marked in blue letters. Ch choline, CHT1 choline transporter type 1, CHAT choline acetyltransferase (CHAT), VACHT vesicular acetylcholine transporter, SYB1 synaptobrevin, SNARE protein, SYT2 synaptotagmin; SNAP25 and VAMP1 are also SNARE proteins. CHR are post-synaptic nicotinergic receptors located at the muscle fiber

patients. *SLC5A7* heterozygous variants lead to a different phenotype: distal hereditary motor neuropathy affecting the upper and lower limbs, and involving the tenth cranial nerve with vocal cord paresis from the second decade onwards [43].

SLC18A3 encodes the vesicular acetylcholine transporter VACHT that loads acetylcholine into synaptic vesicles. Muscle manifestations in these patients worsen in cold water (paramyotonia) [44].

Disorders affecting synaptic vesicle trafficking include defects in exocytosis of synaptic vesicles such as SNAP25 (inhibition of synaptic vesicle release), VAMP1 (vesicle fusion), SYB1 (vesicle exocytosis), SYT2 (calcium evoked acetyl-choline release), MUNC13-1 (vesicle docking). In addition to CMS, other clinical signs are ID, ataxia and congenital contractures in SNAP25 defect, knee contractures in VAMP1 defect, and microcephaly and spastic paraparesis in MUNC-13-1. In these vesicle trafficking defects, pyridostigmine may have some therapeutic effect [40].

Post-synaptic CMS (Fig. 30.4) represent the vast majority of CMS and are caused by mutations in nicotinic receptors: CHRNA1, CHRNE, CHRNB1, CHRND, CHRNG, MUSK and RAPSN. The neuronal nicotinic receptors CHRNA4 and CHRNB2 cause frontal lobe epilepsy and mutations in the muscarinic receptor CHMR3 a distinctive syndrome characterized by pupillary dysfunction and dry mouth and prune-belly syndrome [45]. Additional symptoms include dysmorphism in CHRNA1, and lethal multiple pterygium syndrome (LMPS) or the Escobar variant of multiple pterygia, respiratory distress: (EVMPS) in CHRNG. Fluoxetine has been reported to be beneficial for one patient with CHRNB1, albuterol and salbutamol for CHRNE and albuterol for MUSK [40].

Clinical diagnosis is based on the neurological features and the response to medication. Confirmation of the clinical diagnosis requires DNA sequencing.

30.5 Monoamine Neurotransmitter Disorders

The monoamines, adrenaline, noradrenaline, dopamine, and serotonin, are metabolites of the amino acids tyrosine and tryptophan. The first step in their formation is

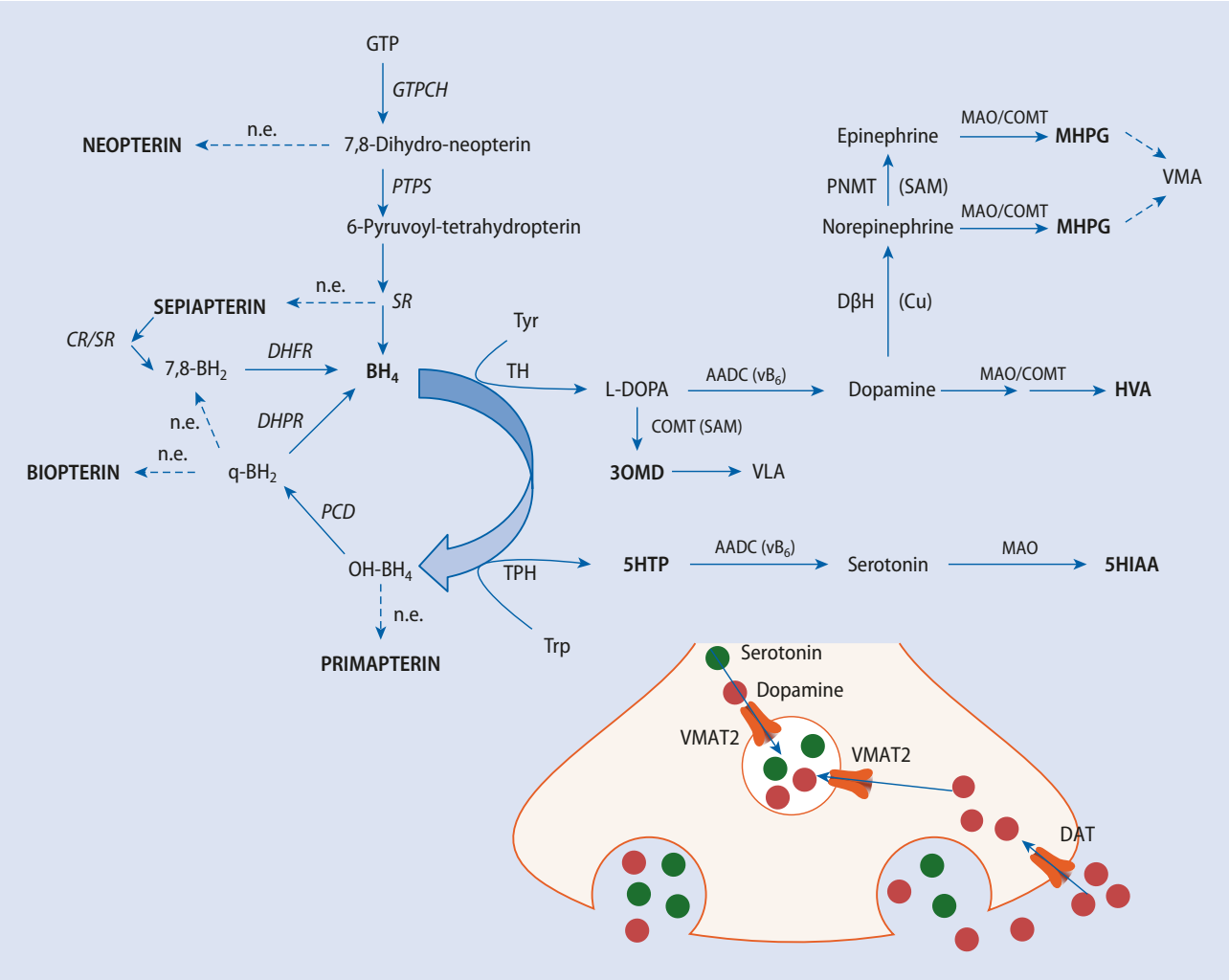


Fig. 30.5 Biochemical pathways of neurotransmitters and pterins. The key metabolites for neurotransmitters and proteins are marked in bold letters. Their simultaneous quantification in CSF is very useful in order to make the correct diagnosis. Abbreviations: AADC aromatic L-amino acid decarboxylase, 7,8-BH_2 7,8-dihydrobiopterin, BH_4 tetrahydrobiopterin, COMT catechol O-methyltransferase, CR carbonyl reductase, DHFR dihydrofolate reductase, DHPR dihydropteridine reductase, DβH dopamine beta-hydroxylase, GTP guanosine triphosphate, GTPCH GTP cyclohydrolase I, 5HTP 5-hydroxytryptophan, 5HIAA 5-hydroxyindoleacetic acid, HVA homovanillic acid, L-DOPA 3,4-dihydroxyphenylalanine, MAO monoamine oxidase, MHPG 3-methoxy-4-hydroxyphenylglycol, n.e. non-enzymatic, 3OMD 3-O-methyldopa, OH-BH_4 hydroxy-tetrahydrobiopterin, PCD pterin-4a-carbinolamine dehydratase, PNMT phenylethanolamine N-methyltransferase, PTPS 6-pyruvoly-tetrahydropterin synthase, q-BH_2 quinoide-dihydrobiopterin, SAM S-adenosylmethionine, SR sepiapterin reductase, TPH tryprophan-5-hydroxylase, TH tyrosine 3-hydroxylase, VLA vanillactic acid, VMA vanillmandelic acid, vB6 vitamin B_6

catalysed by amino-acid-specific hydroxylases, which require tetrahydrobiopterin (BH_4) as a cofactor. BH_4 is also a cofactor of phenylalanine hydroxylase (▶ Chap. 16). Its synthesis from GTP is initiated by the rate-limiting GTP cyclohydrolase-1 (GTPCH-I), which forms dihydroneopterin triphosphate (NH2TP). L-dopa and 5-hydroxytryptophan (5-HTP) are metabolized by a common vitamin B_6-dependent aromatic L-amino acid decarboxylase (AADC) into dopamine (the precursor of the catecholamines, adrenaline and noradrenaline) and serotonin (5-hydroxytryptamine), respectively. Adrenaline and noradrenaline are catabolized into vanillylmandelic acid (VMA) and 3-methoxy-4-hydroxyphenylethyleneglycol (MHPG) via monoamine oxidase A (MAO-A). This enzyme is also involved in the catabolism of both dopamine into homovanillic acid (HVA) via 3-methoxytyramine, and serotonin into 5-hydroxyindoleacetic acid (5-HIAA) (Fig. 30.5). Dopaminergic modulation of ion fluxes regulates emotion, activity, behaviour, nerve conduction, and the release of a number of hormones via G-protein-coupled cell-surface dopamine receptors. Serotonin modulates body temperature, blood pressure, endocrine secretion, appetite, sexual behaviour, movement, emesis, and pain.

Eight genetic disorders of monoamine metabolism are discussed, including enzymes for biosynthesis and catabolism of monoamines (tyrosine hydroxylase, dopamine β-hydroxylase, aromatic L-amino acid decarboxylase, monoamine-oxidase A), synthesis of the cofactor BH_4 (guanosine triphosphate cyclohydrolase-I and sepiapterin reductase, the last two defects causing tetrahydrobiopterin deficiency without hyperphenylalaninemia), and monoamine transporters (dopamine transporter and brain dopamine-serotonin vesicular transporter). Mutations in *DNAJC12* also disrupt monoamine metabolism, but since they cause hyperphenylalaninemia, this disease will be detailed in ▶ Chap. 16. A patient data registry has been launched within the international working group on neurotransmitter disorders (INTD: ▶ https://intd-registry.org).

30.5.1 Tyrosine Hydroxylase Deficiency

Clinical Presentation

About 80 cases of TH deficiency (THD) have been reported worldwide. Patients disclose two main forms of clinical presentation [46]: Type A displays hypokinetic-rigid syndrome (HRS) plus dystonia, with onset in infancy or childhood while Type B presents with complex encephalopathy with neonatal or early infancy onset (HRS plus developmental delay and other movement disorders). Non-progressive ID, tremor, chorea, oculogyric crises (OGC), ptosis, autonomic dysfunction and poor response to L-dopa, can be present in both groups but are more likely in type B [46]. Motor and cognitive prognosis is worse in type B; however, it is likely that there is a phenotypic continuum from A to B phenotype. Atypical clinical cases of THD have also been reported such as spastic paraplegia and dopa responsive myoclonus dystonia [47]. An adult case with task dependent dopa-responsive dystonia has been reported [48].

Metabolic Derangement

TH converts tyrosine into L-dopa, the direct precursor of catecholamine biosynthesis (◘ Fig. 30.5). This enzymatic step is rate-limiting in the biosynthesis of the catecholamines. The enzyme is expressed in the brain and in the adrenals but also in kidney, intestine and lymphoid nodes [46]. BH_4 is the co-factor. The biochemical hallmarks of the disease are low CSF levels of HVA and MHPG, the catabolites of dopamine and norepinephrine, respectively, with normal 5-HIAA levels [49, 50]. Pterin values are normal (◘ Table 30.3).

Genetics and Diagnostic Tests

TH deficiency is an AR disease. Several mutations including promotor region and deletions have been described in *TH*. Patients with promoter mutations had type A TH deficiency [46]. Two common mutations due to founder effects have been detected in the Dutch and the Greek populations [51, 52]. The most important diagnostic test is the measurement of HVA, MHPG, and 5-HIAA in the CSF (◘ Table 30.3). The HVA/5HIAA ratio in CSF is the most sensitive marker not only for diagnosis but also severity [46]. Urinary measurements of HVA and 5-HIAA are not reliable in the diagnosis. Direct enzyme measurement is not a diagnostic option, as there is no enzyme activity detectable in body fluids, blood cells and fibroblasts. Hyperprolactinemia is observed in approximately half of cases.

Treatment and Prognosis

In most cases, TH deficiency can be treated with L-dopa in combination with carbidopa, a L-dopa decarboxylase inhibitor. However, the response is variable, ranging from complete remission (more likely in type A) to mild improvement. Therapy should be started with low doses to prevent dyskinesias. The initiating dose should be <0.5 mg/kg per day, with slow titration (over weeks to months) to 3–10 mg/kg per day, according to response. Patients with poor response to L-Dopa can be treated with the MAO B inhibitor selegiline [53]. Dyskinesias may be triggered by L-dopa increment, intercurrent febrile illness, tiredness, and overexcitement, and may be successfully treated with amantadine [51].

30.5.2 Aromatic L-Amino Acid Decarboxylase Deficiency

Clinical Presentation

Aromatic L-amino acid decarboxylase (AADC) deficiency has been reported in more than 120 patients worldwide [54]. Although neonatal symptoms such as poor sucking, hypotonia, hypothermia and ptosis may be present, clinical presentation of AADC deficiency usually ranges from 4 months to adulthood (although the majority of patients present in childhood). Epilepsy can be associated but it is rarely a single symptom. The most frequent signs are truncal hypotonia associated with limb rigidity, HRS, OGC and developmental delay. Dystonia, ptosis, and autonomic dysfunction (temperature instability with hypothermia, gastro-intestinal

Table 30.3 Biomarkers of monoamine deficiencies

	Biomarkers in the cerebrospinal fluid (CSF)									Biomarkers in urine			Biomarkers in plasma	
DISEASE	**HVA**	**5HIAA**	**HVA/5HIAA ratio**	**3OMD**	**5HTP**	**5MTHF**	**SP**	**NP**	**BP**	**Primapterin**	**NP**	**BP**	**Phe**	**Prolactin**
TH	↓↓	N	↓↓	N	N	N*	N	N	N	N	N	N	N	↑
AADC	↓↓↓	↓	N	↑	↑	N	N	N	N	N	N	N	N	N/↑
GTPCH Recessive	↓↓	↓↓	N	N	N	N	N	↓↓	↓↓	N	↓	↓	↑	N/↑
Dominant	↓	↓/N	N/↓	N	N	N	N	↓	↓	N	N	N	N	N/↑
PTPS	↓↓	↓↓	N	N	N	N	N	↑↑	↓↓	N	↑	↓	↑	N/↑
SR	↓↓	↓↓	N	N	N	N	↑	N	↑	N	N	N	N	N/↑
PCD	↓↓	↓	N	N	N	N	N	N	N	↑	N/↑	N/↓	↑	N
DHPR	↓↓	↓↓	N	N	N	↓	N	N	↑	N	N	↑	↑	N/↑
OTHER	**MAO-A:** HVA ↓, 5HIAA ↓									VLA↓, 3OMD↑, NMN↑			**DBH**: NA i A ↓↓; DA ↑x10; +/– hypoMg; +/– anemia **MAO-A:** Serotonin ↑	
	DAT: HVA ↑, 5HIAA normal, HVA/5HIAA >5													
	VMAT: HVA and 5HIAA↓ Neopterine↑, may be normal.									HVA and 5-HIAA ↑, A and DA ↓				

symptoms, paroxysmal sweating, impaired heart rate and blood pressure regulation, hypoglycaemias, nasal congestion) are also common. A milder AADC phenotype consisting of fatigability, hypersomnolence, dystonia has been reported. Chronic diarrhea has also been reported [54, 55]. A recent study assessed the developmental outcome in a large cohort of patients confirming that OGC were the most common signs in the evolution of patients. More importantly, a high mortality risk was observed for younger patients presenting the severe phenotype [56].

Metabolic Derangement

AADC is implicated in the biosynthesis of catecholamines and of serotonin (Fig. 30.5). The activity of the enzyme requires pyridoxal phosphate as a cofactor, and there is resultant deficiency of the catecholamines and serotonin. The concentrations of the catabolites (HVA from dopamine, 5-HIAA from serotonin, and MHPG in the central nervous system from norepinephrine) are severely reduced in CSF. Another biochemical hallmark of the disease is the increased concentration of metabolites upstvream of the metabolic block: L-dopa, 3-O-methyl-l-dopa, vanillactic acid (VLA), and 5-HTP. In several patients a paradoxical hyperdopaminuria has been noted probably due to production of dopamine and metabolites in non-neural cells (Table 30.3).

Genetics and Diagnostic Tests

AADC deficiency is an AR disorder caused by mutations in *DDC*. Several *DDC* mutations have been reported with a common founder mutation identified in Taiwanese Chinese patients (IVS6+4A>T) [57]. Typical CSF profile consists of markedly decreased HVA and 5-HIAA values, with raised 3-O-methyl-l-dopa and 5-HTP, in the presence of normal pterin concentrations [57]. Increased VLA excretion may be observed in the urinary organic acid profile (Table 30.3). AADC deficiency can be confirmed at the enzyme level, with consistent deficiency in plasma enzyme activity. High throughput newborn screening for AADC deficiency by analysis of concentrations of 3-O-methyldopa from dried blood spots has been validated for early diagnosis [58].

Treatment and Prognosis

Treatment in AADC deficiency may be beneficial but the effects are limited and long-term prognosis is poor. Various strategies have been used including cofactor supplementation in the form of vitamin B_6, (pyridoxine/pyridoxal phosphate), MAO inhibitors (such as tranylcypromine, selegiline, phenelzine), dopamine agonists (pergolide, bromocriptine), high dose L-dopa as "substrate therapy", serotoninergic agents (fluoxetine) or combinations of these and anticholinergic drugs (trihexyphenidyl). Only some patients with relatively mild forms clearly improved on a combined therapy with pyridoxine (B_6)/pyridoxal phosphate, dopamine agonists, and monoamine oxidase B inhibitors. Transdermal rotigotine (a dopamine agonist), may benefit some patients [54]. The most significant advances have been recent gene-therapy approaches, and clinical trials are underway (► www.clinicaltrials.gov), with promising results.

30.5.3 Dopamine β-Hydroxylase Deficiency

Clinical Presentation

Dopamine β-hydroxylase (DBH) deficiency is characterized by lack of sympathetic noradrenergic function. Its clinical hallmark is severe orthostatic hypotension. Most patients complain of fatigue and impaired exercise tolerance. Although DBH deficiency may be present from birth, symptoms become manifest in early childhood and worsen in late adolescence. Perinatal hypoglycaemia, hypothermia and hypotension may occur. There is no obvious intellectual impairment. Additional symptoms in some patients are ptosis, nasal stuffiness, weak facial musculature, hyperflexible joints, brachydactyly and high palate. Impaired kidney function and anemia were present in a recent series of ten patients. Hypomagnesaemia in 5 out of 10. (Wassenberg, JIMD; new [59]). Last clinical descriptions reported in a single patient included hyperinsulinemia, enhanced glucose-stimulated insulin secretion, and insulin resistance [60]. Differential diagnosis includes pure autonomic failure/autonomic neuropathy, familial dysautonomia, and Shy-Drager syndrome or central autonomic failure.

Metabolic Derangement, Diagnostic Tests and Genetics

DBH converts dopamine into noradrenaline. It is present in the synapses of postganglionic sympathetic neurons. A defect in the enzyme should have consequences for (nor-) adrenergic neurons and as well for the adrenals. Pathogenic mutations have been found in *DBH* in all known patients with symptomatic DBH deficiency, and inherited in an AR trait. Tests of autonomic function may provide diagnostic information of great specificity [59]. Patients typically have extremely low plasma noradrenaline and adrenaline levels and increased or high-normal levels of dopamine (◘ Table 30.3). At the enzyme level the diagnosis can be confirmed by the deficiency of DBH activity in plasma. Interestingly, 4% of the population have nearly undetectable DBH activity in plasma with normal concentrations of noradrenaline and adrenaline and without clinical features of DBH deficiency. This is caused by a common allelic variant (1021 C>T) [61].

Treatment and Prognosis

Therapy with L-dihydroxyphenylserine (L-Dops) is available. This compound can be directly converted by AADC into noradrenaline, thereby by-passing the defective enzyme. Administration of 100–500 mg L-Dops orally twice or three times daily increases blood pressure and restores plasma norepinephrine levels, however plasma epinephrine concentration still remains below a detectable level [62]. Droxidopa, an orally available synthetic amino acid precursor of norepinephrine is a new alternative. Long-term, open-label treatment with droxidopa was well tolerated and provided sustained improvement in neurogenic orthostatic hypotension. However, kidney function, anemia, and hypomagnesaemia only partially improved (56 Wassenberg) [63]. Several clinical trials with this drug are completed or ongoing (► www.clinicaltrials.gov). The prognosis on therapy is satisfactory to good.

30.5.4 Monoamine Oxidase-A Deficiency

Clinical Presentation

Monoamine oxidase-A (MAO-A) deficiency has been identified in five generations of one Dutch family [64]. Only males were affected. They showed borderline ID with aggressive and violent behaviour, arson, attempted rape, and exhibitionism. Additionally, a functional polymorphism of the MAO-A gene promoter region may act as a genetic modifier of the severity of autism in males [65]. Other MAO-A polymorphisms have been related to abnormal limbic circuitry for emotion regulation and cognitive control, explaining impulsive aggression and serious delinquency [66]. MAO exists as two X-linked

isoenzymes (A and B). Patients with a contiguous gene syndrome affecting both the MAO-A and -B genes, and also the gene responsible for Norrie disease, have been described with severe intellectual deficiency and blindness [67]. Patients with only the MAO-B and Norrie genes affected had no intellectual impairment or abnormalities in urine catecholamine metabolites.

Metabolic Derangement and Genetics

MAO-A deficiency is a X-linked inherited defect in the catabolism of both serotonin and the catecholamines. Patients have marked elevations of serotonin, normetanephrine, 3-methoxytyramine, and tyramine reported in urine. The concentrations of the metabolites downstream of the metabolic block, VMA, HVA, 5-HIAA, and MHPG, were markedly reduced. A point mutation in the eighth exon of *MAO-A*, causing a premature truncation of the protein, has been reported [64].

Diagnostic Tests, Treatment and Prognosis

Elevated urinary serotonin, normetanephrine, metanephrine, and 3-methoxytyramine is the characteristic pattern in random urine samples (Table 30.3). The ratios in urine of normetanephrine to VMA, normetanephrine to MHPG or HVA/VMA are altered [68]. The discovery of this disorder suggests that it might be worthwhile performing systematic urinary monoamine analysis when investigating unexplained, significant, behaviour disturbances, particularly when these occur in several male family members. In CSF, nearly absent HVA and 5-HIAA are observed, with no accumulation of 3-OMD and 5-HTP (differential diagnosis with AADC deficiency) and normal pterin profile. No effective treatment is known at present. ID and behaviour abnormalities seem to be stable over time.

30.5.5 Guanosine Triphosphate Cyclohydrolase I-Deficiency

Clinical Presentation

This is the most common dopamine-responsive dystonia [69, 70]. Onset is typically about age 6 years, although reported as early as the first week of life or in adulthood, including ages over 50 years [71]. Lower limb dystonia is generally the initial and most prominent symptom, becoming generalized unless treated with L-dopa. Diurnal fluctuation with improvement after sleep is typical. Other clinical features include HRS, OGC, generalized hypotonia, proximal weakness, paroxysmal exercise-induced dystonia, sleep disturbances and impaired cognition [71]. The disease is classified into two types, postural dystonia and action dystonia forms. The dystonia may have a relapsing-remitting course, and be associated with OGC, depression, and migraine. Adult onset patients can start with parkinsonism features and may have mild cognitive impairment and impulsivity [72]. GTPCH can be also inherited as an AR disease and clinical manifestations and very similar to those of sepiapterin reductase deficiency. AR GTPCH-1 deficiency also causes hyperphenylalaninaemia (▶ Chap. 16).

Metabolic Derangement and Genetics

GTPCH-I is the initial and rate-limiting step in BH_4 biosynthesis, the essential cofactor of various aromatic amino acid hydroxylases (Fig. 30.4) with highest affinity for TH. The deficiency is characterized by defective biosynthesis of serotonin and catecholamines. GTPCH-I deficiency can be inherited as both AD and AR trait. The incidence of autosomal dominant GTPCH-I is generally reported to be 2.5–4 fold greater among females than males [71]. A dominant negative mechanism has been proposed [71]. This effect might also account for the phenotypic heterogeneity of DRD, as the degree of enzyme inactivation depends on the specific genetic abnormality. Point mutations and large rearrangements have been reported [71].

Diagnostic Tests

Patients with AD GTPCH-1 deficiency have normal Phe levels in body fluids. The following tests may be helpful in diagnosis (Table 30.3): (1) Measurement of pterins especially in CSF (biopterin and neopterin are decreased from 20% to 50% of normal levels and are the biochemical hallmarks of the disease). (2) Measurement of CSF HVA and 5-HIAA. A normal or slightly low CSF HVA in combination with low 5-HIAA is observed, with no accumulation of biogenic amine precursors (3-OMD and 5-HTP). (3) An oral Phe-loading test. In general, it reveals a 2–6 hour increase in Phe levels and Phe/Tyr ratio. (4) Mutation analysis. (5) Measurement of enzyme activity in fibroblasts. Biochemical alterations of the AR form are described in Table 30.3.

Treatment and Prognosis

Patients have been treated with a combination of low dose L-dopa (4–5 mg/kg/day) and a dopa-decarboxylase inhibitor. There is normally a complete or near-complete response of motor problems soon after therapy initiation. Even when therapy is started after a delay of several years, results are satisfactory. However, for action dystonia and adult onset cases, levodopa does not always show complete effects [71].

30.5.6 Sepiapterin Reductase Deficiency

Clinical Presentation

Sepiapterin reductase deficiency (SRD) is implicated in the final step of BH_4 synthesis. Friedman et al. [73] reported the largest SRD series to date highlighting significant delay in diagnosis, with frequent misdiagnoses of cerebral palsy. Clinical features of SRD are axial hypotonia, motor and language delay, oculogyric crises, weakness, dystonia with diurnal fluctuation, parkinsonism, sleep disturbances, behavioral and psychiatric abnormalities [73]. Tremor of the limbs and head at rest, inhibited by skin contact and spontaneous movement, has been reported as presenting symptoms during infancy [74].

Metabolic Derangement, Genetics and Diagnostic tests

Central nervous system BH_4 depletion contributes to deficient dopamine and serotonin biosynthesis, though with normal availability in peripheral tissues due to alternative metabolic pathways that bypass SR and therefore lack of hyperphenylalaninemia on newborn screening [73]. SRD is AR; different SPR pathogenic variants have been reported [73]. CSF shows elevated biopterin and sepiapterin (the hallmark of the disease) with normal neopterin levels, and very low HVA and 5-HIAA (◘ Table 30.3). Urine pterins are normal. The phenylalanine loading test is frequently positive. Fibroblast enzyme activity is reduced. Accumulation of sepiapterin in urine may be a potential biomarker. Consensus guidelines for the diagnosis and treatment of tetrahydrobiopterin deficiencies (GTPCH-I and SRD) have been recently published [75].

Treatment and Prognosis

For SRD, therapeutic approaches involve dopamine and serotonin precursor supplementation; most patients respond well to L-dopa and 5-hydroxytryptophan combination. Improvement in motor and sleep symptoms have been reported with the combination of L-dopa and carbidopa. Since dyskinesias may appear after treatment, a very low starting dose of L-dopa (around 0.5 mg/kg daily) with slow increment is advised. Regarding 5-HTP treatment (a precursor of serotonin), improvement in sleep, motor, and cognitive aspects have been reported (doses ranging from 1 to 6 mg/kg daily) [75]. Urinary sulphatoxymelatonin has been proposed as a biomarker of serotonin status in biogenic amine-deficient patients, including SRD [76].

30.5.7 Dopamine Transporter Defect

Dopamine transporter (DAT) deficiency syndrome due to *SLC6A3* mutations is an AR disorder presenting as early infantile progressive parkinsonism dystonia with a wide variety of neurological signs [77]. Atypical presentations appear later in childhood including juvenile onset with a milder course [78]. Overall, the clinical picture shows progressive parkinsonism-dystonia that is medically refractory. Other signs are masked facies, eye movement disorders, and some gastrointestinal symptoms. CSF HVA is elevated. *SLC6A3* mutations reduce levels of DAT and the binding affinity of dopamine. Genotype-phenotype analysis suggests that higher residual DAT activity contributes to later presentations. *SLC6A3* missense mutations have been linked to adult parkinsonism and ADHD (attention deficit hyperactivity disorder) [79].

30.5.8 Brain Dopamine-Serotonin Vesicular Transport Defect

Mutations in *SLC18A2*, encoding vesicular monoamine transporter-2 (VMAT2), have been described as an AR cause of severe infantile parkinsonism, autonomic instability, and developmental delay. VMAT2 transports dopamine and serotonin into synaptic vesicles. CSF neurotransmitter metabolites were normal, while depletion of platelet serotonin suggests that these blood cells can be model cells for some pathways relevant for neurological diseases [80]. Treatment with L-dopa caused worsening, whereas dopamine agonists (pramipexole) led to symptomatic improvement [81].

30.5.9 Other Defects

Other extremely uncommon monoamine defects are cytochrome b561 deficiency and norepinephrine transporter deficiency, both associated with orthostatic hypotension. AR b561 deficiency is associated with hypoglycemia and neurological and genitourinary dysfunction. Disrupted ascorbate recycling was suggested to cause functional DBH deficiency and defective norepinephrine synthesis from dopamine [82]. Norepinephrine transporter deficiency seems to be caused by heterozygous *SLC6A2* gene mutations, with orthostatic hypotension and tachycardia [83].

30.6 Synaptic Vesicle Disorders (see also ▶ Chap. 44)

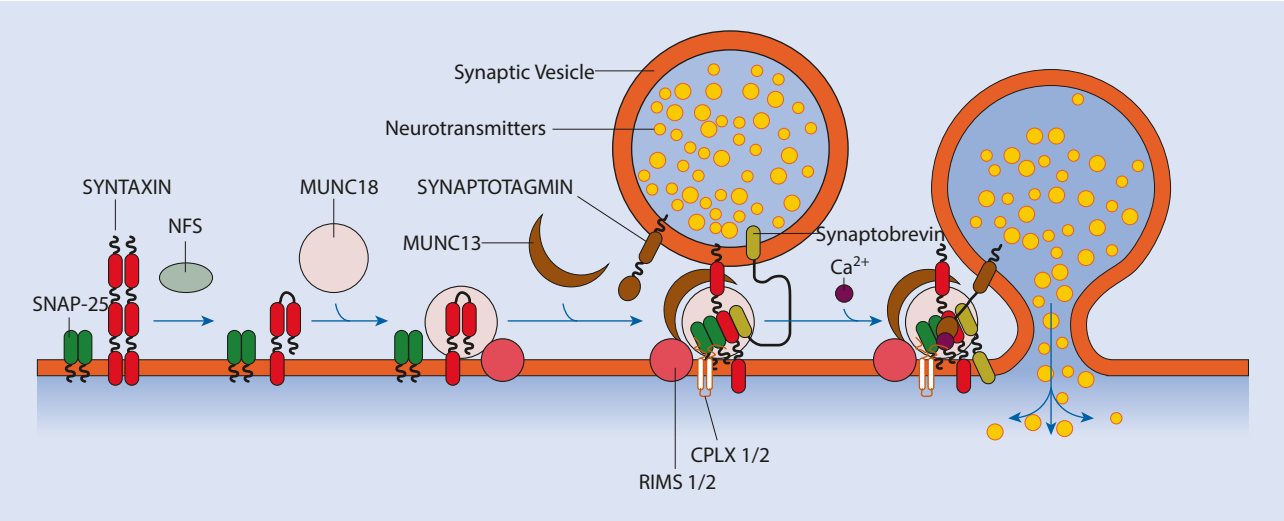
Synapses are equipped with a highly specialized protein machinery to ensure proper neuronal communication. At the presynaptic neuron, neurotransmitter molecules are packed into synaptic vesicles (SVs), ≈40 nm diameter lipid bi-layered organelles containing numerous proteins [84]. SVs are synthesized at the neuronal soma as SV precursors, and travel along the axon to generate mature SVs at synapses. At the synapse, mature SVs translocate to the active zone plasma membrane, where they dock via the interaction of the SV protein synaptobrevin 2 and the plasma membrane proteins SNAP25 and syntaxin 1, that form the SNARE (soluble N-ethylmaleimide–sensitive fusion factor [NSF] attachment protein receptor) complex (◘ Fig. 30.6). When an action potential travelling along the axon invades the presynaptic compartment, membrane depolarization occurs, and massive Ca^{2+} influx evokes an ultrafast SV fusion with the plasma membrane. The vesicular synaptic proteins, as well as the SV membrane, are then retrieved in an endocytotic process that can be clathrin-independent or -dependent [85]. Although mutations in genes that regulate biogenesis and axonal transport of the SVs may affect neurotransmission, disorders of the SV that have been reported to impair neuronal transmission are those involved in exocytosis and endocytosis (◘ Fig. 30.7). There are ~80 genetic defects linked to the SV [86]. We discuss disorders of the pre-synatic terminal (34), all of which impair SV transport, dynamics, and recycling, and therefore considered disorders of cellular trafficking (▶ Chap. 44).

30.6.1 Disorders of SV Exocytosis

Exocytosis of the SV is mediated by SNARE proteins and their key regulators that drive synaptic transmission as an integrated membrane fusion-machine. Syntaxin 1, SNAP25 and synaptobrevin/VAMP2 are the core of this molecular complex. Additional proteins such as MUNC18, MUNC13, synaptotagmins, and complexins regulate the SNARE proteins and therefore participate in the neurotransmission process (◘ Fig. 30.6). Together they constitute an assembly of proteins that interact as a single structure regulated by neuronal activity. The participation of lipids is also important to modulate membrane dynamics and protein interactions.

Clinical Presentation

Mutations in syntaxin 1A (*STX1A*) and 1B (*STX1B*), *SNAP25*, *VAMP2*, synaptotagmin 1 (*SYT1*), *MUNC18–1* (also called *STXBP1*), *UNC13A*, *RIMS1* and *CPLX1* are considered SNAREopathies [87]. They present clinically as neurodevelopmental encephalopathies starting the first year of life, with a constellation of symptoms including global developmental delay, epilepsy (some as severe epileptic encephalopathies), movement disorders (typically hyperkinetic, e.g. dyskinesias, stereotypies), and neuropsychiatric signs including autism spectrum. These symptoms may overlap and present as a combination resulting in a spectrum of "synaptopathies". Other less common signs are neuromuscular dysfunction, ataxia, and ocular abnormalities such as cone-rod dystrophy in *RIMS1* mutations (◘ Table 30.4). The most prevalent are *MUNC18-1*/*STXBP1* with 250+ cases reported [88]



◘ **Fig. 30.6** Synaptic Vesicle exocytosis. SNARE proteins establish interactions between them addressed to synaptic vesicle priming, docking and neurotransmitter release. Syntaxin-1, synaptobrevin and SNAP-25 form the membrane fusion complex. Syntaxin-1, Munc18–1, and 13, NSF, SNAP-25, RIMS and CPLX contribute to exocytosis through membrane fusion. Synaptotagmin-1 acts as a calcium sensor trigging fast neurotransmitter release

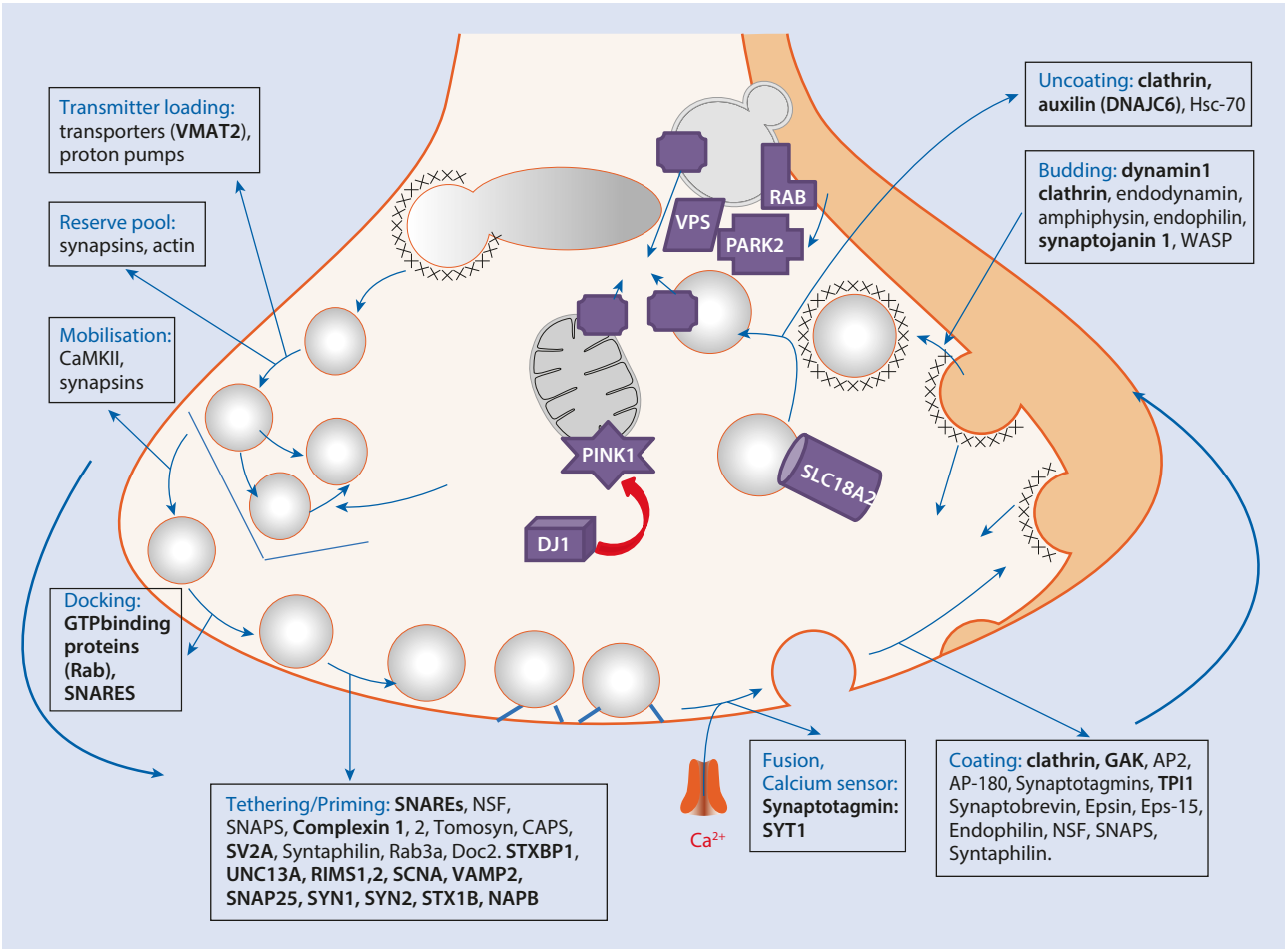


Fig. 30.7 Synaptic Vesicle cycle disorders at the pre-synaptic terminal. Synaptic Vesicles (SV) are filled with neurotransmitters and recruited to sites within the active zone in a process called docking. These SV are primed for release. The rise in cytosolic calcium that occurs after an action potential triggers the opening of a fusion pore between the SVs and plasma membrane. Neurotransmitters are then released. The empty vesicle can be recovered by different ways: (1) a direct reclosing of the fusion pore and reformation of the vesicle ("kiss and run"); (2) complete fusion followed by clathrin-dependent endocytosis, removal of the clathrin coat, and return of the vesicle to the releasable pool; (3) fast, non-clathrin mediated endocytosis: the endocytosed vesicle fuses with an endosome and mature vesicles are formed by budding from the endosome. Proteins marked in bold letters cause SV disorders and are related to their specific action in the different biological processes of the SV cycle including mitophagy and autophagy

and *STX1B* ~50 patients described [89]. There are other genes involved in exocytosis that may alter neurotransmitter homeostasis by interacting with SNARE proteins or through other mechanisms incompletely understood (Table 30.4). This is the case of *NAPB*, *PRRT2*, *SV2A*, *SYN1*, *SYN2*, *GS27* or *GOSR22*, *SYT14*, and *SNCA*. *PRRT2* mutations are a major cause of paroxysmal movement disorders and migraine. *SNCA* mutations lead to late-onset Parkinson disease and dementia [90].

Metabolic Derangement and Genetics

In general, these diseases are caused by heterozygous missense loss of function mutations, although gain of function has been described in the only reported case of *UNC13A* and some *STXBP1* cases [87]. Accordingly, these patients have an increased probability of membrane fusion and a higher number of excitatory postsynaptic currents leading to a hypertransmission state. Little has been reported about biomarkers in these diseases, including studies of CSF neurotransmitter levels. In a personal observation (García-Cazorla), 5-HIAA and GABA levels tended to be high in some *STXBP1* patients, although normal in others; functional studies were not performed. Other -omic approaches may contribute to reveal biomarkers in the future.

Diagnostic tests, treatment and prognosis

Clinical diagnosis is based on the neurological features and confirmation requires DNA sequencing. Treatment is symptomatic. Regarding prognosis, *STXBP1* patients may progress towards parkinsonism over time. Syntaxin-binding protein 1 is a chaperone of alpha-

Table 30.4 Synaptic vesicle disorders and neurological manifestations

Genetic defect and encoded protein [Inheritance] MIM number	Biological function	Other additional Neurological and extra-Neurological signs
NEURODEVELOPMENTAL ENCEPHALOPATHIES WITH EPILEPSY AS PREDOMINANT NEUROLOGICAL MANIFESTATION. Genes responsible of ID/ASD as prominent neurological manifestation are also very likely to produce epilepsy (*SYN1, SYN2*)		
NAPB (N-ethylmaleimide-sensitive factor attachment protein, beta) [AR]	SNARE complex dissociation and recycling: synaptic vesicle docking. EXOCYTOSIS and ENDOCYTOSIS	Early epileptic encephalopathy (multifocal seizures), progressive microcephaly, profound global developmental delay, hypotonia, limb tremulousness and stereotypies
PRRT2 (proline-rich transmembrane protein 2) [AD]	SNARE protein interaction. Regulates EXOCYTOSIS, possibly via interaction with SNAP25.	Benign familial infantile epilepsy, infantile convulsions and choreoathetosis and paroxysmal kinesigenic dyskinesia, migraine, hemiplegic migraine, non-syndromic ID.
SNAP25 (synaptosomal-associated protein, 25-Kd) [AD]	SNARE protein. EXOCYTOSIS	Early epilepsy and developmental delay, hypotonia, ID. Polymorphisms have been related to neuropsychiatric disorders
STXBP1 (syntaxin-binding protein-1, Munc18-1) [AD]	SNARE protein. EXOCYTOSIS	Multiple forms of epilepsy, non-syndromic ID without epilepsy, movement disorders (tremor, ataxia, hyperkinetic movements). Autistic features.
STX1B (syntaxin1B) [AR]	SNARE protein. EXOCYTOSIS	Febrile seizures with or without epilepsy, myoclonic astatic epilepsy.
SV2A (synaptic vesicle glycoprotein 2A) [AD]	SV protein. Regulates EXOCYTOSIS and vesicle fusion by maintaining the SV readily releasable pool	Refractory tonic and myoclonic seizures, microcephaly, growth retardation, optic atrophy, severe hypotonia. Increased T2 signal white matter, thin corpus callosum.
DNM1 (dynamin1) [AD]	SV cycle, Clathrin-mediated ENDOCYTOSIS.	Early onset epileptic encephalopathy, Lennox-Gastaut, West syndrome, ID, hypotonia. Early-onset parkinsonism
TPI1 (triosephosphate isomerase 1) [AR]	SV cycle. ENDOCYTOSIS.	Hemolytic anemia, episodic seizures, periodic dystonia, axonal neuropathy and psychomotor delay (► Chap. 7)
CPLX1 (complexin 1) [AR]	SNARE protein. EXOCYTOSIS	Myoclonic epilepsy, often migrating, severe hypotonia and encephalopathy
DISEASES WITH INTELLECTUAL DISABILITY and AUTISM as the predominant neurological manifestation *(see also VAMP2 and RIMS1)*		
SYN1 (synapsin1) [XLD, XLR] and *SYN2* (synapsin 2) [AD]	Regulation of Neurotransmitter release. EXOCYTOSIS	ASD and epilepsy
UNC13A or *MUNC13–1* (Protein unc-13 homolog A) [AD]	SV docking/priming. EXOCYTOSIS	Dyskinetic movement disorder, developmental delay, and autism.
DISEASES WITH MOVEMENT DISORDERS as the predominant neurological manifestation		
PARKINSONISM (Dystonia-Parkinsonism)		
RAB39B (Ras-related protein Rab-39B) [XLR]	Small GTPases. ENDOCYTOSIS	Waisman Syndrome: delayed psychomotor development, intellectual disability, and early-onset Parkinson's disease (PD). Probable neurodegeneration.
LRRK2 (Leucine-rich repeat serine/threonine-protein kinase 2) [AD]	ENDOCYTOSIS and recycling.	Late onset Parkinson Disease (sporadic and dominant) (PARK8).
SCNA (synuclein alpha) [AD]	SV tethering. EXOCYTOSIS Synaptic protein distribution and SV release.	Late onset Parkinson Disease, Dementia

(continued)

Table 30.4 (continued)

Genetic defect and encoded protein [Inheritance] MIM number	Biological function	Other additional Neurological and extra-Neurological signs
SYNJ1 (synaptojanin 1) [AR]	SV cycle, ENDOCYTOSIS and recycling. Phosphoinositide phosphatase protein involved in SV recycling through lipid metabolism.	Pediatric and Juvenile Parkinsonism, dystonia, and cognitive deterioration. May response transiently to L-dopa. Severe early onset epileptic encephalopathy is other form of presentation. Low CSF HVA levels have been described.
DNM1L (dynamin-like protein 1) [AR]	SV cycle ENDOCYTOSIS. Required for formation of endocytic vesicles.	Severe infantile parkinsonism with tremor in the neonatal period Low HVA in CSF, and findings consistent with lactic encephalopathy might be found. Severe early onset epileptic encephalopathy is other form of presentation
VPS35 (vacuolar protein sorting 35) [AD] #614203; *VPS13C.* [AR]	SV cycle. endosome-trans-golgi trafficking and membrane-protein recycling. ENDOCYTOSIS	*VPS35:* Tremor-predominant, L-dopa-responsive parkinsonism. *VPS13C*. Early-onset parkinsonism
PINK1 (Serine/threonine-protein kinase PINK1, mitochondria) [AR]	SV recycling. Mitochondrial protein. SV mobilization and autophaghy.	Early-onset Parkinson's disease.
DJ1 or *PARK7* (Protein/nucleic acid deglycase DJ-1) [AR]	SV recycling. Interacts with lipid phosphatase to inhibit PINK1	Autosomal recessive early onset Parkinson's disease.
PRKN or *PARK2* (parkin) [AR]	SV recycling, ENDOCYTOSIS. Interacts with RAB, VPS. Mitophagy	Juvenile Parkinson's disease.
ATP13A2 (Cation-transporting ATPase 13A2) and *ATP6AP2* (cation transportin ATPase 6, accessory protein 2). [XLR]	SV endosomal pathway. Cation pump of cell membranes. ENDOCYTOSIS	*ATP13A2:* Kufor-Rakeb syndrome: autosomal recessive hereditary parkinsonism with dementia, and juvenile onset. Some forms of spastic paraplegia. *ATP6AP2:* X-linked ID, epilepsy, and parkinsonism.
GAK (cyclin G-associated kinase) susceptibility gene	SV endosomal pathway. ENDOCYTOSIS	Increases the risk of Parkinson's disease.
DNAJC12 (Heat shock cognate 71 kDa protein) [AR]. *DNAJC13* (DnaJ homolog subfamily C member 13) [AD]. *DNAJC6* (auxilin) [AR]. *DNAJC26, DNAJC10 (both risk factors), DNAJC5 o CSPalpha* [AD]	SV cycle, ENDOCYTOSIS and recycling.Co-chaperone family member. *DNAJC13* is involved in autophagy	*DNAJC12:* HVA and 5-HIAA depletion with hyperphenylalaninemia.Non progressive *DNAJC13:* Late-onset Parkinsonism associated with Lewy body pathology. *DNAJC6*: parkinsonism-dystonia starting from 7 years onwards. Partial response to L-Dopa. Low HVA levels in CSF. *DNAJC26*: early-onset parkinsonism. *DNAJC26 and DNAJC10:* sporadic Parkinson Disease; *DNAJC5:* adult cerebral lipofuscinosis
CLTC (Clathrin). [AD]	SV cycle, ENDOCYTOSIS	ID, hypotonia and early parkinsonism
OTHER ABNORMAL MOVEMENTS (Neurodevelopmental encephalopathies with epilepsy, and ID/ASD have also hyperkinetic movements)		
GS27 or *GOSR2* (Golgi SNAP receptor complex 2) [AR]	SV cycle, SNARE protein Indirectly regulates EXOCYTOSIS	Early-onset ataxia in patients with progressive myoclonic epilepsy. Mild cerebral atrophy. Very mild cognitive impairment. Epilepsy
SYT14 (synaptotagmin 14) [AR]	SV cycle: EXOCYTOSIS	Childhood onset psychomotor delay and progressive spinocerebellar ataxia
VAMP2 (Vesicle-associated membrane protein 2) [AD]	SV cycle: SNARE protein EXOCYTOSIS	Neurodevelopmental encephalopathy with hypotonia, ID, autistic features and hyperkinetic movements.
OTHER symptoms: *RIMS1(SNAREopathy, AD, EXOCYTOSIS): cone-rod dystrophy, one family reported with high intellectual quotient and ASD*		

ID intellectual disability, *AD* autosomal dominant, *AR* autosomal recessive, *ASD* autism spectrum disorder, *XL* X-linked, *XLR* X-linked recessive, *XLD* X-linked dominant

synuclein and could contribute to its misfolding and aggregation [91].

30.6.2 Disorders of SV Endocytosis

The membranes of SVs after neurotransmitter release are retrieved via recycling mechanisms and are regenerated as SVs after refilling with neurotransmitters (◘ Fig. 30.7). Ultrafast endocytosis is clathrin-independent and mediates direct recycling of SVs very rapidly. Clathrin mediated endocytosis is thought to be one of the major mechanisms of endocytosis. Clathrin covers vesicles by polymerization of multiple clathrin molecules. Several proteins and phosphoinositides participate in this endocytic process.

▪ Clinical Presentation

Mutations in *LRRK2*, *ATP13A2*, *ATP6AP2*, diverse *DNAJC* genes (◘ Table 30.4), *SYNJ1* (synaptojanin-1), *Vps13c, Vps35*, *Rab39B*, *GAK, CLTCL1* (clathrin), *DNM1L*, *DJ1* or *PARK7*, *PRK2 or PARK2* (Parkin), *PINK1* and *TPI1* are endocytic-related SV diseases. Parkinsonism is the most common clinical manifestation. Some genes are linked to pediatric and juvenile parkinsonism: *CLTCL1*, *RAB39B*, *SYNJ1*, *DNM1L* [92], *Vps13c*, *PINK1*, *DJ1*, *PRK2*, *ATP13A2*, *ATP6AP2*, *DNAJC12* and *6* [86, 90, 93]. Early-onset parkinsonism is often associated with developmental delay, ID, and other movement disorders. Other genes produce classical late-onset Parkinson disease: *LRRK2*, *Vps35*, *DNAJC* subtypes 10, 13, 26. *DNAJC5* (CSP-alpha) mutations cause adult-onset cerebral lipofuscinosis and may present with parkinsonism (► Chap. 44). Clathrin (*CLTC*) mutations may cause intellectual disability as a single sign and associate with early hypotonia and refractory epilepsy. *SYNJ1* can also present as severe early epileptic encephalopathy. TPI1 (triosephosphate isomerase 1) deficiency causes hemolytic anemia (► Chap. 7) [86, 93] (◘ Table 30.4).

▪ Metabolic Derangement and Genetics

These disorders affect diverse steps of the trafficking processes involved in clathrin-dependent endocytosis (◘ Fig. 30.7), including clathrin removal of coated vesicles (such as in auxilin/*DNAJC6* mutations), endosome-trans-golgi trafficking (vacuolar protein sorting), membrane-protein recycling, and autophagy (parkin). Synaptojanin 1 (*SYNJ1*) is a phosphoinositide phosphatase protein involved in SV recycling through lipid metabolism. *ATP13A2* and *ATP6AP2* genes encode flippases involved in calcium pump (see also traffic Chapter). Inheritance is diverse: AD, AR and X-linked (*SYN1*, *Rab39B*).

▪ Diagnostic tests, treatment, and prognosis

Clinical diagnosis is based on the neurological features and confirmation requires DNA sequencing. Some disorders have been described to have low levels of HVA in the CSF: *DNAJC6* [94], *CLCT* [95, 96], *SYNJ1*, and may respond, at least partially or transiently, to L-dopa treatment, mimicking dopamine biosynthesis defects. *VPS35* also responds to L-dopa. The great majority of these disorders have a neurodegenerative course. However, in most of them only few patients have been described and the long-term outcome is unknown.

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Disorders of Peptide and Amine Metabolism

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Contents

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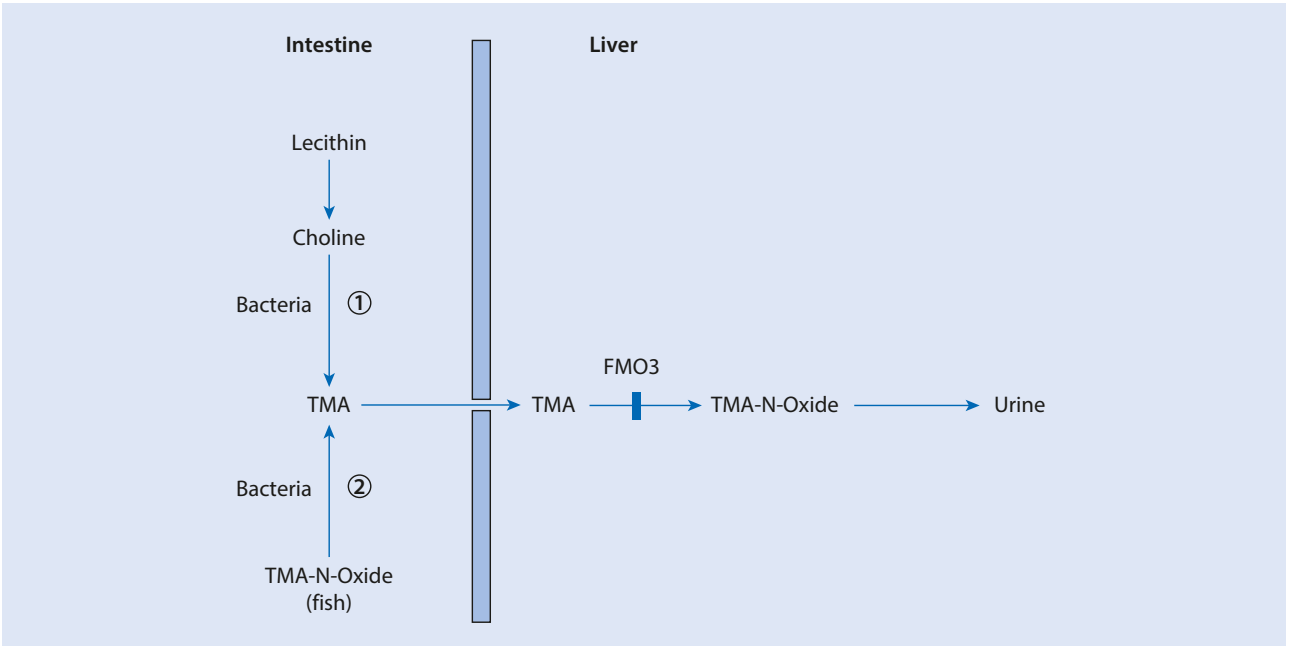

Fig. 31.1 Metabolism of trimethylamine. FMO3 flavin-containing monooxygenase 3, TMA trimethylamine, ①, bacterial choline TMA-lyase, ②, bacterial TMA-N-oxide reductase. The enzyme defect in trimethylaminuria is indicated by a solid blue bar

Trimethylamine Metabolism

Trimethylamine (TMA) is a tertiary amine, a strong base (pKa 9.8), and in its non-protonated free form is volatile and malodorous at ambient temperatures. It is a bacterial metabolite produced in the colon by the action of a wide range of resident micro-organisms on dietary components: free and lecithin-bound choline, trimethylamine-*N*-oxide (TMAO) in salt water fish, carnitine and betaine. The generated TMA is readily absorbed from the colon and transported to the liver where more than 90% is oxygenated by microsomal flavin-containing monooxygenase 3 (FMO3) which requires FAD as a prosthetic group. The product is TMAO which is water soluble, non-odorous and is excreted in urine (Fig. 31.1). FMO3 also catalyses oxygenation of a broad range of drugs [1]. The abundance of liver FMO3 varies with ethnicity and between individuals. Production of FMO3 commences within days of birth but is low throughout childhood. Expression is reduced peri-menstrually, and activity is inhibited by sulfur-containing glucosinolates present in brassica vegetables and by nitric oxide mediated S-nitrosylation. (Reviewed [1–6]).

Introduction

Trimethylamine (TMA) is a volatile tertiary amine which smells of decaying fish. It is a bacterial metabolite which is produced by anaerobes resident in the human colon from choline (free and lecithin-bound), trimethylamine-*N*-oxide (TMAO) in salt water fish, carnitine and betaine. Catabolism of choline occurs within the mitochondria and involves the sequential removal of two methyl groups by dimethylglycine dehydrogenase (DMGDH) and sarcosine dehydrogenase (SDH). Glutathione (GSH) is a tripeptide consisting of glutamate, cysteine and glycine. It is ubiquitous in the eukaryotic organism and plays a role in many fundamental cellular processes. The chapter describes metabolic disorders in trimethylamine metabolism, choline metabolism, the glutathione cycle and peptide metabolism.

31.1 Disorders of Trimethylamine Metabolism

31.1.1 Trimethylaminuria (Fish Malodour Syndrome)

Clinical Presentation

Trimethylaminuria (TMAU) is a metabolic disorder in which TMA accumulates in the body and is excreted in the breath, sweat, urine and vaginal secretions. This causes an unpleasant, pervasive body odour of decaying fish, detectable by most of the population at very low TMA concentrations. Inherited TMAU is pan-ethnic,

with autosomal recessive inheritance. TMAU does not cause physical problems, but frequently impacts seriously on the life of the individual. Affected children may be ridiculed or bullied, become isolated, depressed and have poor educational achievement. Older individuals have low self- esteem, obsessional behavior, become socially isolated and have problems in forming relationships [2–6].

Four forms of TMAU can be recognized [1, 4]: (i) A severe form presents with persisting malodour, generally from infancy or early childhood, sometimes later, which is exacerbated by sweating and menstruation. An estimated incidence is 1 in 40,000 in the UK, but it is probably under-diagnosed [7]. (ii) Mild/moderate TMAU is more common. The incidence is unknown. It presents with episodic malodour starting at any age, often at puberty or peri-menstrually. (iii) Transient TMAU has occurred in preterm neonates when fed on choline-supplemented milk formulae. This resolved with withdrawal of the supplement and was attributed to the normal neonatal FMO3 deficiency coupled with a heavy TMA substrate load. Although transient TMAU has been reported in infants and young children, this resulted from low physiological expression of *FMO3* coupled with polymorphisms of the gene [7, 8], and there may be a life-long risk for recurrence. (iv) Increased excretion of TMA as an occasional consequence of underlying disease. This may contribute to malodour in severe chronic liver disease and rarely viral hepatitis, advanced renal failure with bacterial overgrowth in the small intestine, urine infection with TMAO degrading bacteria, anatomical abnormalities of the intestine such as blind loops with abnormal bacterial colonisation, vaginitis and gingivitis [3, 4].

Metabolic Derangement

TMAU occurs when the quantity of TMA absorbed from the diet exceeds the enzyme capacity of hepatic FMO3. The quantity of TMA depends on the amount and form of TMA precursors ingested and the relative abundance of TMA-producing bacterial species. Enzyme capacity is reduced by mutations of *FMO3*, physiologically decreased expression, or by chemical inactivation of FMO3. In severe TMAU genetic deficiency of FMO3 is the over-riding factor. In mild/moderate TMAU the aetiology is multifactorial, with variable contributions from decreased FMO3 production (genetic or physiological) and/or activity, and/or substrate overload (excess dietary precursors or an abnormal gut microflora). Malodour develops intermittently when an increase in one or more of the risk factors disrupts the finely balanced detoxification system [1–4, 6].

Many of the drugs oxygenated by FMO3 in vitro are also extensively metabolised by the hepatic cytochrome P450 system or detoxified by other routes such as glucuronidation. Exceptions include benzydamine, itopride, ranitidine, cimetidine, ethionamide and sulindac sulfide, the active sulindac metabolite. Genetic variants of FMO3 decreased oxygenation of benzydamine, sulindac sulfide & methimazole in vivo [1].

There is an on-going controversy about whether raised circulating TMAO increases the risk for cardiovascular disease and other life-style illnesses. There is no evidence that a low TMAO in TMAU is protective [1].

Genetics

The *FMO3* gene is highly polymorphic. Up to August 2020, 231 transcript variants were recorded on a shared data base (► https://databases.lovd.nl/shared/genes/FMO3; accessed 25.03.2022). Whole exome sequencing (WES) has extended the list [9]. More than 40 variants cause severe TMAU. Most of these are mis-sense, others being nonsense or small deletions causing a frame-shift mutation [1, 4, 7, 9, 10]. Most of the other variants are single nucleotide polymorphisms (SNPs). A few of these are common (frequency >1%), but most are rare. Of the few which have been investigated, most have little or no effect on FMO3 activity [1, 9]. Two SNPs (p.Glu158Lys) and (p.Glu308Gly) present *in cis* on the same allele reduce FMO3 activity in vitro. This allele is common, with homozygosity estimated at 2–5% and 4% in German & Irish populations, respectively. Whilst this is seldom manifest, mild TMAU may occur in individuals who are homozygous for the allele, or compound heterozygous for the variant and an allele with a severe mutation, when TMA absorption is excessive [8, 10].

WES has raised the possibility that polymorphisms in genes other than *FMO3* may disturb TMA metabolism. Variants with predicted pathogenicity were identified in genes in oxido-reductase pathways in individuals with biochemically substantiated TMAU but no detectable *FMO3* mutations [11].

Diagnostic Tests

Poor body and oral hygiene, gingivitis, vaginosis and urine infection are excluded and the possibility of another malodorous inborn error considered. Urine analysis is the front-line diagnostic approach. This should not be undertaken at the onset of, or during, menstruation. Urine is collected into containers containing 2 ml 6M HCl (urine pH 2.0) to convert free TMA to non-volatile salt and frozen until analysis by proton NMR spectroscopy or mass spectrometric procedures for TMA, TMAO and total TMA [4, 11]. A random urine sample is collected if the odour is obvious. If not, samples should be collected for 2–12 h or for a

timed 6–8 h save, after eating 300 g marine fish or a choline-rich meal (2 eggs +400 g baked (haricot) or soya beans), or after drinking 5 g choline in orange juice. The test should be repeated if the results are negative or border-line.

Malodour may be detectable at TMA concentration >10 μmol/L; 18–20 μmol/mmol creatinine [2]. To estimate FMO3 capacity, free TMA excreted as a percentage of total TMA excreted is calculated from the ratio of TMA to TMA + TMAO. Ratio 0–9% not affected; 10–39% mild/ moderate TMAU (mild 10–19%, moderate 20–39%); ≥40% severe TMAU [6, 10].

Genetic testing (sequencing the entire coding region and intron/exon boundaries of *FMO3*) has been advised if the ratio is ≥10% [7]. Although its additive value in severe cases has been questioned [4], it provides firm evidence of the diagnosis which may accelerate decisions about schooling and psychological support, and enables early testing of asymptomatic siblings. Prenatal diagnosis is possible if the family mutation is known, but is not indicated. In those with mild or intermittent symptoms, knowing the genetic contribution may help understanding and management in some cases [4, 10].

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Treatment

Careful explanation, with genetic counselling if indicated, provides insight. Severely disturbed patients may need psychiatric help. Patients should be warned about increased risk for malodour peri-menstrually, with systemic infections and sweating, and advised about hygiene: low pH soaps (pH 5.5–6.5) to reduce TMA volatility, antiperspirants, light clothing and good ventilation in warm environments, regular laundering of clothes, not to take non-prescribed food supplements such as fish oil capsules, carnitine and health foods with high lecithin & choline and to report possible adverse reactions to prescribed medications promptly. Dietary management aims to reduce the TMA load: avoidance of marine fish and other sea foods, foods rich in choline- (egg yolks, liver, kidneys) and lecithin (brassicas [brussels sprouts, broccoli, cabbage, cauliflower], soy beans and other legumes, rapeseed and sunflower oils). Brassicas are also contra-indicated because they inhibit FMO3 [2]. Choline restriction increases folate requirement, and supplements should be given. Moderate restriction is tolerable and often effective in mild/moderate TMAU. There are published recommendations for more choline- restrictive diets, but these must be supervised by a dietitian because of the risks to the brain and liver [6]. Choline should not be restricted in pregnancy, when breast feeding or in infancy. Riboflavin to activate FMO3 may reduce and even normalise urine TMA excretion of those with residual FMO3 function. For special occasions, TMA production by the gut microflora may be reduced by short courses of antibiotics (neomycin or metronidazole), or lactulose to reduce intestinal transit time, but these should not be taken continuously [4, 6]. Probiotics are not of proven benefit [3]. Ingestion of large amounts of white button mushrooms (*Agaricus bisporus)* and of chlorophyllin decreases TMAO reducing Firmicutes. Activated charcoal and chlorophyllin reduced urine TMA and malodour in one study. None of the above supplements have been systematically evaluated. A novel approach to reducing odour may be to promote diffusion of non-protonated TMA into the acidic core of synthetic vesicles applied as a skin gel [12].

Catabolism of Choline

The catabolism of choline (Fig. 31.2) occurs within the mitochondria and involves the sequential removal of two methyl groups by dimethylglycine dehydrogenase (DMGDH) and sarcosine dehydrogenase. These are related flavin enzymes with covalently linked FAD which use folate as co-factor. The methyl groups from dimethylglycine and sarcosine are transferred to tetrahydrofolate (THF), forming 5,10-methylene THF. The electrons are transferred from FAD to electron transfer flavoprotein (ETF) and thence to the mitochondrial respiratory chain.

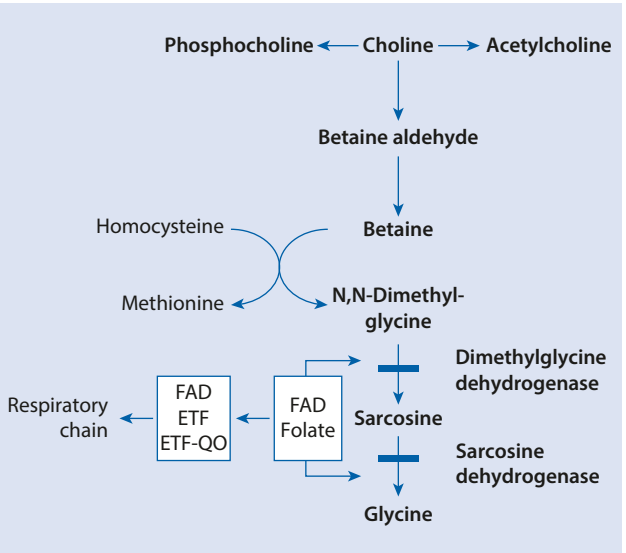

Fig. 31.2 Catabolism of choline. ETF, electron transfer flavoprotein; ETF-QO, ETF-ubiquinone oxidoreductase; FAD, flavin adenine dinucleotide. The enzyme defect in dimethylglycine dehydrogenase deficiency is indicated by a solid bar. Sarcosine dehydrogenase converts sarcosine to glycine

31.2 Disorders of Choline Metabolism

31.2.1 Dimethylglycine Dehydrogenase Deficiency

Clinical Presentation

An adult patient with dimethylglycine dehydrogenase deficiency was investigated for an abnormal body odour resembling fish, which was present from 5 years of age, was increased by stress and effort and caused him major social, psychological and professional problems [13]. The patient had chronic muscle fatigue with persistent elevation of creatine kinase (CK) to around four times normal. Intelligence was normal. Since this first description one further patient with this defect has been reported [14]. This concerns an 11 months old male child with *DMGDH* mutations hospitalised with an upper respiratory tract infection. There was no malodour and CK was 10 times the upper reference range limit. High CK persisted after recovery from the infection. The diagnosis was accomplished using Whole Exome Sequencing. More patients are required to fully understand the clinical presentation and phenotype as well as the clinical significance of *DMGDH* defects [15].

Metabolic Derangement

Dimethylglycine dehydrogenase (DMGDH) is involved in choline- and in 1-carbon metabolism (Fig. 31.2). It catalyzes the oxidation of the tertiary amine N,N-dimethylglycine and the formation of 5,10-methylene tetrahydrofolate. The crystal structure of the enzyme has been published [16]. Dimethylglycine accumulated in body fluids of the adult patient (around 100-fold in plasma and 20-fold in urine) explaining the malodour [13]. Interestingly, data from Magnusson et al. are consistent with a possible causal role of dimethylglycine deficiency in diabetes development [17].

Genetics

The *DMGDH* gene is on chromosome 5q12.2-12.3 [18]. Sequence analysis suggests that the genes for DMGDH and SARDH (sarcosine dehydrogenase) have diverged from a common ancestor. The affected patient is homozygous for an inactivating point mutation (326 A>G leading to H109R at the protein level) of the *DMGDH* gene. From expression studies, this mutated gene codes for a stable protein lacking enzyme activity [19]. The mutated enzyme has a reduced affinity for FAD, a 27-fold decrease in specific activity and a 65-fold increase in Km. This explains the effect on the pathway and the pathogenicity of the mutation [20]. The second patient had a homozygous mutation in a highly conserved nucleotide (c.101+2 T>C) [14]. The paper does not mention body fluid metabolite levels. DMGDH deficiency is inherited as an autosomal recessive trait.

Diagnostic Tests

The diagnosis can be made by exome sequencing (as in the second patient) and at the metabolite level. Against the background of the seemingly mild clinical features of DMGDH deficiency, most future cases will be discovered through whole exome- or genome sequencing. Functional confirmation of the defect can be accomplished by finding raised levels of dimethylglycine in plasma and urine. To this end proton NMR spectroscopy can be used; NMR has the advantage that it can also diagnose trimethylaminuria, another inherited cause of a fishy odour [13; see under 31.1]. Targeted techniques to measure dimethylglycine have been described (GC [21], LC-MS/MS [22], LC-colorimetry [23]) and an untargeted metabolomics strategy may also qualify. Dimethylglycine is currently not detected in routine screening methods for inborn errors of metabolism. Reference values for dimethylglycine are:

- Plasma: healthy adults: 1–5 μmol/L
- Urine:
  - infants (birth to 2 months): <550 μmol/mmol creatinine
  - from 2 months of age (children and adults): <26 μmol/mmol creatinine

Increased serum dimethylglycine levels have also been observed in folate deficiency (up to tenfold), cobalamin deficiency (up to twofold) and renal failure (up to twofold) [13]. DMGDH enzyme activity is present in liver but not in blood cells and fibroblasts.

Treatment

Management is by counselling and minimising the odour by restriction of dietary choline, and avoiding excessive sweating, as outlined for trimethylaminuria. Antibiotics to modify the intestinal microflora are not indicated. The reported adult patient did not benefit from riboflavin supplements. A therapy with folate plus riboflavin was suggested but follow-up is not available [13]. The second patient was treated with high-dose riboflavin with folic acid for 6 months but CK remained increased. At 2 years of age the patient had not (yet) developed further clinical signs or symptoms [14].

31.2.2 Sarcosine Dehydrogenase Deficiency

Clinical Presentation

Several reports have described patients with sarcosine dehydrogenase deficiency (SARDH). Multiple symptoms were reported such as intellectual disability, neurological problems, growth failure, hepatomegaly and cardiomyopathy. Asymptomatic cases have also been described. The association with clinical symptoms may be due to ascertainment bias. Sarcosinaemia is generally considered a benign condition.

Metabolic Derangement

SARDH is a liver mitochondrial matrix flavoenzyme involved in choline- and in 1-carbon metabolism where it catalyzes the oxidative demethylation of sarcosine resulting in glycine formation (Fig. 31.2). In a deficiency situation sarcosine accumulates and will be found increased in body fluids (Sarcosinaemia).

Genetics

The *SARDH* gene on chromosome 9q34.2 encodes the SARDH enzyme. Mutations in *SARDH* have been described in sarcosinemia patients [24]. SARDH deficiency is inherited as an autosomal recessive trait.

Diagnostic Tests

Increased levels of sarcosine in plasma and urine form the hallmark of SARDH deficiency. Sarcosinemia and sarcosinuria also occur in some patients with type II glutaric aciduria and in cases with severe deficiency of folic acid.

Glutathione Metabolism

Glutathione (GSH) is a tripeptide consisting of glutamate, cysteine and glycine. It is ubiquitous in the eukaryotic organism and plays a role in many fundamental cellular processes. Apart from being one of the most important antioxidants, it participates in drug metabolism, free-radical scavenging, biosynthesis of DNA and proteins as well as amino acid transport. GSH is synthesized and metabolized in the γ-glutamyl cycle in which six enzymes take part in its synthesis and turnover (Fig. 31.3). It is synthesised from glutamate by sequential actions of γ-glutamylcysteine synthetase and glutathione synthetase. Degradation of GSH involves four enzymes. γ-Glutamyl transpeptidase initiates the breakdown by catalysing the transfer of its γ-glutamyl-group to acceptors. The γ-glutamyl residues are substrates of the γ-glutamyl-cyclotransferase which converts them to 5-oxoproline and the corresponding amino acids. Conversion of 5-oxoproline to glutamate is catalysed by 5-oxoprolinase. A dipeptidase splits cysteinylglycine, which is formed in the transpeptidation reaction, into glycine and cysteine. The biosynthesis of GSH is feedback regulated, i.e. GSH acts as an inhibitor of γ-glutamylcysteine synthetase. Genetic defects have been described in five of the six enzymes of the γ-glutamyl cycle.

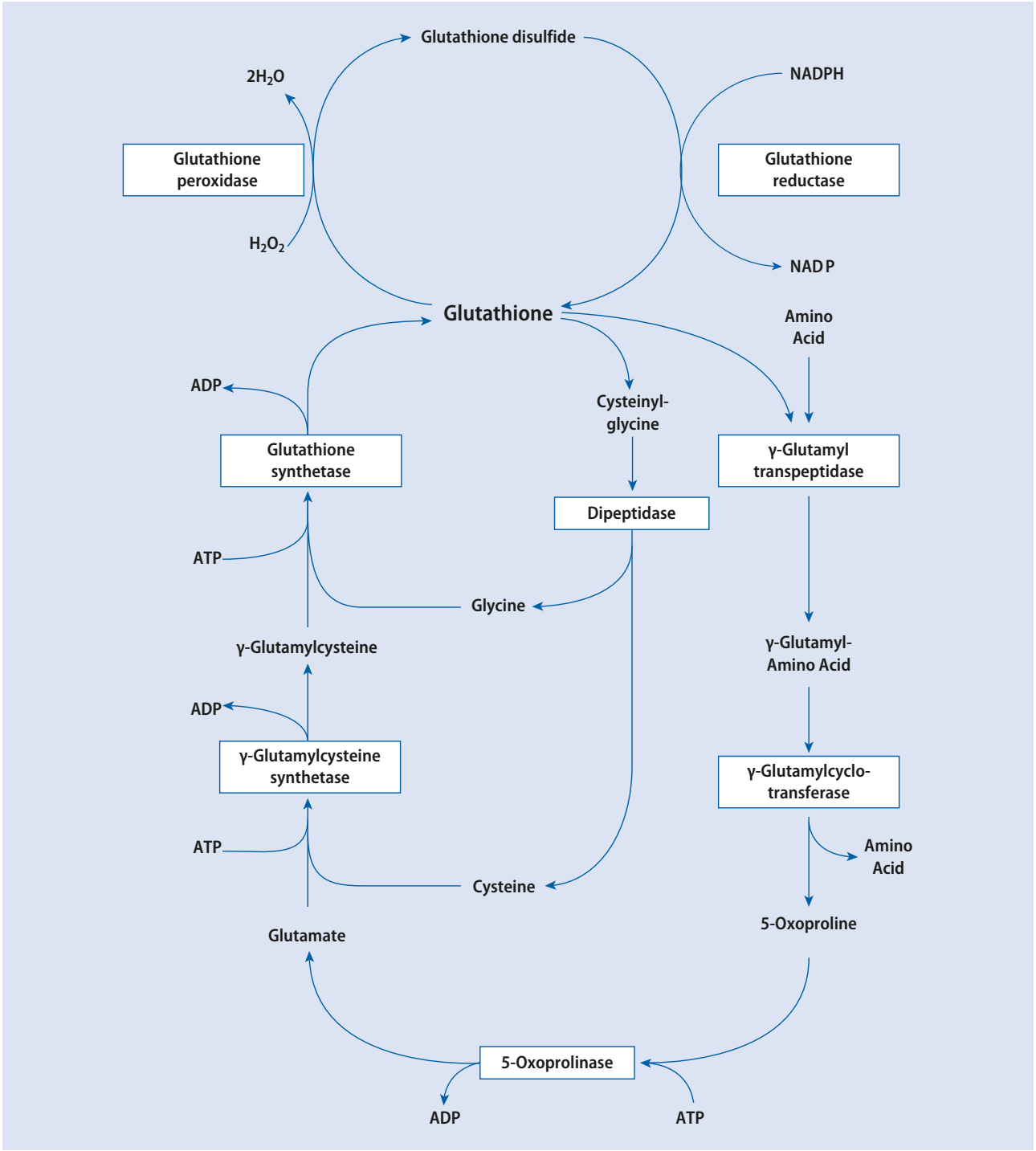

Fig. 31.3 The γ-glutamyl cycle

31.3 Disorders of Glutathione Metabolism

31.3.1 γ-Glutamylcysteine Synthetase Deficiency (Synonym: Glutamate-Cysteine Ligase Deficiency)

Clinical Presentation

Up to now, about nine patients have been identified. The disease is characterized by hemolytic anemia, usually rather mild. In two patients spinocerebellar degeneration, peripheral neuropathy and myopathy have been reported as additional symptoms [25]. Further symptoms included transient jaundice, reticulocytosis, hepatosplenomegaly, and delayed psychomotor development.

Metabolic Derangement

γ-Glutamylcysteine synthetase catalyzes the first and rate-limiting step in the synthesis of GSH (Fig. 31.3). Its deficiency results in low levels of cellular GSH and γ-glutamylcysteine. Knock-down of γ-glutamylcysteine synthetase in rats causes acetaminophen-induced hepatotoxicity [26].

Genetics

γ-Glutamylcysteine synthetase is a dimer consisting of two non-identical subunits encoded by two separate genes which are located on chromosomes 1p21 (light or regulatory subunit) and 6p12 (heavy or catalytic subunit), respectively [27]. Specific mutations in the coding region of *GCLC* have been identified in patients with γ-glutamylcysteine synthetase deficiency. Up to now, four different mutations all in the heavy subunit have been identified in four families. This disease is transmitted as an autosomal recessive trait.

Homozygous γ-glutamylcysteine synthetase knockout mice of the heavy subunit fail to gastrulate already at the embryo state and die before day 8.5 of gestation [28]. Knockout mice of the light subunit are viable and fertile without any significant abnormal phenotype.

Diagnostic Tests

Diagnosis is established by low activity of γ-glutamylcysteine synthetase in erythrocytes, leukocytes and/or cultured skin fibroblasts. Low levels of GSH and γ-glutamylcysteine are found in erythrocytes and/or cultured skin fibroblasts. Mutation analysis confirms the diagnosis.

Treatment and Prognosis

Patients should avoid food and drugs known to precipitate hemolytic crises in glucose-6-phosphate dehydrogenase (G6PD) deficiency, e.g. fava beans, sulfonamides, acetylsalicylic acid, phenobarbital. Prognosis remains to be established.

31.3.2 Glutathione Synthetase Deficiency

Clinical Presentation

Deficiency of glutathione synthetase (GSS) is the most common inborn error of GSH metabolism. More than 80 patients have been reported. According to the severity of clinical symptoms, patients with GS deficiency are classified as mild, moderate or severe [29, 30]. Patients with mild GS deficiency show mild hemolytic anemia as their only clinical symptom. Cellular levels of GSH are usually sufficient to prevent accumulation of 5-oxoproline in body fluids. Patients with the moderate variant usually present during the neonatal period with severe and chronic metabolic acidosis, mild to moderate hemolytic anemia, jaundice and 5-oxoprolinuria. After the neonatal period, the condition usually stabilises, but patients may become critically ill during infections due to pronounced acidosis and electrolyte imbalances. Several patients died during such episodes [31]. Patients with severe GSS deficiency also develop progressive CNS symptoms, e.g. mental retardation, seizures, spasticity, ataxia, and intention tremor. Some patients suffer from recurrent severe bacterial infections, probably due to defective granulocyte function.

Ophthalmological abnormalities, e.g. fundus lesions, retinal dystrophy or crystalline opacities in the lenses have been described in some patients. Antenatal cerebral haemorrhage has been reported in a patient with moderate GSS deficiency, and two further cases of cerebral haemorrhages in two neonates were observed in post-mortem investigations [29, 30]. Although the association between peripartal cerebral haemorrhage and GS deficiency might be coincidental in these cases, in vitro studies suggest that platelet function might be altered in GSS deficiency.

The first pregnancy in a woman with moderate GSS deficiency has been reported to be uneventful resulting in an unaffected infant [32].

Metabolic Derangement

As the enzyme catalyses the last step of GSH synthesis, its deficiency leads to low cellular GSH and excessive production of γ-glutamylcysteine, the metabolite before the enzyme defect. Reduced feedback inhibition of γ-glutamylcysteine synthetase leads to overproduction of γ-glutamylcysteine which is converted into 5-oxoproline by action of γ-glutamyl cyclotransferase. The excessive formation of 5-oxoproline exceeds the capacity of 5-oxoprolinase leading to accumulation of 5-oxoproline causing metabolic acidosis and 5-oxoprolinuria. γ-Glutamylcysteine contains both reactive groups of GSH (i.e. the γ-glutamyl and the sulfhydryl residues). It accumulates in fibroblasts of patients

and may to some extent compensate for lack of GSH. GSH also takes part in the synthesis of leukotriene C_4, the primary cysteinyl leukotrienes. It has been shown that the synthesis of lipoxygenase products is impaired in affected patients [33].

Genetics

GSS deficiency is inherited in an autosomal recessive manner. *GSS* is localised on chromosome 20q11.2 and consists of 13 exons distributed over 32 kb. Since the human genome contains only one *GSS* gene, the various clinical forms of GSS deficiency reflect different mutations as epigenetic modifications in *GSS*. More than 30 different mutations have been identified. Because of the high frequency of splice mutations (approximately 40%), it is recommended that mutation analysis at the genomic level is completed by analyses of RNA transcripts. Heterozygous carriers of GSS deficiency are healthy and show an enzyme activity of about 55% of the normal mean and normal levels of GSH. Although no definite correlation between genotype and phenotype could be established, mutations causing aberrant splicing, frameshift or premature stop codons seem to be associated with the moderate or severe clinical phenotypes, but additional genetic or epigenetic factors seem to alter the phenotypes. The milder forms of the disease are usually caused by mutations mainly affecting the enzyme stability.

Diagnostic Tests

Laboratory findings include increased urinary excretion of 5-oxoproline (up to 1 g/kg/day), low levels of GSH in erythrocytes and/or cultured fibroblasts and decreased activity of GSS in erythrocytes and/or cultured fibroblasts. Enzyme activities of 1–30% of healthy controls are found in affected patients. Mutation analysis confirms the diagnosis. A symptomatic patient with GSS deficiency has been identified through tandem mass spectrometry-based newborn screening [34]. Antenatal diagnosis can be performed by mutation analysis of chorionic villi, analysis of GSS activity in cultured amniocytes or chorionic villi, or by measuring 5-oxoproline in amniotic fluid.

Treatment and Prognosis

The clinical management of GSS deficient patients is aimed at correction of acidosis, prevention of hemolytic crises and support of endogenous defense against reactive oxygen species (ROS). In the neonatal period, correction of metabolic acidosis, electrolyte imbalances, treatment of anemia and excessive hyperbilirubinemia are of crucial importance.

Correction of acidosis can be reached through bicarbonate, citrate or tris(hydroxymethyl)aminoethane (THAM). Doses of up to 10 mmol/kg/day or even higher in episodes of acute infections may be required.

Repeated blood transfusions may be necessary in patients with massive hemolysis. Drugs and foods known to precipitate hemolytic crises in glucose-6-phosphatase dehydrogenase deficiency should be avoided. Successful treatment with erythropoietin has been reported in one patient.

Early supplementation with vitamin E and vitamin C is thought to replenish the lack of GSH as a scavenger of free radicals. Recommended doses are 10 mg/kg/day for vitamin E and 100 mg/kg/day for vitamin C. A long-term follow-up study of 28 patients suggested that early supplementation with both vitamins may prevent CNS damage and improve the long-term clinical outcome [29, 30].

Supplementation with N-acetylcysteine should not be recommended because it was shown at least in cultured fibroblasts that patients with GSS deficiency accumulate cysteine which is known to be neurotoxic in excessive amounts. A therapeutic trial with orally administered GSH showed no lasting benefit in two patients with GSS deficiency. GSH esters, lipid soluble preparations which are easily transported into cells where they are converted into GSH, have been tried in animal models of GSH deficiency and in some patients with GSS deficiency. However, associated toxic effects due to production of alcohols as a by-product during hydrolysis to release GSH make them of limited use. In vitro studies have shown that addition of S-acetylglutathione to the medium of cultured fibroblasts from patients with GSS deficiency normalized intracellular GSH content [35].

Early diagnosis, correction of acidosis and early supplementation with vitamin E and vitamin C appear to be the most important factors regarding survival and long-term outcome.

31.3.3 γ-Glutamyl Transpeptidase Deficiency (Synonym: Glutathionuria)

Clinical Presentation

Up to now, about eight patients have been reported worldwide. Six of them were characterized by CNS involvement. However, two affected siblings presented without any signs of CNS involvement at age of 11 and 13 years, respectively [36]. Therefore, it is yet not clear whether CNS symptoms are a regular part of the clinical picture.

Metabolic Derangement

γ-Glutamyl transpeptidase is a membrane-bound enzyme with subunits of 21 kDa and 38 kDa with its active site. It catalyses the first step in the degradation of

GSH. In addition to high levels of GSH in plasma and urine, the enzyme deficiency leads to increased urinary levels of γ-glutamylcysteine and cysteine as well as a deficiency of leukotriene D_4.

Knock-out mice with γ-glutamyl transpeptidase deficiency present with glutathionuria, glutathionaemia, growth failure, cataracts, lethargy, shortened life span, and infertility [37].

Genetics

The disease is transmitted as an autosomal recessive trait. The human gene for the γ-glutamyl transpeptidase family is composed of at least seven different gene loci, several of them are located on the long arm of chromosome 22. Whole-genome sequencing in two siblings with mild psychomotor developmental delay and mild neurological symptoms revealed the presence of a 16.9 kb homozygous deletion in *GGT1*, one of the genes encoding enzymes with γ-glutamyl transpeptidase activity in the human genome [38]. Further analysis revealed the presence of a 13 bp insertion at the deletion junction.

Diagnostic Tests

High levels of GSH are present in plasma and urine (up to 1 g/day in urine; controls <10 mg) whereas cellular levels of GSH are normal. Decreased activity of γ-glutamyl transpeptidase is found in nucleated cells such as leukocytes or fibroblasts, sometimes also in serum. Erythrocytes are not useful for diagnostic purposes since they also lack γ-glutamyl transpeptidase under normal conditions.

Treatment and Prognosis

There exists no specific treatment or management for a better long-term prognosis. However, administration of N-acetylcysteine to γ-glutamyl transpeptidase deficient mice led to restoration of their fertility [37].

31.3.4 Dipeptidase Deficiency (Synonym: Cysteinylglycinuria)

Clinical Presentation

So far, dipeptidase deficiency has been suggested in only one patient [39]. The 15-year-old boy presented with mental retardation, mild motor impairment, and partial deafness.

Metabolic Derangement

Dipeptidase is a membrane-bound enzyme that hydrolyzes dipeptides, including cysteinylglycine compounds, such as the oxidized γ-glutamyl transpeptidase product cystinyl-bis-glycine and the conversion of leukotriene D_4 to E_4. This leads to cystinylglycinuria and increased excretion of leukotriene D_4.

Genetics

The crystal structure of human membrane-bound dipeptidase has been reported [40]. This enzyme is 42 kDa underglycosylated and 63 kDa when glycosylated. Renal dipeptidase has been mapped to human chromosome 16 at q24. No genetic studies have been performed.

Diagnostic Tests

Diagnosis in the described patient was based on increased urinary excretion of cystinyl-glycine and leukotriene D_4, which is usually not detectable. Leukotriene E_4, the major urinary metabolite in humans, was completely absent.

Treatment and Prognosis

No specific treatment has been proposed. Prognosis remains to be established.

31.3.5 5-Oxoprolinase Deficiency

Clinical Presentation

Up to now, about 15 patients have been described. The clinical symptoms are inconstant and very heterogeneous including renal stone formation, enterocolitis, neonatal hypoglycemia, microcytic anemia, microcephaly and mental retardation. So far, it seems to be a benign biochemical condition and clinical symptoms are merely a coincidence or an epiphenomenon in several pathological conditions [41].

Metabolic Derangement

5-Oxoprolinase catalyses the ring-opening of 5-oxoproline yielding glutamate as a step in the γ-glutamyl cycle. It is the enzyme with the lowest capacity in the γ-glutamyl cycle. Apparently it is composed of two identical 142 kDa subunits. Decreased activity leads to decreased conversion of 5-oxoproline to glutamate resulting in elevated levels of 5-oxoproline in body fluids.

Genetics

The mode of inheritance is autosomal recessive. The condition is caused by mutations of the 5-oxoprolinase (*OPLAH*) gene.

Diagnostic Tests

Elevated levels of 5-oxoproline are found in urine (4–10 g/day; controls <0.1 mol/mol creatinine) and other body fluids. Cellular levels of GSH and acid-base balance are normal. Decreased activity of 5-oxoprolinase in nucleated cells are decreased. Erythrocytes are not a

suitable diagnostic tool because the enzyme is not present under normal conditions.

Treatment and Prognosis

No specific treatment has been proposed. Since it seems to be a benign condition prognosis depends on an underlying pathological condition.

31.3.6 Glutathione Reductase Deficiency

Clinical Presentation

Clinical manifestations of this very rare disease are mainly similar to that of glucose-6-phosphate dehydrogenase (G6PD) deficiency. Congenital glutathione reductase deficiency is associated with acute haemolytic crisis after oxidant drugs or fava beans ingestion (favism) and cataracts [42]. In addition, glutathione reductase deficiency was also found in a newborn mainly presenting with severe neonatal jaundice due to unconjugated hyperbilirubinemia [43].

Metabolic Derangement

Glutathione reductase is a homodimeric flavoprotein that catalyzes the production of GSH from glutathione disulfide (GSSG). As part of the glutathione redox cycle, the enzyme plays a role in the detoxification of reactive oxygen species. Clinical features result from increased cellular susceptibility to oxidative stress due to absence of glutathione reductase activity. Glutathione reductase is almost exclusively responsible for protecting eye lens cells from hydrogen peroxide because these cells are deficient in catalase, an enzyme which catalyzes the breakdown of hydrogen peroxide.

Genetics

Glutathione reductase deficiency is caused by mutations in the glutathione reductase (*GSR*) gene, encoding the enzyme glutathione reductase and leading to its absent activity in erythrocytes [43]. It is transmitted by an autosomal recessive trait.

Diagnostic Tests

Diagnosis is established by absent activity of glutathione reductase in erythrocytes. Mutational analysis confirms the diagnosis. This disease should be distinguished from glutathione reductase deficiency secondary to dietary riboflavin deficiency.

Treatment and Prognosis

Treatment is mainly preventive avoiding oxidative drugs and fava beans. In cases of severe haemolytic crises erythrocyte transfusions may be necessary.

31.3.7 Glutathione Peroxidase 4 Deficiency (Synonym: Spondylometaphyseal Dysplasia, Sedaghatian Type)

Clinical Presentation

This rare lethal neonatal form of spondylometaphyseal dysplasia is characterized by severe metaphyseal chondrodysplasia with mild rhizomelic shortness of the upper limbs, platyspondyly, cardiac conduction defects and central nervous system abnormalities [44, 45]. Intrauterine growth is normal. Subacute myocarditis, cortical necrosis of kidneys, adrenal and pulmonary hemorrhage, absence of the corpus callosum and marked frontotemporal pachygyria have been found at autopsy. Many affected patients have been reported to have a short life span, dying in the first days of life due to cardiorespiratory failure.

Metabolic Derangement

Glutathione peroxidase 4 (GPX4) is deficient [46]. This enzyme is a member of the glutathione peroxidase family of antioxidant defence enzymes and protects cells against membrane lipid peroxidation. GPX4 is essential for early embryo development, regulating anti-oxidative and anti-apoptotic activities. Deficiency of this enzyme suggests possibility of lipid peroxidation inhibition and results in fatal development failure of the cardiac, nervous, and skeletal systems.

Genetics

Recessive truncating mutations in the glutathione peroxidase 4 gene (*GPX4*) are causative for this lethal form of spondylometaphyseal dysplasia.

Diagnostic Tests

The typical clinical and radiologic features are suspicious for this disorder. Diagnosis is established by mutational analysis of the *GPX4*.

Treatment and Prognosis

There exist no treatment options. Death occurs in the perinatal period.

31.3.8 NRF2 Superactivity (Synonym: Immunodeficiency, Developmental Delay, and Hyperhomocysteinaemia)

Clinical Presentation

So far, four patients with this multisystem disorder have been described [47]. These patients display a phenotype with several prominent features including mild developmental delay, failure to thrive from infancy, immunodeficiency and leukoencephalopathy. Additional features,

such as congenital heart defects and liver involvement, are more variable.

Metabolic Derangement

A laboratory hallmark of this disorder is hypohomocysteinemia. This is most likely a direct effect of NRF2 activation as NRF2 positively regulates glutathione synthesis for which homocysteine serves as a precursor. Chronic highly increased levels of NRF2 lead to widespread misregulation of gene expression. Overexpression of enzymes necessary for the generation and regeneration of the two major antioxidants glutathione and thioredoxin leads to an altered cytosolic redox balance and cellular dysfunction by misregulated proteins. These will affect many pathways thereby further enhancing the negative effect of NFR2 accumulation.

Genetics

The disease is caused by de novo activating missense mutations in *NFE2L2*. The causative mutations in *NFE2L2* affect the binding sites of KEAP1 leading to accumulation of NRF2 and consecutive increased expression of genes regulated by NRF2.

Diagnostic Tests

Diagnosis is established by mutation analysis in *NFE2L2*. All patients identified so far showed hypohomocysteinemia and low creatinine levels. G-6-P-dehydrogenase activity seems to be increased.

Treatment and Prognosis

In one patient treatment with luteolin (50 mg/d), a flavone found in leaves, and ascorbic acid (200 mg/d) over a period of 6 months showed positive effects regarding the frequency of infections as well as increased muscle strength and endurance.

31.4 Other Disorders of Peptide Metabolism

31.4.1 Prolidase Deficiency

Clinical Presentation

The exact prevalence of this rare disease is not known. Approximately 90 patients from different ethnic groups have been reported to date [48]. Clinical manifestation and age of onset are quite variable from the first days of life till early adulthood. Skin involvement is very typical, however, this may not be the initial symptom and can appear between 6 months and 30 years of age. Skin lesions, either mild or severe, include chronic, recalcitrant, and painful ulcers of the feet and most frequently of the lower legs. Erythematous, maculopapular or purpuric lesions as well as telangiectasia of the face and hands may precede ulcers. In addition, hyperkeratosis, eczematous lesions and photosensitivity have been reported. Most patients have some degree of intellectual disability, ranging from mild to severe. Further manifestations include a characteristic face, asthma-like chronic reactive airway disease, cystic pulmonary changes, recurrent infections, anemia, thrombocytopenia, hepato- and splenomegaly, short stature, lymphedema, joint laxity, hirsutism or dental dysplasia. Immunological abnormalities are frequently seen including elevated immunoglobulins (especially IgE), abnormal neutrophil chemotaxis or presence of autoantibodies. However, some patients remain asymptomatic. An association between prolidase deficiency and systemic lupus erythematosus has been reported [49].

Metabolic Derangement

The deficiency of the exopeptidase prolidase (or peptidase D), which plays an important role in the biosynthesis and degradation of collagen, leads to significant urinary excretion of a large number of iminopeptides (dipeptides with an N-terminal proline or hydroxyproline, particularly glycylproline).

Genetics

Prolidase deficiency is due to mutations in the *PEPD* gene and inherited as an autosomal recessive trait.

Diagnostic Tests

Diagnosis is based on clinical and laboratory findings, especially massive iminodipeptiduria and confirmed by molecular genetic testing.

Treatment and Prognosis

Treatment is symptomatic. Topical treatment attempts of skin ulcers included proline in combination with glycine ointment, steroids or growth hormone ointment. Oral ascorbate or manganese (a cofactor of prolidase) have been also used. Prognosis varies among patients. However, patients often have a decreased life-expectancy due to severe infections.

31.4.2 X-Prolyl Aminopeptidase 3 Deficiency (Synonym: Nephronophthisis-like Nephropathy Type 1)

Clinical Presentation

This very rare disease has been reported in two consanguineous families with a total number of five patients with a nephronophthisis (NPHP)-like nephropathy. Affected patients were primarily characterized by moderate to early-onset end-stage renal insufficiency [50]. The phenotypic severity and organ involvement varied

widely between the two different families whereas the phenotypic spectrum was concordant for affected members within the same family.

Genetics

This disease is caused by homozygous frameshift and splice-site mutations in the X-prolyl aminopeptidase 3 *(XPNPEP3)* gene.

Diagnostic Tests

Diagnosis is based on clinical and laboratory findings (e.g. serum creatinine, reduced glomerular filtration rate, renal histology) and confirmed by molecular analyses.

Treatment and Prognosis

Treatment and prognosis is mainly depending from the residual kidney function and possible extrarenal manifestations. In 2 affected siblings renal replacement therapy at 8 and 9 years, respectively, was necessary.

31.4.3 Serum Carnosinase Deficiency (Synonym: Carnosinemia)

Carnosinase (CN1), encoded by the *CNDP1* gene, is the only dipeptidase with substrate specificity for carnosine, anserine and homocarnosine [51]. Serum carnosinase deficiency, which means loss of CN1 function, has been reported in a small number of patients with massive increased levels of carnosine in serum [52]. It has been suggested as an inherited metabolic disorder. However, it is uncertain whether the broad and highly variable range of reported clinical features is really due to carnosinase deficiency. Carnosinemia may be an incidental finding of metabolic studies in children with symptoms with a different, independent cause [52]. The genetic basis of carnosinemia has not been clearly confirmed to be caused by *CNDP1* mutations. However, a *CNDP1* polymorphism associated with low CN1 activity correlates with significantly reduced risk for diabetic nephropathy, especially in women with type 2 diabetes. In addition, low CN1 serum activity may also slow progression of chronic kidney disease in children affected with glomerulonephritis [51]. Carnosinemia due to serum CN1 deficiency is suggested to be clinically irrelevant and may be regarded as a non-disease.

31.4.4 Homocarnosinosis

One family of Norwegian origin with homocarnosinosis has been reported [53]. In this family, three affected siblings had spastic paraplegia, retinitis pigmentosa and mental retardation. All three siblings (and the apparently unaffected mother) had 20 times the normal level of homocarnosine in CSF. More than 40 years later genetic analyses were performed post-mortem in this original family [54]. A homozygous pathogenic splice-site variant in *SPG11* was found. The clinical findings in the original family correlate with the heterogeneous *SPG11* phenotype. The same variant was found in other *SPG11* patients, unrelated to the original family, either as homozygous or compound heterozygous constellation. Normal homocarnosine levels were found in the CSF of all unrelated SPG patients. The increased levels of homocarnosine do not seem to be a biomarker for *SPG11*. Only one further case of Russian origin with increased CSF, plasma and urinary content of homocarnosine showing moderate neurological symptoms has been described [55]. At present, homocarnosinosis has still to be regarded as a very rare biochemical aberration with unknown clinical significance.

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Disorders of Purine and Pyrimidine Metabolism

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Contents

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Purine and Pyrimidine Metabolism

Purine nucleotides are essential cellular constituents that are involved in energy transfer, metabolic regulation, and synthesis of DNA and RNA. Purine metabolism can be divided into three pathways (Fig. 32.1).

1. De novo purine synthesis (DNPS) comprises a series of 10 enzymatic reactions that are critical to purine formation [1]. This pathway starts with the formation of phosphoribosyl pyrophosphate (PRPP) and leads to the synthesis of inosine monophosphate (IMP). Purine nucleotide synthesis is completed by the interconversion of IMP to adenosine and guanosine nucleotides. Adenosine monophosphate (AMP) synthesis from IMP requires adenylosuccinate synthase (ADSS) and adenylosuccinate lyase (ADSL), while the synthesis of guanosine monophosphate (GMP) occurs via IMP dehydrogenase (IMPDH) and GMP synthetase. Mononucleotides can further be phosphorylated into di- and trinucleotides and reduced into deoxyribonucleotides. Nucleoside triphosphates (NTPs) and their deoxy counterparts (dNTPs) are incorporated into RNA and DNA, involved in cell signaling or used as energy transfer in cells.
2. The catabolic pathway starts from GMP, IMP, and AMP. Its final product is uric acid, a poorly soluble metabolite excreted in urine, that tends to crystallize once its plasma concentration exceeds 6.5–7.0 mg/dL (0.38–0.47 mmol/L).
3. The salvage pathway allows the recovery of purine bases and nucleosides. Guanine, hypoxanthine, and adenine, which are provided by food intake or catabolic pathways, are reconverted into GMP, IMP, and AMP, respectively. Salvage of the nucleosides adenosine and guanosine, and their deoxy counterparts, is catalyzed by several specific kinases. The salvage pathway additionally converts several pharmacological anticancer agents and antiviral nucleoside analogs into their active forms.

Similar to that of purine nucleotides, the metabolism of pyrimidine nucleotides can be divided into three pathways (Fig. 32.2).

1. The biosynthetic, de novo pathway starts with the formation of carbamoyl phosphate by cytosolic carbamoyl phosphate synthetase II (CPS II), which differs from mitochondrial CPS I, the latter catalyzing the first step of ureagenesis (▶ Chap. 19). This is followed by the synthesis of uridine monophosphate (UMP) and, hence, of (deoxy)nucleoside triphosphates ((d)NTPs) used for RNA and DNA synthesis.
2. The catabolic pathway starts from CMP, UMP, and dTMP and yields β-alanine and β-aminoisobutyrate, which are converted into citric acid cycle intermediates.
3. The salvage pathway, which is composed of several kinases, converts pyrimidine nucleosides cytidine, uridine, and thymidine into their corresponding nucleotides CMP, UMP, and dTMP, respectively. This pathway also converts several pharmacological anticancer and antiviral nucleoside analogs into their active forms.

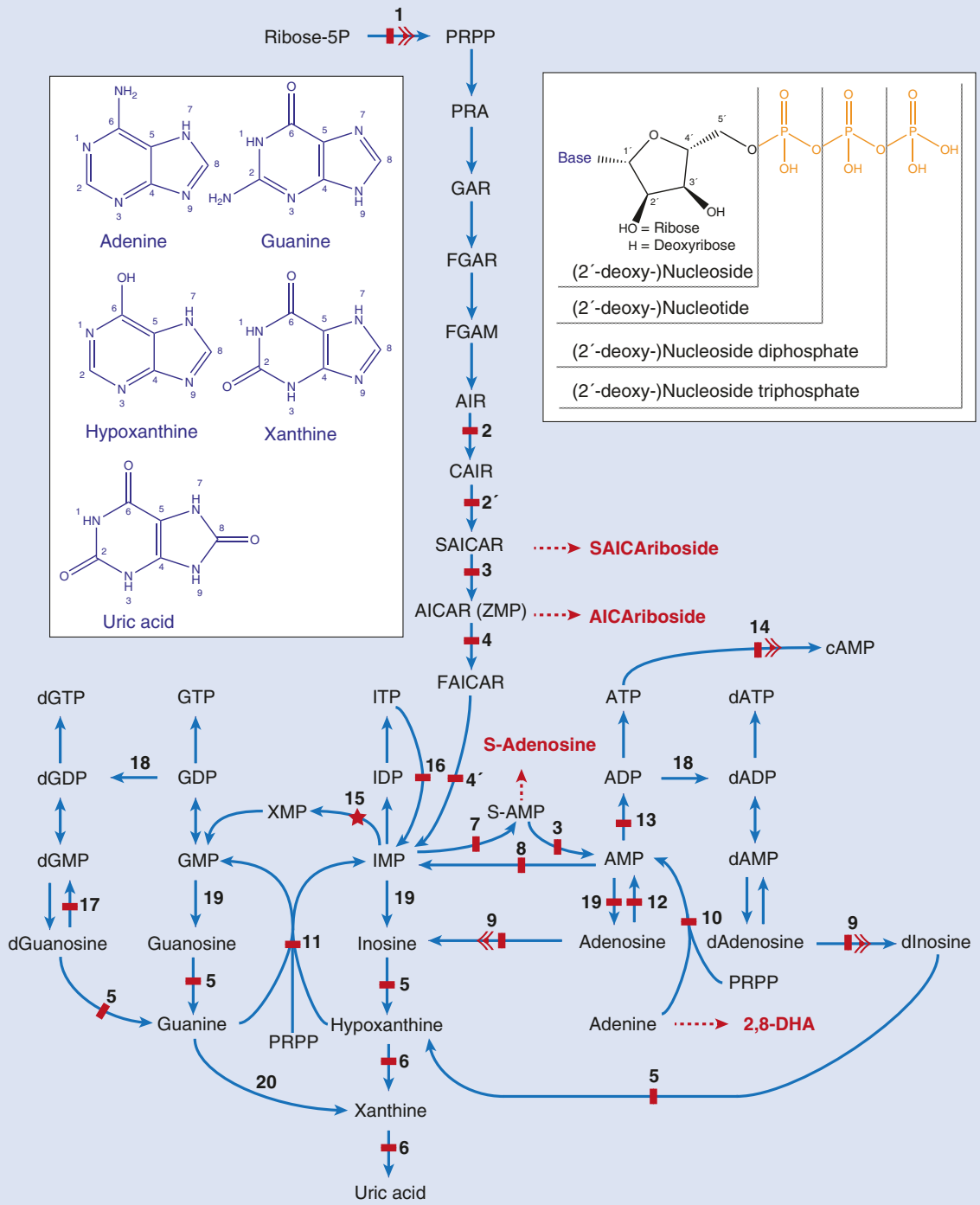

Fig. 32.1 Pathways of purine metabolism. PRPP phosphoribosyl pyrophosphate, PRA phosphoribosylamine, FGAR formylglycinamide ribotide, FGAM formylglycinamidine ribotide, AIR aminoimidazole ribotide, CAIR carboxyaminoimidazole ribotide, SAICAR succinylaminoimidazolecarboxamide ribotide, AICAR aminoimidazolecarboxamide ribotide, FAICAR formylaminoimidazolecarboxamide ribotide, GMP guanosine monophosphate, IMP inosine monophosphate, AMP adenosine monophosphate, S-AMP adenylosuccinate, 2, 8-DHA 2, 8-dihydroxyadenine. **1**, Phosphoribosyl pyrophosphate synthetase (PRPS); **2**, Phosphoribosyl-aminoimidazole carboxylase; **2′**, Phosphoribosyl-aminoimidazole-succinocarboxamide synthetase (**2** and **2′** form PAICS); **3**, Adenylosuccinate lyase (ADSL); **4**, AICAR transformylase; **4′**, IMP cyclohydrolase (**4** and **4′** form ATIC); **5**, Purine nucleoside phosphorylase (PNP); **6**, Xanthine oxidase (XO); **7**, Adenylosuccinate synthase (ADSS); **8**, Adenine monophosphate deaminase (AMPD); **9**, Adenosine deaminase (ADA); **10**, Adenine phosphoribosyltransferase (APRT); **11**, Hypoxanthine-guanine phosphoribosyltransferase (HPRT); **12**, Adenosine kinase (ADK); **13**, Adenylate kinase (AK); **14**, Adenylate cyclase (ADCY5); **15**, IMP dehydrogenase (IMPDH); **16**, Inosine triphosphate pyrophosphatase (ITPase); **17**, Deoxyguanosine kinase (DGUOK); **18**, Ribonucleotide-diphosphate reductase (RRM2B); **19**, 5′-nucleotidase(s); **20** Guanine deaminase. Deficient enzymes are indicated by red solid bars across the arrows and overactive enzymes by double red arrows. Red star indicates another causal mechanism. Unusual metabolites consecutive to enzymatic blocks are highlighted in red

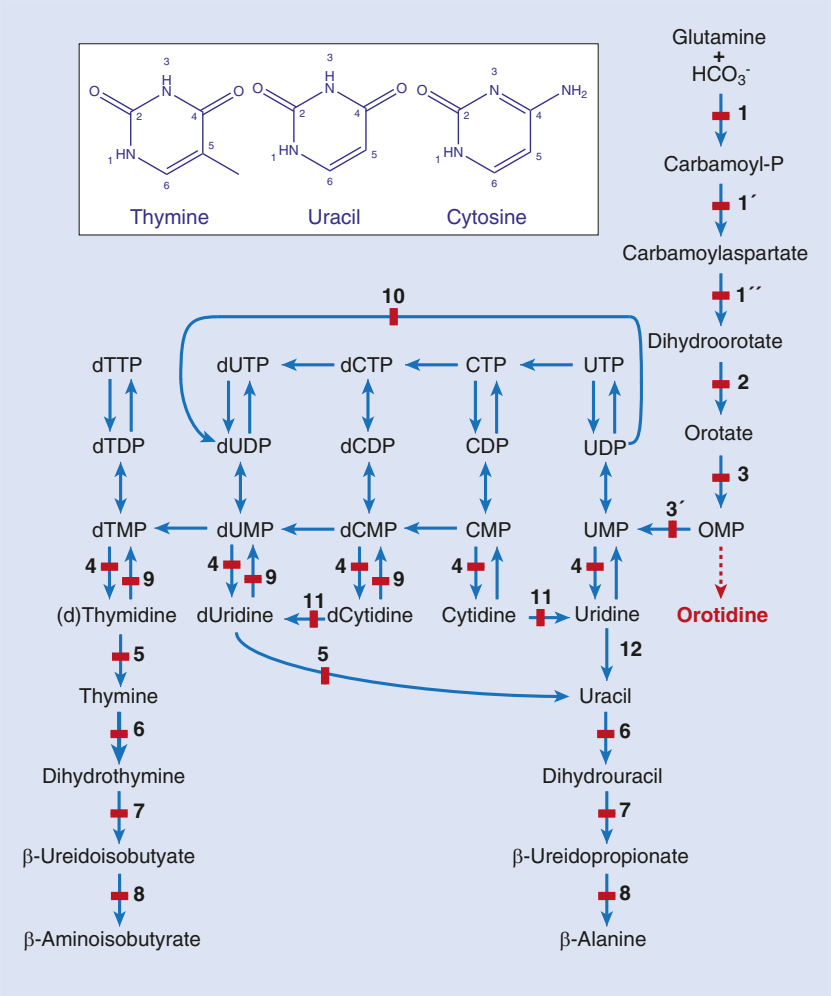

Fig. 32.2 Pathways of pyrimidine metabolism. CMP cytidine monophosphate, OMP orotidine monophosphate, dTMP deoxythymidine monophosphate, UMP uridine monophosphate. **1**, Carbamoylphosphate synthetase II; **1′**, Aspartate transcarbamylase; **1″**, Dihydroorotase (**1**, **1′** and **1″** form CAD trifunctional protein); **2**, Dihydroorotate Dehydrogenase (DHODH); **3**, Orotate phosphoribosyltransferase; **3′**, Orotodine monophosphate decarboxylase (**3** and **3′** form UMPS); **4**, Cytosolic 5′-nucleotidase 3A; **5**, Thymidine phosphorylase (TP); **6**, Dihydropyrimidine dehydrogenase (DPD); **7**, Dihydropyrimidinase (DHP); **8**, β-ureidopropionase (UP); **9**, Thymidine kinase (TK); **10**, Ribonucleotide-diphosphate reductase (RRM2B); **11**, Cytidine deaminase; **12**, Uridine phosphorylase. Deficient enzymes are indicated by red solid bars across the arrows and overactive enzymes by double red arrows. Red star indicates another causal mechanism. Unusual metabolites consecutive to enzymatic blocks are highlighted in red

Introduction

Inborn errors of purine and pyrimidine metabolism comprise essentially deficiencies or overactive enzymes. Many enzymes have isoforms, the mutations of which are responsible for different phenotypes. Given that many purine and pyrimidine metabolism disorders share similar clinical findings, all these defects are presented in this chapter according to the preponderant clinical signs at presentation. An exhaustive list of all purine and pyrimidine defects is shown in Tables 32.1 and 32.2, respectively.

Table 32.1 Inborn errors of purine metabolism

Enzyme number (Fig. 32.1)	Disorder/enzyme defect	Alternative name	Gene Inheritance	Specific biochemical markers	Clinical signs	Age of onset
1	Phosphoribosylpyrophosphate synthetase (PRPS) deficiency	Arts syndrome Charcot-Marie-Tooth disease-X5 CMTX5 X-linked non-syndromic hearing loss DFNX1	*PRPS1* XL	↓ PRPS activity (RBC, F, L) n↓Hypoxanthine (U); n↓ uric acid (P)	Developmental delay Peripheral neuropathy Optic atrophy, deafness	Infancy, childhood
1	Phosphoribosylpyrophosphate synthetase (PRPS1) overactivity	PRPP synthetase superactivity	*PRPS1* XL	↑ Uric acid (U, P) ↑ Hypoxanthine (U)	Gout – Nephrolithiasis and progressive renal failure Developmental delay Deafness	Childhood to adulthood
2	Bifunctional enzyme phosphoribosylaminoimidazole carboxylase/ phosphoribosylaminoimidazole succinocarboxamide synthetase (PAICS) deficiency		*PAICS* AR	↑ AIriboside (expected in body fluids, to be confirmed)	Polymalformative syndrome	Antenatal period
3	Adenylosuccinate lyase (ADSL) deficiency	Adenylosuccinase deficiency	ADSL AR	↑ S-Adenosine (U, CSF) ↑ SAICAriboside (U, CSF)	Developmental delay Autism Seizures	Infancy, childhood
4	AICAR transformylase/IMP cyclohydrolase (ATIC) deficiency	AICA-ribosiduria	*ATIC* AR	↑↑ AICAriboside (U) ↑ SAICAriboside, S-Adenosine (U)	Psychomotor retardation Chorioretinal atrophy Scoliosis	Antenatal period to infancy
5	Purine nucleoside phosphorylase (PNP) deficiency	PNP deficiency	*PNP* AR	n ↓ Uric acid (U, P), ↑ Inosine/d-Inosine (U) ↑ Guanosine/d--Guanosine (U) ↓ PNP activity in RBC	Progressive SCID Ataxia, intellectual deficiency	Infancy to childhood
6	Xanthine oxidase (XO) deficiency	Xanthinuria type I Xanthine dehydrogenase deficiency	*XDH* AR	↑ Hypoxanthine (U), Xanthine (U, P) ↓ Uric acid (U, P)	Nephrolithiasis and progressive renal failure Muscular pain	Any age
6	Combined XO and aldehyde oxidase (AO) deficiencies	Xanthinuria type II Molybdenum cofactor sulfurase deficiency (MOCOS)	*MOCOS* AR	↑ Hypoxanthine (U), Xanthine (U, P) ↓ Uric acid (U, P)	Nephrolithiasis and progressive renal failure Muscular pain	Any age

Table 32.1 (continued)

Enzyme number (Fig. 32.1)	Disorder/enzyme defect	Alternative name	Gene Inheritance	Specific biochemical markers	Clinical signs	Age of onset
6	Combined XO, AO and sulfite oxidase (SO) deficiencies	Xanthinuria type III Molybdenum cofactor deficiency (MOCOD)	*MOCS1, MOCS2 GPHN* AR	↑ Hypoxanthine (U), Xanthine (U, P) ↓ Uric acid (U, P) ↑ Sulfocysteine (U), ↓ Homocysteine (P)	Nephrolithiasis and progressive renal failure Severe neonatal epileptic encephalopathy	Neonatal period
7	Adenylosuccinate synthase like 1 (ADSSL1) deficiency	Distal myopathy-5	*ADSSL1* AR		Distal myopathy	Adolescence
8	Adenosine monophosphate (AMPD1) deaminase 1	Myoadenylate deaminase deficiency	*AMPD1* AR	↓ AMPD1 activity (M)	Cramps Hypotonia Exercice intolerence	Childhood to adulthood
8	Adenosine monophosphate deaminase 2 (AMPD2) deficiency	Pontocerebellar hypoplasia type 9 (PCH9) Spastic paraplegia 63 (SPG63)	*AMPD2* AR		Severe developmental delay, microcephaly, epilepsy Hereditary spastic paraplegia	Infancy to adulthood
9	Adenosine deaminase 1 (ADA1) deficiency	Severe combine immunodeficiency (SCID) ADA-SCID	*ADA1* AR	↑ Adenosine, 2′-deoxyadenosine (U) ↓ ADA1 activity (RBC)	SCID skeletal dysplasia developmental delay, deafness	Infancy to adulthood
9	Adenosine deaminase (ADA) superactivity		*?* AD	↑ ADA1 activity (RBC)	*Non-spherotic hemolytic anemia*	Childhood to adulthood
9	Adenosine deaminase 2 (ADA2) deficiency		*ADA2* AR	↓ ADA2 activity (P)	Systemic vasculitis involving skin Recurrent strokes Anemia, bone marrow failure	Infancy to adolescence
10	Adenine phosphoribosyltransferase (APRT) deficiency	APRT deficiency	*APRT* AR	↑ 2,8-dihydroxyadenine (U) ↑ Adenine (U) ↓ APRT activity (RBC)	Nephrolithiasis and progressive renal failure	Any age
11	Hypoxanthine guanine phosphoribosyltransferase deficiency (HPRT)	Lesch-Nyhan syndrome	*HPRT1* XL	↑ Uric acid (U, P) ↑ Hypoxanthine, Xanthine (U) ↓ HPRT activity (RBC)	Severe developmental delay - behavioural disorder Nephrolithiasis and progressive renal failure Gout	Infancy to adolescence
12	Adenosine kinase (ADK) deficiency		*ADK* AR	↑ Methionine, AdoMet and AdoHcy (P, C) ↑Adenosine (U), n↑Hcy (P) ↑ AICAriboside, SAICAriboside (U)	Liver dysfunction Progressive developmental delay	Infancy

(continued)

Table 32.1 (continued)

Enzyme number (Fig. 32.1)	Disorder/enzyme defect	Alternative name	Gene Inheritance	Specific biochemical markers	Clinical signs	Age of onset
13	Adenylate kinase 1 (AK1) deficiency	Myokinase deficiency	*AK1* AR	↓ AK1 activity (RBC)	Non-spherocytic hemolytic anemia Hepatosplenomegaly Developmental delay	Infancy to adulthood
13	Adenylate kinase 2 (AK2) deficiency	Reticular dysgenesis	*AK2* AR		SCID Sensorineural deafness	Infancy
13	Adenylate kinase 7 (AK7) deficiency	Spermatogenic failure 27 / SPGF27	*AK7* AR		Male infertility	Adulthood
14	ADCY5-related dyskinesia		*ADCY5* AD (AR)		Paroxystic dyskinesia Facial myokimia Alternating hemiplegia childhood-onset chorea	Childhood or early adolescence
15	Inosine-5′-monophosphate dehydrogenase1 (IMPDH1) deficiency	Retinitis pigmentosa-10 (RP10) Leber congenital amaurosis 11 (LCA11)	*IMPDH1* AD		Retinis pigmentosa Leber amaurosis	Infancy to adolescence
15	Inosine-5′-monophosphate dehydrogenase 2 (IMPDH2) deficiency		*IMPDH2* AD		Dystonia-tremor disorder	Childhood to adulthood
16	Inosine triphosphatase (ITPase) deficiency		*ITPA* AR	↑ ITP[a] (RBC) ↓ ITPase activity[a]	Severe developmental delay Cataract Cardiomyopathy	Infancy
17	Deoxyguanosine kinase (DGUOK) deficiency	Mitochondrial DNA depletion syndrome-3 (MTDPS3) Autosomal recessive progressive external ophthalmoplegia with mitochondrial DNA deletions-4 (PEOB4)	*DGUOK* AR		Cholestasis and progressive liver failure Severe hypotonia, psychomotor regression Juvenile onset mitochondrial myopathy	Infancy to adulthood
19	Ecto-5′-nucleotidase (CD73) deficiency		*NT5E* AR		Arterial and joint calcifications, aortic stenosis	Adulthood
	Autosomal-dominant tubulointerstitial kidney diseases (ADTKD)	Juvenile hyperuricemic nephropathy (FJHN), medullary cystic kidney disease (MCKD), Uromodulin-associated kidney disease	*UMOD* AD	↑ Uric acid (P) ↓ Uric acid (U)	Gout Nephrolithiasis and progressive renal failure	Adulthood

Table 32.1 (continued)

Enzyme number (Fig. 32.1)	Disorder/enzyme defect	Alternative name	Gene Inheritance	Specific biochemical markers	Clinical signs	Age of onset
	Hereditary renal hypouricemia type 1 (RHUC1)	Urate transporter 1 deficiency (URAT1)	*SLC22A12* AR	↓ Uric acid (P) ↑ Uric acid (U)	Nephrolithiasis and progressive renal failure Exercise-induced acute kidney injury	Adulthood
	Hereditary renal hypouricemia type 2 (RHUC2)	Urate voltage-driven efflux transporter 1 deficiency (GLUT9)	*SLC2A9* AR	↓ Uric acid (P) ↑ Uric acid (U)	Nephrolithiasis and progressive renal failure Exercise-induced acute kidney injury	Adulthood
	Thiopurine methyltransferase (TPMT) deficiency	Thiopurine toxicity-1 (THPM1)	*TPMT* AR	*TPMT* genotyping	Drug toxicity	
	Nudix hydrolase 15 (NUDT15) deficiency	Thiopurine toxicity-2 (THPM2)	*NUDT15* AD	*NUDT15* genotyping	Drug toxicity	

Treatable diseases are highlighted in grey. *AR* autosomal recessive, *AD* autosomal dominant, *XL* X-linked, *RBC* red blood cells, *U* urine, *P* plasma, *CSF* cerebrospinal fluid, *F* fibroblasts, *L* lymphoblasts, *M* muscle, *ITP* Inosine triphosphate
[a]High levels of ITP and low ITPase activity in RBC do no allow to discriminate between ITPase encephalopathy and *ITPA* variants of pharmacogenetics relevance

Table 32.2 Inborn errors of pyrimidine metabolism

Enzyme number (Fig. 32.2)	Disorder / enzyme defect	Alternative names	Gene Inheritance	Biochemical markers	Clinical signs	Age of onset
1	Carbamoyl phosphate synthetase, Aspartate transcarbamylase, Dihydroorotase (CAD) deficiency	CAD trifunctional protein deficiency Early infantile epileptic encephalopathy-50	*CAD* AR		Developmental delay Drug-resistant epilepsy Anemia with anisopoikilocytosis	Infancy and childhood
2	Dihydroorotate dehydrogenase (DHOD) deficiency	Postaxial acrofacial dysostosis POADS Miller syndrome Genee-Wiedemann syndrome	*DHODH* AR	↑ orotic acid (U) ↑ DHO (P, U)	Postaxial acrofacial dysostosis Severe micrognathia, Cleft lip and/or palate Hypoplasia or aplasia of the postaxial elements of the limbs	Infancy
3	Uridine monophosphate synthase (UMPS) deficiency	Hereditary orotic aciduria	*UMPS* AR	↑ orotic acid, ↑ orotidine (P, U)	Megaloblastic anemia unresponsive to vitamin B_{12} and folic acid Developmental delay Failure to thrive	Infancy and childhood

(continued)

Table 32.2 (continued)

Enzyme number (Fig. 32.2)	Disorder / enzyme defect	Alternative names	Gene Inheritance	Biochemical markers	Clinical signs	Age of onset
4	Cytosolic 5′-nucleotidase 3A deficiency	Pyrimidine 5′nucleotidase 1(P5′N-1) deficiency Uridine 5′-monophosphate hydrolase 1 (UMPH1) deficiency	*NT5CA3* AR		Non spherocytic hemolytic anemia	Childhood
4	Cytosolic 5′-Nucleotidase superactivity			↓ uric acid (U)	Developmental delay, autistic features Growth retardation Seizures, ataxia	
5	Thymidine phosphorylase (TP) deficiency	MTDPS1 (MNGIE type)	*TYMP* AR	↑(deoxy)thymidine, deoxyuridine, uracil, thymine (P, U) ↓ TP activity	Severe gastrointestinal dysmotility, cachexia Peripheral neuropathy, ocular symptoms Asymptomatic diffuse leukoencephalopathy	Adulthood
6	Dihydropyrimidine dehydrogenase (DPD) deficiency	DPD deficiency Hereditary thymine-uraciluria	*DPYD* AR	↑ uracil, thymine (P, U) ↑ 5-OH-methyluracil (U)	Developmental delay Seizures Drug toxicity	Infancy Adulthood
7	Dihydropyrimidinase (DHP) deficiency	Dihydropyrimidinuria	*DPYS* AR	↑↑ dihydrouracil, dihydrothymine (P, U) ↑ uracil, thymine (P, U)	Dysmorphic features, Feeding problems Intellectual disability Drug toxicity	Infancy and childhood Adulthood
8	β-ureidopropionase (UP) deficiency	β-alanine synthase	*UPB1* AR	↑↑ ureidopropionic acid, ureidoisobutyric acid (P, U) ↑ dihydrouracil, dihydrothymine (P, U)	Developmental delay Seizures Asymptomatic	Infancy
9	Thymidine kinase 2 (TK2) deficiency	MTDPS2 (myopathic type) PEOB3	*TK2* AR		Myopathy Progressive external ophthalmoplegia, ptosis	Infancy to adulthood
10	Ribonucleoside-diphosphate reductase subunit M2 B (RRM2B) deficiency	MTDPS8A (encephalomyopathic type with renal tubulopathy) MTDPS8B (MNGIE type) PEOA5	*RRM2B* AR (AD)		Psychomotor delay, failure to thrive, tubulopathy, microcephaly, sensorineural hearing loss Progressive external ophthalmoplegia	Infancy to adulthood
11	Cytidine deaminase deficiency		*CDA* AR		Drug toxicity	Adulthood
	Activation induced Cytidine deaminase (AICDA) deficiency	Hyper IgM syndrome type 2	*AICDA* AR (AD)		Recurrent upper and lower respiratory tract bacterial infections Enlarged lymph nodes	Childhood to adulthood
	Uracil-DNA glycosylase deficiency	Hyper-IgM syndrome type 5	*UNG AR*		Recurrent upper and lower respiratory tract bacterial infections Enlarged lymph nodes	Childhood to adulthood

Treatable diseases are highlighted in grey. *AR* autosomal recessive, *AD* autosomal dominant, *XL* X-linked, *RBC* red blood cells, *U* urine, *P* plasma

Purine and pyrimidine disorders may be classified in group 1 of the simplified classification of IEM (small molecules: ▶ Chap. 1). Indeed, most disorders involve nucleotide synthesis, catabolism or salvage pathways and may be screened by purines and pyrimidines profiles. Clinical symptoms are very diverse and the pathophysiology is sometimes complex (linked to accumulation, deficiency or both) and still badly understood. Many enzymatic defects involve brain development by intricated mechanisms. Some recent works point to relevant functions of purines and pyrimidines in the molecular mechanisms which regulate brain growth and neuronal connectivity [2].

Clinical disease presentations may include birth defects, neurological features, sensory impairment like deafness or retinal damage, immunological, hematological, muscular, and renal features (stones). Several enzymes of purine and pyrimidine metabolism are essential for maintaining the mitochondrial deoxyribonucleoside triphosphate pool and their deficiencies are therefore associated to mitochondrial deoxyribonucleic acid DNA (mtDNA) depletion syndromes (MTDPSs). Additionally, polymorphisms in purine and pyrimidine metabolism genes are responsible for drug toxicity and have a major impact in treatment of some cancers and autoimmune diseases. Disorders may manifest at different times, from the prenatal stage up to advanced age, including patients in their 80s [3–5]. Clinical signs of these diseases are at times nonspecific and, thus, often overlooked. This is gradually being overcome by the use of sensitive biochemical investigations like liquid chromatography (LC) coupled with a photodiode array (PDA) or a mass spectrometer (MS) detector and next-generation sequencing technologies [6].

Most defects are autosomal and recessively inherited, except for diseases that are linked to *HPRT* and *PRPS1* genes (which are X-linked), and those linked to *IMPDH1, IMPDH2 ADCY5, UMOD, NUDT15* and *AICDA* (for which inheritance is autosomal dominant). Only a few neurological disorders are potentially treatable like CAD deficiency that presents with an early severe epileptic encephalopathy responsive to uridine (see below).

32.1 Diseases with Birth Defects, Prenatal or Early Onset of Severe Symptoms with Malformations or Neurological Impairment

Severe phenotypes have been associated with inborn errors of purine and pyrimidine metabolism. This group encompasses several diseases that are mainly characterized by developmental problems, sensory impairments, or both.

32.1.1 Bifunctional Enzyme Phosphoribosyl-Aminoimidazole Carboxylase/Phosphoribosyl-Aminoimidazole-Succinocarboxamide Synthetase Deficiency

Figure 32.1 Enzyme 2.

Phosphoribosyl-aminoimidazole carboxylase/phosphoribosyl-aminoimidazole-succinocarboxamide synthetase (PAICS) deficiency has been recently described in two siblings from the Faroe Islands [7]. The condition is associated with multiple malformations that result in abortion or early neonatal death, polyhydramnios, short neck, short stature, brachycephaly, facial dysmorphism, bilateral choanal atresia, low-set and poorly modulated ears and limbs, external genitalia malformations, esophageal atresia, several costal and vertebral malformations, as well as hypoplasia of the left lung. Whole exome sequencing revealed a homozygous missense mutation in *PAICS* encoding the bifunctional enzyme that is involved in DNPS. Aminoimidazole riboside (AIr) might be elevated in body fluids of affected individuals since a *PAICS* knock-out cell model shown increased levels of AIr in growth medium [8]. Additional reports are necessary to fully understand the clinical course of this disorder.

32.1.2 Adenylosuccinate Lyase Deficiency

Figure 32.1 Enzyme 3.

Approximately 100 patients with ADSL deficiency have been reported, with a prevalence estimated at one in 1.25 million [9–11]. Three phenotypes have been described within a continuum of clinical features. The neonatal form presents with neonatal encephalopathy, a lack of spontaneous movements, respiratory failure, and intractable seizures, all of which result in early death within the first weeks of life [12, 13]. Type I, which is the severe and most common form, presents within the first months of life. This form is characterized by severe psychomotor retardation, early onset seizures, autistic features, growth retardation, and microcephaly. Type II (the mild/moderate form) is characterized by psychomotor retardation and sometimes autistic features and stereotypies [14]. Brain imaging shows unspecific findings with atrophy of the cerebral cortex, corpus callosum, cerebellar vermis, and anomalies of the white matter like delayed or lack of myelination [10, 11, 14]. ADSL catalyzes two non-sequential steps in purine synthesis: the conversion of succinyl-amino-4-imidazolecarboxamide-ribotide (SAICAR) into AICAR and that of S-AMP into AMP. The enzyme's deficiency results in an

accumulation of succinylpurines (SAICAriboside and S-Adenosine) which are the dephosphorylated products of the two substrates of ADSL. The more severe presentations of ADSL deficiency tend to be associated with S-Adenosine/SAICAriboside ratios around one, whereas in milder phenotypes, these ratios are between two and four. This suggests that SAICAriboside is the offending compound and that S-Adenosine likely protects against its toxic effects. The exact mechanism by which ADSL deficiency leads to neurobehavioral dysfunction remains unclear. Diagnosis is based on the presence of succinylpurines in urine or optionally in cerebrospinal fluid, as well as through molecular analysis of *ADSL* [10]. Supplements of adenine with allopurinol, ribose, or S-Adenosylmethionine (SAM) are ineffective. A ketogenic diet could be considered in patients with intractable seizures [10]. The prognosis of survival is variable. Mildly retarded patients have reached adult age, whereas several of those presenting with early epilepsy have died within the first months of life.

32.1.3 AICAR Transformylase/IMP Cyclohydrolase Deficiency

Figure 32.1 Enzyme 4.

AICAR transformylase/IMP cyclohydrolase (ATIC) deficiency has been reported in only three families so far. The clinical description includes profound intellectual deficiencies, epilepsy, marked dysmorphic features (prominent forehead, brachycephaly, wide mouth, thin upper lip, low-set ears), and vision loss [15, 16]. Diagnosis is based on the detection in urines, besides SAICAriboside and S-Adenosine, of high level of 5-amino-4-imidazolecarboxamide (AICA) riboside, the dephosphorylated counterpart of the de novo nucleotide AICAR, also known as ZMP.

32.1.4 Phosphoribosylpyrophosphate Synthetase 1 Deficiency

Figure 32.1 Enzyme 1.

Several variants in the phosphoribosylpyrophosphate synthetase (*PRPS1*) gene give rise to a spectrum of PRPS1 deficiencies, and lower residual activity is associated with increased disease severity. X-linked nonsyndromic hearing loss (DFNX1, formerly DFN2) is the milder form, Arts syndrome is the more severe form, and Charcot–Marie–Tooth disease 5 (CMTX5, also known as Rosenberg–Chutorian syndrome) is in-between [17–19]. Hearing loss is a common feature of the three disorders. CMTX5 and Arts syndrome share neurological anomalies, including ataxia, peripheral neuropathy, and optic atrophy. Arts syndrome is also characterized by moderate intellectual disability and common recurrent infections that result in early death. A dramatic phenotype has been reported in two males with prenatal growth retardation, dysmorphic facial features, severe developmental delay, spastic quadriparesis, macular coloboma-like lesions with retinal dystrophy, short stature, and diabetes insipidus [20]. There is phenotypic overlap between DFNX1, CMTX5, and Arts syndrome, as well as intrafamilial variability depending on gender, X-inactivation ratio, and residual enzyme activity. X-linked retinal degeneration was reported in families in which only female members were affected. Hearing loss and gait abnormalities were observed in some cases [21]. Plasma and urinary uric acid levels are usually in the normal range, suggesting that the defect could be compensated. Since SAM freely crosses the intestinal and blood–brain barriers, it can theoretically replenish nucleotides GTP and ATP, independently of PRPP production. Supplementing the diet with SAM may alleviate some symptoms in Arts syndrome patients, but further studies are warranted to corroborate the effectiveness of SAM supplementation in less severe forms of loss-of-function PRPS-I mutations [18].

32.1.5 AMP Deaminase-2 Deficiency

Figure 32.1 Enzyme 8.

AMPD catalyzes the conversion of AMP to IMP and plays a role in regulating nucleotide metabolism. Three isoforms are encoded by three genes: *AMPD1* (the muscle isoform, ▶ Sect. 32.4.1), *AMPD2* (expressed in liver and brain), and *AMPD3* (the erythrocyte isoform, whose deficiency has been reported in Asia and seems to be completely asymptomatic) [22]. Pathogenic variants in *AMPD2* have been found to be linked to pontocerebellar hypoplasia Type 9 (PCH9) and hereditary spastic paraplegia Type 63 (SPG63) [23, 24]. PCH9 patients display severe developmental delay, postnatal microcephaly, axonal neuropathy, and epilepsy. Neuroimaging exhibits a hypoplastic cerebellum and pons with a typical midbrain figure-eight appearance, callosal hypoplasia or agenesis, and periventricular white matter involvement [25].

32.1.6 Hypoxanthine-Guanine Phosphoribosyltransferase Deficiency

Figure 32.1 Enzyme 11.

Clinical presentations of hypoxanthine-guanine phosphoribosyltransferase deficiency (HPRT) range

from mild to severe forms, depending on the extent of enzyme deficiency [26, 27]. The severe presentation, which is characterized by an enzyme activity of less than 1.5%, is defined as Lesch–Nyhan syndrome. The syndrome's first manifestations are urate overproduction and hypotonia, which occur at 3–6 months of age. Neurological symptoms progress and severe psychomotor retardation is observed by the age of 2–3. This is accompanied by involuntary movements consisting of dystonia, choreoathetosis, and dysarthria. A devastating behavioral disorder is the hallmark of this disease. This includes aggressivity and self-mutilation by biting the fingers, lips, and cheeks, which causes profound disfigurement. Less severe HPRT deficiencies display hyperuricemia with neurological involvement but no behavioral abnormalities, and some rare patients have only isolated hyperuricemia with kidney stones. Macrocytosis is a common feature of HPRT-deficient patients [28]. Brain imaging is usually normal, but a reduction of gray and white matter volume has been described [29].

HPRT catalyzes the recycling of hypoxanthine and guanine into IMP and GMP by transferring the 5′-phosphoribosyl group from PRPP onto the purine base. The uric acid overproduction results from accelerated de novo synthesis, which is caused by the increased availability of PRPP that is not recycled by HPRT. The pathogenesis of the neurological symptoms has still not been satisfactorily explained. Several studies point to dopaminergic dysfunction, which involves 60–90% decreased dopamine concentrations, and to the enzyme activity that is required for its synthesis. However, dopaminergic drugs are not effective [30].

The uric acid overproduction is accompanied by increased serum urate levels, which may reach concentrations as high as 18 mg/dL (1 mmol/L), and by increased urinary production of uric acid, hypoxanthine, and xanthine. Particularly before puberty, uricemia may be in the normal or high-normal ranges. Determining enzyme activity in red blood cells (RBC) provides a definitive diagnosis. This disease is X-linked and recessively inherited. Notably, several females with Lesch–Nyhan syndrome have been reported, due to non-random or skewed inactivation of the X-chromosome [26].

Patients should be treated for hyperuricemia with allopurinol or febuxostat, which inhibit xanthine oxidase (XO), the last enzyme of purine catabolism. This results in decreased production of uric acid, which is replaced by hypoxanthine, which is about ten times more soluble, and by xanthine, which is slightly more soluble than uric acid. Additional measures to prevent crystallization are recommended, including a high fluid intake and low purine diet (i.e. free of red and organ meats, of dried beans and peas, and of fish like anchovy, herring, mackerel, salmon, sardines, and tuna). Since uric acid and xanthine are more soluble at alkaline pH, these measures should also include administrating sodium bicarbonate, potassium citrate, or citrate mixtures to bring urinary pH to 6.0–6.5. Lesinurad and probenecid increase urinary urate excretion but are not indicated in urate overproduction disorders because by further increasing urate excretion they worsen uric acid precipitation. While adequate uricemia control prevents gouty arthritis and urate nephropathy, it does not correct the neurological symptoms. Although no effective and safe therapy for motor or behavioral symptoms is currently available, some treatments have demonstrated improvements in patients. Among these, diazepam, haloperidol, and barbiturates can sometimes improve choreoathetosis, and SAM can improve self-injurious behavior [31]. Patients should be made more comfortable using appropriate restraints, including elbow splints, lip guards, and even tooth extraction, all of which reduce self-mutilation.

32.1.7 Adenylate Cyclase 5-Related Dyskinesia

Figure 32.1 Enzyme 14.

Adenylate cyclase 5 (ADCY5) is encoded by *ADCY5* and converts ATP into cyclic AMP (cAMP), which is an intracellular second messenger crucial for several molecular pathways. ADCY5 is mainly expressed in the striatum, a region implicated in the control of movements, where it is inhibited by dopamine through D2 receptors and activated by adenosine through A_{2A} receptors. *ADCY5* pathogenic variants have been reported in more than 70 patients, with a broad spectrum of hyperkinetic movement phenotypes. Common phenotypes of ADCY5-related dyskinesia include facial dyskinesia, motor exacerbations during drowsiness or prominent sleep-related movements, episodic painful dystonic posturing that increases with stress or illness, and axial hypotonia with delayed developmental milestones. Some variants also cause childhood-onset chorea, dystonic movements, and alternating hemiplegia [32]. Other pathogenic variants in *ADCY5* cause an enzyme gain-of-function, as indicated by in vitro functional studies that demonstrate an increase in intracellular cAMP [33]. In most patients, the inheritance is autosomal dominant, but recessive inheritance has been described in a few patients as well [34]. There is currently no specific treatment for ADCY5-related dyskinesias. However, acetazolamide appears to be the most effective means of controlling chorea and dyskinesia. Caffeine has been reported to improve paroxysmal nocturnal dyskinesia, most likely because of its antagonist effect on A_{2A} receptors [35].

32.1.8 IMP Dehydrogenase Mutations

Figure 32.1 Enzyme 15.

IMP dehydrogenase (IMPDH) catalyzes the first step in the conversion of IMP into GMP, which is subsequently converted into guanine nucleotides. Autosomal-dominant pathogenic variants in *IMPDH1* have been associated with retinitis pigmentosa and, in some cases, with Leber congenital amaurosis, without affecting enzyme activity [36]. Recently, a deleterious heterozygous truncating variant in *IMPDH2* has been associated with a dominant juvenil-onset dystonia-tremor disorder [37].

32.1.9 Inosine Triphosphate Pyrophosphatase (ITPase) Deficiency

Figure 32.1 Enzyme 16.

Profound ITPase deficiency has recently been reported in six families with early infantile encephalopathy, seizures, severe developmental delays, and bilateral cataract (a Martsolf-like syndrome). In most cases, this was also associated with early-onset cardiomyopathy [38, 39]. ITPase catalyzes the conversion of inosine triphosphate (ITP) into IMP and of dITP into dIMP, which thus removes non-canonical nucleotides from the cellular nucleotide pool and avoids their incorporation into nucleic acids. Reduced ITPase activity results in an accumulation of ITP in red blood cells. Incorporation of ITP and dITP into RNA and DNA is believed to cause DNA damage or interference with RNA function, which could result in neuronal apoptosis [38].

32.1.10 Carbamoylphosphate Synthetase II, Aspartate Transcarbamylase, Dihydroorotase Deficiency

Figure 32.2 Enzyme 1.

CAD, combining the enzymatic activities CPS II, Aspartate transcarbamylase, and Dihydroorotase, is a trifunctional enzyme performing the first three of six reactions required for de novo pyrimidine biosynthesis, leading to the formation of UMP. However, UMP can also be formed in a single step from uridine by uridine kinase as part of the pyrimidine recycling pathway. It plays a pivotal role in protein glycosylation, lipid metabolism, and polysaccharide biosynthesis. Early infantile epileptic encephalopathy has been described in CAD-deficient patients, with epilepsy occurring in early childhood (neonatal period–2 years old) along with significant global development delay and even psychomotor regression. Anemia with anisopoikilocytosis is a specific hallmark. The brain volume is initially normal on MRI, which is later followed by progressive cerebral and cerebellar atrophy. However, patients who receive uridine supplementation show obvious benefits manifesting as immediate cessation of seizures, resolved anisopoikilocytosis, and improved development [40–42].

32.1.11 Dihydroorotate Dehydrogenase Deficiency

Figure 32.2 Enzyme 2.

Dihydroorotate dehydrogenase (DHODH) is a mitochondrial enzyme that catalyzes the oxidation of DHO into orotic acid in the catabolic pathway. Pathogenic variants of *DHODH* lead to Miller syndrome, also named Genee–Wiedemann syndrome, which is a very rare genetic condition, also referred to as "postaxial acrofacial dysostosis." This syndrome is characterized by distinctive craniofacial malformations associated with limb abnormalities [43]. Interestingly, the malformations in Miller syndrome resemble those of fetal exposure to methotrexate, an inhibitor of de novo purine synthesis. Thus, defects in purine and pyrimidine biosynthesis likely cause similar birth defects. The mechanism by which the mutations of *DHODH* cause malformations must still be elucidated, though it may involve disturbed fibroblast growth factor signaling [44]. The DHO levels are increased in patients' urine and plasma, although urine analysis fails to reveal DHO presence in some patients. Surprisingly, orotic acid is also elevated in patients' urine but not in their plasma. DHO has been suggested to be oxidized into orotic acid by alternative metabolism upon renal excretion [45]. In theory, dietary supplementation with orotic acid or uridine could by-pass the metabolic block. However, as the principal damage occurs in utero, this therapy is unlikely to be effective.

32.1.12 Dihydropyrimidine Dehydrogenase Deficiency

Figure 32.2 Enzyme 6.

Complete or near-complete deficiency of dihydropyrimidine dehydrogenase (DPD) can be observed in children with growth and psychomotor delays, epilepsy, as well as dysmorphic and autistic features. As DPD catalyzes the catabolism of uracil and thymine into dihydrouracil and dihydrothymine, respectively, its deficiency leads to their accumulation. High amounts of uracil can be detected in urine. How the defect leads to neurologi-

cal symptoms remains elusive, but reduction in the concentration of the neurotransmitter β-alanine is likely to play a role [46]. No treatment is currently available and death in early infancy has been reported (see also ▶ Sect. 32.7.2).

32.1.13 Dihydropyrimidinase Deficiency

Figure 32.2 Enzyme 7.

The clinical phenotype of dihydropyrimidinase (DHP) deficiency is highly variable, ranging from early infantile onset of severe neurological involvement, dysmorphic features, and feeding problems to late onset of mild intellectual disability and even asymptomatic individuals. Around 40 patients have been reported. Dihydropyrimidinase catalyzes the cleavage of dihydrouracil and dihydrothymine into β-ureidopropionate and β-ureidoisobutyrate, respectively. Considerable quantities of dihydrouracil and dihydrothymine, which are normally found in small amounts, are excreted in urine and can be measured by LC/MS-MS (not detectable upon UV examination). There is also a moderate elevation in uracil and thymine excretion. No therapy is available, and prognosis is likely unpredictable. Several variants of *DPYS* have been identified in both symptomatic and asymptomatic individuals [47, 48].

32.1.14 β-Ureidopropionase Deficiency

Figure 32.2 Enzyme 8.

β-ureidopropionase (UP) catalyzes the last step of the pyrimidine degradative pathway with the conversion of β-ureidopropionate and β-ureidoisobutyrate into β-alanine and β-aminoisobutyrate, respectively. Around 40 patients that present with highly variable phenotypes have been reported, ranging from asymptomatic to having severe neurological involvement, including early onset psychomotor retardation with dysmorphic features, epilepsy, optic atrophy, retinitis pigmentosa, severely delayed myelination, cerebellar hypoplasia, or urogenital and colorectal anomalies [49, 50]. The deficiency provokes increased urinary excretion of ureidopropionate (also called N-carbamoyl-β-alanine) and ureidoisobutyrate (also called N-carbamoyl-β-aminoisobutyrate), which may act as neurotoxins. Dihydrouracil, dihydrothymine, uracil, and thymine are only moderately increased. Related studies have indicated that β-ureidopropionate and β-ureidoisobutyrate are weak DHP inhibitors. This may explain the moderately increased levels of dihydrouracil and dihydrothymine, which are observed in these patients.

32.1.15 Cytosolic 5′-Nucleotidase Superactivity

Figure 32.1 Enzyme 19 and Fig. 32.2 Enzyme 4.

Four unrelated children have been described with developmental delay, growth retardation, seizures, ataxia, recurrent infections, autistic features, and hypouricosuria [51]. Studies on the patients' fibroblasts revealed 6- to 20-fold elevations in cytosolic 5′-nucleotidase activity, which was measured either with a pyrimidine (UMP) or a purine (AMP) as the substrate. Based on the possibility that this increased catabolism might cause a deficiency in pyrimidine nucleotides, the patients were treated with uridine at a dose of 1 g/Kg per day. Developmental improvement and a decrease in the frequency of seizures and infections were recorded. However, the existence of the disease has not been established since no other patients or gene alterations have been described until now.

32.2 Diseases with Predominant Kidney Stones or Kidney Involvement

Inborn errors of purine metabolism are rare inherited causes of kidney stones, which are mainly due to increased urinary levels of uric acid, xanthine or 2, 8-dihydroxyadenine (2, 8-DHA). Partial HPRT deficiency may also be responsible for hyperuricemia with kidney stones (see ▶ Sect. 32.1.5 for a description of this disease).

32.2.1 PRPS1 Overactivity

Figure 32.1 Enzyme 1.

This disorder is manifested by the appearance of gouty arthritis or uric acid lithiasis in young adult males, which potentially leads to renal insufficiency [17, 18, 52]. A few patients present in infancy with sensorineural deafness, developmental delays, recurrent infections, short stature, facial dysmorphism, and autistic features [53]. Women are less likely to be affected; some suffer from hyperuricemia, nephrolithiasis, and gout, but are rarely affected by neurologic changes [54]. "PRPS1 superactivity" is the name originally applied to the overall disorder. Two varieties of defects have been described. A gain-of-function point mutation in the open reading frame of *PRPS1*, which results in regulatory defect or an alteration of the catalytic function, in the most severe phenotype. Upregulation of *PRPS1* with an accelerated transcription of an enzyme with normal kinetic properties, constitutes the milder phenotype. For this last

mechanism, no genetic defect has yet been identified. Therefore, it has been suggested that the term "PRPS1 overactivity" should become the overall name and that "superactivity" refers to the phenotype that is associated with the *PRPS1* gain-of-function point mutation [55]. Since PRPP amidotransferase, which is the first rate-limiting enzyme of the de novo pathway, is not saturated by PRPP, the synthesis of purine nucleotides increases, which subsequently enhances the production of uric acid. Uric acid levels are at times very high, reaching 10–15 mg/dL (0.60–0.90 mmol/L) in plasma and up to 2400 mg/24 h (14 mmol/24 h, or 2500 mmol/mol creatinine) in urine. The diagnosis is based on kinetic properties, but this is only performed in a few laboratories. Currently, diagnosis is established using molecular analysis with the identification of a hemizygous *PRPS1* pathogenic variant for more severe forms. Patients should be treated for hyperuricemia by means of XO inhibitors, a low purine diet, high fluid intake, and urine alkalization, as described in HPRT deficiency.

32.2.2 Hereditary Xanthinuria

Figure 32.1 Enzyme 6.

Three types of XO deficiencies are known, all of which cause xanthinuria. Type I classical xanthinuria is caused by isolated XO deficiency (*XDH* gene) [56]. Type II classical xanthinuria is due to deficiencies of both XO and aldehyde oxidase (AO), subsequent to molybdenum cofactor (MoCo) sulfurase deficiency (*MOCOS* gene) when the patient is no longer able to produce the sulfurated form of MoCo, which is essential for XO and AO activities [57]. In Type III, the combined deficiency of XO, AO, and sulfite oxidase (SO) results from the failure to synthesize MoCo, which is common to the three oxidases (see ▶ Chap. 20 for a discussion of the several genes involved in the synthesis of MoCo). Although Type I and Type II xanthinuria can be asymptomatic, kidney stones are formed in about one-third of cases. These stones are usually not visible on X-ray, but they may appear at any age and may cause nephrolithiasis, and even acute renal failure. Muscular pain and stiffness may also be observed, which is primarily caused by crystalline birefringent xanthine deposits in the muscles, and triggered by strenuous exercise. In Type III xanthinuria, the same severe neurological presentation as in isolated SO deficiency is observed (▶ Chap. 20). Hereditary xanthinuria is characterized by hypouricemia, hypouricosuria, and high levels of hypoxanthine and xanthine in the urine. Plasma hypoxanthine is not elevated or only minimally elevated, owing to its efficient reutilization by HPRT. In contrast, plasma xanthine, which is normally below 1 μM, may rise up to 10–40 μM. The very limited solubility of xanthine explains the formation of kidney stones and its deposition in the muscles. An allopurinol loading test enables disambiguation of Types I and II, as allopurinol is not converted into oxopurinol in the case of combined XO and AO deficiency, whereas it is still converted into oxopurinol in the event of isolated XO deficiency [58]. Classical xanthinuria types I and II are mostly benign, but a low purine diet should be prescribed, and fluid intake should be increased so as to prevent renal stones.

32.2.3 Adenine Phosphoribosyltransferase Deficiency

Figure 32.1 Enzyme 10.

Adenine phosphoribosyltransferase (APRT) deficiency is characterized by urinary gravel, small stones, and crystals, which are frequently accompanied by abdominal pain, dysuria, hematuria, and urinary tract infections [59]. Chronic progressive renal disease is the most common presentation, whereas some patients may exhibit acute anuric renal failure. More than 400 patients have been reported. The disease can occur at any age, with half of patients showing first symptoms in adulthood. This deficiency results in suppression of the adenine salvage, provided by food and by the polyamine pathway. Consequently, adenine is oxidized (by XO) into 2,8-DHA, which is a very poorly soluble compound. The resulting precipitates are characteristically radiolucent, which produces a "bright" kidney pattern. Increased levels of adenine and 2, 8-DHA are detected in urine [6, 60]. Distinctive DHA crystals may sometimes be detected in the urine using microscopic examination. Measurement of APRT activity in red blood cells confirms the diagnosis. In symptomatic patients, XO inhibitors prevent the formation of 2, 8-DHA. Purine restriction in the diet and high fluid intake are recommended. Alkalization of the urine is not advised because, unlike that of uric acid, the solubility of 2, 8-DHA does no longer increase up to pH 9. The ultimate prognosis depends on renal function at diagnosis. After kidney transplantation, it is essential to continue XO inhibitor treatment [61].

32.2.4 Uric Acid Transport Defects: Hypo- and Hyperuricemia

See also ▶ Sect. 1.5.2.

Renal hypouricemia (RHUC) is caused by defective renal tubular urate transport. RHUC Type 1, which is more common in Asia, is linked to pathogenic variants in *SLC22A12*, which encodes the urate transporter 1 (URAT1). Pathogenic variants in *SLC2A9*, which encodes GLUT9, are responsible for RHUC Type 2.

These defects are characterized by a decreased serum uric acid levels and increased fractional uric acid excretion, which may lead to severe complications, including nephrolithiasis and exercise-induced acute kidney injury [62].

As a reminder, pathogenic variant in *UMOD*, the most common cause of autosomal-dominant tubulointerstitial kidney diseases (ADTKD) is frequently associated with hyperuricemia and gout. ADTKD are characterized by a slowly progressive chronic kidney disease including tubulopathy and interstitial fibrosis that appears in adolescence, which leads to end-stage renal disease [63].

32.3 Diseases with Predominant Immunologic or Hematological Symptoms

Cellular and humoral immunity impairment is a major sign of adenosine deaminase 1 (ADA1), purine nucleoside phosphorylase (PNP), and adenylate kinase (AK) 2 deficiencies. These features are also observed in adenosine deaminase 2 (DADA2) deficiency. Hemolytic anemia is presenting sign of cytosolic 5′-nucleotidase 3A deficiency while megaloblastic anemia is characteristic finding in AK1 deficiency and uridine monophosphate synthase (UMPS) deficiency. Diamond-Blackfan-like anemia is observed in DADA2 and finally, anemia with anisopoikilocytosis is a specific hallmark of CAD deficiency (see ▶ Sect. 32.1.9). Of note, FAMIN (also called LACC1) has recently been presented as a multifunctional purine enzyme [64]. Its deficiency (AR disease) has been associated with juvenile idiopathic arthritis, (Still's disease) and early-onset inflammatory bowel disease [65].

32.3.1 Adenosine Deaminase 1 Deficiency

Figure 32.1 Enzyme 9.

The clinical spectrum of ADA1 deficiency is broad, including profound impairment of both humoral and cellular immunity in infants (severe combined immunodeficiency-SCID), delayed onset and less severe in older children, and even benign partial ADA1 deficiency in adults [66–68]. These presentations account for about 40% of North American patients with SCID and 10–20% of European patients with SCID. Its incidence is estimated at ~1/500,000 in the U.S. [69]. Approximately 80–85% of patients display multiple, recurrent, opportunistic infections within the first weeks or months of life, which rapidly become life-threatening. Infections are mainly localized in the skin, and the respiratory and gastrointestinal tracts. In the latter case, they often lead to intractable diarrhea, malnutrition, and growth retardation. Suggestive signs include hypoplasia and an apparent absence of lymphoid tissue (tonsils, lymph nodes, or a thymus shadow on X-ray). Patients might develop skeletal dysplasia (scapular spurring and costochondral cupping), developmental delays, sensory-neural hearing defects, respiratory distress, hepatic failure, and neutropenia [70]. Approximately 15–20% of ADA1-deficient children display clinical symptoms during the first years of life. Infections in delayed-onset patients may initially be less severe than in those with ADA-SCID. Recurrent otitis, sinusitis, and upper-respiratory-tract infections are common. At diagnosis, these patients often display chronic respiratory insufficiency. The very rare individuals who survive into the first decade of life or beyond without being diagnosed often display deteriorated immune function and chronic sequelae of recurrent, particularly respiratory infections and pulmonary alveolar proteinosis (PAP). There is an increased frequency of *dermatofibrosarcoma protuberans* in these patients [71]. This deficiency results in bodily fluids accumulating adenosine and 2′-deoxyadenosine (which are normally undetectable) [66]. These compounds, and their metabolites, induce the premature death of lymphoid progenitor cells, which subsequently profoundly impairs the generation of T, B, and natural killer (NK) lymphocytes. SCID should be suspected in the presence of lymphopenia (<500 total lymphocytes per mm^3) involving B, T, and NK cells, and hypogammaglobulinemia. The disease is progressive since residual B and T cell function at birth disappears later. The enzymatic diagnosis is confirmed on RBCs. The disease severity often correlates with the ADA1 activity loss.

Diagnosis of SCIDs (including ADA1 deficiency) have been significantly changed since the introduction of genetic newborn screening in several countries using a combination of T cell receptor excision circles (TRECs) as a surrogate marker for new T cell production, and kappa-deleting recombination excision circles (KRECs) as a surrogate marker of freshly formed, naive B cells [72]. ADA1 deficiency diagnosis is then confirmed by demonstrating absent or very low (<1% of normal) ADA activity in RBC. Newborn TREC values may be above the cut-off levels in late-onset ADA1 deficiency, which may impede the identification of these patients. Conversely, newborn screening using detection of adenosine and 2′-deoxyadenosine would be positive [73].

Severe ADA1-SCID invariably leads to death within the first year of life, unless specific treatment is administered or drastic steps are taken, such as rearing the patient in strictly sterile conditions from birth. Multiple treatment options have been developed, including allo-

geneic hematopoietic stem cell transplantation (aHSCT), enzyme replacement therapy (ERT), and, more recently, autologous HSCT with gene therapy (GT). Choosing between treatments is difficult as it depends on the treatment's accessibility, response to ERT, and potential short- and long-term risks [74]. According to the last consensus, the care standard of aHSCT with a human leukocyte antigen (HLA)-matched sibling donor (MSD) without conditioning has survival rates exceeding 80%. The transplantation should be performed as soon as possible to prevent acquired infections and minimize other toxic effects due to the high systemic levels of adenine metabolites. ERT should be started prior to transplant. In the absence of an HLA-compatible donor, HSCTGT may be an alternative therapy [75].

32.3.2 Adenosine Deaminase 2 Deficiency

Figure 32.1 Enzyme 9.

In 2014, deficiency of adenosine deaminase 2 (DADA2) was described as a monogenic autoinflammatory disease that is characterized by stroke and systemic vasculitis in young children [76, 77], following recessive loss-of-function variants in *ADA2* (previously *CECR1* [cat eye syndrome chromosome region, candidate 1]). Recently, the clinical DADA2 spectrum has been expanded based on reports of hematologic and immunologic abnormalities like pure red cell aplasia (PRCA), BMF, hypogammaglobulinemia, and lymphopenia [78, 79]. The disease onset usually occurs in childhood, with a quarter of patients presenting symptoms before 1 year of age, and the remaining three-quarters presenting symptoms before the age of 10. Vasculopathy of small- and medium-sized arteries, which commonly involves the skin and central nervous system, is DADA2's major clinical feature. Cutaneous manifestations are found in more than 75% of patients. These manifestations include *livedo reticularis*, *polyarteritis nodosa*, digital necrosis, and nonspecific skin rash. More than 50% of patients experience one or more neurological events, sometimes at disease onset in infancy. A typical MRI image consists of acute or chronic lacunar ischemic infarcts that are located in the deep-brain nuclei and/or the brain stem, but the subcortical white matter is spared. Gastrointestinal manifestations are observed in one-third of patients, consisting of abdominal pain, inflammatory bowel disease, elevated transaminases, hepatosplenomegaly, and portal hypertension. A fever and increased erythrocyte sedimentation rate or C-reactive protein levels are reported in 50% of patients. Other features include arterial hypertension, renal vessel artery aneurysm or stenosis, kidney inflammation, arthralgia, myalgia, and arthritis that mainly affects small joints. Immunological and hematological manifestations are described in 25% of patients. PRCA that mimics Diamond–Blackfan anemia occurs in the first year of life. Patients with BMF present later in childhood with recurrent infection, gingivitis, and hepatosplenomegaly, even without vasculitis or systemic inflammation. Lymphoproliferation is another essential DADA2 feature with generalized lymphadenopathy and splenomegaly. Autoimmune lymphoproliferative syndrome, Hodgkin lymphoma, and idiopathic multicentric Castleman disease have likewise been reported [80, 81]. The large clinical spectrum renders early diagnosis difficult. Without early diagnosis and specific treatment, mortality resulting from recurrent stroke or infections remains significant in young adults.

In contrast to ADA1 deficiency, adenosine and 2′-deoxyadenosine levels are normal. Diagnosis can be suggested by low or absent ADA2 activity in plasma/serum and confirmed using molecular analysis. Genotype–phenotype correlations in DADA2 have been established with *ADA2* variants. Missense mutations with at least 3% residual enzymatic activity are associated with vasculitis. PRCA and BMF are associated with missense mutations with minimal residual enzyme activity, nonsense variants, and insertions/deletions that result in complete function loss [82].

Anti-TNF- agents (etanercept, infliximab, adalimumab, and golimumab) effectively control inflammation, resulting in a major reduction in stroke occurrence without serious undesirable effects, thereby enabling glucocorticoid weaning [83]. Unlike the standard care for stroke patients, discontinuing treatment with acetylsalicylic acid and other anticoagulants is recommended because hemorrhagic stroke is a potential complication [76]. Since PRCA and BMF are proven to be refractory to anti-TNF agents, these patients likely benefit from aHSCT with myeloablative or reduced-intensity conditioning [84].

32.3.3 Purine Nucleoside Phosphorylase Deficiency

Figure 32.1 Enzyme 5.

PNP deficiency is characterized by a progressive SCID form (about 4% of SCIDs) with decreased T cell numbers. Recurrent infections usually occur later, starting from the end of the first year and up to the age of five to six; infections are initially less severe than in ADA1 deficiency [85]. Neurodevelopmental problems develop toward the end of the second year of life, including ataxia, intellectual deficiency, and muscle spasticity [86]. One-third of patients display increased risk of autoimmune disorders like autoimmune hemolytic ane-

mia, immune thrombocytopenia, neutropenia, thyroiditis, and lupus. The disorder is much less common than ADA1 deficiency, with only about 80 patients reported so far. PNP is found in most body cells, with the highest expression in lymphoid tissue. It degrades guanosine, deoxyguanosine, inosine, and deoxyinosine [87]. PNP deficiency leads to the accumulation of its substrates, which results in intracellular accumulation of deoxyguanosine triphosphate (dGTP), especially in the thymus, where high cell turnover occurs. Additionally, dGTP may directly interfere with DNA synthesis or repair through inhibition of ribonucleotide reductase activity, which thus prevents the cellular proliferation that is required for immune responses. The profound impairment of cellular immunity, which characterizes PNP deficiency, has been expounded by the greater T cell ability to accumulate dGTP, compared to B cells. The normally ubiquitous expression of PNP accounts for non-immunologic symptoms being present in PNP deficiency cases. SCID can be suspected by a marked reduction in T cell numbers. B-lymphocyte function is deficient in about one-third of patients. Low plasma uric acid may be used as a marker for PNP deficiency, but is not always reliable. PNP enzyme activity should be measured either in RBC lysates, blood spots, or leukocytes. Diagnosis is confirmed using molecular PNP gene analysis. The late onset of the immunodeficiency in PNP deficiency may enable normal newborn screening using combined TRECs–KRECs. Mass spectrometry on dried blood spots identifies PNP metabolites (guanosine, inosine, dGuo, and dIno), which appears to be a more accurate neonatal screening method [88]. Most initially diagnosed patients have died due to overwhelming infections. The only successful treatment is aHSCT, but its effectiveness for neurological and autoimmune problems is unclear, due to the small number of transplanted patients. However, no cases reported further neurological deterioration after aHSCT, with improvement even reported in some patients [89].

32.3.4 Adenylate Kinase Deficiencies

Figure 32.1 Enzyme 13.

Adenylate kinases (AK) are phosphotransferases that catalyze the interconversion of adenine nucleotides. They also play a crucial role in energetic metabolism. Among the nine AK isoenzymes, three are known to be associated with diseases [90].

AK1, the major cytosolic form, is highly expressed in skeletal muscles, brain, and erythrocytes. Its deficiency results in chronic non-spherocytic hemolytic anemia, which is associated with hepatosplenomegaly and psychomotor retardation.

Deficiency of the mitochondrial AK2, which is the unique form expressed in neutrophils, T lymphocytes, and the inner ear, cause immunodeficiencies of varying severity, ranging from reticular dysgenesis (a form of SCID, with severe T cell depletion, severe neutropenia, and sensorineural deafness) to less severe immune deficiency [91].

Patients with mutations in AK7, which is a cytosolic enzyme expressed in ciliary cells, have been reported manifesting primary ciliary dyskinesia (sinusitis, otitis, chronic cough, and infertility) or isolated masculine infertility [92].

32.3.5 Adenosine Deaminase 1 Overactivity

Figure 32.1 Enzyme 9.

Diamond-Blackfan anemia, which is a rare autosomal-dominant hereditary disease characterized by red blood cell aplasia and usually associated with heterozygous pathogenic variants in genes encoding ribosomal proteins, is associated in most cases with an increase of erythrocyte ADA1 activity [93, 94]. Historically a very high erythrocyte ADA1 overactivity (approximately 50-fold elevation) has been reported to cause non-spherocytic hemolytic anemia [95].

32.3.6 Uridine Monophosphate Synthase Deficiency

Figure 32.2 Enzyme 3.

This deficiency is a very rare disorder that presents within the first months of life with megaloblastic anemia unresponsive to iron, folic acid, or vitamin B_{12}. When undiagnosed, the disorder leads to failure to thrive and psychomotor delay. Some patients without anemia but with epilepsy have been reported [96]. Uridine monophosphate synthase is a bifunctional enzyme of the de novo pyrimidine synthesis. The first enzymatic reaction by orotate phosphoribosyltransferase converts orotic acid into orotidine 5′-monophosphate (OMP), and the second reaction by OMP decarboxylase converts OMP into UMP. The defect provokes a massive overproduction of orotic acid (alternative name hereditary orotic aciduria), attributed to the ensuing decrease in the feedback inhibition exerted by the pyrimidine nucleotides on the first enzyme of their de novo synthesis, CPS II, and a deficiency of pyrimidine nucleotides [97]. This deficiency of pyrimidine nucleotides leads to impairment of cell division, which results in megaloblastic anemia, failure to thrive, and psychomotor delay. Urinary analysis reveals a marked increased excretion of orotic acid, reaching, in infants, 200- to 1000-fold the

normal value. These levels are usually much higher than those in urea cycle defects. Occasionally, orotic acid crystalluria is observed, particularly in the dehydration setting. The level of orotidine, the dephosphorylation product of OMP, is also increased in the urine, yet usually below that of orotic acid, although this may vary among some patients. It should be noted that mild orotic aciduria may be observed in individuals carrying a heterozygous *UMPS* variant without any clinical consequences. This should be considered upon differential diagnosis [98]. Uridine, which is converted by uridine kinase into UMP, is an efficient treatment if started early in life. An initial dose of 100–150 mg/Kg, divided over the day, induces prompt hematologic response and growth acceleration. Further dosage should be adapted in order to obtain the lowest possible orotic acid output. In some cases, normal psychomotor development can be achieved, whereas this is not obtained in others, possibly owing to delayed therapy onset.

32.3.7 Cytosolic 5′-Nucleotidase 3A Deficiency

Figure 32.2 Enzyme 4.

Cytosolic 5′-nucleotidase 3A deficiency is characterized by the accumulation of pyrimidine nucleotides, resulting in pronounced basophilic stippling on peripheral blood smear and chronic hemolytic anemia. This enzyme is restricted to erythrocytes and its deficiency is the third most common cause of hereditary non-spherocytic hemolytic anemia after glucose 6-phosphate dehydrogenase and pyruvate kinase deficiencies. Its clinical presentation is similar to lead intoxication because of its inhibitory effect on cytosolic 5′-nucleotidase 3A. Several pathogenic variants have been identified, whereas the mechanisms by which the increased pyrimidine nucleotides cause hemolysis remain unknown [99, 100].

32.3.8 Hyper-IgM Syndromes

Cytidine deaminase is part of a superfamily that also comprises activation-induced cytidine deaminase (AICDA). This RNA-editing enzyme is specifically expressed in B-lymphocytes, and its deficiency causes autosomal recessive Type II hyper-IgM syndrome (HGIM2), which is defined by markedly diminished serum IgG and IgA levels with normal or increased serum IgM levels. Patients present with recurrent bacterial sino-respiratory and gastrointestinal tract infections, with associated lymphoid hyperplasia. Early initiation of intravenous immunoglobulin and antibiotic prophylaxis most often drastically improve the symptoms [101, 102]. Additionally, AICDA is involved in a rare autosomal dominant form of HIGM [103, 104].

Uracil-DNA glycosylase removes uracil from DNA due to deamination of cytosine or replicative incorporation of dUMP instead of dTMP. Previously, pathogenic variants in *UNG* were detected in three unrelated patients with a phenotype resembling HIGM2, including susceptibility to bacterial infection, lymphoid hyperplasia, increased serum IgM concentration, and profoundly decreased serum IgG and IgA concentrations (HIGM5) [105].

32.3.9 Ecto-5′-Nucleotidase (NT5E) Deficiency

NT5E, which encodes CD73 (Figure 32.1 Enzyme 19), is involved in the synthesis of adenosine on the surface of various types of cells. Its deficiency has been reported in several families characterized by symptomatic arterial and joint calcifications during adulthood [106]. Homozygous variants in this gene have also been reported in two adult siblings affected by late-onset severe aortic stenosis [107].

32.4 Diseases with Predominant Muscular Involvement

32.4.1 AMP Deaminase 1 Deficiency

Figure 32.1 Enzyme 8.

The deficiency of muscle AMP deaminase (AMPD1, frequently referred to as myoadenylate deaminase) is observed in 1–2% of the Caucasian population [108]. The vast majority of deficient individuals are asymptomatic. Some subjects, in whom the AMPD1 defect is termed primary, exhibit isolated muscular weakness, cramps, or post-exercise myalgias. This is sometimes accompanied by an increase in serum creatine kinase and myoglobinuria [109]. AMPD1 deficiency is defined as "double trouble," or coincident AMPD1 deficiency, when genetically proven AMPD1 coexists with another disease, generally a metabolic myopathy (e.g., glycogenosis) or a neuromuscular disorder (e.g., amyotrophic lateral sclerosis, facioscapulohumeral myopathy, [110]). This emphasizes the need to ascertain the absence of another disease when an AMPD1 deficiency is found. Screening for this defect can be performed by exercise testing or forearm ischemic exercise testing. This disease can thus be differentiated from McArdle disease (glycogen storage disorder type V, ▶ Chap. 5) because the normal rise in venous ammonia is absent in AMPD1 deficiency, whereas the normal

rise in lactic acid is absent in McArdle disease [111]. Diagnosis can be made using histochemical or biochemical muscle assays and molecular analysis of *AMPD1*. Patients may display a gradual symptom progression, with a major impact on their daily lives. Ribose administration has been reported to improve muscular strength and endurance [112].

32.4.2 Muscle-Specific Adenylosuccinate Synthase Deficiency

Figure 32.1 Enzyme 7.

The *ADSSL1* gene encodes the adenylosuccinate synthase (ADSS)-like 1-protein, a muscle-specific enzyme responsible for first step in the conversion of IMP to AMP. Compound heterozygous variants in *ADSSL1* are associated with adult-onset distal myopathy [113]. Mild facial-muscle weakness and predominant distal muscle weakness appear during adolescence. Muscle weakness and atrophy progress slowly in the lower legs and, to a lesser extent, distally in the upper limbs. Proximal myopathy with contractures and muscle atrophy, respiratory dysfunction, dysphagia and cardiopathies has also been reported [114, 115]. Serum CK levels are mildly increased (four to eight times the normal values). In the early disease stage, diffuse fatty infiltration is observed on MRI of the gastrocnemius muscles, as well as in the soleus or tibialis anterior muscles. Muscles in the thigh are involved in later stages. Predominant fatty replacement of tongue muscles is a pathognomonic radiological sign. Muscle biopsies display nemaline bodies and lipid droplets in myofibers [113].

32.5 Diseases with Predominant Liver Involvement

32.5.1 Adenosine Kinase Deficiency

Figure 32.1 Enzyme 12.

Around 20 patients with adenosine kinase (ADK) deficiency have been reported, almost all of whom displayed neonatal onset liver dysfunction of varying severity, with microvesicular steatosis. All patients exhibited developmental delays, hypotonia, and dysmorphic features (frontal bossing often accompanied by hypertelorism). Other features include sparse hair, slender hands and feet, epilepsy, hyperinsulinemic hypoglycemia, optic nerve glioma, and dysplasia of the hips [116]. Biochemical analysis showed increased plasma levels of methionine, SAM, and S-adenosylhomocysteine (SAH), but normal or mildly elevated homocysteine levels, indicating a block in the methionine cycle (▶ Chap. 20). Urinary hyperexcretion of adenosine was observed, and accumulation of AICAriboside and SAICAriboside, have also been described in one patient [117]. A low methionine diet has been proven to improve liver function in several patients, whereas only one patient showed neurological improvement. Diazoxide is recommended for hyperinsulinism.

32.5.2 Deoxyguanosine Kinase Deficiency

Figure 32.1 Enzyme 17.

DGUOK deficiency leads to a multisystemic mtDNA depletion syndrome, which has a predominantly hepatocerebral presentation (MTDPS3, see below). However, some patients with isolated liver disease have been reported, which is sometimes accompanied by renal disease.

32.6 Mitochondrial DNA Depletion Syndromes

Mitochondrial deoxyribonucleic acid DNA depletion syndromes (MTDPSs) are autosomal recessive disorders characterized by severe reduction in mtDNA content in the affected tissues. These syndromes are associated with defects in mtDNA maintenance caused by mutations in the nuclear genes that are involved in mitochondrial deoxyribonucleoside triphosphate (dNTP) synthesis or mtDNA replication (▶ Chap. 10). Several enzymes of purine and pyrimidine metabolism are essential for maintaining the mitochondrial pool of dNTPs, including thymidine kinase, ribonucleotide reductase M2 B subunit, deoxyguanosine kinase, and thymidine phosphorylase. These defects likely produce an imbalance in mitochondrial nucleotides, which disturbs the replication of mtDNA.

32.6.1 Deoxyguanosine Kinase Deficiency

Figure 32.1 Enzyme 17.

Mitochondrial deoxyguanosine kinase (DGUOK) phosphorylates the deoxy- counterpart of guanosine into dGMP and plays an essential role in supplying the precursors of mtDNA. DGUOK deficiency leads to a multisystemic mtDNA depletion syndrome (MTDPS3), which has predominantly a hepatocerebral presentation. However, some patients with isolated liver disease have been reported, which is sometimes accompanied by renal disease. Within weeks of birth, patients present with cholestasis and progressive liver failure,

neurological abnormalities (severe hypotonia, developmental regression, abnormal ocular movement), hypoglycemia, and increased lactate levels [118]. Less common presentations have also been reported, including infantile cirrhotic portal hypertension, juvenile-onset mitochondrial myopathy, and adult-onset chronic progressive external ophthalmoplegia and myopathy [119]. Liver transplantation may be considered in children with hepatic or hepatorenal forms, without significant neurological symptoms. In vitro studies suggest that administrating deoxynucleotides or NAD may be potential treatments for mtDNA depletion syndrome [120, 121].

32.6.2 Ribonucleotide Reductase Deficiency

Figure 32.1 Enzyme 18; Fig. 32.2 Enzyme 10.

Ribonucleotide reductase catalyzes the rate-limiting step in the de novo reduction of ribonucleoside diphosphates to dNTPs during DNA synthesis and repair in the nucleus and mitochondria. Ribonucleotide reductase M2B (Rrm2b), also known as p53R2, is a subunit of the ribonucleotide reductase complex and is encoded by *RRM2B*. Several *RRM2B* pathogenic variants have been reported in severe MTDPS with early onset (neonatal or infantile). Affected individuals typically present within the first months of life with hypotonia, lactic acidosis, failure to thrive, tubulopathy, microcephaly, psychomotor delay, sensorineural hearing loss, and profound mtDNA depletion in muscle (MTDPS8A). The disease leads to death within few months. The RRM2B mutations have also been reported to cause a mitochondrial neurogastrointestinal encephalopathy (MNGIE)-like phenotype (MTDPS8B) with mtDNA depletion and autosomal-dominant progressive external ophthalmoplegia (PEOA5) with multiple mtDNA deletions [122, 123].

32.6.3 Thymidine Kinase 2 Deficiency

Figure 32.2 Enzyme 9.

Mitochondrial thymidine kinase 2 (TK2) plays an essential role in the pyrimidine nucleoside salvage pathway. It mediates the first (and rate-limiting) step in the phosphorylation of pyrimidine nucleosides in the mitochondrial matrix. MTDPS Type 2 (MTDPS2) is caused by pathogenic variants in *TK2*. Primarily, TK2 deficiency manifests as a myopathy and includes extremely severe and rapidly progressive early-onset forms with survival of less than 2 years, to milder forms with late or very late-onset, and slower progression rate, but with almost invariable respiratory involvement than shortens life expectancy. A new therapy based on oral administration of deoxynucleosides has been proposed in an effort to induce the synthesis of pyrimidine dNTPs via alternative enzyme pathways by means of supplementation with their precursors [124]. This treatment has striking effects on early-onset severe myopathy patients, resulting in improvement in muscle strength, reduction or discontinuation of mechanical ventilation and gastrostomy feeding, and regaining the ability to walk without any major undesirable effects. During treatment, growth differentiation factor 15, a biomarker for mitochondrial diseases significantly declined and it was accompanied by a clinically-relevant improvement [125].

32.6.4 Thymidine Phosphorylase Deficiency

Figure 32.2 Enzyme 5.

Thymidine phosphorylase (TP) deficiency causes mitochondrial neurogastrointestinal encephalomyelopathy (MNGIE), an autosomal recessive disease associated with multiple deletions of muscle mtDNA (MTDPS1) (▶ Chap. 10). TP deficiency results in marked accumulation of (deoxy)thymidine and deoxyuridine, which most likely provokes imbalance in mitochondrial nucleotides, and hence compromises the replication of mtDNA. The prevalence of MNGIE is estimated at 1–9 in 1000,000 world-wide. MNGIE is a progressive and degenerative disease, characterized by a complex clinical picture, involving multiple organ systems. The major clinical features are severe gastrointestinal dysmotility, cachexia, peripheral neuropathy, ocular symptoms, and asymptomatic diffuse leukoencephalopathy. Other signs include certain neurological, muscular, cardiac, and endocrine features. Clinical variability has been reported among MNGIE patients and among members of the same family. The mean mortality age has been estimated at 37.5 years [126]. Biochemical diagnosis relies on increased blood and urinary levels of (deoxy)thymidine and deoxyuridine but also uracil and thymine and severely reduced TP enzyme activity in leukocytes. The last two metabolites are probably produced by bacterial degradation or by another phosphorylase [127]. Symptomatic management of MNGIE consists of nutritional support, infection prevention, and pain relief. Platelet infusions, peritoneal/hemodialysis, and enzyme replacement therapy using recombinant erythrocyte encapsulated TP could be used to provide some clinical and biochemical correction. aHSCT is currently

the available treatment for MNGIE, yet with varying success. Liver transplantation is a promising emerging treatment and should be the first choice for patients with pre-existing liver failure. Adeno-associated viruses (AAV) gene therapy and lentiviral-mediated hematopoietic stem cell gene therapy (HSCGT) are potential future curative options. Gastrointestinal complications are the main mortality factor in MNGIE patients, and they are also the least treatable with the currently available therapies [128].

32.7 Pharmacogenetics

32.7.1 Thiopurine S-Methyltransferase and Nudix Hydroxylase 15 Deficiencies

Purine analogs, such as 6-mercaptopurine, 6-thioguanine, and azathioprine, are used to treat various diseases, including cancers, rheumatoid arthritis, and Crohn's disease, and for immunosuppression following organ transplantation. Phosphoribosylation by HPRT converts purine analogs into active thionucleotides, which exert their therapeutic action through incorporation into DNA and RNA. The balance between activation and inactivation pathways determines the level of active metabolites of these analogs. The wide variations in therapeutic response and occurrence of toxic undesirable effects in patients receiving thiopurines are related to the genetic polymorphism of inactivating enzymes. Thiopurine S-methyltransferase (TPMT, not shown) catalyzes the S-methylation of thiopurines, which results in their inactivation. Genetic TPMT polymorphisms is a well-known determinant in the wide therapeutic response variations**.** Patients with no or less efficient thiopurine methylation display more extensive conversion to active thionucleotides, which leads to severe, potentially fatal myelosuppression. Recently, the Nudix hydrolase 15 gene (*NUDT15*) has been identified as another essential pharmacogenetic gene for thiopurines, particularly in Asian and Hispanic populations. NUDT15 converts cytotoxic thioguanine triphosphate (tGTP) into the less toxic monophosphate tGMP. In patients with defective NUDT15, treatment with a standard mercaptopurine dose may lead to significant DNA damage and cytotoxicity, due to excessive tGTP/tdGTP accumulations. Other gene polymorphisms, including the ITPA gene, have also been associated with 6-mercaptopurine-induced toxicity, but with controversial reports. International guidelines for thiopurine treatment suggest that the *NUDT15* genotypes should be considered in addition to *TMPT*, prior to drug administration [129].

32.7.2 Dihydropyrimidine Dehydrogenase Dihydropyrimidinase and Cytidine Deaminase Deficiencies

Figure 32.2 Enzyme 6, Fig. 32.2 Enzyme 7 and Fig. 32.2 Enzyme 11.

Partial deficiency in DPD should be considered in patients who receive fluoropyrimidines (5-fluorouracil, capecitabine, and tegafur), which are classic treatments for various cancers. It is characterized by severe toxicity manifesting as profound neutropenia, stomatitis, diarrhea, and neurological symptoms, including ataxia, paralysis, and stupor. In the Caucasian population, approximately 3–8% of individuals display partial DPD enzyme deficiency, and 0.1–0.5% complete DPD enzyme deficiency. Carriers of one *DPYD* pathogenic variant constitute the majority of deficient patients and homozygous or compound heterozygous carriers result in complete deficiency [130, 131]. Generally, DPD testing (quantification of the dihydrouracil/uracil ratio in plasma and/or *DPYD* genotype) identifies patients at higher risk for toxicity who should be treated more safely using a lower drug dosage. DPD testing before fluoropyrimidine therapy is now strongly recommended by the European Medicines Agency.

Likewise, patients with partial deficiency of dihydropyrimidinase (DHP) are also liable to develop 5-fluorouracil toxicity [132].

Cytidine deaminase catalyzes the conversion of cytidine into uridine. This enzyme plays a key role in the inactivation of gemcitabine, which is a cytidine analog with activity against several solid tumors. Cytidine deaminase deficiency is linked with a number of genetic polymorphisms, associated with the risk of developing severe toxicity, and constitutes another example of predictive pharmacogenetics [133].

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Disorders of Haem Biosynthesis

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Contents

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The Haem Biosynthetic Pathway

Haem (iron protoporphyrin) is a metalloporphyrin with iron as the central metal atom, and is the prosthetic group for many haemoproteins. The largest amounts of haem are produced in the bone marrow, for formation of haemoglobin, and in the liver, primarily for cytochrome P450 enzymes (CYPs). The pathway for haem synthesis (Fig. 33.1) consists of eight enzymes and their substrates and products. The first and last three enzymes are located in mitochondria and the other four in the cytosol. The pathway is regulated differently in bone marrow and liver. The first enzyme of the pathway, 5-aminolevulinic acid synthase (ALAS), also known as δ-aminolevulinic acid synthase, is the only enzyme in this pathway for which erythroid and housekeeping forms of the enzyme are encoded by separate genes. The housekeeping enzyme (termed ALAS1) is rate limiting in the liver, is subject to negative feedback by haem, which represses its synthesis and its import into mitochondria, and is induced by a variety of drugs, steroids and other chemicals that also induce CYPs. By contrast, the erythroid-specific enzyme (ALAS2), which is encoded by a separate gene located on the X chromosome, is induced by haem and iron, providing for erythroid-specific regulation of haem synthesis [1].

Abbreviations: AD, autosomal dominant; ADP, ALA dehydratase porphyria; AIP, acute intermittent porphyria; ALA, 5-aminolevulinic acid; ALAD, ALA dehydratase; ALAS, 5-aminolevulinic acid synthase; ALAS1, housekeeping form of ALAS; ALAS2, erythroid-specific form of ALAS; AR, autosomal recessive; CEP, congenital erythropoietic porphyria; CPOX, coproporphyrinogen oxidase; FECH, ferrochelatase; HMB, hydroxymethylbilane; HMBS, HMB synthase; IV, intravenous; PBG, porphobilinogen; PBGD, PBG deaminase; PPOX, protoporphyrinogen oxidase; UROD, uroporphyrinogen decarboxylase; UROS, uroporphyrinogen III synthase.

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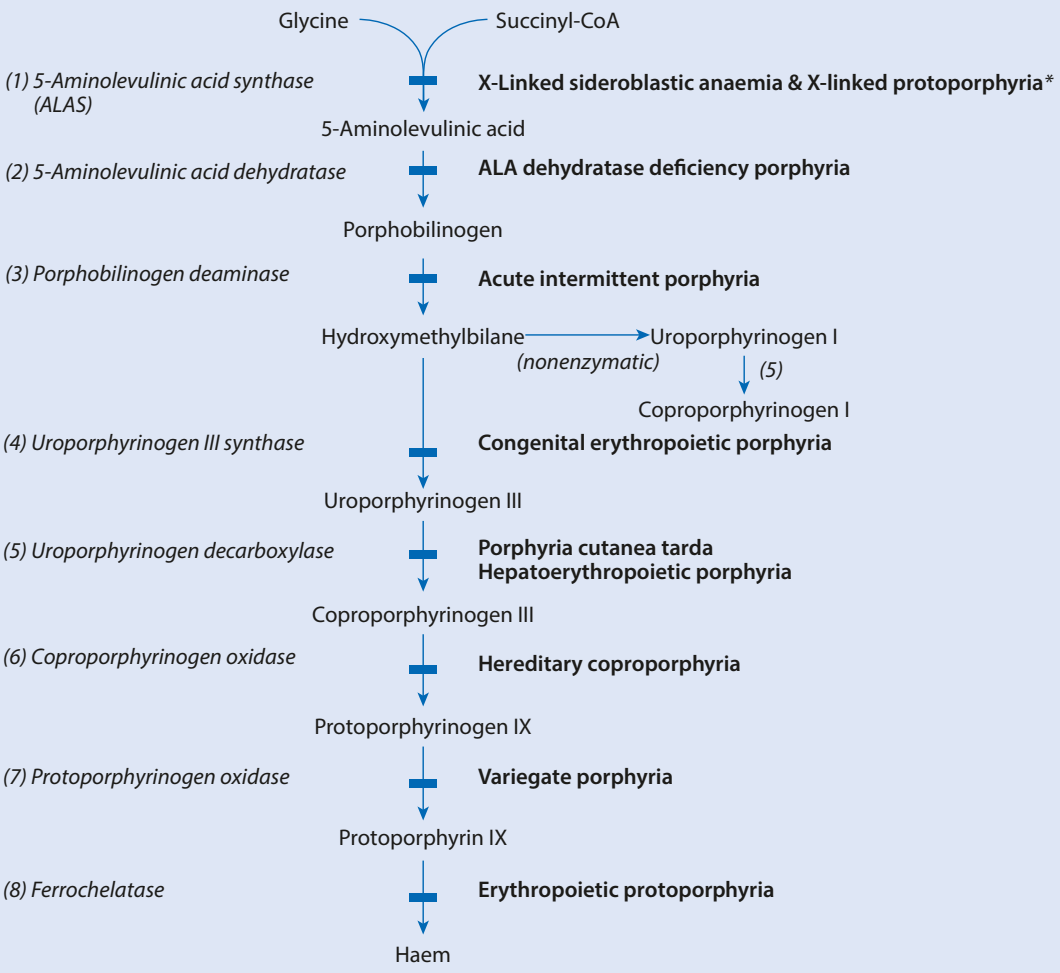

Fig. 33.1 Pathway of haem biosynthesis. ALA 5-aminolevulinic acid, CoA coenzyme A. Diseases caused by the various enzyme alterations (indicated by solid bars across the arrows) are given in bold. *Gain of function mutations cause X-linked protoporphyria (XLP)

Introduction

X-Linked sideroblastic anaemia (XLSA) is often due to loss of function mutations of *ALAS2*. Characteristics of the disease include childhood- or adult-onset anaemia, ineffective erythropoiesis with formation of ring sideroblasts, iron accumulation and pyridoxine responsiveness. Porphyrias are due to altered activity of enzymes of this pathway, and are associated with striking accumulation of haem pathway intermediates and their oxidised products.

Of the three most common porphyrias, porphyria cutanea tarda (PCT) presents with chronic blistering photosensitiviry, acute intermittent porphyria (AIP) presents with acute neurovisceral symptoms that can be exacerbated by certain drugs, hormones and nutritional changes, and erythropoietic protoporphyria (EPP) with acute, nonblistering photosensitity. All porphyrias are inherited, with the exception of PCT, which is mostly due to an acquired enzyme deficiency in the liver.

33.1 X-Linked Sideroblastic Anaemia

Clinical Presentation

X-Linked sideroblastic anaemia (XLSA) (▶ Chap. 1 ◘ Table 1.37) is suggested by hypochromic anaemia in the presence of increases in serum iron concentration and transferrin saturation. Males are more commonly affected than females. The bone marrow contains nucleated erythrocyte precursors with iron-laden mitochondria surrounding the nucleus (ring sideroblasts). Progressive iron accumulation may result from ineffective erythropoiesis, leading to organ damage [2].

Metabolic Derangement

These features reflect a deficiency of haem synthesis, which is due to a deficiency of ALAS2. Acquired forms have been attributed to alcohol, chemotherapy and the early stages of a myelodysplastic syndrome, which might affect one or more steps in haem synthesis. However, mutations of *ALAS2* or other mediators of mitochondrial iron metabolism have not been excluded in many of these cases (see ▶ Chap. 1 ◘ Table 1.37).

Genetics

X-Linked sideroblastic anaemia (XLSA) due to loss-of-function mutations of *ALAS2* is the most common cause of congenital sideroblastic anemia [2–4], and its genotypes and phenotypes are heterogeneous [5, 6]. More than 60 different *ALAS2* mutations have been reported in 120 families with XLSA [6]. Some point mutations occur in the pyridoxine binding site of the enzyme, and in such cases enzyme activity may be at least partially restored and anaemia corrected by high doses of this vitamin.

Diagnostic Tests

Hypochromic anaemia with evidence of iron overload suggests this diagnosis. Ring sideroblasts in the bone marrow and pyridoxine responsiveness is further evidence. Detection of an *ALAS2* mutation and demonstration of its X-linked inheritance is important for a definite diagnosis. Concurrent haemochromatosis (*HFE*) mutations increase risk for iron accumulation.

Treatment and Prognosis

Treatment consists in administration of pyridoxine and folic acid. The starting dose of pyridoxine is 100–300 mg/day, and a maintenance dose of 100 mg/day. Phlebotomy to remove excess iron not only prevents organ damage, which is the primary cause of morbidity in this disease, but also may increase responsiveness to pyridoxine [2].

33.2 The Porphyrias

Porphyrias result from altered activity of haem synthetic pathway enzymes and are characterised by accumulation of pathway intermediates and their oxidised products. Cutaneous manifestations are caused by excitation of excess porphyrins by light with a wavelength near 400 nm (within the lower part of the visible range), leading to generation of singlet oxygen and cell damage [7].Neurological effects are poorly explained, but are associated with increase in the porphyrin precursors ALA and PBG.

Patterns of excess intermediates in these disorders are characteristic for each type of porphyria and important for diagnosis. ALA and PBG are water soluble, colourless and nonfluorescent. They are excreted almost entirely in urine, as are porphyrins with a large number of carboxylic side chains (e.g. uroporphyrin, an octacarboxyl porphyrin). Protoporphyrin (a dicarboxyl porphyrin) is not soluble in water and is excreted entirely in bile and faeces. Coproporphyrin (a tetracarboxyl porphyrin) is excreted in both urine and bile, and its urinary excretion increases when hepatobiliary function is impaired. Most of the porphyrin intermediates are porphyrinogens (reduced porphyrins), which undergo autooxidation if they accumulate and leave the intracellular environment, and are then excreted primarily as the corresponding porphyrins. Porphyrinogens are colourless and nonfluorescent but porphyrins are reddish and display red fluorescent when exposed to light with a wavelength near 400 nm.

Classification and Diagnosis

Porphyrias are classified according to the tissue where intermediates initially accumulate (the liver in hepatic porphyrias and the bone marrow in erythropoietic porphyrias), or by their clinical presentation (acute neurovisceral or cutaneous porphyrias) (◘ Table 33.1).

Table 33.1 X-linked sideroblastic anemia and porphyrias and causative alterations in enxymes in the haem biosynthetic pathway

Disease	Enzyme	Gene	Inheritance and disease classification				
			Inheritance	Hepatic	Erythropoietic	Acute	Cutaneous
X-Linked sideroblastic anaemia	ALAS2 loss of function	*ALAS2*	XL	NA	NA	NA	NA
X-Linked protoporphyria	ALAS2 gain of function		XL		X		X
5-Aminolevulinic acid dehydratase porphyria	5-Aminolevulinic acid dehydratase	*ALAD*	AR	X		X	
Acute intermittent porphyria	Porphobilinogen deaminase[a]	*HMBS*	AD	X		X	
Congenital erythropoietic porphyria	Uroporphyrinogen III synthase	*UROS*	AR		X		X
Porphyria cutanea tarda	Uroporphyrinogen decarboxylase[b]	*UROD*	AD (but mostly acquired)	X			X
Hepatoerythropoietic porphyria	Uroporphyrinogen decarboxylase		AR	X	X		X
Hereditary coproporphyria	Coproporphyrinogen oxidase	*CPOX*	AD	X		X	X
Variegate porphyria	Protoporphyrinogen oxidase	*PPOX*	AD	X		X	X
Erythropoietic protoporphyria	Ferrochelatase	*FECH*	AR		X		X

NA not applicable, *ALAS2* 5-aminolevulinic acid synthase, erythroid specific form, *AR* autosomal recessive, *AD* autosomal dominant, *XL* X-linked
[a]The enzyme is also known as hydroxymethylbilane (HMB) synthase (HMBS), and formerly as uroporphyrinogen I synthase
[b]Inherited deficiency of uroporphyrinogen decarboxylase is partially responsible for familial (type 2) porphyria cutanea tarda

A diagnosis of porphyria should be considered in patients with unexplained neurovisceral symptoms or cutaneous photosensitivity. Laboratory tests can be both sensitive and specific, if properly chosen and interpreted [1, 8, 9]. However, some tests, particularly urinary porphyrin measurements, may be abnormal in other diseases. Measurements of deficient enzymes and especially DNA studies are important for diagnostic confirmation, genetic counselling and screening of family members.

The clinical presentation determines the type of initial laboratory testing (Table 33.2). Prompt diagnosis of acute porphyrias is important because treatment is more successful if started soon after the onset of symptoms. The three most common acute porphyrias are AIP, HCP and VP. Because these are often clinically latent with no excess accumulation of pathway intermediates even throughout life, the family history is often negative. Diagnosis of active cases is based on measurement of porphyrin precursors and porphyrins in urine, plasma and faeces. All major medical centres should have capabilities for rapid screening of spot urine samples for excess PBG, and total porphyrins should be measured later on the same sample [8]. Finding normal levels of these compounds excludes all acute porphyrias as the cause of current symptoms.

Total urine and plasma porphyrins are increased in all patients with blistering skin lesions due to porphyrias. A fluorescence emission scan of diluted plasma at neutral pH is useful for differentiating several cutaneous porphyrias [10]. Measurement of erythrocyte protoporphyrin is necessary for the diagnosis of protoporphyrias, which cause nonblistering photosensitivity [9].

Table 33.2 First-line laboratory tests for screening for porphyrias and second-line tests for further evaluation when initial testing is positive

Testing	Acute neurovisceral symptoms	Cutaneous photosensitivity
First-line	Urinary porphobilinogen and total porphyrins[a] (quantitative; random or 24-h urine)	Blistering skin lesions: Total plasma or urine porphyrins Nonblistering: Erythrocyte porphyrins[c]
Second-line	Urinary 5-aminolevulinic acid, porphobilinogen and total porphyrins[a] Total faecal porphyrins[a] Erythrocyte porphobilinogen deaminase Total plasma porphyrins[b] Mutation analysis	Erythrocyte porphyrins[c] Urinary 5-aminolevulinic acid, porphobilinogen and total porphyrins[a] Total faecal porphyrins[a] Mutation analysis

[a]Fractionation of urinary and faecal porphyrins is usually not helpful unless the total is increased
[b]The preferred method is direct fluorescence spectrophotometry
[c]Erythrocyte porphyrins are generally expressed as protoporphyrin; however, preferred methods also detect other porphyrins. This test lacks specificity, because erythrocyte protoporphyrin is increased in many erythrocytic disorders. Testing should include measurement of erythrocyte total protoporphyrin and, if elevated, metal-free and zinc protoporphyrin [9]. Porphyrins other than protoporphyrin are increased particularly in congenital erythropoietic porphyria

33.2.1 5-Aminolevulinic Acid Dehydratase Porphyria

Clinical Presentation

Only 8 reported cases of this porphyria are documented by molecular methods [11]. Symptoms resemble those of AIP, including abdominal pain and other neuropathic symptoms, usually beginning near puberty. In one severe case disease onset was in early childhood, with failure to thrive and anaemia. An adult-onset case was associated with polycythaemia vera. It is unexplained why all 8 cases have been males, whereas most symptomatic patients with other acute porphyrias are female.

Metabolic Derangement

5-Aminolevulinic acid dehydratase (ALAD) porphyria (ADP) is due to markedly deficient activity (usually <5% of normal) of ALAD, the second enzyme in the haem biosynthetic pathway (Fig. 33.1; Table 33.1). This porphyria is commonly classified as hepatic, and in one case hepatic exosomal ALAS1 mRNA (measured in exosomes in plasma and urine) was found to be increased, as in other acute porphyrias [11]. But increased erythrocyte zinc protoporphyrin and a recent report of improvement after suppression of erythropoiesis suggests the disease may have a significant erythropoietic component [12].

Genetics

ADP is an AR disorder with severe enzyme deficiency in affected individuals and half normal activity in both parents. Seven of the 8 described cases had compound heterozygous mutations, and one with late onset ADP had a myeloproliferative disorder (polycythemia vera) and a heterozygous inherited mutation.

Diagnostic Tests

Characteristic findings include elevations in urinary ALA and coproporphyrin III and erythrocyte zinc protoporphyrin, normal or only slightly increased urinary

PBG, and a marked decrease in erythrocyte ALAD activity. Excess coproporphyrin III probably results from metabolism of ALA via the haem biosynthetic pathway in a tissue other than the site of its initial accumulation. Increased erythrocyte zinc protoporphyrin is found in other homozygous types of porphyria. The diagnosis of this porphyria should always be confirmed by DNA studies [11].

Lead poisoning can be distinguished by showing reversal of inhibition of ALAD activity in erythrocytes by dithiothreitol added in vitro. Hereditary tyrosinaemia type 1 leads to accumulation of succinylacetone (2,3-dioxoheptanoic acid), a potent inhibitor of ALAD (▶ Chap. 17). Other heavy metals and styrene can also inhibit this enzyme.

Treatment and Prognosis

It is prudent to avoid drugs that are harmful in other acute porphyrias. In general, treatment of acute attacks is the same as in AIP. Haemin therapy is generally more effective than glucose loading [11]. Blood transfusions and hydroxyurea (hydroxycarbamide) to suppress erythropoiesis was beneficial in one case [12]. A Swedish child with severe early-onset disease showed no response to haemin therapy or liver transplantation [13].

33.2.2 Acute Intermittent Porphyria (AIP)

Clinical Presentation

This AD condition is the most common of the acute porphyrias worldwide. Due to incomplete penetrance, most heterozygotes remain clinically asymptomatic for all or most of their lives. Triggering factors include certain drugs, steroid hormones and nutrition. Symptoms are very rare in children, and more common in adult women than men. Acute attacks of neurovisceral symptoms and signs are the most common presentation, although subacute and chronic manifestations also occur [14]. Attacks usually last for several days or longer, often require hospitalisation and are usually followed by complete recovery. Severe attacks are sometimes fatal, especially if the diagnosis is delayed. Abdominal pain, the most common symptom, is usually steady and poorly localised, but is sometimes crampy. Tachycardia, hypertension, restlessness, fine tremors and excess sweating reflect sympathetic overactivity. Other common manifestations may include nausea, vomiting, constipation, pain in the limbs, head, neck or chest, muscle weakness and sensory loss. Dysuria and bladder dysfunction as well as ileus, with abdominal distension and decreased bowel sounds, may accompany an attack. However, increased bowel sounds and diarrhoea may occur. Tenderness, fever and leukocytosis are mild or absent. A peripheral neuropathy that is primarily motor can develop, and is manifested by muscle weakness that most often begins proximally in the upper extremities. It may progress to involve all extremities and the respiratory muscles, and even lead to bulbar paralysis and mimic Guillain Barré syndrome. Tendon reflexes are usually decreased or absent with advanced neuropathy. Muscle weakness is sometimes focal and asymmetrical. Cranial and sensory nerves can be affected. Advanced motor neuropathy and death are now rare. Seizures may occur as a result of hyponatraemia, or as a neurological manifestation of porphyria. Hyponatraemia can be due to electrolyte depletion from vomiting or diarrhoea, poor intake, renal sodium loss, or inappropriate antidiuretic hormone secretion. Psychiatric manifestations such as agitation, hallucinations, delerium and depression are most common during attacks. In some patients, MRI findings have resembled the reversible posterior leukoencephalopathy syndrome. Other severe complications may include acute kidney failure and rhabdomyolysis [15, 16]. A rare form of childhood-onset leukoencephalopathy with slowly progressive spastic paraparesis, cerebellar ataxia, peripheral neuropathy, and optic atrophy has been related to AIP [17].

Persistent hypertension and impaired renal function may develop over the long term. A common variant of peptide transporter 2 (PEPT2), a transporter for ALA in the kidney, can predispose to development of renal disease in AIP [18]. Chronic abnormalities in liver function tests, particularly transaminases, are common, although few patients develop significant hepatic impairment. The risk of liver cancer (hepatocellular carcinoma or cholangiocarcinoma) is increased in this and other acute porphyrias, and also in porphyria cutanea tarda [19].

Metabolic Derangement

AIP is due to reduced activity of PBGD (◘ Fig. 33.1 and ◘ Table 33.1). Most heterozygotes remain asymptomatic with normal levels of urinary porphyrin precursors. Clinical expression of the disease is accompanied by accumulation of haem pathway intermediates initially in liver, followed by their excretion primarily in urine.

The deficient enzyme can become limiting for haem synthesis when certain drugs, hormones, or nutritional factors increase the demand for hepatic haem by increasing the synthesis of CYPs and ALAS1 in the liver, causing ALA and PBG to accumulate. Excess porphyrins originate non enzymatically from PBG, and perhaps enzymatically from ALA transported to tissues other than the liver [1].

Genetics

Inheritance is AD; more than 300 different mutations of the PBGD gene (*HMBS*) have been identified in unrelated families [1]. Two forms of this enzyme, an erythroid-specific and a housekeeping form are derived from the same gene. A deficiency of the housekeeping form in the liver is essential for causing AIP. Mutations located in or near the first of the 15 exons in this gene can cause AIP by impairing the synthesis of the housekeeping form but not of the erythroid-specific form of PBGD. Homozygous cases of AIP are extremely rare but should be suspected in early childhood cases [20]. Bi-allelic *HMBS* variants may also cause early onset leukoencephalopathy and slower disease progression at an older age [17].

Diagnostic Tests

The finding of a substantial increase in urinary PBG is a sensitive and specific indication of either AIP, HCP or VP (◘ Table 33.1) [8]. PBG usually remains increased between attacks of AIP unless there have been no symptoms for a prolonged period. Faecal porphyrins are generally normal or minimally increased in AIP, and markedly increased in active cases of HCP and VP. VP is also characterised by increased total plasma porphyrins [10]. Urinary coproporphyrin is generally more increased in HCP and VP than in AIP, but this does not reliably differentiate these 3 acute porphyrias. Urinary uroporphyrin can be increased in all these disorders, especially when PBG is increased. Other biomarkers to predict greater disease activity are sought. Decreased erythrocyte PBGD helps to confirm a diagnosis of AIP and diagnose clinically latent disease. However, DNA studies are preferred [8, 21].

Treatment and Prognosis

Haemin (haem arginate or haematin, 3–4 mg/kg body weight, infused IV once daily for 4 days or longer) represses hepatic ALAS1 and markedly reduces levels of ALA and PBG [8]. Carbohydrate loading with IV 10% glucose (at least 300 g daily), also represses ALAS1. It is much less effective than haemin, but may be started initially until haemin is obtained. Haem arginate is the preferred form of haemin for IV administration. Haematin (haem hydroxide) commonly causes phlebitis at the site of infusion and has a transient anticoagulant effect, but can be reconstituted with human albumin, which stabilises the haem as haem albumin and confers some of the advantages of haem arginate [8]. Repeated haemin infusions can lead to iron overload [22]. Small volume phlebotomies should be instituted if the serum ferritin becomes elevated, as measured at least a week after the last dose of haemin. Overdoses of haemin can cause acute kidney and liver failure [23].

Most acute attacks require hospitalisation for administration of IV haemin or glucose and observation for neurological complications, respiratory impairment and electrolyte imbalances. Narcotic analgesics are commonly required for abdominal, back or extremity pain, and a phenothiazine or ondansetron as an antiemetic; metoclopramide has been listed as unsafe. Benzodiazepines in low doses are safe if a minor tranquilliser is required. Bladder distension may require catheterization. Abdominal pain may disappear within hours, and paresis begins to improve within days. After a prolonged attack with severe motor neuropathy, muscle weakness resolves more gradually and there may be some residual weakness.

Treatment of seizures is problematic because many anticonvulsants can exacerbate acute porphyrias. Gabapentin, levetiracetam and vigabatrin can be given safely. β-Adrenergic blocking agents may control tachycardia and hypertension in acute attacks of porphyria. Advanced renal disease may be accompanied by increased plasma porphyrins and, rarely, blistering photosensitivity. Renal failure may be treated by haemodialysis or renal transplantation [24].

Precipitating factors such as harmful drugs, dietary indiscretions, smoking, endogenous or exogenous hormones (particularly progesterone and progestins) and intercurrent infections must be addressed. With prompt treatment and precautions to prevent further attacks, the outlook for patients with AIP is usually excellent [8, 25]. However, some patients continue to have attacks and may develop chronic pain and become narcotic dependent. Close monitoring is important because there is often coexisting depression and an increased risk of suicide.

Additional prevention strategies are important for patients who experience repeated attacks even when avoiding harmful drugs and dietary habits. Haemin administered prophylactically (e.g. once weekly) is sometimes effective [26]. GnRH analogues can be considered for preventing frequent attacks due to progesterone elevation during the luteal phase of the menstrual cycle [27, 28].

Givosiran, an interfering RNA therapeutic, was recently approved for prevention of frequent acute attacks of AIP and other acute hepatic porphyrias. This drug lowers hepatic ALAS1 mRNA (measured in exosomes in urine or plasma) and urine ALA and PBG for a month or longer after subcutaneous dosing, and in double blind, placebo controlled studies markedly reduced annualized attack rates and requirements for haemin therapy [29].

Allogeneic liver transplantation for patients with frequent attacks refractory to haemin and other medical therapies is effective if accomplished before there is advanced motor paralysis [24, 30, 31]. Some patients with renal failure have undergone combined liver and kidney transplantation. Domino transplantation, whereby the explanted AIP liver is used as a donor liver, leads to development of AIP in the recipient [24].

33.2.3 Congenital Erythropoietic Porphyria (CEP) (Gunther Disease)

Clinical Presentation

This is usually a severe disease presenting soon after birth, or even in utero as non-immune hydrops [32]. Rarely, milder symptoms first appear during adult life. Cutaneous features resemble those in PCT, but are much more severe in most cases. Lesions include bullae and vesicles on sun-exposed skin, hypo- or hyperpigmented areas, hypertrichosis, and scarring. Digits and facial features may be lost due to infection and scarring. The teeth are reddish brown (erythrodontia) because of porphyrin deposition and fluoresce when exposed to long-wave ultraviolet light. Porphyrins are also deposited in bone. Haemolysis is almost invariably present in severe cases, resulting from ineffective erythropoiesis and the markedly increased erythrocyte porphyrin levels, and is often accompanied by splenomegaly. Severe cases may be transfusion dependent. Life expectancy is often shortened by infections or haematological complications. There are no neurological manifestations [33].

Metabolic Derangement

This rare disorder is due to a severe deficiency of uroporphyrinogen III synthase (also known as cosynthase) (UROS) (Fig. 33.1 and Table 33.1). HMB (the substrate of the deficient enzyme) accumulates and is converted nonenzymatically to uroporphyrinogen I, a nonphysiological intermediate, which is partially metabolized to coproporphyrinogen I, and the corresponding porphyrins (uroporphyrin I and coproporphyrin I) accumulate in bone marrow, plasma, urine and faeces. Porphyrin accumulation in erythroid cells results in intramedullary and intravascular haemolysis. To compensate, erythropoiesis and haem synthesis are actually increased in spite of the severe deficiency of UROS. Adult-onset cases are likely to be associated with a myeloproliferative disorder and clonal expansion of erythroblasts with UROS deficiency [34].

Genetics

CEP is an AR disorder. Patients have either homozygous or compound heterozygous *UROS* mutations. At least 39 different *UROS* mutations and an X-linked *GATA-1* mutation have been identified in CEP [35, 36]. Parents and other heterozygotes are asymptomatic and display half-normal UROS activity. A coexisting *ALAS2* gain of function mutation can lead to more severe disease, indicating that *ALAS2* is a modifying gene in CEP [37].

Diagnostic Tests

Erythrocyte, plasma and urine porphyrins are markedly increased. Uroporphyrin I, coproporphyrin I and even zinc protoporphyrin are increased in erythrocytes. Porphyrins in urine are primarily uroporphyrin I and coproporphyrin I, and in faeces mostly coproporphyrin I. Porphyrin precursors are not increased. Erythrocyte UROS activity is markedly deficient, but this assay is not widely available. The diagnosis should be confirmed by mutation analysis. The disease can be diagnosed in utero by porphyrin measurements and DNA studies.

Treatment and Prognosis

A multidisciplinary approach is emphasized [38]. Intrauterine transfusion is possible, and severe photosensitivity can be prevented in neonates by avoiding phototherapy for hyperbilirubinaemia. Protection of the skin from sunlight must be emphasized to patients, who may experience little immediate pain from sunlight and not understand that cumulative skin damage is occurring over time. Minor trauma should be avoided and secondary bacterial infections treated promptly to prevent scarring and mutilation. Haemolysis may improve after splenectomy. Oral charcoal may be helpful by increasing faecal excretion of porphyrins. High-level blood transfusions and hydroxyurea may be effective by suppressing erythropoiesis and porphyrin synthesis [39]. Improvement was reported in one patient with iron deficiency (resulting from spontaneous gastrointestinal bleeding and then maintained by iron chelation) suggesting that iron deficiency can reduce ALAS2 activity [40]. Studies of differences in disease severity among 3 affected siblings suggested modulation of their phenotype by iron status, and phlebotomies in another patient, reduced hemolysis, plasma and urine porphyrins and photosensitivity [41]. Further studies of iron reduction as a strategy for treatment of CEP are needed.

Bone marrow or hematopoietic stem cell transplantation can be curative, but it is associated with morbidity and mortality, and is currently recommended for transfusion-

dependent patients, preferentially during infancy [36]. Hepatic dysfunction has developed after transplantation in some patients and is poorly understood [42].

Future therapies for CEP may include pharmacological chaperones, proteasome inhibition and gene therapy. Ciclopirox is a repurposed drug under study as a chaperone to stabilize the mutated enzyme and enhance its activity [43]. Proteasome inhibitors may rescue UROS enzyme that is subject to premature degradation due to missense mutations [44]. Gene therapy is under study in human cell lines [45] and immune-deficient mice transplanted with human erythroid bone marrow cells in which UROS was made deficient by RNA interference [46].

33.2.4 Porphyria Cutanea Tarda (PCT)

Clinical Presentation

This is the most common and readily treated form of porphyria and causes chronic, blistering skin lesions, especially on the dorsal hands, forearms, face and (in women) the dorsal feet. Neurological effects are not observed. Sun-exposed skin becomes friable, and minor trauma may precede the formation of bullae or cause denudation of the skin. Small white plaques (›milia‹) may precede or follow vesicle formation. Hypertrichosis and hyperpigmentation are also noted. Thickening, scarring and calcification of affected skin may be striking, and is referred to as pseudoscleroderma. Skin lesions are indistinguishable clinically from those in all other blistering cutaneous porphyrias, but distinct from the painful, nonblistering photosensitivity in protoporphyrias (see later discussion).

A normal or increased amount of hepatic iron is required to develop PCT [47]. Acquired and inherited susceptibility factors include moderate or heavy alcohol intake, hepatitis C and less commonly HIV infection, oestrogen use, smoking, *HFE* (hemochromatosis gene) mutations, uroporphyrinogen decarboxylase (*UROD*) mutations and low levels of ascorbic acid and carotenoids [48]. A large outbreak of PCT occurred in Turkey after ingestion of seed wheat treated with hexachlorobenzene as a fungicide, but such toxic exposures are seldom evident in isolated cases of the disease [47].

Metabolic Derangement

This porphyria is caused by a profound deficiency of hepatic UROD (◘ Fig. 33.1 and ◘ Table 33.1). As a result, highly carboxylated porphyrinogens (with 5–8 carboxyl groups) accumulate in the liver and are autoxidized to the corresponding porphyrins. A specific inhibitor of hepatic UROD has been characterised as a uroporphomethene in a murine model of PCT [49].

Genetics

PCT is primarily due to an acquired inhibition of UROD specifically in the liver, with a heterozygous *UROD* mutation contributing in only ~20% of patients. PCT is classified as types 1–3 based on the presence or absence of *UROD* mutations and a family history of the disease [47]. These types have few clinically important differences, and hepatic UROD inhibition occurs in all overt cases. More than 100 mutations have been identified in type 2 disease [50]. PCT is an iron-related disease, so *HFE* mutations can increase disease susceptibility.

Diagnostic Tests

The complex patterns of excess porphyrins in plasma, urine and faeces are important for diagnosis of PCT, because the blistering skin lesions and skin histopathology, while characteristic, are not specific. Therefore, the diagnosis should be established by laboratory testing before instituting therapy. Plasma and urine porphyrins are increased in all porphyrias that cause blistering skin lesions. PCT is confirmed by increased total urinary or plasma porphyrins with a predominance of highly carboxylated porphyrins, especially uroporphyrin and heptacarboxyl porphyrin. However, urine porphyrin patterns in other porphyrias, such as variegate porphyria, are sometimes similar to PCT [51]. In contrast to erythropoietic porphyrias, erythrocyte porphyrins are normal or only modestly elevated. Urine PBG is normal, and ALA may be slightly elevated.

The fluorescence spectrum of plasma porphyrins can rapidly distinguish VP from PCT (◘ Table 33.2) [10]. Cases of so-called pseudoporphyria have skin lesions resembling PCT but no significant increases in porphyrins; sometimes a photosensitising drug is implicated.

Treatment and Prognosis

Iron depletion by phlebotomy is standard treatment at most centres, although low-dose hydroxychloroquine (or chloroquine) is also effective [52]. Patients are also advised to discontinue alcohol, oestrogens, iron supplements and other contributing factors. Repeated phlebotomy stimulates erythropoiesis and utilisation of storage iron for haemoglobin formation, and gradually reduces the serum ferritin to a target range of 15–20 ng/ml. At this point phlebotomies are stopped. Hepatic UROD activity then gradually increases to its genetically determined level, and porphyrin levels gradually normalize. Removal of only 5–6 units (450 ml each) of blood at 2-week intervals may be needed unless there is marked iron overload.

After remission, ferritin may increase without recurrence, in most cases, and maintenance phlebotomies are seldom needed. Postmenopausal women can usually resume oestrogen replacement if needed. The disease recurs especially in patients who resume alcohol intake but is expected to respond to another course of phlebotomies. The serum ferritin should be maintained below about 100 ng/ml in patients who also have haemochromatosis, and perhaps in other patients who experience multiple relapses.

A low dose of hydroxychloroquine (100 mg twice weekly) or chloroquine (125 mg twice weekly) gradually removes excess porphyrins from the liver. This is a suitable alternative when phlebotomy is contraindicated or difficult and is the preferred treatment in some centres [52]. Standard doses of these 4-aminoquinolines should not be used because they exacerbate photosensitivity and cause hepatocellular damage. Risk of retinal damage is very low, and may be lower with hydroxychloroquine than chloroquine. The mechanism by which these drugs remove porphyrins from the liver in PCT is not well understood, and they are not effective in other porphyrias.

33.2.5 Hepatoerythropoietic Porphyria

Clinical Presentation

This rare disease is clinically similar to CEP and usually presents with blistering skin lesions shortly after birth. Mild cases may present later in life and more closely resemble PCT. Concurrent conditions, such as viral hepatitis, may accentuate porphyrin accumulation.

Metabolic Derangement

Hepatoerythropoietic porphyria (HEP) is the homozygous form of familial (type 2) PCT and is due to a substantial deficiency of UROD [47, 53]. Intermediate deficiencies of the enzyme are found in the parents (Fig. 33.1 and Table 33.1). The disease has features of both hepatic and erythropoietic porphyrias. Although usually a more severe disease than PCT, mild and atypical forms of the disease also occur [53].

Diagnostic Tests

The excess porphyrins found in urine, plasma and faeces in this condition are similar to those in PCT. In addition, erythrocyte zinc protoporphyrin is substantially increased, as in other AR porphyrias. Erythrocyte porphyrins in CEP are usually mostly uroporphyrin I and coproporphyrin I, but in some cases there is a predominance of zinc protoporphyrin. It is important to document the diagnosis by molecular methods.

Genetics

HEP results from homozygous or compound heterozygous *UROD* mutations. *UROD* mutations found in this disease markedly decrease enzyme activity, but some activity remains, so haem formation can occur [47].

Treatment and Prognosis

Therapeutic options focus on protection from sunlight, as in CEP.

33.2.6 Hereditary Coproporphyria and Variegate Porphyria

Clinical Presentation

These acute porphyrias can present with acute attacks that are identical to those in AIP. However, they are also cutaneous porphyrias, because especially in variegate porphyria (VP) blistering skin lesions can occur that are indistinguishable from PCT [54]. Symptoms in heterozygotes almost never occur before puberty. Factors that exacerbate AIP are important in both of these porphyrias. Homozygous cases of hereditary coproporphyria (HCP) and VP have been described, and in such cases clinical manifestations may begin in childhood and resemble homozygous AIP. Harderoporphyria is a variant phenotype of homozygous HCP in which hematological features are prominent [55].

Metabolic Derangement

HCP and VP result from heterozygous mutations of coproporphyrinogen oxidase (CPOX) and of protoporphyrinogen oxidase (PPOX), respectively, (Fig. 33.1 and Table 33.1). Heterozygotes have approximately 50% deficiencies of these enzymes, and most remain latent, without symptoms or porphyrin elevations. In active HCP, coproporphyrin III (derived from autooxidation of coproporphyrinogen III) is increased in urine and faeces, and urinary ALA, PBG and uroporphyrin are increased particularly with acute attacks. Similar abnormalities are seen in VP, but in addition protoporphyrin (derived from autooxidation of protoporphyrinogen) is increased in faeces and plasma porphyrins are increased. A close association of CPOX and PPOX in the mitochondrial membrane may explain the accumulation of coproporphyrinogen (in addition to protoporphyrinogen) in VP. These porphyrinogens can inhibit PBGD, which along with induction of hepatic ALAS1, may account for the increase in ALA and PBG during acute attacks of HCP and VP [56].

Genetics

In these AD conditions, affected individuals and latent carriers have approximately 50% activity of the affected

enzyme due to heterozygous *CPOX* or *PPOX* mutations. VP is particularly common in South Africa, where a founder *PPOX* mutation (R59W) accounts for many descendants of Dutch ancestry with VP [54]. Harderoporphyria, a variant of homozygous HCP with a distinct phenotype that includes neonatal hemolytic anemia and jaundice, results from certain *CPOX* mutations that prematurely release harderoporphyrinogen (the tricarboxyl intermediate of the CPOX reaction) [55].

Diagnostic Tests

Urinary ALA and PBG are increased during acute attacks of these porphyrias, although the increases may be smaller and more transient than in AIP. Urinary coproporphyrin increases may be more prominent and prolonged than in AIP, but this alone is a highly nonspecific finding.

A marked, isolated increase in faecal coproporphyrin III is distinctive for HCP, whereas faecal coproporphyrin III and protoporphyrin are about equally increased in VP. Plasma porphyrins are commonly increased in VP, and the fluorescence spectrum of plasma porphyrins is characteristic and very useful for rapidly distinguishing this disease from all other porphyrias [10].

Identification of the familial mutation confirms the diagnosis and enables screening of family members. CPOX and PPOX are mitochondrial enzymes, so are not measurable in erythrocytes; assays using cultured fibroblasts or lymphocytes are available only in a few research laboratories.

Treatment and Prognosis

Attacks of neurological symptoms are treated and prevented as in AIP. Cutaneous symptoms are more difficult to treat, and therapies that are effective for PCT (phlebotomy and low-dose hydroxychloroquine) are not effective in these conditions. Protection from sunlight is important. As in other acute porphyrias, screening of active cases for hepatocellular carcinoma is recommended [25].

33.2.7 Erythropoietic Protoporphyria and X-Linked Protoporphyria

Clinical Presentation

Erythropoietic protoporphyria (EPP) is the third most common porphyria, and the most common in children. X-linked protoporphyria (XLP) is less common and has the same phenotype. Cutaneous symptoms begin in early childhood and are much more prominent than observed skin changes. Pain can affect sun-exposed areas within minutes of exposure, and with prolonged exposure is followed by erythema and oedema that may resemble angioneurotic oedema. Chronic skin changes may include lichenification, leathery pseudovesicles, labial grooving and nail changes, but are not apparent in most patients because they have learned to avoid sunlight. In contrast to other cutaneous porphyrias, blistering, milia, friability, scarring and hypertrichosis are seldom present. There is no fluorescence of the teeth and, in the absence of hepatic failure (see below), no neuropathic manifestations. Mild anaemia with hypochromia and microcytosis due to iron deficiency is common and is poorly understood [57, 58].

The severity of symptoms is generally stable over time. Patients adjust their lifestyles and occupations in order to avoid painful sunlight exposure, which has a substantial effect on quality of life [57, 59]. The disease is especially difficult in children with unexplained symptoms before diagnosis, which is often much delayed. Drugs that exacerbate hepatic porphyrias are not known to worsen this disease. Gallstones containing protoporphyrin may develop. Some patients develop liver disease, referred to as protoporphyric hepatopathy, which can progress rapidly and require liver transplantation. Operating room lights have produced severe skin and peritoneal burns in some patients with protoporphyric hepatopathy. A motor neuropathy may further complicate the course of liver decompensation in this disease and is unexplained [60].

Metabolic Derangement

EPP is due to an inherited deficiency of ferrochelatase (FECH), the eighth and last enzyme in the haem biosynthetic pathway, (◘ Fig. 33.1 and ◘ Table 33.1). FECH deficiency is substantial (10–30% of normal) in EPP, leading to increases in protoporphyrin in the bone marrow. Bone marrow reticulocytes are the primary source of the excess protoporphyrin. Circulating erythrocytes, which have accumulated but are no longer synthesising protoporphyrin, probably contribute smaller amounts. Excess protoporphyrin is transported in plasma and excreted in bile and faeces. Because zinc protoporphyrin is also a product of FECH activity, the excess protoporphyrin found in erythrocytes in EPP is mostly metal-free. Young circulating erythrocytes appear fluorescent when a blood smear from a patient with this condition is examined by fluorescence microscopy.

XLP, which has the same phenotype, results from gain-of-function mutations affecting ALAS2 [61–63]. Because ferrochelatase is not deficient, the proportion of zinc protoporphyrin in erythrocytes is greater (15 ~ 50% of the total) than in EPP (0 ~ 15%). On average porphyrin levels are higher and liver disease may be more common in XLP. Large amounts of

protoporphyrin originating from the bone marrow is excreted in bile in EPP and XLP, may undergo enterohepatic circulation, and can cause cholestasis and liver failure.

Genetics

At least 125 *FECH* mutations have been identified in EPP [61–63], and most mutant alleles express little or no ferrochelatase activity (disabling or null mutations). Most patients with manifest disease have inherited a severe *FECH* mutation from one parent and a weak, or hypomorphic, *FECH* allele from the other [64]. This hypomorphic allele is found in ~10% of normal Caucasians and has no consequence unless trans to a severe *FECH* mutation. This pattern of inheritance, which is found in the great majority of cases, is best described as autosomal recessive, and explains why FECH activity is only 10–30% of normal in patients with manifest EPP. Rarely, families have two disabling *FECH* mutations, where at least one expresses some FECH activity to allow some heme synthesis in those who are compound heterozygotes [61]. For unknown reasons, seasonal palmar keratoderma occurs in some of these families [65]. Late-onset protoporphyria may develop in the presence of myeloproliferative disorders, with clonal expansion of erythropoietic cells with a *FECH* mutation [66].

In families with XLP, the disease is likely to be more common and severe in males and more variable in severity in females, presumably reflecting the degree of X chromosome inactivation. In one family, protoporphyria resulted from mutation of ClpX, which is involved in inactivation of ALAS2. This mutation prolongs ALAS2 activity, thereby leading to accumulation of protoporphyrin [67].

Diagnostic Tests

The recommended screening test for these protoporphyrias is a determination of total erythrocyte protoporphyrin. Finding an elevation is not specific, so fractionation is required to discern whether the protoporphyrin is predominantly metal-free, which establishes the diagnosis [9]. Metal-free protoporphyrin comprises 85 ~ 100% of the total in erythropoietic protoporphyria, 50–85% in XLP, and less than ~50% in other conditions (e.g., iron deficiency, anaemia of chronic disease and lead poisoning).

The plasma porphyrin concentration is usually but not always increased. Moreover, the excess protoporphyrin found in plasma in this condition is particularly sensitive to light exposure, which may increase the chance of a falsely normal measurement [68]. Total faecal porphyrins may be normal or increased in protoporphyria, with a predominance of protoporphyrin. Urinary porphyrins and porphyrin precursors are normal. Urinary coproporphyrin is elevated with protoporphyric hepatopathy, as with other liver diseases.

DNA testing establishes the pathogenic *FECH* mutation and the presence or absence of the hypomorphic *FECH* allele, which confirms the diagnosis of EPP and enables genetic counseling. Likewise, a gain of function *ALAS2* mutation confirms a diagnosis of XLP [61–63].

Treatment and Prognosis

Patients manage photosensitivity by avoidance of sunlight, which impairs quality of life. Oral β-carotene or cysteine improve tolerance to sunlight in some patients, perhaps by quenching singlet oxygen or free radicals. Narrow-band ultraviolet light therapy can increase skin pigmentation and sunlight tolerance. Afamelanotide, a synthetic agonistic analogue of α-melanocyte-stimulating hormone that increases melanin formation and darkens the skin and also has anti-inflammatory and anti-oxidative effects, significantly increases sunlight tolerance and improves quality of life in patients with protoporphyria [69].

Vitamin D and calcium supplements and vaccinations for hepatitis A and B are recommended. There is agreement that iron replacement is beneficial in XLP [70]. Although there are indications that iron deficiency may benefit EPP [71], correction of iron deficiency has been beneficial in some patients [72]. Patients with late-onset EPP may improve with treatment of an underlying myelodysplastic disorder [73].

Treatment of liver complications is difficult. Transfusions and intravenous haemin may suppress erythroid protoporphyrin production. Plasma exchange, cholestyramine (which may reduce the enterohepatic circulation and plasma levels of protoporphyrin), ursodeoxycholic acid and vitamin E are also administered. Phlebotomy was reportedly beneficial in one patient with hepatopathy but other interventions or spontaneous improvement may have contributed. Liver transplantation is sometimes required [30]. Sequential bone marrow transplantation can prevent recurrence of hepatopathy in the transplanted liver [74].

Gene therapy is being studied in this and other erythropoietic porphyrias [75]. Because ALAS2 may be increased and contribute to protoporphyrin accumulation in EPP, ALAS2 mRNA is a potential therapeutic target, using RNA interference [76]. Iron availability influences correct and aberrant FECH mRNA splicing and thereby FECH enzyme activity [77]. Isoniazid, which can decrease ALAS2 activity by depleting pyridoxine, appeared beneficial in a murine model but not in humans with EPP; higher doses of this drug were not investigated given known risks of liver toxicity [78].

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Disorders in the Transport of Copper, Iron, Magnesium, Manganese, Selenium and Zinc

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Contents

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Introduction

Metals, as well as selenium, are indispensable elements in cell biology. They function as cofactors for many specific proteins and are involved in all major metabolic pathways. The number of recognised IEM involving the absorption, transport, or metabolism of these elements is rapidly growing. Clinical presentations can involve all organs and systems including the liver and the central nervous system. Deficiency of metals results mostly in loss of function of metal-dependent proteins while excess can result in unregulated oxidation of proteins, lipids and other cellular components. Treatments rely on daily supplementation of the deficient metal at pharmacological doses and on chelating drugs where there is excess.

34.1 Copper

Copper metabolism

Each day approximately 2 mg copper is absorbed from the intestine, which is subsequently removed from the portal circulation by the hepatocytes. Excretion of copper by the liver into the bile is the only mechanism of copper elimination and in physiological conditions the amount of copper excreted into the bile is equivalent to that absorbed from the intestine.

The uptake of copper, both by enterocytes and hepatocytes, is done through the copper transporter CTR1, a protein residing in the plasma membrane. Excretion in these two cell types is done by two closely related ATPases, ATP7A and ATP7B (Fig. 34.1). As copper is essential for cellular metabolism, but also potentially toxic, it is bound within the cell to small proteins, copper chaperones, which direct this metal to specific proteins, such as superoxide dismutase. In the circulation over 90% of plasma copper is bound to serum ceruloplasmin.

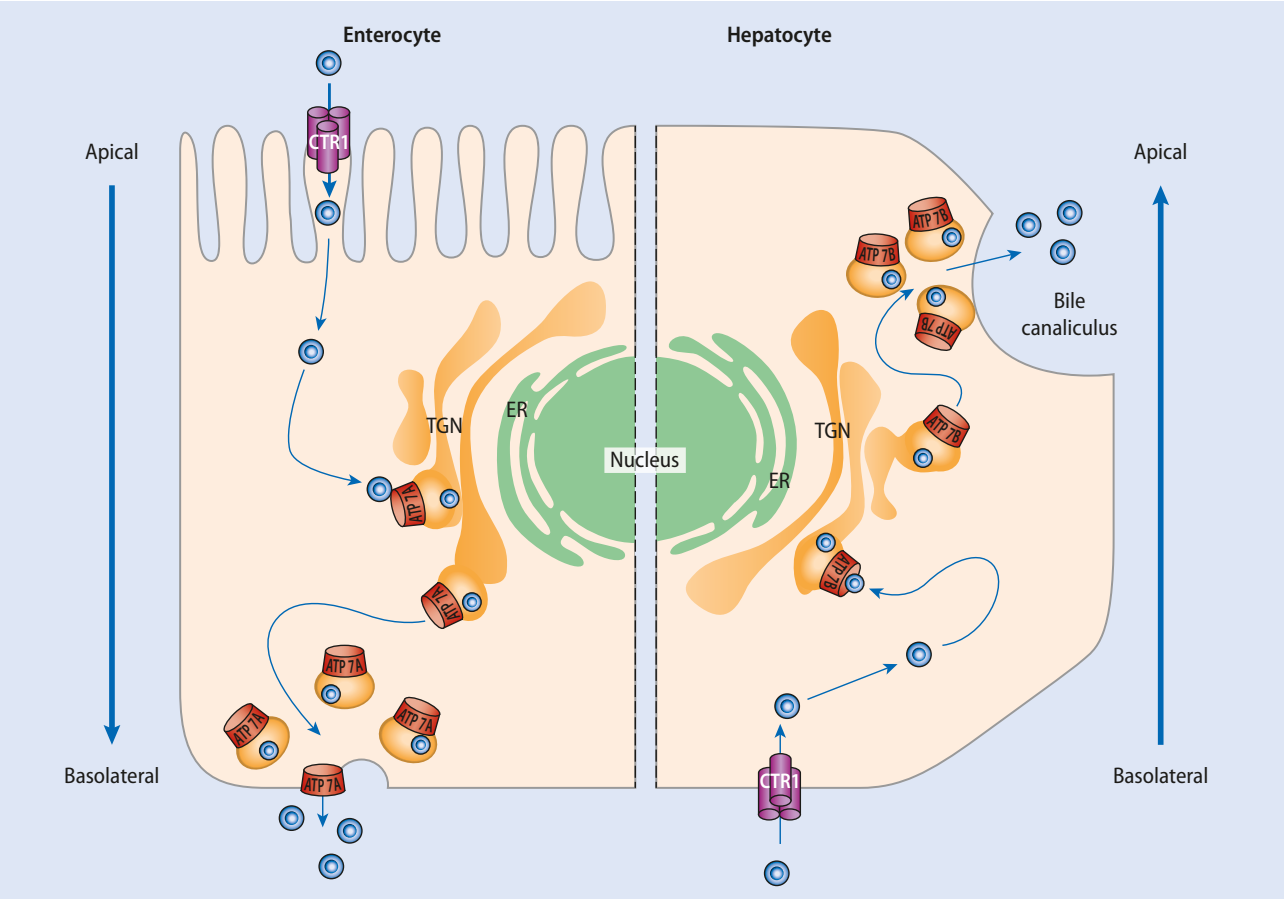

Fig. 34.1 Cellular copper metabolism. In both the enterocyte (left panel) and the hepatocyte (right panel) copper enters the cell through CTR1, the main copper transporter, and is then transported to ATP7A (in the enterocyte) or ATP7B (in the hepatocyte) . These proteins are synthesized in the endoplasmatic reticulum (ER), but subsequently reside in the Trans Golgi Network (TGN). Upon a rising intracellular copper concentration they relocalise to the cell periphery (ATP7A and ATP7B), and also the plasma membrane (ATP7A), which process is essential for the excretion of copper. Through these proteins copper is excreted by the enterocyte to the blood (ATP7A), while in the hepatocyte copper is excreted to the bile (ATP7B). (Figure modified from [1])

Introduction

Copper balance is disturbed in two inborn errors: Wilson disease and Menkes disease. Wilson disease, or hepatolenticular degeneration is due to mutations in *ATP7B*, and is characterized by a gradual accumulation of copper in the liver and, secondarily, in other organs, such as brain, kidney and cornea. Clinical symptoms result from the copper accumulation in the liver and/or the brain. Early treatment with copper chelators or zinc is generally effective.

Menkes disease is an X-linked disorder due to mutations in *ATP7A*. The disorder is characterized by a general copper deficiency. Patients manifest progressive neurodegeneration, which is usually fatal in infancy or childhood. Early therapy with copper histidine can be effective in selected patients. Occipital horn syndrome and a rare phenotype, X-linked distal hereditary motor neuropathy, are also due to *ATP7A* mutations and can be observed in older children or adults.

Indian Childhood Cirrhosis (ICC), also known as Idiopathic Copper Toxicosis (ICT), is a rare copper storage disease seen in infants susceptible to high oral copper intake. Several other rare disorders are recognized with a low serum copper and ceruloplasmin: MEDNIK syndrome and AT-1 deficiency, both primarily characterized by severe mental retardation. Also, some Congenital Disorders of Glycosylation (CDG) can present with a low serum ceruloplasmin and copper as well as mild liver dysfunction. In some patients this could be attributed to PGM-1-CDG mutations (▶ Chap. 43), but in most no cause has been found (CDG-X).

34.1.1 Wilson Disease

Clinical Presentation

The overwhelming majority of cases display hepatic and/or neurological symptoms, and the disease should be suspected in any patient with liver disease without obvious cause or with a movement disorder [2–4]. In addition, the diagnosis is often made when siblings of a patient are screened. Occasionally, Wilson disease presents with isolated raised transaminases, Kayser-Fleischer rings, haemolysis or psychiatric symptoms.

Patients with hepatic symptoms generally present between 8 and 20 years of age but may be as young as 3 or over 50. The presentation can be acute and severe with hepatitis, jaundice and impending liver failure. Transaminases, although raised, generally are much lower than in autoimmune or viral hepatitis. While liver disease is rapidly progressive in some patients, in others jaundice can persist for months without progression to liver failure, or even subside. These patients ultimately develop liver cirrhosis and may present several years later with neurological disease.

Neurological symptoms usually develop in the second or third decade, although patients may be as young as 8 years of age. Symptoms include dysarthria and diminished control of movements, accompanied in a later stage by tremors, rigidity and drooling in combination with swallowing problems. A frequent early sign is a deterioration in the quality of handwriting. In some patients psychiatric symptoms predominate, ranging from behavioural disturbances, often characterized by impulsivity and irritability, to frank psychosis.

Most patients have aminoaciduria in combination with excessive loss of bicarbonate, calcium and phosphate, and may develop renal stones or osteoporosis. Coombs negative haemolytic anaemia can be present.

The greenish brown Kayser-Fleischer ring, located in the membrane of Descemet at the limbus of the cornea, can be seen with the naked eye in the majority of patients with full-blown neurological disease. Careful slit lamp examination will reveal this ring in almost all these patients. In contrast, in a substantial proportion of the patients presenting with liver disease and in most presymptomatic patients, the Kayser-Fleischer ring is absent. Conversely, a Kayser-Fleischer ring is occasionally found in patients with cholestatic liver disease. Consequently, its absence does not rule out Wilson disease, nor does its presence confirm the disorder.

Metabolic Derangement

Wilson disease is caused by reduced excretion of copper into bile, resulting in a gradual accumulation of copper in the liver and, secondarily, in the brain, kidneys and eyes. A number of patients exhibit severe liver disease, while others redistribute copper to the brain, especially the basal ganglia, causing neurological disease. Copper excess exerts its hepatic toxicity by generating free radicals that oxidize the mitochondrial membranes, resulting in their swelling and loss of oxidative phosphorylation capacity. The characteristic Kayser-Fleischer ring is a deposit of copper and sulphur. The renal dysfunction is a consequence of copper accumulation in the renal tubules. The increased urinary copper excretion, characteristic for Wilson disease, is due to the loss of unbound, dialysable copper through the kidneys. This unbound copper can cause haemolysis in some patients.

The primary defect in Wilson disease is a lesion of a protein localized in the trans-Golgi network, ATP7B, an adenosine triphosphatase (ATPase), which is responsible for the excretion of copper [1] and for the incorporation of copper into ceruloplasmin. Because of the latter and the reduced half-life of ceruloplasmin without copper, the concentration of serum ceruloplasmin is sub-

normal in Wilson disease. Some rare patients, although unable to excrete copper into bile, can incorporate copper into ceruloplasmin and have a normal serum ceruloplasmin.

Genetics

Wilson disease in an autosomal recessive condition caused by mutations in *ATP7B* [1–4]. Its transcript, ATP7B, has six copper binding domains and is expressed predominantly in liver and kidney. ATP7B is highly homologous to ATP7A, the protein defective in Menkes disease.

Almost 800 mutations in *ATP7B* have been described so far [5]. Interestingly the genetic prevalence of Wilson disease, calculated at 1:7000, is at least 5 times higher than the population frequency, which is estimated at approximately 1:40,000 [5, 6]. While some of this difference might be due to missed diagnoses of Wilson disease, other factors likely contribute, such as incomplete penetrance and the suboptimal performance of computer programs that predict whether a variant is disease causing or not. For example, patients heterozygous for the P1379S variant, long considered to be pathogenic, in combination with a well established disease causing mutation, turned out to have some ATP7B protein expression [7]. Without treatment these patients remained symptom free, with normal liver parameters and urinary copper excretion. The implications of these observations for patients who have two supposedly pathogenic mutations, eg when detected after birth, are unclear at present. Currently it is still common practice to initiate treatment in these cases, although it could be considered to delay treatment until liver biochemistry or 24 hour urinary copper becomes abnormal [8].

Diagnostic Tests

Wilson disease is characterized by low serum ceruloplasmin and serum copper, elevated urinary copper, and increased liver copper. These laboratory results should only be interpreted in combination, because each individual parameter can be abnormal in situations other than Wilson disease [2–4]. For example, liver copper is raised in liver cirrhosis, whereas serum ceruloplasmin is low in a substantial proportion of heterozygotes for Wilson disease, and in patients with hereditary aceruloplasminemia. Conversely, serum ceruloplasmin is normal in a small proportion of patients with Wilson disease. Urinary copper excretion is determined in a 24 hour collection but is sensitive to contamination. Excretion is always increased in symptomatic patients but may be normal or only borderline elevated in presymptomatic individuals. In addition, patients with liver disease may have a somewhat raised urinary copper excretion.

Given these diagnostic difficulties a scoring system has been developed, which uses both clinical symptoms, the laboratory parameters mentioned above, as well as genetic analysis [2]. With a score of 4 or more a diagnosis of Wilson disease is established; below that threshold more tests are needed, eg a liver biopsy (Table 34.1).

Table 34.1 Diagnostic scoring system in suspected Wilson disease

Clinical signs and symptoms		Laboratory results	
Kayser-Fleischer rings		Urinary copper (μmol/24 hr)	
Present	2	<0.6	0
Absent	0	0.6–1.6	1
Neurologic symptoms or typical MRI		>1.6	2
Severe	2	Serum ceruloplasmin (mg/l)	
Mild	1	>200	0
Absent	0	100–200	1
Coombs negative haemolytic anaemia		<100	2
Present	1	Mutation analysis	
Absent	0	2 mutations	4
		1 mutation	1

Modified from [2]
A score of 4 or more establishes the diagnosis of Wilson disease. With 3 points more test are needed, eg a liver biopsy with liver copper analysis. A copper content >250 μg/g dry weight adds another 2 points to the score; 1 point is added when rhodanine positive granules are found, or a liver copper content between 50 and 250 μg/g dry weight

When Wilson disease is diagnosed in a family, siblings should be investigated. Increasingly the partner of a Wilson disease patient is investigated, and, if found to be a carrier of a pathogenic *ATP7B* mutation, the children are screened for mutations too. The results are not always straightforward however, as the status of some DNA changes can be unclear as to whether they are pathogenic or a variant of uncertain significance (VUS). In these cases classical biochemical analysis of copper metabolism might be clarifying, although it remains difficult to distinguish between carriers and patients who still have a low copper load.

Treatment and Prognosis

The prognosis is excellent for patients who start treatment before severe tissue damage has occurred, i.e. when presymptomatic or diagnosed at an early stage [2–4, 9]. The prognosis can still be good for those with more advanced disease, provided decoppering treatment is instituted

immediately after diagnosis. To this aim several therapeutic agents are available: penicillamine, trien and zinc.

The largest experience is with penicillamine, the first agent to be introduced. Penicillamine chelates copper by forming a stable complex that is subsequently excreted in urine. Maintenance dose for adults is 750–1500 mg/day, divided in 2–3 doses, together with 25 mg/day of pyridoxine. Dosing in children is 20 mg/kg/day. With this therapy the majority of patients with liver disease will recover, although liver transplantation cannot be avoided in all [2, 3]. Of patients with neurological disease 80% will recover, but the majority will have some residual disabilities. In the remainder no improvement is seen, or there may even be a deterioration. In this group mortality is not uncommon [2, 9]. A significant proportion of patients with neurological disease will have initial worsening of symptoms after starting penicillamine therapy. In addition, side effects and toxic reactions are seen in up to 25% and therapy has to be stopped in half [2, 9]. Tolerability might be better when starting with a lower dose, i.e. 250 mg/day, with 250 mg increments each 4–7 days. Given this suboptimal safety profile, alternatives for penicillamine have been sought, with trien (trientine) being the first to be introduced. This agent is also a copper chelator, with an efficacy that seems to be similar to penicillamine but with less side effects [10]. The starting dose is 900–2500 mg/day in adults, divided in 2–3 doses, initially in the lower end of this range, with a maintenance therapy of 900–1500 mg/day [2, 3]. Tetrathiomolybdate, as its bis-choline moiety, is another chelator that might be used as it seems to be able to quickly reduce copper load and induce significant early improvements in neurological function [11].

Oral zinc has been used in the treatment of Wilson disease for more than 40 years. It induces metallothionein synthesis in the small intestinal epithelium. Since metallothionein binds copper preferentially over zinc, copper balance will become negative through faecal excretion, as villus cells are lost into the intestinal lumen. As compared to penicillamine, zinc does not have any serious side effects, although some patients experience gastric complaints on zinc sulphate. This can generally be solved by switching to zinc gluconate or zinc acetate. Given its favourable side effect profile, zinc is the agent of choice in presymptomatic individuals. In patients with symptomatic disease (particularly with neurological symptoms) a small non-randomized, non-blinded trial showed similar outcomes for zinc and penicillamine [12]. Given the side effects of penicillamine and the frequency of initial deterioration in patients with neurological disease, zinc should be considered in this group [2, 9]. In patients with hepatic disease, which can evolve rapidly, zinc is less appropriate as it may have a slower effect on copper overload. The initial dose for adults is 150 mg elemental zinc per day, divided in three doses [2, 3]. Urinary copper excretion should be followed; it should fall rapidly initially, and more slowly thereafter. A reasonable goal is to achieve an excretion below 1.2 μmol/day on maintenance treatment [3]. Copper depletion should be avoided: after a few years 75 mg/day of elemental zinc, or even less can be sufficient.

In patients presenting with severe liver disease, sufficient experience is only available for penicillamine. In this group the revised King's score will predict who will recover and who will need a liver transplant [13].

34.1.2 Menkes Disease

Clinical Presentation

Symptoms are generally noted in male infants by the age of 2–3 months, when neurodegeneration becomes manifest with seizures, hypotonia and loss of developmental milestones [14]. Usually, nonspecific signs are already present at birth, including prematurity, large cephalhaematomas, skin laxity and hypothermia. The hair, if present, breaks easily in areas exposed to the mild pressure of lying down and has a sandpaper feel. It can already exhibit the characteristic pili torti, which will appear later on in all cases. A typical facial appearance, with sagging cheeks, gradually becomes prominent. Over time, hypotonia is replaced by spasticity. Feeding difficulties, vomiting and/or chronic diarrhoea are common. Weight gain is generally insufficient while linear growth is relatively well preserved. The loose skin, particularly prominent at the back of the neck and on the trunk, is a consequence of defective collagen crosslinking, as are the vascular tortuosity and bladder diverticula, which are present in virtually all patients. The latter are a frequent source of infection. Umbilical or inguinal hernias and/or a pectus excavatum are also commonly encountered.

Attenuated forms occur in ≈10% of the patients. Of these, occipital horn syndrome is characterised by connective tissue abnormalities much like those in Menkes disease but with less severe effects on neurodevelopment [15]. Exostoses, particularly at the occipital insertion of the paraspinal muscles (hence its name) are characteristic. In addition skin and joint laxity are common, as are urinary tract diverticuli with secondary recurrent urinary tract infections. Generally patients also have orthostatic hypotension and chronic diarrhoea, likely due to autonomic neuropathy.

A third and rare phenotype, X-linked distal hereditary motor neuropathy, presents with symptoms of distal muscular atrophy and weakness in older children or adults [16]. Motor neurons are particularly sensitive to minor perturbations in copper homeostasis, which, however, take years to develop. These symptoms are reminiscent of those observed in acquired copper deficiency, for example due to gastric bypass treatment for obesity.

Metabolic Derangement

In Menkes disease, cellular copper uptake is normal but copper cannot be exported from cells due to a defect in the ATP7A protein [14]. Copper efflux from the intestinal cells into the circulation is severely reduced, and insufficient copper is available for incorporation into the ~20 cuproenzymes for proper functioning. Affected copper-requiring enzymes include lysyloxidase, a critical enzyme in collagen crosslinking, and tyrosinase, which is necessary for melanin formation and copper-zinc superoxide dismutase. Copper-requiring enzymes in the brain are dopamine β-hydroxylase, essential for catecholamine biosynthesis, peptidyl glycine monooxygenase, involved in the processing of neuropeptide precursors, and cytochrome-c-oxidase, involved in the respiratory chain. Deficient activity of these enzymes is probably responsible for a significant part of the cerebral pathology in Menkes disease.

Genetics

Menkes disease is a rare condition with an incidence of approximately 1:250,000 [14]. Although inherited as an X-linked recessive trait it should be noted that approximately one third of patients have de novo mutations. The disease is caused by mutations in *ATP7A* which is expressed in all tissues, except liver. The mutation spectrum in Menkes disease is wide, ranging from single base-pair changes to intragenic deletions encompassing one or more exons. Chromosomal abnormalities, mostly X-autosome translocations have also been reported [17]. The vast majority of these changes are predicted to result in a truncated, nonfunctional protein. Splice site mutations that potentially permit small amounts of *ATP7A* to be transcribed have been described in almost 50% of the patients with occipital horn syndrome [15]. In patients with X linked distal hereditary motor neuropathy a small set of missense mutations have been reported that are associated with even higher residual ATP7A activity [16].

Diagnostic Tests

A level of serum copper (<11 μmol/l) and serum ceruloplasmin (<200 mg/l) below the usual range supports the diagnosis of Menkes disease, but is not specific in the first 3 months of life as these low levels are normal in this age category. Abnormal levels of catecholamines and their metabolites, however, are quite specific, especially the ratio between dopamine and norepinephrine and the ratio of dihydroxyphenylacetic acid to dihydroxyphenylglycol in plasma [18]. The final diagnosis requires identification of the mutation. Larger deletions, responsible for approximately 15% of Menkes cases, may be missed by routine screening for mutations and thus require specific attention. Prenatal diagnosis is preferably made by mutation analysis. Carrier detection should also be undertaken by DNA analysis.

Treatment and Prognosis

Classically, most patients died before 3 years of age due to infections or vascular complications, but with current medical care, especially through improved feeding techniques, longer survival is not uncommon. Moreover, parenteral treatment with copper histidine can bypass the intestinal block, making copper available for incorporation into cuproenzymes. Initial results of this therapy, which is given by daily or twice-daily subcutaneous injection, were generally disappointing, but in the majority treatment was only started after the third month. When started early, i.e. in the first weeks of life, and continued, survival beyond 3 years of age is the rule [18]. Nevertheless, the better outcomes, both with respect to survival and with respect to intellectual and motor development, are seen in patients that have some residual activity of the ATP7A protein [18, 19].

34.1.3 Other Copper Storage Disorders

Indian Childhood Cirrhosis (ICC) is characterized by a normal or slightly elevated serum ceruloplasmin and an extremely high liver copper (800–6500 μg/g dry weight) [20]. It is seen solely in young children. The usual outcome is liver failure, although this can be prevented by early decoppering therapy. The disorder is supposedly caused by an increased dietary copper intake in genetically susceptible individuals, due to the use of copper utensils when cooking milk. Eliminating this practice has virtually eradicated ICC. Although the disease is confined to India (hence its name) a similar disease has been seen in Tyrol (Endemic Tyrolean Infantile Cirrhosis, ETIC), which also seemed to be caused by using copper vessels when preparing milk [21]. Sporadic cases from all over Europe and Northern America have been described (generally labelled Idiopathic Copper Toxicosis, ICT), mostly associated with a high copper content of water in certain wells. All three entities are probably the same disease. As many of these patients are from consanguineous families, a genetic cause of this disorder is likely.

34.1.4 Other Disturbances of Copper Metabolism with a Low Serum Copper

Every disorder with a low serum ceruloplasmin, the main serum copper binding protein, secondarily also displays a low serum copper. Conversely a disturbed intracellular transport of copper, resulting in a secretion defect, will result in a lowered serum ceruloplasmin. Several of these disorders have been described: MEDNIK syndrome, SLC33A1 deficiency, and aceruloplasminemia. In addition, within the large group of con-

genital disorders of glycosylation, some subtypes present with a low serum ceruloplasmin as well as some other abnormalities in copper metabolism.

MEDNIK syndrome is caused by mutations in *AP1S1*, which encodes the small subunit σ1A of the adaptor protein-1 (AP1) complex. When deficient a low serum copper and ceruloplasmin, in combination with mental retardation, variable intestinal pseudo-obstruction, deafness, ichthyosis and raised transaminases is seen [22]. The AP-1 complex is necessary for the intracellular trafficking of ATP7A and possibly other membrane proteins such as ATP7B, through its involvement in clathrin coated vesicle assembly (▶ Chap. 44). When disturbed this secondarily results in abnormal intracellular copper metabolism with aspects of both Menkes and Wilson disease. Zinc-acetate therapy may improve clinical and biochemical abnormalities [22]. A very similar phenotype is seen with homozygous loss of function mutations in *AP1B1*, encoding the large β subunit of the AP-1 complex [23].

AT-1 deficiency (Huppke-Brendle syndrome) is a lethal autosomal recessive disorder, caused by mutations in *SLC33A1,* which encodes this highly conserved acetyl-CoA transporter. Clinical symptomatology, most notable a severe psychomotor retardation with cerebellar and cerebral atrophy, hearing loss and congenital cataracts, may be primarily due to hypoacetylation of proteins crucial for normal brain development, with the hypoceruloplasminemia, resulting in a low serum copper, being another secondary effect of insufficient acetylation [24].

Aceruloplasminaemia is characterized by a very low serum ceruloplasmin and serum copper. It primarily causes a disturbance of iron metabolism and is discussed in the following section [25].

Some congenital disorders of glycosylation, can also present with a low serum ceruloplasmin, disturbed transaminases, a low to normal serum copper and normal or slightly elevated liver copper and urinary copper excretion, resembling Wilson disease, but not fulfilling the relevant diagnostic criteria [2, 26] (▶ Chap. 43).

34.2 Iron

Iron Metabolism

Iron is essential for the synthesis of haem and other metalloproteins. Among these metalloproteins, the iron sulfur protein cluster is especially important, as it plays a crucial role in mitochondrial metabolism, as evidenced by the inherited defects of Fe-S protein biosynthesis (▶ Chap. 10). For all these functions more than 20 mg of iron per day is required, with only 1–2 mg derived from intestinal absorption, the remainder being re-used.

Absorption of iron occurs primarily in the duodenum (◘ Fig. 34.2) through the divalent-metal transporter DMT1, which is encoded by *SLC11A2*. The major recycling route for iron is its removal from erythrocytic haem by haemoxygenase, both in macrophages and enterocytes. Ferroportin, encoded by *SLC40A1*, is responsible for the import of iron into the circulation, both from enterocytes and macrophages. When exported, oxidation of ferrous (Fe^{2+}) to ferric iron (Fe^{3+}), is done by hephaestin at the enterocyte; in other cell types this function is mediated by membrane bound ceruloplasmin. In the circulation iron in its ferric state is bound to apo-transferrin forming holo-transferrin. Transferrin receptor 1, encoded by *TFRC*, mediates the uptake of transferrin. Subsequently iron is released from transferrin in an endosomal cell compartment, reduced by STEAP3 and transported into the cytoplasm by DMT1. Iron recycling is essential to meet iron requirement in cells, e.g. for haem production in erythroid precursors. In several cell types, including macrophages, iron can be stored bound to ferritin until needed. As iron, especially when unbound, is toxic its homeostasis is strictly regulated. Hepcidin, the key regulator of circulating iron levels encoded by *HAMP*, inhibits iron release by ferroportin when high, and facilitates iron export into the circulation when low. The synthesis of hepcidin in turn is regulated by other proteins, including HFE encoded by *HFE1*, hemojuvelin encoded by *HJV,* and transferrin receptor 2 encoded by *TRF2* [27, 28].

Introduction

Iron cannot be actively excreted. An excess of iron leads to an increased concentration of circulating free iron, which is primarily taken up by the liver, the pancreas and the heart [27, 28]. This is why syndromes associated with iron overload – or haemochromatosis – when fully developed, manifest with a triad of cirrhosis, diabetes and cardiomyopathy. The archetypal hereditary haemochromatosis (type 1) is caused by mutations in *HFE*, causing hepcidin deficiency and resulting in systemic iron overload which only becomes manifest during the fourth or fifth decade, if at all. In juvenile haemochromatosis (type 2), which is caused by mutations in *HJV* or in *HAMP*, hepcidin deficiency is more prominent and patients present already in young adulthood with symptoms of systemic iron overload. In TFR2-related haemochromatosis (type 3), caused by mutations in *TFR2*, symptoms are virtually identical to those of type 1 haemochromatosis. While the first three subtypes are autosomal recessively inherited, ferroportin disease (type 4) is autosomal dominant. Ferroportin disease type A is due to loss of function mutations in *SLC40A1* which hamper the export of iron, giving iron accumulation in macrophages along

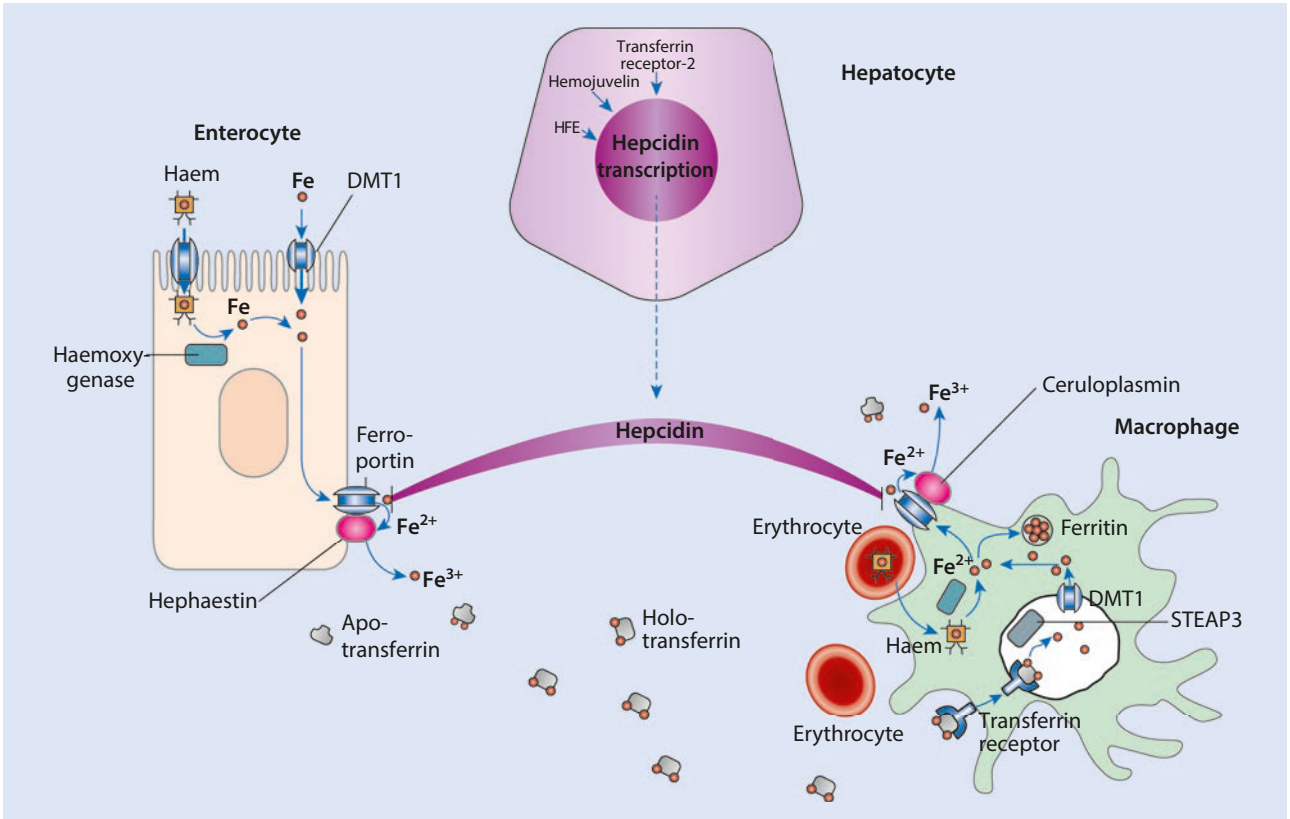

Fig. 34.2 Cellular iron metabolism. Iron is imported into the enterocytes through the divalent metal transporter, DMT1. Iron can also be acquired through direct uptake of haem, from which it is released through haemoxygenase. Export of iron into the circulation is accomplished by ferroportin. At export iron is oxidized to the ferric state by hephaestin (enterocytes) or ceruloplasmin (other cell types) to allow binding to apo-transferrin, thereby forming holo-transferrin. The transferrin receptor binds transferrin and is internalized in target tissues, such as erythroid precursor cells and macrophages. Iron is subsequently set free from transferrin in an endosomal cell compartment, reduced by STEAP3 and made available to the intracellular iron pool by DMT1. In several cell types, such as the macrophage, excess iron can be bound to ferritin, and stored until needed. Iron homeostasis is controlled through hepcidin, which inhibits ferroportin. Hepcidin is synthesized in the hepatocytes and its transcription is regulated by several other proteins, including HFE, hemojuvelin and transferrin receptor-2

with borderline microcytic anemia. Ferroportin disease type B is caused by gain of function mutations in *SLC40A1* as a consequence of which ferroportin activity – and thus iron export – is no longer inhibited by hepcidin. Clinically, this gives rise to a more classical hemochromatosis phenotype.

In five disorders, iron deficiency anemia is a prominent sign. Iron-refractory iron deficiency anaemia (IRIDA) is caused by mutations in *TMPRSS6*. It results in high hepcidin levels, which prevent the release of iron from enterocytes and macrophages, causing microcytic anaemia. Mutations in *TFRC*, encoding transferrin receptor 1, cause mild iron-refractory deficiency anaemia, in combination with severe combined immune deficiency. Patients with atransferrinaemia, due to *TF* mutations, present with hypochromic microcytic anaemia. A deficiency of DMT1 is caused by mutations in *SLC11A2*, hampering the uptake of iron by erythroblasts and resulting in microcytic anaemia. Patients also exhibit liver iron overload. Patients with mutations in *STEAP3* present with a similar phenotype.

In ten neurodegenerative disorders brain iron accumulation can be found, particularly in the basal ganglia [29]. Besides these well described genetic disorders signs of iron accumulation on brain MRI can be found in various other chronic and neurodegenerative disorders. In addition it is linked to aging. This might be due to chronic oxidative stress and aggregation of misfolded protein (▶ Chaps. 1 and 2).

Only two of these disorders have a clear link to iron homeostasis. Aceruloplasminaemia is caused by mutations in *CP*. Patients accumulate iron in the brain, especially the basal ganglia, and in the islets of Langerhans, and present with extrapyramidal symptoms and/or diabetes. Neuroferritinopathy is caused by mutations in *FTL*, resulting in deposition of iron and ferritin in the basal ganglia. In the remaining 8 disorders, iron accumulation is poorly understood, may be variable and appears to be only partially responsible for the symptomatology. Of these, pantothenate kinase deficiency, caused by mutations in *PANK2*, and Coenzyme A synthetase deficiency, encoded by *COASY*, are involved in

the synthesis of Coenzyme A, an important player in lipid metabolism. Interestingly, mutations in *PPCS*, involved in an early step of Coenzyme A synthesis, are not associated with neurodegeneration or brain iron accumulation but with dilated cardiomyopathy. The product of two other genes, *PLA2G6* (▶ Chap. 35) and *FA2H* (▶ Chap. 40), mutations in which are responsible for neuroaxonal dystrophy and fatty acid hydroxylase associated neurodegeneration (FAHN) respectively, also involve lipid metabolism. Mutations in *C19ORF12*, which encodes a mitochondrial membrane protein, may also affect lipid metabolism. Other disorders that can be associated with iron accumulation in the brain are Woodhouse-Sakati syndrome, caused by mutations in *DCAF17*, beta propellor protein-associated neurodegeneration (BPAN), an X-linked dominant disorder caused by mutations in *WDR45,* and ATP13A2 deficiency, which can give a wide range of neurological abnormalities. The latter two genes appear to be involved in autophagosome pathways (▶ Chap. 44).

34

34.2.1 Systemic Iron Overload Syndromes (Haemochromatosis)

34.2.1.1 Classic Hereditary Haemochromatosis (Type 1)

Clinical Presentation

Classic hereditary haemochromatosis, also called type 1 or HFE related haemochromatosis, is an autosomal recessive disorder, characterized by a slow but progressive accumulation of iron in various organs, which becomes clinically apparent during the fourth or fifth decade of life [30–32]. The initial symptoms are nonspecific and include fatigue, weakness, abdominal pain, weight loss and arthralgia. Given the increased awareness of this condition, and the improved diagnostic possibilities, the classic symptoms of full-blown haemochromatosis, such as liver fibrosis and cirrhosis, diabetes, cardiomyopathy, hypogonadotrophic hypogonadism, arthropathy and skin pigmentation are now seen only rarely [28, 30, 31].

Metabolic Derangement

Classic hereditary haemochromatosis is caused by a disturbance in iron homeostasis associated with hepcidin deficiency and systemic accumulation of iron. It is caused by a dysfunction of HFE, which is involved in sensing serum iron levels and thus indirectly for regulating hepcidin synthesis [28, 31].

Genetics

As many as 0.5% of the Northern European population are homozygous for the C282Y mutation in *HFE*, yet only a fraction will develop clinically significant iron overload, especially males [32, 33]. Other mutations in *HFE* are also described, e.g. H63D, with compound heterozygosity for H63D and C282Y being associated with iron overload [30].

Diagnostic Tests

When blood work, initiated because of HFE related complaints or during screening of first degree family members of an established patient, indicates elevated transferrin saturation (above 45%) and/or serum ferritin (>200 ng/ml in adult females and >300 ng/ml in adult males) genetic testing of *HFE* is performed to establish a final diagnosis [33].

Treatment and Prognosis

Treatment by phlebotomy is generally initiated when serum ferritin is elevated, although end organ damage is unlikely when serum ferritin is below 1000 ng/ml. Yet this group with moderately elevated serum ferritin also benefits from iron removal as fatigue improves and mortality due to cardiovascular events and extrahepatic cancers is reduced [33]. In adults, initially 500 ml blood is removed weekly or bi-weekly. Phlebotomy frequency is usually reduced to once every 3–6 months when serum ferritin levels are around 50 ng/ml [31–33].

34.2.1.2 Juvenile Hereditary Haemochromatosis (Type 2)

Juvenile hereditary haemochromatosis, also called type 2 haemochromatosis, is the most severe type of hereditary haemochromatosis, probably because hepcidin deficiency is more pronounced. Patients present in the second and third decades, mostly with hypogonadotropic hypogonadism and cardiomyopathy as a result of iron overload. Type 2A is caused by mutations in the *HJV* gene encoding hemojuvelin, which is necessary for proper regulation of hepcidin expression, and type 2B from mutations in the *HAMP* gene encoding hepcidin [34]. In juvenile hereditary haemochromatosis serum ferritin is high and transferrin iron saturation elevated, as in classic HFE-related haemochromatosis. A final diagnosis is made by mutation analysis. Treatment is by phlebotomy [28].

34.2.1.3 *TFR2*-Related Hereditary Haemochromatosis (Type 3)

The transferrin receptor 2, encoded by *TFR2*, is thought to be important for sensing iron status [28]. Mutations in this gene result in hepcidin deficiency and an iron overload phenotype which resembles classic, HFE-related haemochromatosis, although patients generally are somewhat younger [35]. Elevated ferritin and transferrin saturation in combination with a high liver iron content are present. Mutation analysis, usually undertaken because of the absence of the classic haemochro-

matosis genotype, leads to the correct diagnosis. Phlebotomy is the treatment of choice [35].

34.2.1.4 Ferroportin Related Hereditary Haemochromatosis (Type 4A and 4B)

Haemochromatosis type 4, ferroportin disease, differs in several aspects from the other three subtypes of haemochromatosis. It is autosomal-dominantly inherited and caused by mutations in *SLC40A1*, encoding ferroportin, which is not only expressed at the enterocyte, but also at the cellular membrane of the macrophages. In type 4A, characterized by loss of function mutations in *SLC40A1,* the export of iron from macrophages is impaired, causing iron overload in this cell type and secondarily a high serum ferritin. Patients may have mild microcytic anaemia with low transferrin saturation due to the reduce iron export from the macrophages. In this subtype tolerance to phlebotomy is limited by the concurrent anaemia [28, 36]. In contrast, type 4B, characterized by gain of function mutations in *SLC40A1*, cause resistance to feedback inhibition by hepcidin. These patients present with a more classic hepatic iron overload haemochromatosis phenotype, which is amenable to phlebotomy [28, 36].

34.2.1.5Neonatal Haemochromatosis

Neonatal haemochromatosis (NH) was once thought to be an autosomal recessive inherited disorder. It is now recognized as being acquired, and almost always the result of a maternal alloimmune reaction to the infant liver which starts in utero: Gestational Alloimmune Liver Disease or GALD. The resulting liver injury leads to a decrease in hepcidin production, causing severe iron overload of both the liver and extrahepatic organs. Patients present in the first few weeks of life with severe liver failure. The diagnosis is made in any child with neonatal liver failure in combination with high serum ferritin and extrahepatic siderosis, as evidenced by MRI and/or oral mucosal biopsy, which will demonstrate iron deposits in the minor salivary glands. Therapy is by exchange transfusion in combination with intravenous immunoglobulins (IVIGs) [37]. The risk of recurrence in a subsequent pregnancy from a mother who has given birth to an affected child is as high as 90% but may be prevented by giving IVIGs during pregnancy [37].

34.2.2Iron Deficiency and Distribution Disorders

34.2.2.1 Iron-Refractory Iron Deficiency Anaemia (IRIDA)

This disease is caused by a deficiency of matriptase-2, which is encoded by *TMPRSS6*. If a mutation in both copies of this gene is present the normal cleavage of hemojuvelin is suppressed, resulting in high hepcidin levels [28]. This will result in iron deficiency, low transferrin saturation (<10%) and microcytic anaemia at a young age [38]. Oral iron supplementation is not effective, as high hepcidin levels will prevent iron release from the enterocytes, necessitating intravenous iron therapy.

34.2.2.2 Mild IRIDA with Severe Combined Immune Deficiency

Patients from the two consanguineous families described so far had mild anemia, resistant to iron supplementation. In these patients a homozygous mutation in *TFRC*, encoding Transferrin Receptor 1 (TfR1) was shown to interfere with the internalization of TfR1 [39]. The relatively mild hematological consequences of this problem were probably caused by the protecting effect of high STEAP3 expression in erythroid precursor cells, a protein that facilitates the uptake of iron. In contrast, lymphocytes, who are dependent on iron too, do not have this mechanism, and all patients displayed severe immune deficiency with hypogammaglobulinemia, T cell defects and severe childhood infections, sometimes leading to death [39].

34.2.2.3Atransferrinaemia

So far 16 patients with congenital atransferrinaemia or hypotransferrinaemia have been described. Patients with this autosomal recessive disorder, caused by mutations in the *TF* gene, present with moderate to severe hypochromic microcytic anaemia and growth retardation along with signs of iron overload. Serum transferrin levels are very low, serum ferritin levels are elevated. Plasma infusions to increase the transferrin pool, represent an effective treatment [40].

34.2.2.4 Hypochromic Microcytic Anaemia with Iron Overload Type 1

Hypochromic microcytic anaemia with iron overload type 1 is caused by mutations in *SLC11A2*, encoding DMT1. DMT1 is required for iron uptake by enterocytes and one of the isoforms of DMT1 is responsible for removing iron from absorbed transferrin in erythroid precursor cells. Consequently these patients present at a young age with microcytic anaemia, high serum transferrin saturation and a variable hepatic iron overload. Erythropoietin (EPO) treatment seems to be effective [41, 42].

34.2.2.5 Hypochromic Microcytic Anaemia with Iron Overload Type 2

This subtype is caused by mutations in *STEAP3*. The encoded protein, STEAP3, is an endosomal ferrireductase which facilitates the transferrin mediated uptake of iron. In the 3 siblings reported thus far, anaemia was present from early childhood, while patients became transfusion dependent several years later, usually in late

childhood. High ferritin levels, and an increased transferrin saturation were found. Although the degree of iron overload varied, all 3 had hypogonadism [43].

34.2.3 Neurodegeneration with Brain Iron Accumulation (NBIA)

34.2.3.1 Aceruloplasminaemia

Aceruloplasminaemia is an autosomal recessive disorder characterised by accumulation of iron in the liver, islets of Langerhans and the brain, in particular the basal ganglia and the retina [25]. Clinically the disease consists of adult-onset neurological disease (chorea, cerebellar ataxia, dystonia, tremors and psychiatric signs), retinal degeneration and diabetes mellitus. It is characterized by a deficiency of ceruloplasmin, both the soluble form (serum ceruloplasmin) and its membrane anchored isoform. The latter is, through its ferroxidase activity, essential for iron export in brain, retinal, hepatic and pancreatic cells. More than 60 aceruloplasminaemia-causing mutations in the ceruloplasmin (*CP*) gene have been identified. The diagnosis is made by a combination of clinical symptoms (► Chap. 2), iron overload in liver and brain, and a nondetectable level of serum ceruloplasmin. In addition, transferrin saturation is low in combination with a high serum ferritin. Desferrioxamine, a high-affinity iron chelator, reduces body iron stores, but its effect on neurological symptoms is controversial [25].

34.2.3.2 Neuroferritinopathy

Neuroferritinopathy is an autosomal dominant disease characterised by accumulation of deposits of iron and ferritin in the brain, most prominently in the basal ganglia, where it can even result in cavitation. Most patients present their first symptoms in early adulthood and over decades develop the full clinical picture, consisting of chorea, ataxia, rigidity and dystonia, as well as mixed complaints of cognitive dysfunction (► Chap. 2). Thus far, all mutations that have been described in patients with this disease affect the light chain of ferritin, encoded by *FTL*. Biochemical indicators of iron metabolism are normal, with the exception of serum ferritin, which is in the low to low-normal range. There is currently no effective treatment [44].

34.2.3.3 Pantothenate Kinase-Associated Neurodegeneration (PKAN)

In typical patients this disease presents before the age of 6 years with dystonia, rigidity and chorea-athetosis. Symptoms are slowly progressive, with involvement of the corticospinal tract and development of spasticity. Affected children lose the ability to walk within 10–15 years [45, 46]. In atypical patients the onset is later and progression is slower. On MRI iron accumulation in the basal ganglia can be seen, showing up as an area of hyperintensity in the globus pallidus with surrounding hypointentsity (›eye of the tiger‹ sign). This autosomal recessive disease is caused by mutations in *PANK2* encoding pantothenate kinase 2, which is a key enzyme in the biosynthesis of coenzyme A [45, 47]. A dysfunction of this enzyme will hinder the beta oxidation of fatty acids, giving oxidative stress and resulting in pathological changes at the sites that are most vulnerable, i.e. the basal ganglia. Diagnosis is made by MRI and genetic testing in a child presenting with extrapyramidal symptoms. Treatment is symptomatic [45].

34.2.3.4 COASY Associated Neurodegeneration (CoPAN)

Recessive mutations in *COASY*, which encodes Coenzyme A synthetase, a bifunctional enzyme that catalyzes the last two steps in Coenzyme A synthesis have been described in several patients. If some protein activity is still present it seems to cause COASY associated neurodegeneration (CoPAN), with a clinical picture very similar to PKAN [48]. An almost total absence of Coenzyme A synthetase is associated with pontocerebellar hypoplasia and arthrogryposis [49].

34.2.3.5 Phosphopantothenoylcysteine Synthetase Deficiency

Phosphopantothenoylcysteine synthetase, encoded by *PPCS,* catalyzes the second step in the biosynthesis of Coenzyme A. Biallelic mutations in *PPCS* were found in five individuals from two unrelated families, presenting with severe dilated cardiomyopathy, but apparently without neurodegeneration. Circumventing the missing step in Coenzyme A biosynthesis by giving panthetine might be used as a treatment [50].

34.2.3.6 PLA2G6-Associated Neurodegeneration (PLAN)

PLAN is a heterogeneous group of disorders, comprising infantile neuroaxonal dystrophy (INAD), atypical neuroaxonal dystrophy (ANAD) and *PLA2G6*-related dystonia-parkinsonism. All are characterized by mutations in *PLA2G6*, encoding the phospholipase enzyme A2 group VI, which catalyzes the release of free fatty acids from phospholipids. A deficiency of this enzyme probably changes the lipid composition in neuronal cellular and subcellular membranes, disturbing their remodeling, and giving the characteristic formation of axonal spheroids, which are the neuropathological hallmark of this disease in childhood [29, 47, 51] (see ► Chap. 35 for a detailed description).

34.2.3.7 Fatty Acid Hydroxylase Associated Neurodegeneration (FAHN)

Mutations in *FA2H* may also lead to iron accumulation in the brain. The encoded protein, fatty acid 2-hydroxylase, is involved in the synthesis of

sphingolipids, a major component of myelin [29, 47, 52] (see ► Chap. 40 for a detailed description).

34.2.3.8 Mitochondrial Protein Associated Neurodegeneration (MPAN)

Mutations in *C19ORF12*, encoding a mitochondrial protein with an unknown function at present, appears to be a frequent cause of iron deposition in the basal ganglia [53]. Patients present at a mean age of 11 years with a progressive movement disorder, followed by spasticity, and neuropsychiatric symptoms with cognitive decline. Optic atrophy and motor axonal neuropathy are other frequent clinical findings [53].

34.2.3.9 Woodhouse-Sakati Syndrome

Patients with this syndrome, an autosomal recessive disorder caused by mutations in *DCAF17*, present with hypogonadism, diabetes mellitus, partial alopecia along with varying degrees of mental retardation and progressive extrapyramidal involvement. Brain MRI can show white matter abnormalities along with basal ganglia iron deposition [54]. Progression of the disease is variable even within families [54].

34.2.3.10 Beta Propellor Protein-Associated Neurodegeneration (BPAN)

BPAN, formerly referred to as Static Encephalopathy of Childhood with neurodegeneration in Adulthood (SENDA), is an X linked disorder caused by mutations in *WDR45*, which are generally de novo. *WDR45* encodes a protein with a 7-bladed propeller structure that has a role in autophagy (see ► Chap. 44) [29, 55]. Patients exhibit a global developmental delay and a Rett-like phenotype throughout childhood with progressive cognitive decline in early adulthood. Movement disorders and epilepsy are common. However patients have a very large phenotypic variability, probably due to differences in X-chromosome inactivation. In addition almost all patients described to date are female, suggesting reduced survival of affected male embryos. Iron accumulation in the globus pallidus and substantia nigra can be seen on MRI, especially in the later stages of the disease [55].

34.2.3.11 ATP13A2 Deficiency

Recessive mutations in *ATP13A2* can give symptoms classified as Kufor-Rakeb syndrome, ie juvenile onset Parkinson disease with cognitive impairment and pyramidal signs, with iron deposition in the basal ganglia present in some, but can also be found in patients with neuronal ceroid lipofuscinosis (NCL) (► Chap. 40), or complicated spastic paraplegia type SPG78. It seems that the compromised function of ATP13A2 in the different regions of the brain predominates in each of these phenotypes, which may have some overlap, but the actual pathophysiology behind this phenomenon remains unclear at present. The exact function of ATP13A2 is not known, but it is thought to be a lysosomal transporter for divalent transition metals, required to maintain intracellular manganese homeostasis. Its deficiency seems to cause lysosomal and mitochondrial dysfunction through disturbed autophagy, affecting especially neuronal cells (► Chap. 44) [56].

34.3 Magnesium

Magnesium Metabolism

Magnesium is the second most abundant intracellular cation and plays an essential role in many biochemical processes as well as neuromuscular excitability. Normal serum magnesium concentration (0.75–1.4 mmol/l) is maintained by adapting the urinary magnesium excretion to the uptake in the small intestine.

Magnesium is absorbed from the gut through paracellular transport in the small intestine as well as transcellular transport in the colon. Urinary excretion of magnesium is carefully regulated by modulating the reabsorption of the huge quantities of magnesium that are filtered each day. While most magnesium is passively reabsorbed in the loop of Henle, the active reabsorption that takes place in the distal convoluted tubule determines the actual magnesium balance.

Many genetic defects can lead to symptomatic hypomagnesaemia, the consequences of which are diverse and may vary from hyperexcitability to convulsions, intellectual disability and even to death. In all cases treatment is by magnesium supplementation [57].

■■ Introduction

Primary hypomagnesemia with secondary hypocalcemia generally presents in the first months of life with increased neuromuscular irritability or even frank convulsions. It is caused by mutations in *TRPM6*, reducing uptake of magnesium from the gut. Magnesium supplementation is highly effective.

Isolated dominant hypomagnesaemia can provoke generalized convulsions and is associated with mutations in either *FXYD2, CNNM2* or *KCNA1*.

Isolated autosomal recessive hypomagnesaemia is caused by mutations in *EGF* or *EGFR*.

34.3.1 Primary Hypomagnesaemia with Secondary Hypocalcaemia

■ Clinical Presentation

Primary hypomagnesaemia with secondary hypocalcaemia (HSH) is a rare autosomal recessive disorder. Patients commonly present in the first months of life

with generalized seizures or other symptoms of increased neuromuscular excitability such as irritability, muscle spasms and/or tetany [58].

Metabolic Derangement

Primary hypomagnesaemia is caused by impaired magnesium uptake from the gut [59]. A lowered renal threshold for magnesium, causing a significant renal magnesium leak, is a contributing factor and also generally prevents serum magnesium from completely normalizing during supplementation [59]. The disease is caused by a defect of the TRPM6 protein, which acts as an ion-channel for magnesium.

Severe hypomagnesaemia blocks synthesis and/or release of parathormone (PTH). In addition, when hypomagnesaemia is present, the administration of PTH fails to induce a rise in serum calcium. The hypocalcaemia in HSH is thus secondary to low PTH levels in combination with some form of end organ resistance.

Genetics

Primary hypomagnesaemia is an autosomal recessive disorder that is caused by mutations in *TRPM6* [59]. This gene is expressed in the intestine as well as in the cells lining the distal tubules. To date almost 40 mutations have been identified [59, 60].

Diagnostic Tests

Primary hypomagnesaemia is characterized by a very low serum magnesium (0.24 ± 0.11 mmol/l; normal 0.75–1.40 mmol/l) in combination with a low serum calcium (1.64 ± 0.41 mmol/l; normal 2.12–2.70 mmol/l) [58]. In the presence of serum hypomagnesaemia, the urinary excretion of magnesium is reduced, and PTH levels are inappropriately low. Renal magnesium wasting only becomes apparent when supplementation has started [59].

Treatment and Prognosis

Untreated, the disorder will result in permanent neurological damage or death. However, magnesium supplementation corrects all clinical symptoms. During the initial stage this should be given intravenously, with concurrent parenteral supplementation of calcium. After stabilization, magnesium therapy can be continued orally in an amount that is adjusted to the clinical response. The individual dosage varies greatly between patients (between 0.4 and 3.9 mmol/kg/day of elemental magnesium) [58, 59]. With this regimen, serum calcium normalizes, but serum magnesium will generally remain just below normal. Dividing oral magnesium supplementation in three to five doses will reduce fluctuations of serum magnesium and will prevent the development of chronic diarrhoea in many, but not all patients.

The prognosis of primary hypomagnesaemia is good if the diagnosis is made early; with treatment both growth and development are normal. However, patients who have frequent hypomagnesaemia/hypocalcaemia-induced convulsions, either before or after the diagnosis is made, are at risk for developing psychomotor retardation [58, 59].

34.3.2 Isolated Dominant Hypomagnesemia

Autosomal dominant hypomagnesaemia is caused by a reduced tubular threshold for magnesium and may be associated with a lowered urinary calcium excretion [61–63]. Patients have hypomagnesaemia and may display symptoms associated with a low serum magnesium such as generalized convulsions, muscle cramps or weakness, headaches, or may have no symptoms at all.

The disorder can be caused by mutations in any of the following genes: *CNNM2* [61], *FXYD2* [62] or *KCNA1* [63], with only one or a few families described for each defect. *CNNM2* encodes cyclin M2 (CNNM2), and is expressed in the distal convoluted tubules, where it is involved in magnesium transport, although the exact mechanism has still to be elucidated [61]. *FXYD2* encodes the γ-subunit of Na^+K^+-ATPase, which is expressed both in the proximal and distal tubules [62]. For this defect too the exact mechanism that causes the hypomagnesaemia is unclear. *KCNA1* encodes the potassium channel Kv1.1, localised alongside TRPM6 at the distal tubules. Two specific *KCNA1* dominant mutations were found to be associated with severe hypomagnesemia, while all other mutations in this gene described to date are associated with ataxia and/or epilepsy. The reason for this divergent phenotype is unknown at present [63].

34.3.3 Isolated Autosomal Recessive Hypomagnesaemia

Isolated autosomal recessive hypomagnesaemia has been described in two children from a consanguineous family. Apart from the hypomagnesaemia, no biochemical abnormality was present, and specifically calcium excretion in the urine was normal. In this family a homozygous mutation in *EGF*, the gene for epidermal growth factor was found. Through its receptor EGFR, this secondarily reduces TRPM6 function, which is essential for renal tubular magnesium reabsorption [64]. In another consanguineous family a homozygous loss of function mutation in *EGFR* resulted in hypomagnesemia, as well as severe epithelial inflammation [65].

34.3.4 Hypomagnesaemia with Other Serum Electrolyte Abnormalities and/or Congenital Malformations or with Nephrocalcinosis

Hypomagnesemia can also be present in combination with more prominent abnormalities in other serum electrolytes, as seen in the Gitelman and EAST syndromes, and in Bartter syndrome subtypes as well [57, 66]. It can also be present in familial hypomagnesaemia with hypercalciuria and nephrocalcinosis syndrome (FNHHC), in the Kenny-Caffey syndrome, which is characterized by severe short stature and in patients with renal malformations and/or maturity onset diabetes of the young (MODY) caused by *HNF1B* or *PCBD1* mutations [57, 66]. In these disorders too, hypomagnesemia is not the main symptom.

A final diagnosis of a genetic cause of hypomagnesemia can be made by targeted sequencing of the genes involved; for a clinical diagnosis a structured approach can be helpful [67].

34.4 Manganese

Manganese Metabolism

Manganese is an essential trace element and acts as a co-factor for multiple enzymes, such as hydrolases, ligases, galactosyltransferases, lyases and oxidoreductases. As manganese is potentially toxic its concentration is carefully controlled. This involves transporters involved in its uptake into cells, including the divalent metal transporter DMT1 (SLC11A2), ZIP8 (SLC39A8), ZIP14 (SLC39A14), and excretion from cells, which is facilitated by ferroportin (SLC40A1) and ZnT10 (SLC30A10). Importantly iron competes with manganese at a number of transporters, including DMT1 and ferroportin, so iron can be used for treatment of hypermanganesaemia.

Introduction

Manganese toxicity can be seen in situations where intake escapes normal control mechanisms, such as exposure to manganese dust in adults working in the mining industry, or in patients with a deficiency of key transporters in manganese excretion. These patients present with extrapyramidal motor symptoms such as dystonia and rigidity due to deposits of manganese in the basal ganglia, which can be seen on an MRI of the brain.

Manganese deficiency presents as a type II congenital disorder of glycosylation due the essential role of this trace element in normal glycosylation or as Leigh disease because of defective activity of manganese superoxide dismutase.

34.4.1 Hypermanganesaemia with Dystonia Type 1 (HMNDYT1)

Hypermanganesaemia with dystonia type 1 is caused by bi-allelic mutations in *SLC30A10*. Patients typically present in childhood, generally with dystonia, at a mean age of onset of 7 years. The dystonia can be both focal and generalized with a characteristic "cock-walk" gait, dysarthria, fine tremor and bradykinesia [68–71]. Occasionally spastic paraplegia is seen, and in one family adult onset parkinsonism. Hepatomegaly is usually present and a liver biopsy shows a variable degree of fibrosis or cirrhosis, which may progress to liver failure. In addition, in most patients polycythaemia is present.

The hypermanganesaemia in this syndrome is caused by a deficiency of SLC30A10, which is essential for the excretion of manganese by the liver and also by the brain in high manganese conditions [68–70]. Consequently dysfunction of SLC30A10 leads to accumulation of manganese in the liver, blood and brain. This causes inflammation and cellular dysfunction in hepatocytes and neurons, especially the dopaminergic cells in the basal ganglia, and giving the characteristic symptoms of this disease. The polycythaemia is thought to be the result of an induction of erythropoietin gene expression by manganese [70].

HMNDYT1 is an autosomal recessive disease that is caused by mutations in *SLC30A10*. Most described patients have homozygous mutations, including missense mutations, nonsense mutations and small deletions [70, 71].

Very high blood manganese concentrations are found: 3345 ± 2575 nmol/L (normal: <320 nmol/L). Typical findings on T1-weighted MRI of the brain include a bilateral hyperintense signal in the basal ganglia, the midbrain and cerebellar nuclei as well as the cerebral and cerebellar white matter [70, 71].

Intravenous chelation therapy with $Na_2CaEDTA$ for 5–8 days every 4 weeks, in combination with iron supplementation is the treatment of choice [70]. The first induces excretion of manganese in the urine, while oral iron competitively inhibits manganese uptake in the intestine. This treatment can result in improvement of neurological symptoms, MRI abnormalities and serum manganese levels and may interrupt cirrhosis progression. Treatment should be monitored by measurement of calcium and trace elements as zinc, copper and iron. Some patients have required zinc supplementation because of a fall in plasma zinc or a reduction in iron supplementation because of a high serum iron level.

34.4.2 Hypermanganesaemia with Dystonia Type 2 (HMNDYT2)

Deficiency of SLC39A14 gives a rapidly progressive dystonia with spasticity, developmental delay and parkinsonism, due to manganese deposition in the brain and especially the basal ganglia [70–72]. Median age of onset is 16 months. Unlike type 1 patients, no liver dysfunction or polycythemia is seen. The pathogenesis is probably due to a disrupted manganese import by SLC39A14 into the liver, so excess manganese cannot be excreted [72]. The resulting high manganese blood levels, with an average of 2898 ± 2532 nmol/l, are similar to those seen in type 1 patients. Treatment with $Na_2CaEDTA$ can effectively reduce blood manganese levels. In one patient this has been associated with clinical improvement, but in others treatment has been less successful, either due to the type of mutation or the stage at which treatment was initiated [71, 72].

34.4.3 CDG2N-SLC39A8 Deficiency

Bi-allelic mutations in *SLC39A8*, which encodes a transporter for manganese, are associated with a disorder characterized by low levels of plasma manganese and a secondary defect in glycosylation, probably due to the impaired function of manganese containing transferases [70, 73]. Patients present in infancy with profound hypotonia, severe developmental delay and cerebellar atrophy. The presentation may resemble Leigh's disease [74]. SLC39A8 deficiency, which is treatable with manganese supplementation, is further discussed in ► Chap. 42.

34.5 Selenium

Selenium is an essential micronutrient. Its biological role is mainly mediated through selenocysteine, which is incorporated into the 25 known human selenoproteins, such as the glutathione peroxidases, mediating the removal of cellular reactive oxygen species, and deiodinases, which are involved in thyroxine metabolism. Selenocysteine is not encoded in the DNA itself, but its incorporation into a selenoprotein is mediated through a cotranslational process involving a specialised SElenoCysteine Insertion Sequence, or SECIS element, in the 3′ untranslated region of the mRNA. Interaction of this element with proteins such as the SECIS-binding protein 2, encoded by *SECISBP2,* directs a specific tRNA to incorporate selenocysteine at an UGA codon. The final step in the synthesis of this tRNA, i.e. switching a serine residue for a selenocysteine residue, is mediated through SepSecS, encoded by *SepSecS*.

Three rare inborn errors of metabolism are identified within this system. The first, involving mutations in *SECISBP2*, gives a reduced synthesis of selenoproteins and has a multisystem expression with oligospermia, myopathy and an increased dermal photosensitivity. At least part of this phenotype seems to be related to a reduced ROS defence. In addition, the deficiency of deiodinases gives an abnormal thyroid hormone profile with lowered serum T_3 and increased T_4 [75, 76]. The second inborn error involves mutations in *SepSecS*, giving progressive microcephaly, profound mental retardation and severe spasticity, an autosomal recessive clinical entity known as progressive cerebello-cerebral atrophy [77]. The third involves a bi-allelic mutation in the coding sequence of the selenocysteine transfer RNA, giving muscle weakness and thyroid dysfunction [78].

In addition several single gene disorders affecting a specific selenoprotein are known, all very rare: SEPN1 deficiency, causing an early onset myopathy, which generally makes night time ventilation inevitable before adulthood; GPX4 deficiency, causing a syndrome with severely abnormal skeletal and brain development, which is lethal perinatally and TXNRD2 deficiency, giving endocrinological abnormalities [79].

34.6 Zinc

Zinc Metabolism

Zinc is a cofactor for over 100 enzymes and, as such, is involved in all major metabolic pathways. It is also essential for nucleic acid metabolism and protein synthesis and their regulation through so called zinc-finger proteins. Zinc deficiency, either hereditary or acquired, has major detrimental effects, whereas high serum zinc has few, probably because of binding to albumin and α2-macroglobulin.

Homeostasis of zinc is maintained through the coordinated action of two families of zinc transporters: SLC30 (ZnT) and SLC39 (Zip). These transporters have opposing roles in cellular zinc metabolism. The SLC30 family transporters decrease intracellular zinc concentration by promoting zinc efflux from cells (or to intracellular vesicles), while the SLC39 family increases intracellular zinc concentration by promoting zinc influx into cells (or the release of zinc from intracellular vesicles).

■■ Introduction

Acrodermatitis enteropathica is due to mutations in *SLC39A4*, encoding ZIP4, the major zinc importing carrier in the intestine. Symptoms typically start in infancy after the introduction of bottle feeding, and

include periorificial and acral dermatitis, diarrhoea, infections, and growth retardation. Therapy with zinc is very effective.

Bi-allelic mutations in *SLC39A13*, encoding ZIP13, cause dysfunction of this zinc transporter. With a deficiency of this protein the distribution of zinc between cellular organelles and the cytoplasm is skewed, giving abnormal collagen modification and a disturbed bone morphogenetic protein signaling, leading to a specific subtype of spondylodysplastic Ehlers-Danlos syndrome.

In a single family homozygous mutations in *SLC30A9*, encoding the intracellular zinc transporter ZnT9, caused a cerebro-renal syndrome.

Zinc deficiency in breast fed babies presents with the same dermatological symptoms as acrodermatitis enteropathica. It is caused by maternal heterozygous mutations in *SLC30A2*, encoding the zinc transporter ZnT2. Oral zinc therapy is highly effective.

Hyperzincemia with hypercalprotectinaemia is characterized by extremely elevated levels of calprotectin thought to cause uncontrolled, harmful inflammatory reactions.

Familial hyperzincaemia without symptoms is most likely a non-disease.

34.6.1 Acrodermatitis Enteropathica

Clinical Presentation

Children with acrodermatitis enteropathica (AE) are healthy at birth but develop symptoms some weeks after breast feeding has been stopped. The most striking clinical feature is a severe dermatitis, classically localized at the acral and periorificial sites [80, 81]. At onset, these skin lesions are erythematous, while after the first year of life pustular and hyperkeratotic changes become more prominent. Secondary infection with *Candida albicans* and/or *Staphylococcus aureus* is not uncommon. In addition to the skin lesions, seen in almost all patients, intermittent diarrhoea can develop, which in more advanced stages can progress to intractable watery diarrhoea and failure to thrive. If untreated, a significant fraction of the patients will have a gradual downhill course, although the majority seems to be able to survive without treatment into adulthood. Mood changes are an early sign of zinc deficiency, presenting as apathy and irritability in infancy and later on as depression. Infections are also frequent, and can be life threatening. Other clinical features include alopecia and nail deformities, as well as ophthalmological symptoms such as blepharitis, conjunctivitis and photophobia.

Metabolic derangement

AE is caused by insufficient intestinal absorption of zinc. The reduced zinc absorption results in zinc deficiency with impairment of the function of many enzymes that have zinc as cofactor. Tissues with a high cellular turnover, such as skin, intestine, and lymphoid system are most severely affected.

Genetics

AE is an autosomal recessive disease caused by mutations in *SLC39A4* [82]. *SLC39A4* encodes a zinc transporter, ZIP4, with eight transmembrane domains, which probably form a zinc channel, and is expressed at the apical membrane of the enterocytes. Over 40 mutations have been identified so far, mainly in families from Europe, the Middle-East and North-Africa [83].

Diagnostic Tests

In most patients, serum zinc levels are lower (5.8 ± 3.2 μmol/l) than normal (12.6 ± 2.3 μmol/l), although values within the normal range can be found [84]. Measurements of zinc in other tissues, such as hair and red or white blood cells, do not seem to improve diagnostic accuracy. In addition, several conditions, such as chronic diarrhoea due to other causes, can present with low serum zinc. Therefore, the diagnosis of AE can never be based solely on serum zinc levels. Other tests may contribute to a certain extent: low urinary zinc excretion (reflecting a low serum zinc level), low serum alkaline phosphatase activity, changes in the serum fatty acid profile, hypobetalipoproteinemia, and a reduction of serum vitamin A. In many patients, both humoral and cell-mediated immunity are depressed. Small bowel biopsy generally shows partial to subtotal villous atrophy and Paneth cell inclusions on electron microscopy.

A practical approach is to start zinc therapy when the clinical diagnosis is suspected; improvement should occur within 1 week. When the clinical signs of acrodermatitis were equivocal one may consider to temporarily withdraw zinc therapy after some time to provoke a relapse, and in this way differentiate between true AE (which will relapse quickly) and acquired zinc deficiency. Direct investigation of *SLC39A4* for mutations is theoretically superior, but in more than half of the patients with clinically confirmed AE no mutations in the coding region of *SLC39A4* could be found [85].

Treatment and Prognosis

Zinc therapy for AE was introduced in 1975 and is now used in all patient. The usual dose is 35–90 mg elemental zinc in divided doses during the day, on which patients will start to show clinical improvement within days [80]. Simultaneously, laboratory abnormalities such as serum zinc levels, urinary zinc excretion and alkaline phosphatase activity will normalize. Generally, the initial dose can be maintained throughout childhood, although some patients may need an increase during their growth spurt. After puberty, the requirements for zinc may be lower, but during pregnancy and lactation 400–500 mg zinc sulphate/day is needed. If the preparation causes

gastric problems it may be encapsulated, or alternatively zinc gluconate or other zinc salts may be used. As zinc therapy will decopper patients it is necessary to monitor serum copper, and either reduce the dose of zinc or supplement copper if a deficiency is found. With zinc supplementation prognosis is excellent.

34.6.2 Spondylocheirodysplastic Ehlers-Danlos Syndrome

This very rare subtype of Ehlers-Danlos syndrome, with only four families described to date, is caused by biallelic mutations in *SLC39A13*, encoding the zinc transporter ZIP13. This protein is involved in zinc transport from intracellular organelles to the cytoplasm (▶ Chap. 44). Its deficiency supposedly causes low cytosolic zinc, leading to abnormal bone morphogenetic signaling and disturbed collagen modification. This in turn gives the characteristic clinical symptoms, i.e. short stature, hyperelastic skin and joint hyper mobility [86].

34.6.3 Birk-Landau-Perez Syndrome

A single family has been described with a homozygous mutation in *SLC30A9*, encoding the zinc transporter ZnT9. This disturbes intracellular zinc homeostasis and signaling. SLC30A9 is highly expressed in fetal brain, the cerebellum, and the kidney, concordant with the symptomatology in this family, which includes severe neurological disability, profound ataxia and nephropathy [87].

34.6.4 Transient Neonatal Zinc Deficiency

Rarely, zinc deficiency with acrodermatitis can occur in breast-fed babies, especially in premature infants, as they have an increased zinc requirement in combination with a reduced capacity for zinc uptake in the gut [88]. Although this condition responds rapidly to oral zinc supplements, it is clearly different from AE, as it is seen exclusively during breast feeding and no impairment of intestinal zinc uptake can be found. The deficiency is caused by reduced levels of zinc in maternal milk, due to heterozygous mutations in *SLC30A2*, encoding the zinc transporter ZnT2 in the mother [89].

34.6.5 Hyperzincaemia with Hypercalprotectinaemia

A very high serum zinc (77–200 μmol/l) can be encountered in combination with an extreme elevation of serum calprotectin (up to 1000 times the upper limit of normal) [90]. These patients present with recurrent infections, hepatosplenomegaly, arthritis, anaemia and persistently raised concentrations of C-reactive protein. It is speculated that the very high concentration of calprotectin, the major zinc binding protein of phagocytes, results in the uncontrolled and harmful inflammatory reactions which characterize this syndrome, while the hyperzincaemia is caused by the zinc capturing properties of calprotectin. Whether this syndrome is genetically determined is not clear yet.

34.6.6 Familial Hyperzincaemia Without Symptoms

Elevated serum zinc (40–70 μmol/l) was described in seven family members from one large pedigree [91]. In this family the condition seemed to be inherited in an autosomal dominant fashion. In another family two siblings had serum zinc levels that were clearly abnormal (63 μmol/l and 128 μmol/l), with no abnormalities in the parents [92]. In all individuals investigated zinc concentrations in hair and erythrocytes were normal, as was urinary zinc excretion. Most of the excess zinc seemed to be bound to albumin. There were no clinical symptoms, nor additional biochemical abnormalities, so this condition appears to be benign.

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Complex Molecule Disorders and Cellular Trafficking Disorders

Contents

Disorders of Intracellular Triglyceride and Phospholipid Metabolism

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Contents

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Introduction

Acylglycerols play many organ-specific roles in cell structure, biochemistry and signalling. Inborn errors of acylglycerol metabolism cause a wide array of phenotypes. Many of these conditions were described recently in only a few patients and many steps of acylglycerol metabolism are not yet associated with an inborn error. Exome and genome sequencing is revealing many genetic defects of the biosynthesis, remodelling and degradation of triglycerides (TGs) [1] and phospholipids (PLs) [2] and of the phosphorylation of phosphatidylinositol (PI). These disorders are usually not detectable by current biochemical diagnostic techniques. Biochemical diagnosis for such patients is an important unmet challenge that hopefully will be met with developments in lipidomics and enzymology, especially important for patients with DNA variants of unknown significance. Recurrent features found in several inborn errors of acylglycerol metabolism include enzyme redundancy (i.e., different enzymes or isoforms that catalyse the same biochemical step, often in cell-specific fashions), molecular remodelling by cleavage and re-esterification of fatty acids (FAs), and enzymes, substrates and/or products with roles in other metabolic pathways, cell signalling or transcription regulation. Some conditions described as autosomal recessive show marked heterozygote effects. Some produce striking clinical syndromes in infants; others mimic common adult conditions like metabolic syndrome and type II diabetes. Inborn errors of intracellular TG metabolism [1] often affect adipose tissue, but muscle, liver or skin may dominate their clinical presentations. Inborn errors of PL metabolism [3] involve the synthesis, remodelling or degradation of phosphatidyl-choline (PC), -ethanolamine (PE), -serine (PS) and –inositol (PI). They often affect the central and peripheral nervous systems, but also muscle, eye, skin, bone, cartilage, liver, kidney and the immune system. Symptomatic treatments are often useful but mechanism-based treatment is not available for most of these conditions.

35.1 Inborn Errors of the Common Pathway and of Triglyceride Synthesis and Degradation

Introduction

Much of lipid metabolism occurs upon the 3-carbon backbone of glycerol, a derivative of glycolysis and gluconeogenesis. This chapter describes inborn errors of intracellular acylglycerol metabolism. Starting with inborn errors of the "common pathway", i.e., until synthesis of phosphatidic acid (PA), a diacylglycerol with a phosphate group (P) in the *sn*-3 position (▶ Sect. 35.1), it proceeds to TG synthesis and degradation (▶ Sect. 35.2), PL synthesis and head group rearrangements in cytoplasm and mitochondria (▶ Sect. 35.3), cleavage and remodelling of the R1 and R2 positions of PLs (▶ Sect. 35.4) and ends with inborn errors of the phosphorylation of one PL, phosphatidylinositol (PI, ▶ Sect. 35.5). For inborn errors of extracellular acylglycerol and lipoprotein metabolism, see ▶ Chap. 36.

35.1.1 Glycerol-3-Phosphate Dehydrogenase 1 (GPD1) Deficiency

Figure 35.1, step 1

About 20 patients are reported. Four cardinal liver-related features are fasting hypertriglyceridemia (2.5–70 mmol/L at presentation), fatty liver, hepatomegaly from birth (sometimes marked), and 4–8 fold elevations of serum aminotransferase and γ-glutamyltransferase levels. Short stature is common. Patients tended to improve with age although fibrosis and cirrhosis occurred in some [6] and the full clinical spectrum is unknown. Heterozygotes were asymptomatic [7]. GPD1 deficiency should be considered in patients with fatty liver, especially in infants and nonobese older patients. The differential diagnosis includes glycogen storage diseases I and III, citrin deficiency, lysosomal acid hydrolase deficiency and partial lipodystrophy.

Diagnosis is by gene sequencing. Growth failure may improve with a hypercaloric low fat, high carbohydrate diet and medium chain triglyceride supplementation. The causes of fatty liver and hypertriglyceridemia are not obvious from the acylglycerol pathway (Fig. 35.1) or from the redox role of GPD1 (not shown), in which its glycerol-3-phosphate product is oxidized at the mitochondrial inner membrane by GPD2, reducing coenzyme Q and regenerating DHAP [8].

35.1.2 Glycerol Kinase Deficiency

Figure 35.1, step 2

This condition is described in ▶ Sect. 7.8.

35.1.3 1-Acylglycerol-3-Phosphate O-Acyltransferase 2 *(AGPAT2)* Deficiency

Figure 35.1, step 3

Autosomal recessive deficiency of AGPAT2 is a common cause of severe congenital generalized lipodystrophy [9] (Lipodystrophies). Leptin replacement therapy may improve some metabolic complications [10, 11].

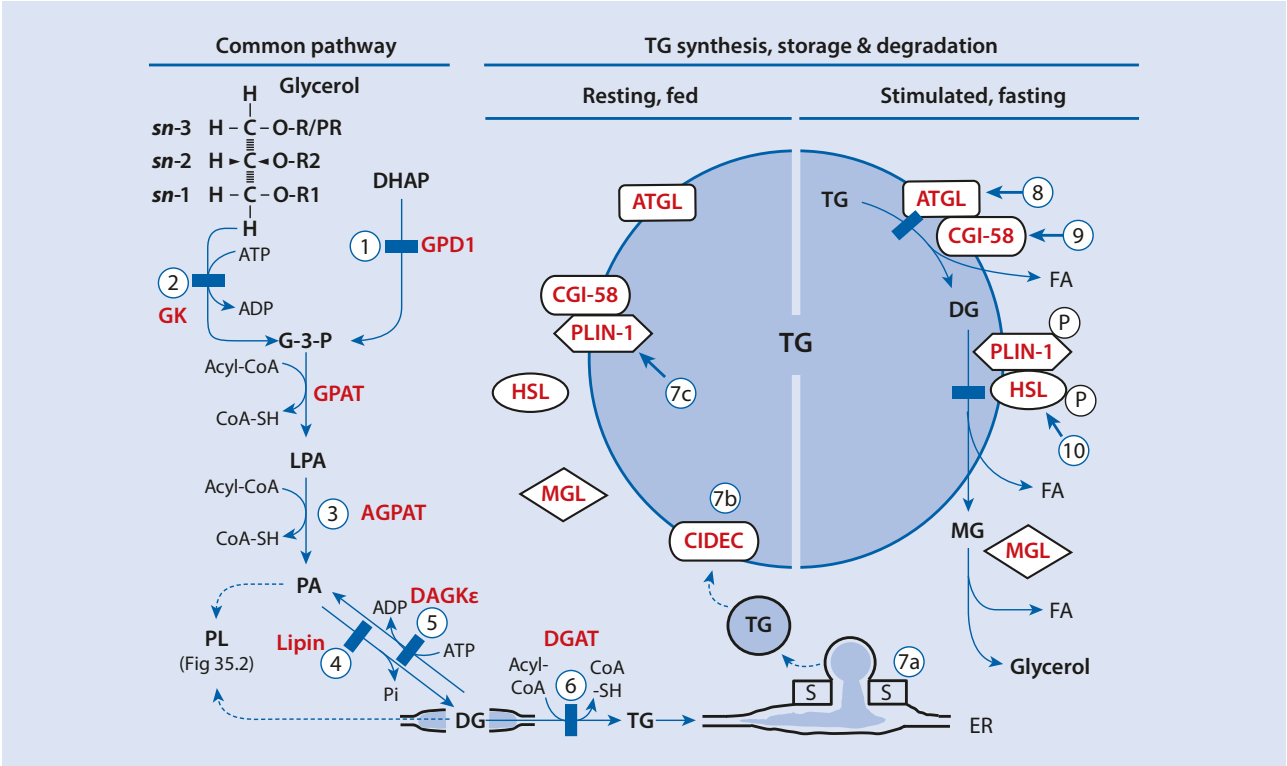

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Fig. 35.1 The common pathway and triglyceride synthesis, storage and lipolysis. Glycerol (upper left) forms the core of all acylglycerols. In acylglycerols, R1 and R2 are fatty acids (FA). Typically, R1 is a saturated and R2 is an unsaturated FA (e.g., arachidonic). The *sn*-3 oxygen binds to a FA in triglycerides (TGs), to a phosphate group in phosphatidic acid (PA) and to a phosphate linked to a polar head group in phospholipids (PLs). PLs form the bilayer of cell membranes and the monolayer surrounding lipid droplets (LDs) containing hydrophobic neutral lipids like TGs. Some steps of TG and of PL metabolism are mediated by several different enzymes or isoforms, in tissue- or organelle-specific ways. *De novo* acylglycerol synthesis begins from glycerol-3-phosphate (G3P), formed from glycerol or from dihydroxyacetone phosphate (DHAP), an intermediate of both glycolysis and of gluconeogenesis. G3P is esterified twice. First, glycerolphosphate acyltransferases (GPATs) in endoplasmic reticulum (ER) and mitochondria add a FA at the *sn*-1 position to form lysophosphatidic acid (LPA). Second, acylglycerol phosphate acyltransferases (AGPATs) form PA. PA can be converted into an *sn*-1,2-diacylglycerol (DG) by PA phosphatases (lipins-1, 2 and 3). DG can be phosphorylated to PA by diacylglycerol kinases (DAGKs), or can produce either PLs (Fig. 35.2) or TGs. Diacylglycerol acyltransferases (DGATs) catalyse the synthesis of TGs. DGs and TGs localize between the leaflets of the ER membrane. Seipin (S), a circular dodecamer that interacts with AGPAT2 and other proteins [4], permits the budding of LDs from the ER. CIDEC and other CIDE proteins are felt to facilitate fusion to form large LDs [5]. One or more of perilipins (PLIN) 1–5 are found on the surface of all LDs. Perilipin 1 is abundant in adipocytes. Lipolysis is most studied in adipocytes, illustrated here, but important inter-tissue differences exist. Adipocyte lipolysis is activated by fasting, providing FAs and glycerol for fuel. The main TG lipase in adipocytes, adipocyte triglyceride lipase (ATGL), is on the LD surface. Interaction with CGI-58 enhances lipolytic cleavage of TGs by ATGL, producing *sn*-1,3-DGs more than *sn*-2,3-DGs. DGs are hydrolysed by hormone-sensitive lipase (HSL), producing mainly *sn*-1-monoacylglycerols (*sn*-1-MGs). MG hydrolase(s) produce a FA and glycerol. In adipocytes, β-adrenergic stimulation and low circulating insulin levels both favour PLIN1 and HSL phosphorylation by protein kinase A (PKA), reorganising the LD surface from quiescent (left side of LD in figure) to lipolytically active (right). Circled numbers indicate metabolic blocks discussed in the text

Lipodystrophies

Several diseases in this chapter are among the commonest genetic lipodystrophies [9]. Two forms, generalized and partial, are distinguishable clinically. Acquired forms are often related to antiretroviral therapy or autoimmunity [9]. **Generalized lipodystrophy** (e.g. AGPAT2, Seipin deficiencies) is often autosomal recessive and usually evident from birth, with near total absence of fat tissue in subcutaneous, intermuscular, intraabdominal and intrathoracic white adipose tissue (WAT) depots, bone marrow and often mechanical fat depots (palms, soles, orbits, periarticular). Bone cysts may occur. In **partial lipodystrophy** (e.g., Perilipin, CIDEC, HSL, CCTα deficiencies), subcutaneous fat loss, noticed in childhood or early adulthood, occurs mainly in limbs and gynecoid regions, conferring an android appearance. Most identified patients are female. Polycystic ovary syndrome is common. Many partial lipodystrophy patients are likely diagnosed as metabolic syndrome. Lipomas and adipose hypertrophy can occur in the nuchal region, face, trunk [12]; this pattern of fat redistribution greatly increases suspicion of partial lipodystrophy. In both forms, insulin resistance, fatty liver, hypertrophic cardiomyopathy, low circulating levels of leptin and adiponectin, hypertriglyceridemia and pancreatitis are frequent. Muscle definition is high in regions lacking subcutaneous fat and some level of muscular hypertrophy occurred in 21/21 (100%) of generalized and 25/34 (75%) of partial lipodystrophy patients [13]. Diagnosis is molecular, by multigene panels. Symptomatic treatment is offered for complications.

35.1.4 Phosphatidic Acid Phosphatase (PAP; Lipin) Deficiencies

Figure 35.1, step 4

The pathophysiology of Lipin deficiencies is incompletely understood. Lipins mediate a key step at which the TG and PL synthetic pathways diverge, and they moonlight as nuclear transcriptional co-activators for genes of metabolic or immunological importance such as PPAR-α, PPAR-γ and nuclear factor of activated T-cells c4 (NFATc4) [14].

35.1.4.1 Lipin-1 (*LPIN1*) Deficiency

Lipin-1 deficiency should be considered in any child with rhabdomyolysis, especially between 2–6 years of age with severe recurrent episodes and normal plasma acylcarnitines (autosomal recessive; gene, *LPIN1*). It accounts for ~10% of patients with severe recurrent childhood rhabdomyolysis [15, 16]. Creatine kinase levels often exceed 10,000 IU/L after febrile illnesses, exercise, anaesthesia or fasting. Between episodes, evaluation is usually normal. Rarely, chronic myolysis with proximal weakness, cardiac arrest, sometimes with hyperkalaemia and cardiac dysfunction and cardiac steatosis [17] are reported. WAT mass and distribution, peripheral nerve function and plasma levels of cholesterol, TGs and adiponectin have been normal. Rarely, heterozygotes develop rhabdomyolysis under stress (e.g., statin therapy) [15].

Molecular testing, including deletion analysis for a common deletion mutation, is the preferred diagnostic method. If muscle biopsy is performed, e.g., to rule out glycogenosis or respiratory chain disorders, histology is nonspecific, although ragged red fibres are described in lipin-1 deficiency. If muscle biopsy is necessary, it should be obtained after recuperation from, not during, rhabdomyolytic episodes.

Rhabdomyolysis can cause renal failure or be fatal [18]. During febrile infections, avoid other stresses like exercise [17]. Treatment includes aggressive intravenous fluid administration to stimulate diuresis, with close monitoring of electrolytes and of renal and heart function, and haemodialysis if necessary [18]. High glucose intake may be beneficial [19]. Suppression of inflammation may have therapeutic potential, but this remains to be formally tested [20].

35.1.4.2 Lipin-2 (*LPIN2*) Deficiency

Majeed syndrome is a rare autosomal recessive disease with a characteristic clinical triad [21]: (i) Chronic recurrent multifocal osteomyelitis (CRMO) of early onset (3 weeks to 2 years) with high fever, severe pain, and tender periarticular soft tissue swelling often recurring every 3–4 weeks. X-rays show irregular radiolucent osteolytic lesions with surrounding sclerosis. MRI of active bone lesions reveals high signal intensity on T_2-weighted images, and low intensity with T_1-weighting. (ii) Congenital dyserythropoietic anaemia, presenting during the first year. (iii) In some cases, transient inflammatory pustular dermatosis (Sweet syndrome). Transient hepatomegaly, neutropenia, and cholestatic jaundice have occurred in neonates. Erythrocyte sedimentation rate is elevated. Cultures of blood, bone or skin lesions are sterile.

Differential diagnosis includes infectious osteomyelitis, other forms of CRMO (onset usually later at about 9 years) [22] and other periodic fever and autoinflammatory syndromes [21]. Dyserythropoeitic anaemia provides a useful clue to diagnosis.

Diagnosis is possible clinically if all three elements occur. In the presence of even one, the diagnosis should be considered. Diagnosis is by molecular testing of *LPIN2*.

Treatment with IL-1 receptor antagonists or anti IL-1β antibody is promising [22, 23]: suppression of NLRP3 inflammasomes [24] can reduce interleukin-1 (IL-1) release, making Majeed syndrome a treatable form of CMRO [22]. Nonsteroidal anti-inflammatory agents are provided, plus physical therapy to counter the disuse atrophy for which patients are at risk. Chronic corticosteroid treatment is avoided.

35.1.5 Diacylglycerol Kinase Epsilon (*DGKE*) Deficiency

Figure 35.1, step 5

Mutations in *DGKE* cause autosomal recessive atypical haemolytic-uremic syndrome (aHUS), usually starting in the first year of life [25]. Affected individuals had repeated episodes of microangiopathic haemolytic anaemia, thrombocytopenia and acute renal failure. Renal filtration recovered but hypertension, microhaematuria and proteinuria persisted. Chronic kidney disease was common by the second decade, with progressive worsening long after the last acute episode of aHUS. Useful diagnostic clues for DGKE deficiency include the early onset and the development of nephrotic syndrome 3–5 years after disease onset, both of which are rare in other forms of HUS. Some patients develop membranoproliferative glomerulonephritis with or without HUS.

Other causes of HUS include the typical postinfectious form, hereditary disorders of the complement cascade and untreated cblC disease.

Anticomplement therapy, used in HUS caused by primary complement disorders, is ineffective in *DGKE*-related aHUS. In contrast, unlike individuals with soluble complement defects, renal transplantation can be efficacious in aHUS caused by *DGKE* mutations [25].

35.2 Inborn Errors of Cytoplasmic Triglyceride Synthesis, Storage and Degradation

Figure 35.1

Disorders of extracellular TG-containing lipoproteins are discussed in ▶ Chap. 36.

35.2.1 Diacylglycerol O-Acyltransferase (DGAT) Deficiencies

Figure 35.1, step 6

The two DGAT enzymes are evolutionarily unrelated, with distinct properties and distributions.

35.2.1.1 Diacylglycerol O-Acyltransferase 1 (*DGAT1*) Deficiency

Severe congenital watery diarrhoea and protein-losing enteropathy sometimes requiring long-term parenteral nutrition has been described in several patients with biallelic *DGAT1* mutations [26, 27]. Endoscopic examinations are normal. Biopsies can be normal or show lipid droplets in enterocytes, sometimes suggestive of microvillus inclusion disease. Molecular analysis of *DGAT1* is the most convenient diagnostic method. Treatment is mostly symptomatic; at least one patient received small bowel transplantation [27].

35.2.1.2 Diacylglycerol O-Acyltransferase 2 (*DGAT2*) Deficiency

In a father and son, slowly progressive Charcot-Marie-Tooth disease with early-onset sensory ataxia and tremor was attributed to a missense mutation, p. Tyr233His, *de novo* in the father's *DGAT2* [28]. This interesting observation requires confirmation in other patients.

35.2.2 Diseases Related to Structural Proteins of Lipid Droplet (LD) Production, Fusion and Maintenance

Figure 35.1, steps 7a, 7b and 7c

LD require structural proteins for their formation and maintenance, as illustrated by the following three conditions affecting Seipin (gene *BSCL2*), necessary for LD budding from the ER; CIDEC, felt to facilitate LD fusion and Perilipin 1 (PLIN1), an adipocyte LD surface protein that coordinates the shift between non-lipolytic and lipolytic states. All three cause forms of lipodystrophy (see blue box Lipodystrophies).

35.2.2.1 Seipin (*BSCL2*) Deficiency

Figure 35.1, step 7a

Seipin mutations produce two distinct clinical phenotypes (1) Congenital generalized lipodystrophy (Berardinelli-Seip syndrome) and (2) neurodegenerative disease with or without lipodystrophy.

Combined, AGPAT2 and seipin deficiencies account for about half of severe congenital generalized lipodystrophy patients [29] (Lipodystrophies). Patients can develop hypertrophic cardiomyopathy, mild to moderate intellectual deficiency and bone cysts [30].

BSCL2 mutations can also produce isolated neurological disease, most commonly progressive spastic paraparesis and distal motor neuropathy [31], entering the differential diagnosis of axonal neuropathies (Charcot-Marie-Tooth), amyotrophic lateral sclerosis

and spastic paraplegia. Of note, patients with lipodystrophy do not usually develop such upper or lower neuron signs [30]. One mutation, c.985C>T, is associated with a rapidly progressive, infantile- or childhood-onset neurodegeneration affecting cortex and basal ganglia (Celia's encephalopathy) [32].

35.2.2.2CIDEC Deficiency

Figure 35.1, step 7b

The only reported patient, a 19-year-old woman with partial lipodystrophy (Lipodystrophies), insulin-resistant diabetes, hypertriglyceridemia and pancreatitis, was homozygous for a premature stop mutation in CIDEC [33].

35.2.2.3Perilipin 1 (*PLIN1*) Deficiency

Figure 35.1, step 7c

Heterozygous frameshift mutations in *PLIN1* cause familial partial lipodystrophy 4 (Lipodystrophies) [34, 35]. Body mass index may be normal because skeletal muscle hypertrophy balances fat loss. Most lack the striking facial and nuchal fat accumulation seen in Dunnigan partial lipodystrophy [36] but facial and cervical fat accumulation can occur [35]. Some have acromegalic features [35].

35.2.3Neutral Lipid Storage Diseases (NLSDs): ATGL and CGI-58 Deficiencies

Two forms of NLSDs occur: NLSD with lipid myopathy and/or cardiomyopathy (NLSDM) and NLSD with ichthyosis (NLSDI) [37]. NLSDM results from ATGL deficiency and NLSDI, from CGI-58 deficiency [38]. In both, the peripheral blood smear reveals Jordan's anomaly, i.e., vacuolated polymorphonuclear leucocytes. Vacuoles appear "empty" on Giemsa staining and red on Oil Red O staining for neutral lipid. Both are considered autosomal recessive diseases but symptomatic heterozygotes are reported.

35.2.3.1Adipocyte Triglyceride Lipase (ATGL, *PNPLA2*) Deficiency

Figure 35.1, step 8

ATGL deficiency [39, 40] typically presents in young adults, with weakness and fatty infiltration of muscle, cardiomyopathy or both. About 100 patients are described [41–43]. The clinical spectrum is broad. Weakness is usually proximal but is distal in about 15% [41, 43]. It is progressive and often asymmetrical, usually starting in the right arm. Symptoms may begin in infancy [44] but some patients are athletic in childhood. Exercise intolerance, muscle pain and cramps may occur. Nearly all patients show 1.5- to 25-fold elevations of serum creatine kinase [40–43]. Rhabdomyolytic crises are not reported. Muscle MRI shows high fat content particularly in gastrocnemius (median head), soleus and biceps femoris. Cardiomyopathy affects about half of patients. It may be severe and may require transplantation. Hypertrophy is commonest but dilated forms occur. Coronary artery obstruction by triglyceride-rich deposits [39] is principally reported in Japanese patients; visceral and pancreatic fat contents are high [45]. Other observations recurring in two or more patients include diabetes, hepatomegaly with steatosis, hypertriglyceridemia and acute pancreatitis, hearing loss, short stature and kyphosis [40–42]. In patients with myopathy or cardiomyopathy, the diagnosis of NLSDM is suggested by high neutral lipid levels in muscle, the presence of Jordan's anomaly and normal plasma acylcarnitine levels, excluding fatty acid oxidation disorders (► Chap. 12). It is confirmed by molecular testing of *PNPLA2*.

Treatment is symptomatic. Some modalities are effective in Atgl-deficient mice but are not tested in humans [46]. High protein diet and avoidance of fasting are predicted from mice [47] and from basic nutritional considerations to be beneficial in ATGL deficiency.

One series revealed many symptomatic heterozygotes [48]: skeletal myopathy, 16/21 (76%); cardiomyopathy, 9/21 (42%), with ventricular tachycardia in one; hepatomegaly, 4/21 (19%); insulin resistance or diabetes, 3/21 (14%). Siblings and parents should be evaluated for heart dysfunction, muscle weakness and glycaemia. Testing for Jordan's anomaly, and molecular testing if the causal *PLPNA2* mutations are known, should be discussed with at-risk family members. Disease related to the paralogous gene, *PNPLA1*, is discussed in Notes added in proof, at the end of this chapter.

35.2.3.2α,β-Hydrolase Domain-Containing 5 (CGI-58, *ABHD5*) Deficiency

Figure 35.1, step 9

ABHD5 mutations cause NSLDI (Chanarin Dorfman Syndrome). Nonbullous congenital ichthyosis occurs, often affecting skin flexures, scalp and face, with hyperkeratosis of palms and soles and pruritus. Neonates can present as collodion babies [49]. Liver involvement is frequent, with steatosis and two- to four-fold elevations of plasma aminotransferases; steatohepatitis, fibrosis and sometimes, cirrhosis [50]. Myopathy may occur, with elevated serum creatine kinase, abnormal electromyography and neutral lipid in types 1 and 2 fibres [51, 52]. Neurosensory deafness, cataract, nonprogressive psychomotor retardation, ataxia, spasticity and cardiomyopathy are reported.

Lipids are essential for normal skin barrier function; lipid metabolic disorders cause a substantial fraction of ichthyoses [53]. Jordan's anomaly should be searched for carefully; its presence in an ichthyotic patient greatly raises suspicion of NLSDI. Skin biopsy, part of the evaluation of congenital ichthyosis, is typically negative for neutral fat droplets because standard fixation procedures remove fat. Molecular analysis of *ABDH5*, often as part of a con-

genital ichthyosis panel, is the main diagnostic technique. Treatment is symptomatic. Case reports describe skin improvement with retinoid treatment [49, 54].

Heterozygotes with a single severely-deficient *ABDH5* allele can develop fatty liver and/or dyslipidaemia [55].

35.2.4 Hormone-Sensitive Lipase (HSL, *LIPE*) Deficiency

Figure 35.1, step 10

The 9 reported HSL-deficient patients [56, 57], each of whom had homozygous frameshift mutations in *LIPE,* had adult-onset partial lipodystrophy (Lipodystrophies) with multiple progressive symmetrical lipomas in nuchal regions and truncal and abdominal fat accumulation. Four had muscle symptoms, 5 had hyperCKemia (438–771 IU/L) and 4 had myopathy on biopsy. Diabetes (7/9 patients, 78%), hepatic steatosis (5/7, 71%) and hypertriglyceridemia (1.7–6.0 mmol/L) occurred. Adipose biopsies revealed small adipocytes, impaired lipolysis, increased DG content and inflammation [57].

In a population study of the Amish founder mutation, heterozygotes tended to insulin resistance and high plasma TG levels (1.24 ± 0.80, versus 0.96 ± 0.67 mmol/L in controls), but had normal fat mass and blood pressure [57].

35.3 Inborn Errors of Phospholipid Biosynthesis and Mitochondrial Phospholipid Metabolism

Figure 35.2

Introduction

In this chapter, PL metabolism is discussed as follows: head group and PL synthesis in the ER and mitochondrial PL metabolism in ▶ Sect. 35.3, FA cleavage and esterification (remodelling) in ▶ Sect. 35.4 and PI phosphorylation and dephosphorylation in ▶ Sect. 35.5. These inborn errors produce a broad clinical spectrum, as expected from the important roles of PL in every tissue. Features recurring in two or more conditions include: myopathy, sometimes with cardiomyopathy; chorioretinal degeneration and hypoacusis, sometimes diagnosed as Usher syndrome; hypogonadal hypogonadism and cataracts. Patients with two or more of these features raise clinical suspicion of PL diseases. Neurological signs are frequent and can affect nearly all systems: progressive spastic paraplegia with or without ataxia, dystonia with basal ganglia changes on MRI, upper and lower motor neuron disease, epilepsy and intellectual deficiency recur among these diseases. Syndromic skeletal dysplasias with any of the above signs should raise the possibility of inborn errors of PL metabolism.

35.3.1 Choline Kinase β (CHKβ) Deficiency

Figure 35.2, step 1

CHKβ deficiency is an autosomal recessive megaconial congenital muscular dystrophy, with early-onset hypotonia, muscle wasting, mild to moderate intellectual disability and abnormal mitochondrial morphology [63]. Some patients have autistic features, ichthyosis and pruritus and dilated cardiomyopathy, a major cause of mortality. Recently, two patients with congenital neurogenic muscular atrophy progressing to a combined neuropathic and myopathic phenotype were described [64]. Brain MRI is often normal but thin corpus callosum and cerebral atrophy can occur. Serum creatine kinase is a useful diagnostic marker, being mildly (<200 IU/L) but constantly elevated in CHKβ deficiency. Muscle histology showed enlarged mitochondria (megaconial), especially at the periphery of fibres, but sparse in the centre. One patient requiring heart transplantation showed a partial defect of complex V assembly in heart and muscle. No specific treatment is presently known. Diagnosis is by molecular analysis. Biochemically, a complete loss of CHK enzyme activity was associated with decreased levels of PC and of the PC/PE ratio in muscle biopsies [63]. No phenotype-genotype correlation is known.

35.3.2 Choline-Phosphate Cytidylyltransferase α (CCTα, *PCYT1A*) Deficiency

Figure 35.2, step 2

Loss-of-function mutations in *PCYT1A*, encoding CCTα are reported in 3 independent manuscripts, associated with two distinct autosomal recessive phenotypes: (1) spondylometaphyseal dysplasia (SMD) with cone-rod dystrophy [65, 66] and (2) partial lipodystrophy (Lipodystrophies) [67]. (1) SMD with cone-rod dystrophy, reported in 12 patients, causes postnatal growth deficiency, short stature with platyspondyly and shortening of all tubular bones, rhizomelia and bowing of the long bones of the legs. Patients had progressive early-onset visual impairment, related to pigmentary maculopathy and cone-rod dystrophy. An apparently-isolated infantile-onset retinal dystrophy has been described in three patients from two Italian families, clinically resembling Leber congenital amaurosis [68]. (2) Two unrelated females with *PCYT1A* mutations presented partial lipodystrophy, but no skeletal or visual abnormalities [67]. CCTα is the rate-limiting enzyme in the main synthetic pathway of PC. The disease mechanisms are unknown although PLs are important for mineralization because their anionic phosphate binds calcium. Low HDL cholesterol levels were seen in both clinical forms. Diagnosis is by molecular analysis.

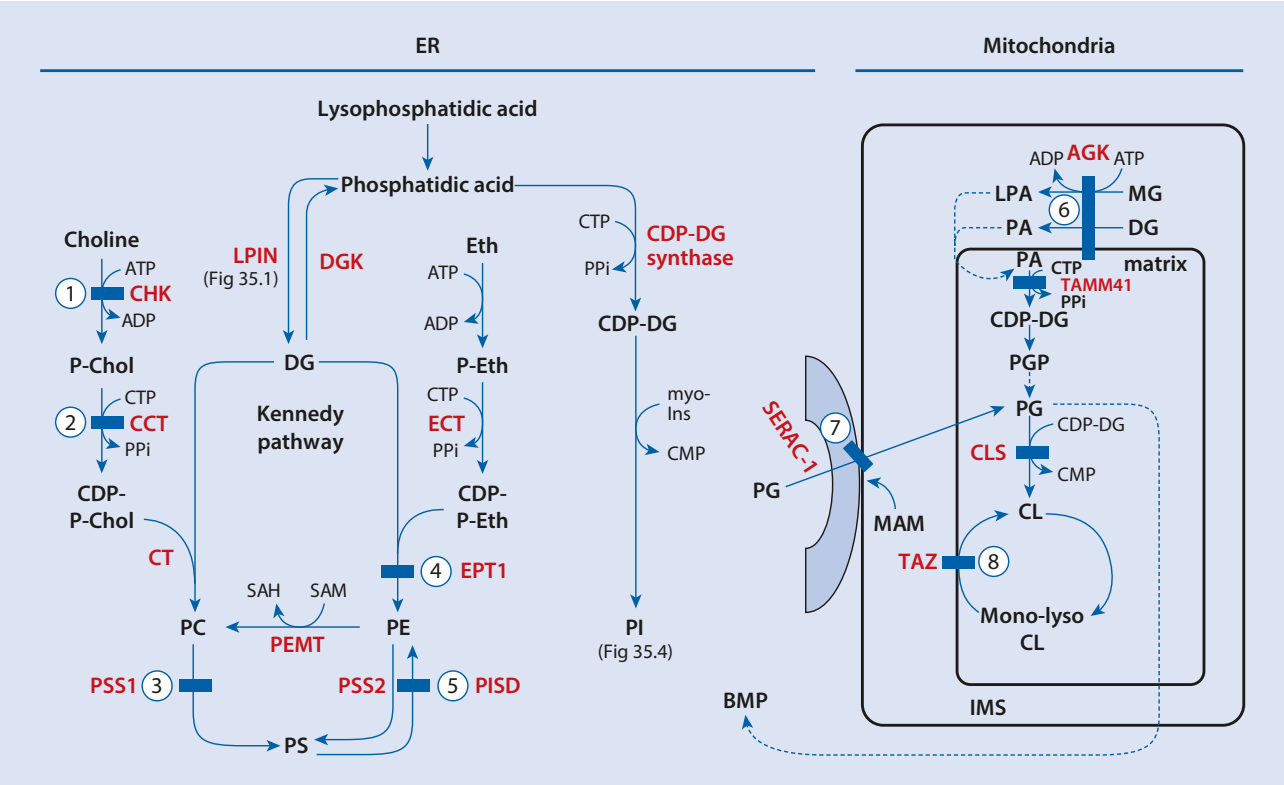

Fig. 35.2 Phospholipid biosynthesis. Phospholipids (PL) are dynamic components of cell membranes with many roles in cell physiology [58, 59]. The four most abundant PLs [58] are phosphatidylcholine (PC, about 50% in most membranes), phosphatidylethanolamine (PE, ~20%), phosphatidylserine (PS, ~5%) and phosphatidylinositol (PI, 6–9%); the mitochondrial inner membrane differs, most strikingly by the presence of cardiolipin (CL, ~16%) and high abundance of PE. **Endoplasmic reticulum (ER)**. *De novo* biosynthesis of PC requires the phosphorylation of choline into phosphocholine (P-Chol), catalysed by choline kinase (CHK). Using cytidine-triphosphate (CTP), P-Chol is converted into CDP-phosphocholine (CDP-P-Chol) by phosphocholine cytidylyltransferase (CCT), rate limiting for PC synthesis. The final step of PC biosynthesis is catalysed by choline transferase (CT), by which choline is transferred from CDP-P-Chol to DG. PC can also be synthesised by methylation of PE by phosphatidylethanolamine N-methyltransferase (PEMT). *De novo* biosynthesis of PE can occur by analogous reactions catalysed by different enzymes including ethanolamine phosphotransferase (EPT1), located in the Golgi. This sequence, using DGs and CDP derivatives of the head group, is termed the "Kennedy pathway". CEPT1 (not shown), located in ER and nucleus, catalyses the last reaction for both PC and PE synthesis [60]. PE can also be synthesised from PS by phosphatidylserine decarboxylase (PISD). PISD is a mitochondrial enzyme shown here for simplicity with other enzymes acting on PS. PS is not synthesised *de novo,* but rather from PC or PE, by substitution of their head group by serine, catalysed by PSS1 or PSS2, respectively. Phosphatidic acid (PA) and lysophosphatidic acid (LPA) are mainly produced as in ◘ Fig. 35.1. **Mitochondria**. Some steps of PL metabolism proceed only in mitochondria and fluxes of PLs among the mitochondrial membranes and ER occur by mechanisms still under study at regions of closeness such as the mitochondria-associated membrane (MAM) of the ER [61]. PA and LPA are synthesised in mitochondria by acylglycerol kinase (AGK). A mitochondrial protein import role of AGK has been recently discovered [62]. CDP-diacylglycerol (CDP-DG) is converted in mitochondria to phosphatidylglycerophosphate (PGP) and then into phosphatidylglycerol (PG). SERAC1, located in the MAM, is essential for remodelling and transport of PG. PG is a precursor of bis(monoacylglycero)-phosphate (BMP), an important PL of late endosomes, and of cardiolipin (CL). The final step of CL synthesis is condensation of PG with CDP-DG, catalysed by cardiolipin synthase (CLS). CL formation and maintenance in a mature and symmetric conformation requires remodelling enzymes including monolysocardiolipin acyltransferase (gene *TAZ*)

35.3.3 Phosphatidylserine Synthase 1 (PSS1, *PTDSS1*) Gain of Function

◘ Figure 35.2, step 3

Heterozygous *PTDSS1* mutations cause Lenz-Majewski syndrome (Lenz-Majewski hyperostotic dwarfism) [69]. The 10 reported patients have sclerosing bone dysplasia with brachydactyly and symphalangism. Patients have a progeroid appearance and intellectual disability. Severe growth retardation and cutis laxa are present at birth [70]. Hyperostosis is progressive, affecting the cranium, vertebrae, and diaphyses of tubular bones. Patients should be evaluated for hydrocephalus, which is frequent and treatable. To date, all known mutations were *de novo* and all impaired product inhibition by PS, activating phosphatidylserine syn-

thase 1 (PSS1). PS is important for bone mineralization, binding calcium within matrix vesicles and enhancing hydroxyapatite crystal formation [71]. Furthermore, these *PTDSS1* mutations impair the metabolism of PI 4-phosphate [72]. Interestingly, quantitative urine amino acids in one Lenz-Majewski patient showed a six-fold increase of phosphoserine [73]. If confirmed in other patients, urinary phosphoserine may become a useful marker for this disease. Diagnosis is molecular. Of note, although mutations to date have all been *de novo*, a small risk of parental germinal mosaicism exists and future pregnancies of each parent should be followed.

35.3.4 Ethanolamine Phosphotransferase (EPT1, *SELENOI*) Deficiency

Figure 35.2, step 4

The first human disorder due to defective CDP-ethanolamine biosynthesis linked to mutation in *SELENOI* was reported in four Omani children aged 19 months to 15 years [74]. One additional 4-year-old subject has been reported since the first description [75]. Subjects presented a complex phenotype, classified as a complicated form of hereditary spastic paraplegia (SPG81). Onset was in infancy/early childhood with motor developmental delay then regression, and progressive spastic paraplegia. All patients had mild, apparently non-progressive intellectual impairment. Language delay was present, later complicated by dysarthria. Variable features included microcephaly, seizures, bifid uvula with or without cleft palate and retinitis pigmentosa [74]. *SELENOI* encodes ethanolamine phosphotransferase (EPT1), also called selenoprotein 1. Two pathways can produce PE: the PS decarboxylation pathway (▶ Sect. 35.3.5) and the Kennedy pathway. The final step of the Kennedy pathway can be catalysed by EPT1 or CEPT1, which transfer phosphoethanolamine from CDP-ethanolamine to DG. EPT1, located in the Golgi apparatus, is involved in the synthesis of long chain PE. PE plays an important role in membrane fusion, properties conferred by the shape of PE and its ability to form reverse non-lamellar structures [76].

35.3.5 Phosphatidylserine Decarboxylase (*PISD*) Deficiency

Figure 35.2, step 5

Mutations in *PISD* were reported in two remotely related Indian children [77] and five patients of Portuguese ancestry (Liberfarb syndrome) [78]. Patients had low birth length and progressively developed severe short stature (−4 to −9 SD) due to a spondyloepimetaphyseal dysplasia producing marked kyphoscoliosis and associated with joint hyperlaxity with multiple dislocations (hip, knee, elbow). The Portuguese patients had progressive acquired microcephaly with mild to moderate intellectual disability (IQ 46 to 70), chorioretinal degeneration and sensorineural hearing loss. MRI showed bilateral optic nerve atrophy and cerebellar atrophy.

PISD encodes a PS decarboxylase that decarboxylates PS to PE in mitochondria. In addition to EPT1 (▶ Sect. 35.3.4, Golgi) and CEPT1 (ER/nucleus), this is a third pathway of PE biosynthesis. PE levels are high in the inner mitochondrial membrane [79], and are essential for mitochondrial fusion and biogenesis. Cultured *PISD*-deficient fibroblasts from humans and mice showed fragmented mitochondria [77, 80]. The Indian patients were homozygous for a point mutation and the Portuguese patients, for an intronic deletion felt to affect splicing.

35.3.6 Acylglycerol Kinase (AGK) Deficiency: Sengers Syndrome

Figure 35.2, step 6

Sengers syndrome [81] is an autosomal recessive mitochondrial disorder associated with cataracts. Four forms occur: (1) late onset with hypertrophic cardiomyopathy, congenital cataract, skeletal myopathy, exercise intolerance, hyperlactacidemia and normal mental development, with survival until the fourth or fifth decade and death principally from cardiomyopathy [82, 83]; (2) fatal neonatal-onset encephalomyopathy including abnormal basal ganglia, brainstem and cerebellar hypoplasia as well as cortical infarction; (3) fatal neonatal liver dysfunction [84]; and (4) isolated cataract [85]. Of 29 patients reviewed [83], 17 died (59%), 15 between 2 days and 11 months of age; tachydyspnoea and elevated lactate, presumably reflecting poor cardiac and mitochondrial function, correlated with high risk of death.

AGK encodes mitochondrial acylglycerol kinase [82], localized to the mitochondrial intermembrane space. AGK phosphorylates MG and DG to produce LPA and PA, respectively. LPA and PA are substrates for mitochondrial PL biosynthesis [82]. AGK is a subunit of the TIM22 complex for import of transmembrane proteins, that may explain the ANT1 deficiency observed in Sengers syndrome [62].

Plasma and urinary lactate are usually elevated except in late onset patients [83]. Urinary 3-methylglutaconic acid is high in 70% of patients [83]. Lipids and glycogen frequently accumulate in heart and skeletal muscle. Various respiratory chain deficien-

cies occur in biopsied muscle; complex I deficiency is a constant finding in myocardium [82]. Genotype-phenotype correlations have been suggested [83], with homozygous nonsense mutations in infantile Sengers syndrome and one or more splice site or start codon variants in milder forms. No effective mechanism-based treatment is available. Supportive treatments like cataract surgery and heart transplantation can be useful.

35.3.7 *SERAC1* Mutations: MEGDEL Syndrome

Figure 35.2, step 7

"MEGDEL" describes an autosomal recessive syndrome with 3-methylglutaconic aciduria, sensorineural deafness, encephalopathy and Leigh syndrome. Patients can have hepatic involvement, ranging from neonatal hypoglycaemia, transient cholestasis to fulminant hepatic failure ("MEGD(H)EL" [86]). During the first year of life, failure to thrive and truncal hypotonia occur and by 2 years of age, deafness, dystonia, spasticity, psychomotor delay, and/or a loss of acquired skills often develop. Most patients have a homogeneous clinical phenotype, with severe neonatal liver dysfunction with hypoglycaemia (48%), followed by muscular hypotonia (91%), progressive spasticity and dystonia (82%) with orofacial dyskinesia in 58%, moderate to severe intellectual disabilities (88%) with 58% nonverbal, bilateral basal ganglia and putamen involvement on cerebral MRI (98%) and optic atrophy (25%). Epilepsy can begin in the neonatal period or later [87]. Milder phenotypes occur, with juvenile slowly progressive complex spastic paraplegia and non-progressive mild cognitive deficit [88].

MEGDEL is caused by mutations in *SERAC1* [89]. The SERAC1 protein is at the interface between the ER and mitochondria (mitochondria-associated membrane, MAM), and is felt to be involved in the remodelling of phosphatidylglycerol 36:1 (PG36:1), a precursor for bis(monoacylglycerol) phosphate (BMP). In fibroblasts with mutations in *SERAC1*, the level of PG34:1 is high and PG36:1 and BMP are low, with accumulations of free intracellular cholesterol producing a positive filipin test and abnormal CL species in mitochondria [90]. The diagnosis can be suspected clinically and by elevated urinary 3-methylglutaconic acid or abnormal CL species in cultured fibroblasts. In one cohort, 61/62 patients (98%) had increased 3-methylglutaconic acid [87]. Diagnosis is by molecular testing of *SERAC1*.

35.3.8 Cardiolipin Remodelling Enzyme (*TAZ*) Deficiency: Barth Syndrome

Figure 35.2, step 8

Barth syndrome, an X-linked recessive mitochondrial disorder, classically presents with cardiomyopathy, skeletal muscle weakness, neutropenia, growth retardation [91], elevated urinary 3-methylglutaconic acid and hypocholesterolemia [92]. There is marked variability in severity, ranging from pregnancy loss of affected male foetuses or neonatal death to isolated mild cognitive defects in about half of patients [93]. Cardiomyopathy is the greatest threat to health, presenting either as biventricular dilatation or as left-ventricular non-compaction occurring before 1 year of life. Episodes of sudden cardiac deterioration are common, often followed by unexplained remissions. Cardiac function often improves in children and adolescents but adults can become symptomatic, about 13% requiring an implantable defibrillator for ventricular arrhythmia [94].

Barth syndrome is due to mutations in *TAZ* (encoding tafazzin) on chromosome Xq28, causing abnormalities in the mitochondrial PL, cardiolipin (CL). After synthesis, CL acyl chains are remodelled to achieve their final mature composition. *TAZ* encodes monolysocardiolipin acyltransferase-1 (MLCLAT-1), essential for CL remodelling and maintenance. While acyltransferases typically use an activated acyl-CoA as the acyl donor, tafazzin uses a FA linked to a PL producing a symmetrical reaction. In Barth syndrome, mitochondrial CL levels are low, with accumulation of monolysocardiolipin [95]. No genotype-phenotype correlation is described [96]. Barth syndrome was previously classified as 3-methylglutaconic aciduria type II with normal 3-methylglutaconyl-CoA hydratase activity (see ▶ Chap. 18). Urinary 3-methylglutaconic and 3-methylglutaric acid levels are normal in some patients [90], but elevated levels should raise suspicion of Barth syndrome. Cells from patients, including lymphocytes, fibroblasts and muscle, show a high monolysocardiolipin/cardiolipin ratio (MLCL/CL). A rapid screening method that measures this ratio in bloodspots by tandem mass spectrometry is specific, sensitive and clinically available [97]. Diagnosis is confirmed by molecular analysis of *TAZ*. No specific treatment is available. Prognosis is highly variable. Survival depends primarily on heart function. Cardiomyopathy may respond to medical therapy but some patients require heart transplantation. Granulocyte colony-stimulating factor treatment can improve neutropenia. Carrier females are asymptomatic unless a chromosome abnormality inactivates their normal allele [98].

35.4 Inborn Errors of Phospholipid Remodelling

Figure 35.3

35.4.1 α/β Hydrolase Domain-Containing Protein 12 (ABHD12) Deficiency

Figure 35.3, step 1

PHARC (polyneuropathy, hearing loss, ataxia, retinitis pigmentosa and cataract) syndrome is an autosomal recessive neurodegenerative disease [100]. Patients may present during adolescence with slowly progressive cataracts, hearing loss and demyelinating peripheral neuropathy. The presence and severity of ataxia are variable. Retinitis pigmentosa with cone-rod dysfunction typically develops in the second or third decade. Some families with PHARC show earlier onset. Behaviour was normal in adult patients. Cerebral cortical function is generally spared, although single patients with intellectual disability and myoclonic seizures are reported. Most patients display cerebellar atrophy on brain imaging [101]. *ABHD12* mutations have also been found in patients with non-syndromic retinitis pigmentosa or Usher syndrome [102]. ABDH12, a serine hydrolase, cleaves the endocannabinoid 2-arachidonoyl glycerol (2-AG), releasing arachidonic acid. It also hydrolyses lysoPS, with preference for molecules with very long chain FAs at the *sn*-2 position [103]. Intriguing observations hint at possible pathogenic mechanisms in PHARC. 2-AG and N-arachidonoylethanolamine (anandamide) are endogenous ligands of cannabinoid receptors CB1 and CB2, which mediate many physiological functions [104]. Clinically, PHARC syndrome resembles Refsum disease, but patients have normal phytanic and pristanic acid levels in plasma and normal peroxisomal function including α-oxidation. Also, the combination of retinitis pigmentosa and ataxia can resemble spinocerebellar ataxia type 7 (SCA7) and mitochondrial NARP syndrome. There is no known biochemical marker for PHARC syndrome. Diagnosis relies upon molecular analysis [105].

35.4.2 Phospholipase A_2 (PLA2G6, *PNPLA9*) Deficiency

Figure 35.3, step 2

PLA2G6-associated neurodegeneration (PLAN) [106] is a neurodegeneration involving upper and lower neurons, the cerebellum, the basal ganglia especially the globi pallidi, in which iron may accumulate, and the optic nerve. One or more of these may dominate the clinical presentation, and the diagnostic search may begin from one of many neurological categories (neuroaxonal dystrophy, dystonia-parkinsonism, and recently, pontocerebellar hypoplasia). The three main presentations are as follows. (1) Infantile neuroaxonal dystrophy (INAD) begins between 6–36 months with psychomotor regression or delay, hypotonia, then progressive spasticity, cognitive decline, visual impairment and death before 10 years. Seizures are not reported but electroencephalography shows characteristic high-voltage fast rhythms [107]. (2) Atypical NAD has later onset and greater variability. It may first resemble non-specific stable mild encephalopathy/cerebral palsy, with unsteady gait, ataxia, speech delay or autistic features,

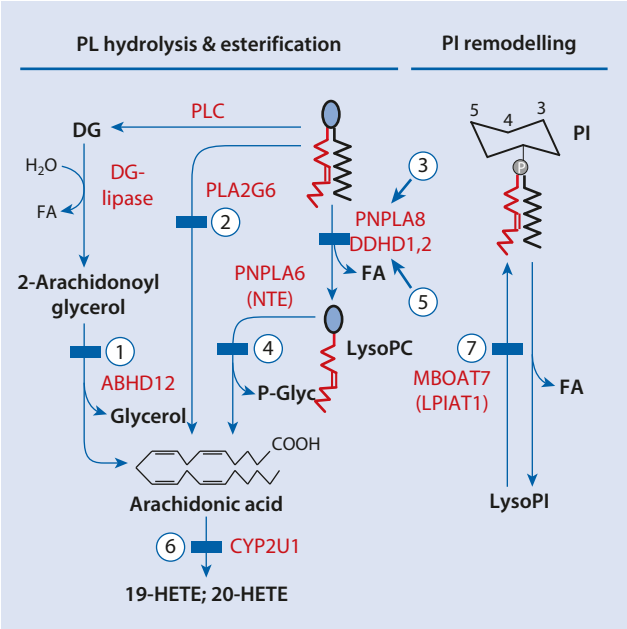

Fig. 35.3 Phospholipid hydrolysis and re-esterification. PLs can produce bioactive lipids, released from membranes after PL cleavage by large groups of phospholipases. For instance, as illustrated in this figure by a PC molecule, arachidonate-containing PLs can be cleaved by phospholipase C (PLC) to DG, then cleaved by DG lipase into 2-arachidonoylglycerol, which can be hydrolysed into arachidonic acid by α,β hydrolase containing-domain 12 (ABHD12). Alternatively, PLs can be hydrolysed directly at the *sn*-2 position by a phospholipase A_2 (e.g., PLA2G6), or at *sn*-1 by enzymes with a phospholipase A_1 activity, (e.g., DDHD1, DDHD2; PNPLA8), releasing a FA and a lysoPL. Lysophospholipases, such as PNPLA6 (NTE) then can cleave lysoPLs into phosphoglycerol (P-Glyc) and FA. Arachidonic acid is the precursor for additional bioactive lipids including prostaglandin, thromboxane, leukotriene and eicosanoids (▶ Chap. 42). CYP2U1, a cytochrome P-450, catalyses the formation of 19- and 20-hydroxyeicosatetraenoic acid (19- and 20-HETE), from arachidonic acid [99]. Membrane PLs are continually remodelled by deacylation and reacylation in the "Land's cycle", illustrated on the right side of the figure by LysoPI acyl transferase (LPIAT1, *MBOAT7*), which incorporates a FA, preferentially arachidonic acid, into lysoPI, forming PI. PI is a component of membrane PLs and the precursor of signalling lipid phosphoinositides

attention deficit. Patients then deteriorate between 7 and 12 years. (3) *PLA2G6*-related dystonia-parkinsonism (PARK14, autosomal recessive early-onset parkinsonism, AREP) typically presents in adolescents or young adults, beginning with psychological or walking difficulties and progressing to dystonia (affecting the extremities in particular) and parkinsonism, sometimes with mental decline. In all forms, strabismus, nystagmus, and optic atrophy often occur. In all, cerebral MRI frequently shows T_1 hypointensities in the globi pallidi, substantia nigra and cortex (reflecting iron accumulation), atrophy of the cerebellar vermis and hemispheres and thin corpus callosum [107]. A fourth presentation, infantile- or childhood-onset pontocerebellar hypoplasia (PCH1), associated with anterior horn cell degeneration, was recently described in two siblings with homozygous *PLA2G6* mutations. Cerebellar signs were the first manifestation; they developed neurogenic weakness and psychomotor regression after 1 year of age. Cerebral MRIs showed reduced size of the vermis and cerebellar hemispheres and enlarged fourth ventricle and cisterna magna and no detectable iron accumulation [108]. Neuropathology was a principal diagnostic technique before molecular diagnostics were available. Characteristic spheroids occur throughout the brain [109, 110] and can be seen in axons on biopsies of conjunctiva, skin, rectum, muscle, or sural nerve; they can be absent in patients presenting with dystonia/parkinsonism.

PLA_2 is also known as $iPLA_2\beta$ and as patatin domain containing protein-9 (gene, *PNPLA9*) [111, 112]. PLA_2 can hydrolyse PLs at the *sn*-2 position, releasing arachidonic or docosahexaenoic acid (DHA) and a lysoPL. These products serve numerous functions, including PL remodelling and release of bioactive lipids that serve in cell signalling and membrane remodelling. Genetic testing for *PLA2G6* mutations is the preferred diagnostic technique. Mutations causing INAD/ANAD result in loss of PLA2G6-mediated FA release. In contrast, the mutations responsible for DP and AREP do not appear to decrease catalytic function, but may modify substrate preferences or regulatory mechanisms for PLA2G6 [113].

35.4.3 Mitochondrial Calcium Independent Phospholipase $A_2\gamma$ ($iPLA_2\gamma$, *PNPLA8*)

Figure 35.3, step 3

PNPLA8 mutations were first described in a 2-year-old girl with progressive muscle weakness, dystonia, seizures, poor weight gain and hyperlactacidemia, suspected to have mitochondrial myopathy [114]. Two other unrelated patients were reported with biallelic loss-of-function *PNPLA8* mutations. One had microcephaly, spasticity and seizures at birth. The second was normal until 1 year then showed neuro-regression, focal epilepsy, progressive weakness and involuntary movements [115]. *PNPLA8* encodes the mitochondrial calcium-independent phospholipase $A_2\gamma$ ($iPLA_2\gamma$), the predominant phospholipase of the inner mitochondrial membrane. Its preferred substrates are PC and PE that contain arachidonate at the *sn*-2 position, producing 2-arachidonoyl-lysoPC and 2-arachidonoyl-lysoPE, precursors of leukotriene and eicosanoid synthesis by lipooxygenases [116].

35.4.4 Deficiency of Neuropathy Target Esterase (NTE, *PNPLA6*)

Figure 35.3, step 4

Neuropathy target esterase (NTE), encoded by *PNPLA6*, was initially identified as the target that is irreversibly inhibited by organophosphorus compound intoxication, causing organophosphate-induced delayed neuropathy. *PNPLA6* mutations cause several autosomal recessive conditions initially felt to be unrelated, such as childhood-onset progressive spastic paraplegia (HSP), peripheral neuropathy and distal muscle wasting designated SPG type 39 [117]. Four neuroendocrine syndromes with hypogonadotrophic hypogonadism (HH) also result from *PNPLA6* mutations: Boucher-Neuhauser (BNHS), Gordon Holmes (GHS) [118], Oliver-McFarlane and Laurence-Moon syndromes [119]. The first two show HH plus spinocerebellar ataxia beginning between the second and fourth decades, with chorioretinal dystrophy detected between the first and the sixth decades, and cerebellar atrophy and small pituitary on MRI (BNHS), or HH with progressive cognitive decline, dementia and variable adult-onset movement disorders (GHS). Both Oliver-McFarlane and Laurence-Moon syndromes have chorioretinal atrophy, typically noted before 5 years of age, deficiencies of multiple pituitary hormones (growth hormone, thyroid-stimulating hormone, with HH in nearly all patients) and spinocerebellar involvement in half. Oliver-McFarlane but not Laurence-Moon syndrome patients also have trichomegaly (long eyelashes), possibly with bushy eyebrows [119].

NTE, a serine hydrolase with phospholipase B activity, can hydrolyse PLs, particularly membrane lysoPC, generating glycerophosphocholine and FA [120]. This reaction is felt to be important for normal vesicular trafficking and release, which may explain the wide range of multisystemic symptoms associated with *PNPLA6* mutations. There is no specific treatment although

symptomatic measures like hormone replacement are of benefit. Diagnosis is by molecular testing.

35.4.5 *DDHD1* and *DDHD2* Mutations

Figure 35.3, step 5

DDHD1 and *DDHD2* each encode DDHD domain-containing A_1 type phospholipases although their substrate specificities differ. A_1 type phospholipases are serine hydrolases able to cleave the *sn*-1 acyl group of PLs. Deficiencies of DDHD1 and DDHD2 each produce autosomal recessive hereditary spastic paraplegias (HSP), often associated with other neurological signs. Few patients are described and phenotypic spectra are not established. Diagnosis is by molecular genetic testing of *DDHD1* and *DDHD2*. There is no specific treatment for either condition; symptomatic treatments improve quality of life.

35.4.5.1 *DDHD1* Mutations (SPG28)

DDHD1 mutations were first identified in three families with adolescent-onset isolated HSP, classified as SPG28 [121]. Patients reported later showed widespread neurological symptomatology, including axonal neuropathy with distal sensory loss, cerebellar oculomotor disturbance with saccadic eye pursuit [122], retinal dystrophy, thin corpus callosum and brain iron accumulation (NBIA) [123].

DDHD1 has been designated as a phosphatidic acid preferring phospholipase A_1, and recently was suggested to hydrolyse PI and PS at the *sn*-1 position, producing lysoPI and lysoPS [124].

35.4.5.2 *DDHD2* Mutations (SPG54)

DDHD2 mutations can be associated with very early onset progressive and complex HSP, classified as SPG54 [125]. Among eight patients with *DDHD2* mutations, six presented with congenital or infantile HSP and two showed onset of HSP in the fourth decade [126]. In early onset SPG54, the onset of HSP is accompanied by or preceded by intellectual and motor delay [125], cerebellar ataxia in 90% of patients, and abnormal eye movement in 50%. Neuroimaging reveals optic nerve hypoplasia, with marked thinning of the corpus callosum (90%), subtle periventricular white-matter hyperintensities on T_2-weighted MRI images (70%) and an abnormal lipid peak on cerebral proton magnetic resonance spectroscopy in 90% of cases. Of note, cognitive function has been normal in patients with adult onset [126]. Another clinical pattern described recently is midlife-onset, slowly progressive HSP with cerebellar ataxia but normal intellectual abilities [127].

DDHD2 can hydrolyse a range of PL substrates in vitro. Recent experiments suggest that it may act as a triglyceride hydrolase in the CNS [124, 128].

35.4.6 *CYP2U1* Mutations (SPG56)

Figure 35.3, step 6

Patients with *CYP2U1* mutations have presented with autosomal recessive early-onset (birth to 8 years) HSP, frequently involving the upper limbs and sometimes associated with dystonic postures or cognitive alterations [121]. Degenerative pigmentary maculopathy was reported in three patients with HSP [129]. On brain MRI, thinning of the corpus callosum and delayed myelination with white matter lesions occur in some patients [130]. Globus pallidus hypointensities on T_1-weighted cerebral MRI are reported, corresponding to areas of calcification. Spinal cord MRI revealed hydromyelia of the thoracic spinal cord in one patient [131]. Clinical severity varied widely even within families, with no obvious genotype-phenotype relationship. For example in one family, two patients never walked, while a third was fully autonomous in his fourth decade but had difficulty with running [121]. Initially classified as SPG49 [121], this condition is now designated SPG56.

CYP2U1 encodes CYP2, a cytochrome P450 enzyme. Cytochromes P450 act in the tissue-specific conversion of substrates into locally active compounds, including arachidonic acid derivatives. CYP2U1 is a brain specific enzyme involved in ω1- and ω2- hydroxylation of arachidonic acid and related long-chain FAs, producing 19- and 20-hydroxyeicosatetraenoic (HETE) acids. Structural abnormalities of the mitochondrial membrane were observed in patient cells. *CYP2U1* mutation should be suspected in HSP patients with basal ganglia calcifications. Definitive diagnosis is by molecular genetic testing of *CYP2U1*.

35.4.7 Lysophosphatidylinositol Acyltransferase (LPIAT1, *MBOAT7*) Deficiency

Figure 35.3, step 7

Over 30 patients are reported with mutations in *MBOAT7* encoding lysophosphatidylinositol acyltransferase (LPIAT1) [132–134]. Clinically, patients present moderate to severe intellectual disability, epilepsy and autistic features, with hypotonia. Cerebral MRI can be normal but cerebellar atrophy involving the folia, thin corpus callosum, enlargement of perivascular spaces and hyperintensity of the globi pallidi and dentate nuclei on T_2-weighted MRI are reported. A common genetic variant of *MBOAT7* (*rs641738*) has been associated with increased risk of fatty liver disease and alcoholic liver cirrhosis [135, 136], suggesting that MBOAT7 might regulate hepatic fat accumulation and the whole body adiposity.

MBOAT7 is a lysophosphatidylinositol acyltransferase 1 (LPIAT1) belonging to the membrane bound O-acyltransferase family. LPIAT1 participates in the "Lands' Cycle" of PL acyl-chain remodelling of the membranes, through sequential deacylation and reacylation reactions. It catalyses a desaturation of the second acyl-chain of PLs and specifically transfers a PUFA, in form of acyl-CoA to lysoPI and other lysoPLs. Arachidonoyl-CoA is its preferred substrate. MBOAT7 thus contributes to levels of free arachidonic acid, a potent trigger for inflammation due to eicosanoid production from arachidonate [137].

35.5 Inborn Errors of Phosphoinositide Phosphorylation

Figure 35.4 and Table 35.1

Phosphatidylinositol (PI) biosynthesis occurs in the ER from CDP-DG, catalysed by PI synthase. Glycosylphosphatidylinositol (GPI) is a glycosylated PI, which anchors a plethora of proteins to the cell surface (▶ Chap. 43). Moreover, PI is a source of highly bioactive phosphoinositide (PItd) molecules that account for about 10–15% of cell PLs. PItds are phosphorylated derivatives of PI generated by different kinases and phosphatases [138, 139]. Phosphorylation at one or more hydroxyl groups, at positions 3, 4 and 5 of the inositol ring, defines the seven known PItds: PI-3P, PI-4P, PI-5P, PI-3,4P_2, PI-3,5P_2, PI-4,5P_2, and PI-3,4,5P_3 (Fig. 35.4). Use of different acyl chains produces further complexity, resulting in over 75 different PItds species [140].

PI and PItds are located on the cytoplasmic membrane leaflet of ER, Golgi, endosomes, lysosomes and nucleus. Different PItds are enriched at specific locations; multiple cellular stimuli may alter PItds metabolism, modifying the cellular location or activity level of the enzymes involved [141]. PItds act as second messengers, interacting with several protein domains to recruit and activate protein complexes involved in receptor signalling, secretion, endocytosis, ion channel regulation, intracellular vesicular trafficking, cytoskeletal organization and apoptosis [141, 142] (▶ Chap. 44). The

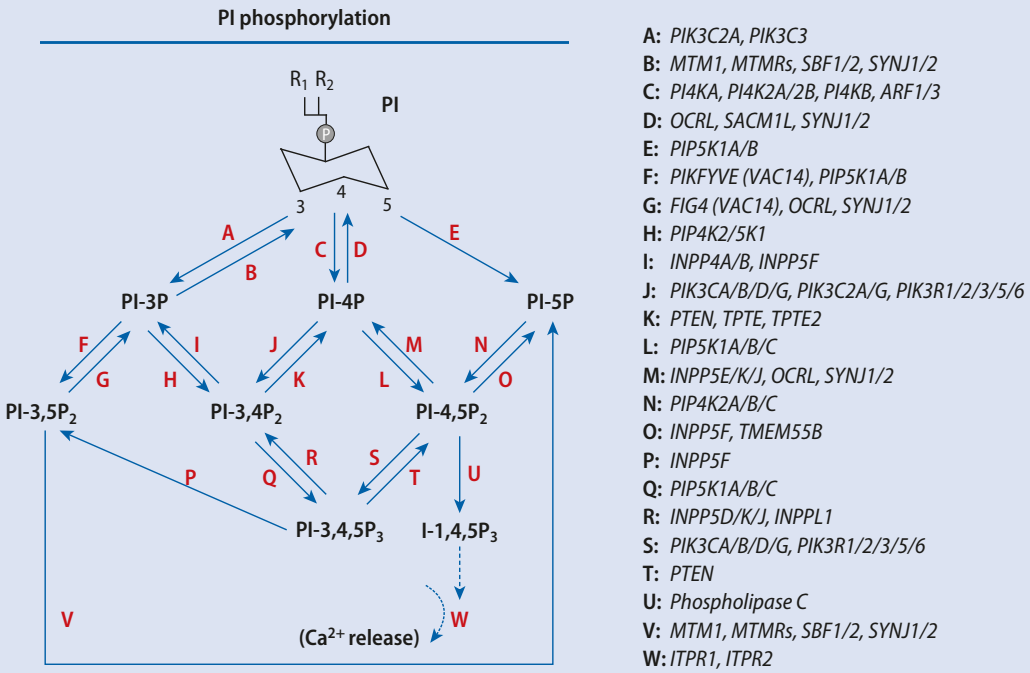

Fig. 35.4 Phosphoinositide metabolism. Phosphatidylinositol (PI) is a membrane PL composed of DG and a D-*myo*-inositol head group. Most typically, phosphoinositide acyl chains are stearic (C18:0) and arachidonic (C20:4) acids in the *sn*-1 and *sn*-2 positions respectively. Arachidonate-rich phosphoinositides are believed to be one source of PLA_2-mediated arachidonate release for the synthesis of prostaglandins and leukotrienes. PI can be phosphorylated and dephosphorylated by several kinases (downward arrows) and phosphatases (upward arrows). Phosphorylation of the inositol ring to form phosphoinositides (PItds) occurs only at positions 3, 4 and 5. Seven PItds are possible, containing one, two or three phosphates (PI-3P, PI-4P, PI-5P, etc). PItd metabolism differs among cellular compartments (ER, Golgi, endosome, etc). The same reaction may involve different enzymes in different organelles. Some enzymes have multiple substrates. PItd degradation by phospholipase C (U) produces inositol-1,4,5-triphosphate (I-1,4,5P_3). I-1,4,5P_3 interacts (dashed arrow) with various receptors, including ITPR1 and ITPR2 (W), involved in calcium release (dotted arrow) from the ER

Table 35.1 Disorders of phosphoinositide phosphorylation

Gene	Disorder	Inh	O/T	Im	N	Dv	M	Sk	K	Ey	C
PIK3CA	PIK3CA-related overgrowth spectrum	So	+		+			+			+
PIK3CD	Activated PI3Kδ S 1	AD	+	+							
	PI3Kδ deficiency S	AR		+							
PIK3R1	Activated PI3Kδ S 2	AD	+	+							
	SHORT S	AD				+		+		+	+
	Agammaglobulinemia	AR		+							
PIK3R2	Megalencephaly ± polymicrogyria	AD, So	+		+						
PIK3R5	Ataxia with oculomotor apraxia	AR			+						
PIK3C2A	Oculocerebrodental S	AR			+	+		+	+	+	
PI4K2A	ID, epilepsy, myoclonus & akathisia S	AR			+	+					
	PI4K2A-related cutis laxa S	AR			+	+					+
PI4KA	Hypomyelinating leucodystrophy	AR		+	+	+			+		
PIKFYVE	Fleck corneal dystrophy	AD								+	
PIP5K1C	Lethal contractural S 3	AR					+				
PTEN	PTEN-hamartoma tumour S	AD, So	+		+	+					+
FIG4	Yunis-Varón S	AR			+	+		+			
	CMT 4J	AR			+						
	Familial epilepsy with polymicrogyria	AR			+						
	Amyotrophic lateral sclerosis	AD			+						
VAC14	Yunis-Varón S	AR			+	+		+			
	Striatonigral degeneration	AR			+	+				+	
OCRL	Oculocerebrorenal S of Lowe	XL			+	+		+	+	+	
	Dent disease 2	XL							+		
SYNJ1	Atypical juvenile parkinsonism	AR			+						
	Early-onset epileptic encephalopathy	AR			+	+					
MTM1	X-linked centronuclear myopathy	XL				+	+				
MTMR2	CMT 4B1	AR			+						
SBF2	CMT 4B2	AR			+					+	
SBF1	CMT 4B3	AR			+	+					
INPP5E	Joubert S	AR			+	+				+	
INPP5K	CMD, cataracts & ID	AR				+	+				
INPPL1	Opsismodysplasia	AR						+			
PLCB1	Epileptic encephalopathy	AR			+	+					
PLCB3	SMD & corneal dystrophy	AR				+		+		+	
PLCB4	Auriculocondylar S	AD, AR						+			
PLCG2	PLAID / APLAID	AD		+							+
PLCD1	Hereditary leukonychia	AD, AR									+
	Hereditary trichilemmal cysts	AD	+								+

Table 35.1 (continued)

Gene	Disorder	Inh	O/T	Im	N	Dv	M	Sk	K	Ey	C
PLCE1	Steroid-resistant nephrotic S	AR							+		
ITPR1	Gillespie S	AD, AR			+	+				+	
	Spinocerebellar ataxia 15	AD			+						
	Spinocerebellar ataxia 29	AD			+	+					
ITPR2	Generalized isolated anhidrosis	AR									+

(A) PLAID (Auto-immunity) PLCG2-associated antibody deficiency and immune dysregulation, *CMD* congenital muscular dystrophy, *CMT* Charcot-Marie-Tooth, *ID* intellectual deficiency, *S* syndrome, *SHORT* short stature (S), hyperextensibility of joints, and/or inguinal hernia (H), ocular depression (O), Rieger anomaly (R), and teething delay (T); *SMD* spondylometaphyseal dysplasia, *Inh* inheritance, *AD* autosomal dominant, *AR* autosomal recessive, *So* somatic, *XL* X-linked, *O/T* overgrowth/tumours, *Im* immune, *N* neurologic, *Dv* development, *M* muscular, *Sk* skeletal, *K* kidney, *Ey* eye, *C* cutaneous

two main routes of PItd degradation are dephosphorylation by PItd phosphatases and hydrolysis by PI-specific phospholipase C (PLC). PLC-mediated cleavage of PI-4,5P_2 produces inositol 1,4,5-triphosphate which stimulates calcium release in the ER, another mechanism of inositol action [143].

Inborn errors of phosphoinositide metabolism (Table 35.1) create localized imbalances of intracellular membrane lipids [144, 145]. Highly diverse clinically, their commonest features involve overgrowth and tumour formation, immune dysfunction, neurologic defects, intellectual disability, muscular dystrophy, skeletal dysplasia and renal, ophthalmologic and cutaneous abnormalities. Clinically available biochemical assays cannot usually identify these disorders, and diagnosis is usually molecular.

Notes added in proof

PNPLA1, a paralogue of *PNPLA2*, is expressed specifically in keratinocytes. The PNPLA1 protein interacts with CGI-58, and as in CGI-58 deficiency, PNPLA1 deficiency causes autosomal recessive congenital ichthyosis. This is attributed to lack of PNPLA1-mediated fatty acylation and the resulting low level of acylglucosylceramides, essential compounds for skin barrier function (see ► Chap. 40, ► Sect. 40.1.8)

Descriptions of two inborn errors of mitochondrial cardiolipin (CL) synthesis appeared recently. Cytidine diphosphate diacylglycerol (CDP-DAG) is synthesized from phosphatidic acid (PA) and cytosine triphosphate (CTP) by two families of enzymes, CDS1/2 in the ER, which lead to PI synthesis, and TAMM41 in mitochondria (Fig. 35.2). In three patients, deleterious *TAMM41* variants caused autosomal recessive neonatal lethargy and hypotonia, with developmental delay, myopathy and ptosis. Low levels of phosphatidylglycerol and cardiolipin, and decreased levels of subunits from respiratory chain complexes I to V, occurred in muscle but not in fibroblasts [146].

In four patients, mutations in *CRLS1*, encoding cardiolipin synthase 1 (CLS, Fig. 35.2), were associated with autosomal recessive progressive encephalopathy, with variable bull's eye maculopathy, nystagmus, deafness, diabetes insipidus and cardiomyopathy including left ventricular noncompaction in one patient [147]. Three of these patients died between 3-10 months of age and the fourth had microcephaly and severe encephalopathy at age 19 years. Patients' fibroblasts showed high levels of the CLS substrate, phosphatidylglycerol and low levels of its product, cardiolipin.

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Inborn Errors of Lipoprotein Metabolism Presenting in Childhood

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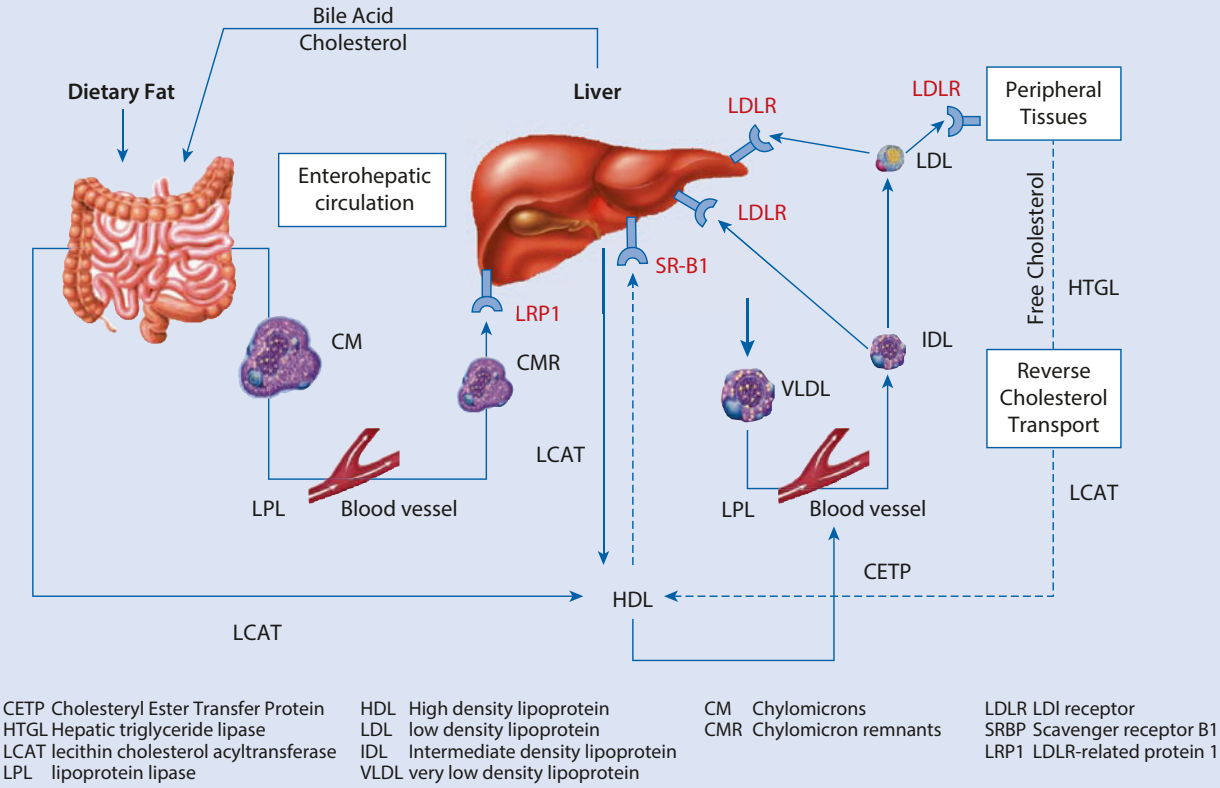

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Fig. 36.1 Schematic diagram of lipid and lipoprotein metabolism. This figure demonstrates five major lipoprotein classes. These are chylomicrons, very low-density lipoprotein (VLDL), intermediate density lipoprotein (IDL), low-density lipoprotein (LDL) and high-density lipoprotein (HDL). Chylomicrons are triglyceride-rich particles produced by the intestine. They are the largest of the lipoproteins. Their primary function is to transport dietary triglyceride and cholesterol from the intestinal lumen to sites of storage or metabolism. Chylomicrons are rapidly cleared and are usually absent after fasting. The clearance of chylomicrons occurs through the action of lipoprotein lipase (LPL), which creates chylomicron remnants. These chylomicron remnants are cleared from the circulation by the liver. These remnants are thought to be atherogenic by damaging the endothelium. (*Adapted from Daniels SR. Pediatric Cardiology 2003*)

Lipid and Lipoprotein Metabolism (Fig. 36.1)

Very low-density lipoprotein (VLDL) particles are relatively large particles, which are produced in the liver. The function of VLDL is to transport endogenously synthesized triglycerides (TG) and cholesterol to the peripheral tissue. Intermediate density lipoproteins (IDL) are created with the metabolism of VLDL by lipoprotein lipase. IDL particles may be removed by the liver or are converted to low-density lipoprotein (LDL) particles by hepatic triglyceride lipase. LDL particles contain ~45% cholesterol and they are the major carrier of cholesterol to peripheral tissues. LDL particles are heterogeneous. Small dense LDL particles have been associated with increased risk for cardiovascular disease. Increased concentrations of small dense LDL particles are associated with male gender and diabetes, particularly in adults. LDL particles are recognized by specific LDL receptors that are highly expressed in the liver. Once LDL particles bind to the receptor they are internalized into the cell. This pathway removes approximately 75% of LDL particles. The remaining LDL particles are removed by macrophages.

High density lipoproteins are produced by the liver and the gastrointestinal tract, as well as by peripheral catabolism of chylomicrons and VLDL particles. HDL particles are also heterogeneous. HDL2 is the subfraction that is associated with protection against atherosclerosis. HDL3 is a smaller particle and increased in alcohol consumption, obesity, diabetes, cigarette smoking, uraemia and hypertriglyceridemia. HDL particles are involved in reverse transport of free cholesterol from peripheral tissues to the liver providing an explanation for the protective effect of HDL particles against atherosclerosis.

Lipoprotein (a) or Lp(a) has also been found to be associated with the atherosclerotic process. The structure of Lp(a) is similar to LDL, but with the addition of a large 'little a' protein bound to apoB via a single cysteine-mediated disulphide bond. Its plasma levels are regulated independently from LDL, and risk of coronary heart disease is greatly increased if both LDL and Lp(a) are elevated. One way Lp(a) may be related to atherosclerosis is because the (a) protein has structural similarities to plasminogen, and it may inhibit the thrombolytic activity of plasminogen.

Introduction

Lipids are highly diverse molecules that are traditionally best known for their role in the formation of biological membranes and cellular systems and as a way to store energy. In the last decade, lipids have taken a more centre stage in apoptosis, cell signaling, inflammation, immunity and inborn errors of metabolism (IEMs). Inborn errors of lipoprotein metabolism are a group of genetic disorders exemplified by changes in plasma lipids due to defects in the protein lipid-carriers (lipoproteins), lipoprotein receptors, or enzymes responsible for the hydrolysis and clearance of lipoprotein-lipid complexes [1]. The proteins responsible for the maintenance of normal plasma and tissue lipids, which are primarily triglycerides and free and esterified cholesterol, include the apolipoproteins A-I, A-II, A-IV, A-V, B, C-I, C-II, C-III, and E with key enzymes including lipoprotein lipase (LPL), hepatic triglyceride lipase (LIPC), lecithin cholesterol acyltransferase (LCAT), and cholesterol ester transfer protein (CETP); and key receptors being the low-density lipoprotein receptor (LDL-R) for LDL-cholesterol (LDL-c), and the ATP-binding cassette transporter 1 (*ABC1A*) for HDL-cholesterol (HDL-c) levels [2, 3]. A number of genetic abnormalities of lipoprotein metabolism have been described in childhood (Lipid and lipoprotein metabolism; Fig. 36.1, Table 36.1).

Plasma Lipid and Lipoprotein Metabolism

The major classes of lipids circulating in plasma are cholesterol, cholesteryl ester, triglycerides and phospholipids. Lipids are important components of many of the body's tissues. They serve as building blocks for hormones and are a vital component of cell membranes (▶ Chap. 35). However, lipids are insoluble in water. Thus, to be transported in the blood stream, they must be packaged into large, complex water-soluble molecules called lipoproteins. The structure of a lipoprotein is made up of a core consisting of cholesteryl ester and triglyceride covered by a polar surface layer consisting of phospholipids, free cholesterol and protein moieties called apolipoproteins. These lipoprotein particles have differing densities, which are determined by the relative content of protein and lipid. The apolipoproteins perform functions related to transport and uptake into cells.

Lipoprotein Disorders Presenting in Childhood

A number of genetic abnormalities that results in dyslipidemia in childhood have been described, of which heterozygous familial hypercholesterolaemia is the most common inherited lipid disorder with a prevalence of roughly 1 in 250 [5]. The responsible genes, inheritance and the observed plasma lipoprotein patterns for lipoprotein disorders manifesting in childhood are listed in Table 36.1.

36.1 Disorders of Low Density Lipoprotein Metabolism

In the majority of these disorders, the atherosclerotic process begins in childhood, and the extent and rate of progression has a direct relationship with increases in lipid levels. Secondary causes of hyperlipidemia, including obesity, hypothyroidism, metabolic syndromes are not discussed in this chapter but form an important differential diagnosis in disorders of lipoprotein metabolism presenting in children.

Whilst healthy lifestyles and a lower saturated fat intake is the cornerstone of treatment of lipid disorders in children, lipid-lowering therapies are becoming increasingly more important, with minimal adverse effects and no short-term effect on growth and development. There are many established therapies and emerging therapies for lipoprotein disorders and these are detailed in Tables 36.2 and 36.3.

There are five known genetic disorders causing elevated LDL-C that are expressed in children and that cause early atherosclerosis and premature coronary artery disease (CAD). These include familial hypercholesterolaemia (FH), familial ligand defective apo-B (FLDB), autosomal recessive hypercholesterolaemia, sitosterolemia, and mutations in proprotein convertase subtilisin-like kexin type 9 (PCSK9). These disorders arise from either gene mutations that affect LDL receptor activity or abnormalities in the LDL receptor itself. The presence of these disorders indicates a significantly elevated risk for premature atherosclerosis and CAD in adulthood. Of these genetic disorders affecting LDL receptor activity, FH is the most common disorder diagnosed in childhood, and usually identified by cascade screening.

Identifying children and adolescents at enhanced risk for atherosclerosis is important for long-term car-

Table 36.1 Monogenic lipoprotein associated disorders presenting in childhood

Disorder	Inheritance	Protein & gene responsible	Observed plasma lipoprotein pattern	Frequency; ethnicity	Key references
Disorders affecting low density lipoprotein metabolism					
AD familial hypercholesterolemia	AD[a]	LDL receptor – *LDLR* (heterozygous mutations) May have additional polygenic component	↑ LDL	1 in 250	[4–6]
AR familial hypercholesterolemia	AR	LDL receptor – *LDLR* (homozygous/compound heterozygous mutations)	↑ LDL	1 in 10^6	[4, 5]
AD familial hypercholesterolemia	AD[a]	Proprotein convertase subtilisin/kexin 9 – *PCSK9*	↑ LDL	In the UK – 2% of mutation positive FH	[4, 5, 7]
Familial ligand-defective apo-B, FLBD	AD[a]	Apo-B – *APOB* May have polygenic component	↑ LDL	Two four common mutations: p.(R3527Q) in Caucasians. In the UK: ~5% of mutation positive FH patients; common allele p.R3527W in East Asians Identical phenotype to LDLR mutation FH	[4, 5, 8, 9]
AD familial hypercholesterolemia	AD	Apolipoprotein E *APOE*. One variant only described, p.(Leu167del)	↑ LDL	Unkown	[10]
AR familial hypercholesterolemia	AR[a]	LDL-receptor adaptor protein 1 – *LDLRAP1* Commonly truncation mutations	↑ LDL	Rare except in Sardinia	[6, 11]
AR familial hypercholesterolemia	AR[a]	Homozygosity for pathogenic lysosomal acid lipase variants– *LIPA*[b]	↑ LDL		[12, 13]
Familial Hypobetalipoproteinemia (FHBL)	AD[a]	Apo-B – *APOB* subjects generally have truncatng mutations. May have polygenic component.	↓ Apo-B lipoproteins (chylomicrons, VLDL, LDL)	Rare	[14–16]
Abetalipoproteinemia	AR[a]	Large sub-unit of microsomal triglyceride transfer protein – *MTTP*	↓ Apo-B lipoproteins, no chylomicrons, ↓ HDL	<1 in 100,000 pan ethnic	[14–16]
Chylomicron retention disorder	AR	Deficiency of a GTPase (Sar1b) –*SAR1B*	↓ apo-B48 lipoproteins (chylomicrons), ↓ HDL	Rare Consanguinity frequent	[17]
Disorders affecting triglyceride metabolism					
Type-I hyperlipidaemia – Familial lipoprotein lipase deficiency	AR[a]	Lipoprotein lipase – *LPL*	↑ Chylomicron, VLDL	Consanguinity 1 in one million (homozygous) Founder effect in French Canadian population in Quebec; carrier frequency 1 in 40	[2, 13]
Type-1 hyperlipidaemia – Apolipoprotein C-II deficiency	AR[a]	Apo-C-II – *APOC2*	↑ Chylomicrons, VLDL	Very rare	[18]

Table 36.1 (continued)

Disorder	Inheritance	Protein & gene responsible	Observed plasma lipoprotein pattern	Frequency; ethnicity	Key references
Type-I hyperlipidaemia – Apo AV deficiency	AR[a]	Apo-A5 – *APOAV*	↑ Chylomicrons, VLDL	Very rare Consanguinity	[19, 20]
Hypertriglyceridaemia	AD[a]	Angiopoietin-like proteins −3 -4 and -5 *ANGPTL3/ANGPTL4/ ANGPTL5*	↑ Chylomicrons, VLDL	Rare	[21]
Type-I hyperlipidaemia	AR[a]	Glycosylphosphatidylinositol-anchored high-density lipoprotein-binding protein 1 – *GP1HBP1*	↑ Chylomicrons, VLDL	Very rare	[22]
Combined lipase deficiency	AR[a]	Lipase maturation factor –*LMF1*	↑ Chylomicrons, VLDL		[2, 13]
Disorders affecting high density lipoprotein					
Familial LCAT deficiency	AR[a]	Lecithin cholesterol acyl transferase – *LCAT*	↓HDL	Rare <100 patients reported	[23, 24]
Sterol storage disorders					
Wolman disease	AR[a]	Lipase A, lysosomal acid, cholesterol esterase – *LIPA*	Dyslipidaemia infrequent: ↑ LDL ↓↓ HDL ↑ to normal TG	1 in 500,000 Null allelic variants with no residual enzyme function	[25]
Cholesterol ester storage disease	AR[a]	Lipase A, lysosomal acid, cholesterol esterase – *LIPA*	↑ LDL ↓↓ HDL ↑ to normal TG	Precise prevalence unknown; 90 to 170,000; German Cohort 1 in 50,000 Nearly all individuals with CESD reported in the published literature are compound heterozygous or homozygous for c.894G>A.	[26]

[a]Clinical phenotypes expressed in childhood
[b]Mutations in *LIPA* are known to cause cholesterol ester storage disease

diovascular health and prevention of early coronary heart disease from both inherited and secondary causes of lipoprotein disorders (Fig. 36.2).

Homozygous FH is a severe and rare genetic disorder of lipid metabolism, resulting in extremely elevated LDL-C levels and increased cardiovascular risk. Although the clinical phenotype of this disease is highly variable, recommended target LDL-C levels are hardly ever reached even with widely used lipid-lowering medication. In these children, early lipoprotein apheresis can provide further time-averaged LDL-C level reductions by up to 48% but this therapy may not be appropriate for all children and there are emerging therapies discussed in Table 36.2 which may be beneficial. Liver transplant is a therapeutic option that should be considered in all children with homozygous FH and appropriate and early assessment is recommended.

Heterozygous FH is more common and early intervention with lipid lowering therapies should be considered by the age of 10 years as is recommended by the UK National Institute for Health and Care Excellence (NICE) guidelines to prevent early onset coronary artery disease.

Dietary and healthy lifestyle measure, with particular emphasis on exercise and avoidance of smoking are the first form of treatment. Monitoring carotid intima media thickness (CIMT) may be a way forward for all children with heterozygous FH to determine the rate of disease

Table 36.2 Lipoprotein disorders presenting in childhood: clinical manifestations and therapies

Disorder	Key manifestations	Therapies		Emerging therapies	Key references
		Diet	Drugs/other		
Autosomal dominant familial hypercholesterolaemia (FH) due to heterozygous *LDLR* mutation	Diagnostic criteria[a] Screening criteria[b] Increased carotid intima-media thickness (CIMT) and distal coronary artery calcification detected by computed tomography (CT) scanning are confirmed markers of early atherogenesis. Coronary calcification is present in 25% of 11–23 year olds with phenotypic heterozygous FH **Laboratory:** lipid profile – elevated total and LDL-C; decreased HDL-C; increased non-HDL-C; lipoprotein 9a) [lp(a)] for risk stratification. For children aged 8–10 years, LDL-C is ideally reduced by 50% from pre-treatment levels. For children aged ≥10 years, especially if there are additional cardiovascular risk factors, including elevated Lp(a), the target LDL-C should be <3.5 mmol/L (130 mg/dL). **Monitoring:** Hepatic aminotransferases, creatine kinase (CK) and creatinine levels should be measured before starting treatment and regularly during statin therapy.	Culturally acceptable heart healthy diets from early childhood. Healthy lifestyle advice from early childhood. Encourage physical activity. Smoking to be strictly discouraged.	Lipid lowering therapies in both boys and girls. Early treatment of FH can reduce LDL-C burden, improve endothelial function, substantially attenuate the progression of atherosclerosis, and improve coronary outcomes. The benefits of LDL-C reduction should be balanced against the long-term risk of treatment side effects	PCSK9 monoclonal antibodies evolocumab and alirocumab	[7, 27–31]
Autosomal dominant familial hypercholesterolaemia heterozygous *PCSK9* mutations	<10% of heterozygous FH are due to *PCSK9* mutations. Management as above.	As above	As above		

Table 36.2 (continued)

Disorder	Key manifestations	Therapies		Emerging therapies	Key references
		Diet	Drugs/other		
Autosomal recessive familial hypercholesterolaemia due to homozygous/compound heterozygous, *LDLR* mutations	Early cardiac features: aortic stenosis and regurgitation; coronary ostial stenosis Angina pectoris, myocardial infarction and death in early childhood have been reported. But first major cardiovascular events usually occur during adolescence, depending on the severity of the mutation.		An aggressive cholesterol-lowering approach should be initiated as soon as possible to prevent or delay the development of CHD: lipoprotein apheresis Extensive cardiovascular evaluation is imperative: paediatric cardiologist assessment and follow up; coronary CT angiography and MRI to evaluate coronary arteries and aorta is recommended. Invasive coronary angiography case by case as indicated. Lipid lowering drugs Liver transplant	**(a)** Oral MTTP inhibitor (Lomitapide) especially for patients intolerant to LDL apheresis (>12 years of age) **(b)** Antisense RNA therapy (Injectable Mipomersin) Both drugs decrease hepatic production of apo B lipoproteins, which are atherogenic (>18 years of age) PCSK9 targeted therapy inhibiting PCSK9 activity and reducing LDL-C especially in LDLR mutations (Evolocumab) Cholesterol transfer protein (CETP) inhibitors Pre-HDL infusion	[32]
Familial ligand-defective apo-B (FLDB)	**Phenotype:** similar to LDLR heterozygous FH with risk of coronary heart disease but less severe. FLDB homozygotes have later onset and less severe CHD than FH homozygotes **Laboratory:** similar to LDLR heterozygous FH with increased LDL	From the age of 8 onwards: 30% of total calories from fat; <10% of total intake of unsaturated fat	Lipid lowering therapies Liver transplant (homozygous FLDB)		[33]
Autosomal recessive familial hypercholesterolaemia	Phenocopy of LDLR homozygous FH but less severe. **Childhood**: 97% developing planar, tuberous, tendon xanthomas; nearly 50% have coronary artery disease; arcus cornea. Aortic valve disease; aortic root disease; ascending atherosclerosis rare and presents later in life Parents usually have normal lipid profile	Low fat diet	Lipid lowering drugs LDL apheresis Liver transplant		[34]

(continued)

Table 36.2 (continued)

Disorder	Key manifestations	Therapies		Emerging therapies	Key references
		Diet	Drugs/other		
Familial hypobetalipoproteinaemia (FHBL)	Heterozygous FHBL: **Infancy:** asymptomatic; **Childhood and adults:** partial fat malabsorption; gallstones in adults; hepatic steatosis and fatty liver. **Homozygous FHBL:** similar to abetalipoproteinaemia **Laboratory:** <5th percentile plasma total cholesterol, LDL cholesterol, triglycerides and total apolipoprotein B; truncated apoB on lipid intake. Homozygous/compound heterozygous FHBL: similar to abetalipoproteinaemia	Low fat diet in homozygous/ compound heterozygous FHBL	Heterozygotes: Vit. E to prevent adult onset neuropathy. Homozygous/ compound heterozygous FHBL: adequate fat soluble vitamins		[35]
Abetalipoproteinaemia	**Infancy:** fat malabsorption; faltering growth; hypocholesterolaemia; ApoB deficiency **Childhood and adults:** atypical retinitis pigmentosa; ataxia; posterior column neuropathy; myopathy; loss of night vision; hepatomegaly; cirrhosis **Laboratory:** acanthocytosis; hepatic steatosis; elevated transaminases; hypocholesterolaemia; apo B deficiency coagulopathy; significant fat soluble vitamin deficiency	Low fat diet	Fat soluble vitamins with high dose vitamin A and E Liver transplant	Microsomal transfer protein (MTTP) inhibitors	[35]
Chylomicron retention disorder	**Infancy:** fat malabsorption with chronic diarrhoea, vomiting and abdominal distension; cardiomyopathy; hepatomegaly; retinopathy; delayed light adaptation; micro-nystagmus; Endoscopy – fat laden enterocytes; **Childhood and adults**: hepatic cirrhosis rare; poor bone mineralisation; areflexia; proprioception abnormalities; ataxia; myopathy; sensory neuropathy; **Laboratory:** hypocholesterolaemia (>50% reduction); normal triglycerides; Vit E deficiency invariable; elevated creatine kinase; macrovesicular hepatic steatosis common; elevated transaminases; essential fatty acid deficiency; normal lipids in parents	Low long chain fat diet	Adequate fat soluble vitamins supplement		[17]
Familial lipoprotein lipase disease (LPL)	**Infancy:** abdominal pain; vomiting; faltering growth; hepatosplenomegaly; eruptive xanthomas; lipaemia retinalis; life threatening acute pancreatitis frequent; fever; hyperviscosity syndromes; intestinal bleeds and nephromegaly are rare. Homozygous LPL: 25% present before 1 year of age and majority are symptomatic by 10 years of age. **Adult:** heterozygous LPL **Laboratory:** lactescent plasma/milky serum appearance; hypertriglyceridaemia (HTG); hypercholesterolaemia (HC); very low HDL-C; hyperchlomicronaemia; normocytic anaemia	Very low fat diet; Medium chain fats used as a source of fat as not reliant on chylomicron formation	Lipid lowering drugs – fibrates as primary therapy Thiazolidinedione drugs (e.g. pioglitazone) Fat soluble vitamins and essential fatty acids Plasmapheresis	Gene therapy – withdrawal of marketing authorisation in Europe in 2017	[13, 36–38]

Table 36.2 (continued)

Disorder	Key manifestations	Therapies		Emerging therapies	Key references
		Diet	Drugs/other		
Apolipoprotein C-II deficiency	Clinical heterogeneity common. **Infancy and childhood:** severe cases have similar phenotype to familial LPL deficiency. **Adults:** persistent hepatosplenomegaly; pancreatitis, eruptive xanthomas. Risk of atherosclerosis rare. **Laboratory**: severe hypertriglyceridemia; hyperchylomicronaemia; raised total cholesterol and VLDL; decreased LDL-C and HDL-C	Severe cases and homozygous mutations: As for familial LPL deficiency	Lipid lowering drugs – fibrates as primary therapy Essential fatty acids Plasmapheresis		[39]
Apo AV deficiency	**Childhood:** recurrent abdominal pain; eruptive xanthomas; hepatosplenomegaly; faltering growth; pancreatitis **Laboratory:** severe hypertriglyceridaemia (>10 mmol/l)	Low fat diet, MCT containing diet ω-3 fatty acid supplements (accelerates triglyceride clearance by increased LPL activity)	Lipid lowering drugs – fibrates as primary therapy Essential fatty acids Plasmapheresis		[40]
Hypertriglyceridaemia (angiopoietin-like proteins (*ANGTL3, −4, −5*)	Clinical presentation: As above **Laboratory:** Suppression of LPL activity; Hypertriglyceridaemia; increased non esterfied fatty acids	Low fat diet, MCT containing diet ω-3 fatty acid supplements	Lipid lowering drugs – fibrates as primary therapy		[21]
Glycosylphosphatidylinositol-anchored high-density lipoprotein-binding protein 1 deficiency (*GPI-HBPI*)	**Later childhood and adults: homozygous GPI-HBPI** relapsing, severe pancreatitis resistant to conventional treatment; early coronary artery disease **Laboratory:** severe triglyceridaemia; hyperchylomicronaemia	Low fat diet, MCT containing diet ω-3 fatty acid supplements	Lipid lowering drugs – fibrates Essential fatty acids Plasmapheresis		[41]
Familial LCAT deficiency (α & β LCAT deficiency) Norum-Gjone (severe form of LCAT deficiency)	**Childhood and adults:** proteinuria; retinal haemorrhage; aneurysmal dilatation of retinal vessels; angioid streaks; corneal opacities; early atherosclerosis; hepatosplenomegaly; end stage renal failure is a common symptom of disease progression **Laboratory:** normocytic anaemia; increased red cell free cholesterol and lecithin; reduced red cell half-life; moderate haemolysis; sea blue histiocytes on bone marrow examination Serum cholesterol ester (<50%); increased serum nonesterified cholesterol; increased serum phospholipids; decreased lysophosphatidylcholine; decreased high-density lipoproteins (HDL); increased triglycerides		Supportive treatment for anaemia and renal disease Corneal transplant		[23, 24]

(continued)

Table 36.2 (continued)

Disorder	Key manifestations	Therapies		Emerging therapies	Key references
		Diet	Drugs/other		
Wolman disease	Rapidly progressive infantile disorder presenting as early as day 1 of life to a few months old, with death in early infancy in untreated infants. Infantile-onset malabsorption. Persistent vomiting, steatorrhoea, abdominal distension. Severe malnutrition/cachexia. Profound growth failure. Hepatosplenomegaly, liver failure. Adrenal calcification: not always present with absence of adrenal calcification associated with delayed diagnosis. Adreno cortical insufficiency (rare). **Laboratory:** anaemia, liver dysfunction, hyperlipidaemia (unusual and more typical of CESD); abdominal X Ray/CT abdomen – adrenal calcification (absence does not exclude Wolman).	Early metabolic dietetic involvement recommended: Electrolyte replacement Total parental nutrition Liver specific formula feeds for liver failure and malabsorption	**Supportive:** corticosteroid & mineralocorticoid replacement for adrenal insufficiency. Vitamin and mineral supplementation Specific: haematopoetic stem cell transplantation (variable success) Liver transplantation	Enzyme replacement therapy (Sebelipase Alfa)	[42–45]
Cholesteryl ester storage disorder	Liver disease is common. It may present as altered liver function with or without jaundice, hepatomegaly, splenomegaly, heaptic steatosis, fibrosis, or cirrhosis. Liver disease can lead to esophageal varices, which are associated with risk of hemorrhage and can be life-threatening, with liver failure and hepatocellular carcinoma experienced in some patients. Lipid deposition in the wall of the intestinal tract results in diarrhoea and weight loss. With significant hyperlipidaemia, xanthelasma in the palpebral fissures, atherosclerosis and coronary heart disease are well recognised manifestations. Enlarged adrenal glands with punctate calcifications has been described in severe disease. Gall bladder disease with cholesterol gall stones has been noted with sever hyperlipidaemia. **Laboratory:** ↑ LDL, ↓↓ HDL, ↑ to normal TG, liver dysfunction; liver ultrasound – fatty liver (differential diagnosis of NAFLD); liver histology: microvescicular steatosis with foam cells. Immunohistochemistry: lysosomal markers cathepsin D, lysosomal-associated membrane protein 1 (LAMP1), LAMP2, and lysosomal integral membrane protein 2	Low fat diet (variable response)	**Supportive:** treatment for liver dysfunction and liver failure as in Wolman disease. Lipid lowering therapies (limited response) Specific: liver transplantation	Enzyme replacement therapy (Sebelipase Alfa)	[44–47]

Table 36.2 (continued)

Disorder	Key manifestations	Therapies		Emerging therapies	Key references
		Diet	Drugs/other		
Sitosterolaemia	Tendon and tuberous xanthomas, premature atherosclerosis **Laboratory:** haematological abnormalities invariable and maybe only clinical feature in the early stages: abnormally shaped erythrocytes (stomatocytes), thrombocytopenia, giant platelets (macrothrombocytopenia); elevated plant sterols and elevated LDL	Low-plant-sterol diet: restriction of shellfish intake, which contains high amounts algae-derived plant sterol, brassicasterol	Bile acid binding resins; statins; sitostanol; Ezetimibe: NPC1L1 transporter has a role in absorption of plant sterols. Ezetimibe specifically inhibits intestinal cholesterol absorption, targeting NPC1L1 Ileal bypass surgery		[46]

Identification of children with HeFH is shown in ▶ Fig. 36.2 [28] and management of children with HeFH is detailed in Tables 36.2 and 36.3 [28]

Family history of premature CHD plus high LDL-C levels are the two key selective screening criteria

A family history of premature CHD in close relative(s) and/or baseline high cholesterol in one parent, together with an LDL-C ≥ 4 mmol/L (160 mg/dL) indicates a high probability of FH

If the parent has a genetic diagnosis, an LDL-C ≥ 3.5 mmol/L (130 mg/dL) suggests FH in the child

Secondary causes of hypercholesterolaemia should be ruled out

DNA testing establishes the diagnosis

If a pathogenic *LDLR*/*APOB*/*PCSK9* mutation is identified in a first-degree relative, children may also be genetically tested

If a parent died from CHD, a child even with moderate hypercholesterolaemia should be tested genetically for FH and inherited elevation in Lp(a)

If DNA testing is available, cascade screening of families is recommended using both a phenotypic and genotypic strategy

If DNA testing is not available, a phenotypic strategy based on country, age- and gender-specific LDL-C levels should be used

Children with suspected HeFH should be screened from the age of 5 years; screening for HeFH should be undertaken when clinically suspected (both parents affected or xanthoma present) and as early as possible

Age at screening should be similar for boys and girls. † Universal screening in childhood may also be considered

Unit conversion from mmol/L to mg/dL:

For total, HDL, and LDL cholesterol multiply mmol/L by 38.67, e.g. 3.5 mmol/L = 3.5 mmol/L * 38.67 = 135 mg/dL

For triglycerides multiply mmol/L by 88.57, e.g. 1.9 mmol/L = 1.9 mmol/L * 88.57 = 168 mg/dL

Unit conversion from mg/dL to mmol/L:

For total, HDL, and LDL cholesterol divide mg/dL by 38.67, e.g. 135 mg/dL = 135 mg/dL/38.67 = 3.5 mmol/L

For triglycerides divide mg/dL by 88.57, e.g. 168 mg/dL = 168 mg/dL/88.57 = 1.9 mmol/L

[a]**Diagnosis of familial hypercholesterolaemia in children and adolescents** [27–30]

[b]**Screening criteria for paediatric FH (children and adolescents)** [27, 28]

Table 36.3 Lipid lowering drugs

Drug class	Drug function	Drug names licensed in childhood[a]	Benefits	Possible side effects[a]
Statins	Inhibits the enzyme the body needs to make cholesterol	Lovastatin[a] Rosuvastatin[a] Fluvastatin[a] Atorvastatin[a] Pravastatin[a] (preferred choice under 10 years of age) Simvastatin[a]	Decrease LDL and triglycerides; slightly increase HDL	Constipation, nausea, diarrhea, stomach pain, cramps, muscle soreness and possible damage, memory loss, forgetfulness, confusion, pain and weakness, increased risk of diabetes; possible interaction with grapefruit juice
Bile acid binding resins	Prevents bile from being reabsorbed into the circulatory system	Colestipol[a] Cholestyramine[a] Colesevelam[a]	Decrease LDL	Constipation, bloating, nausea, gas; may increase triglycerides
Cholesterol absorption inhibitors	Blocks the amount of cholesterol that is absorbed by the small intestine	Ezetimibe[a]	Decrease LDL; slightly decrease triglycerides; slightly increase HDL Often used as an adjunctive therapy with statins	Stomach pain, fatigue, muscle soreness
Combination cholesterol absorption inhibitor and statin	Inhibits production of cholesterol and blocks absorption of cholesterol by the small intestine	Ezetimibe and simvastatin[a]	Decreases LDL and triglycerides, increases HDL	Stomach pain, fatigue, gas, constipation, abdominal pain, cramps, muscle soreness, pain and weakness; possible interaction with grapefruit juice
Fibrates	Reduces production of triglycerides	Bezabifrate Fenofibrate[a] Gemfibrozil	Decrease triglycerides; increase HDL	Nausea, stomach pain, gallstones
Omega-3 fatty acids	Inhibits production of triglycerides in the liver	Lovaza (prescription omega-3 fatty acid supplement)	Decreases triglycerides	Belching, fishy taste, increased infection risk
Lomitapide	Microsomal transfer protein inhibition	Lomitapide[a]	Decreases cholesterol Used as an adjunctive therapy and for patients who are unable to tolerate lipoprotein apheresis.	Abnormal liver function tests, gastrointestinal.

[a]Reference to Summary of Product Characteristics (SPC) recommended prior to licensed indication of lipid lowering therapies in children and for complete list of side effects

progression and to objectively influence treatment options, in addition to careful attention given to family history of early CAD. Dyslipidemic lipoprotein levels in adolescence have been shown to be associated with an increased risk of high CIMT in young adulthood [23].

Some children and adolescents with more marked dyslipidemia will require drug treatment. Treatment with both diet and drugs appear safe and efficacious in childhood, but longer-term safety data are needed. Disease registries are important to collate data on the natural history, family history, life style choices, therapeutic benefits of lipid lowering benefits and side effects of lipid lowering drugs.

36.2 Disorders of Triglyceride (TG) Metabolism

Hypertriglyceridemia may present with life-threatening pancreatitis, a well-recognised and medical emergency in children. Inborn errors of triglyceride metabolism may present early in childhood with faltering growth,

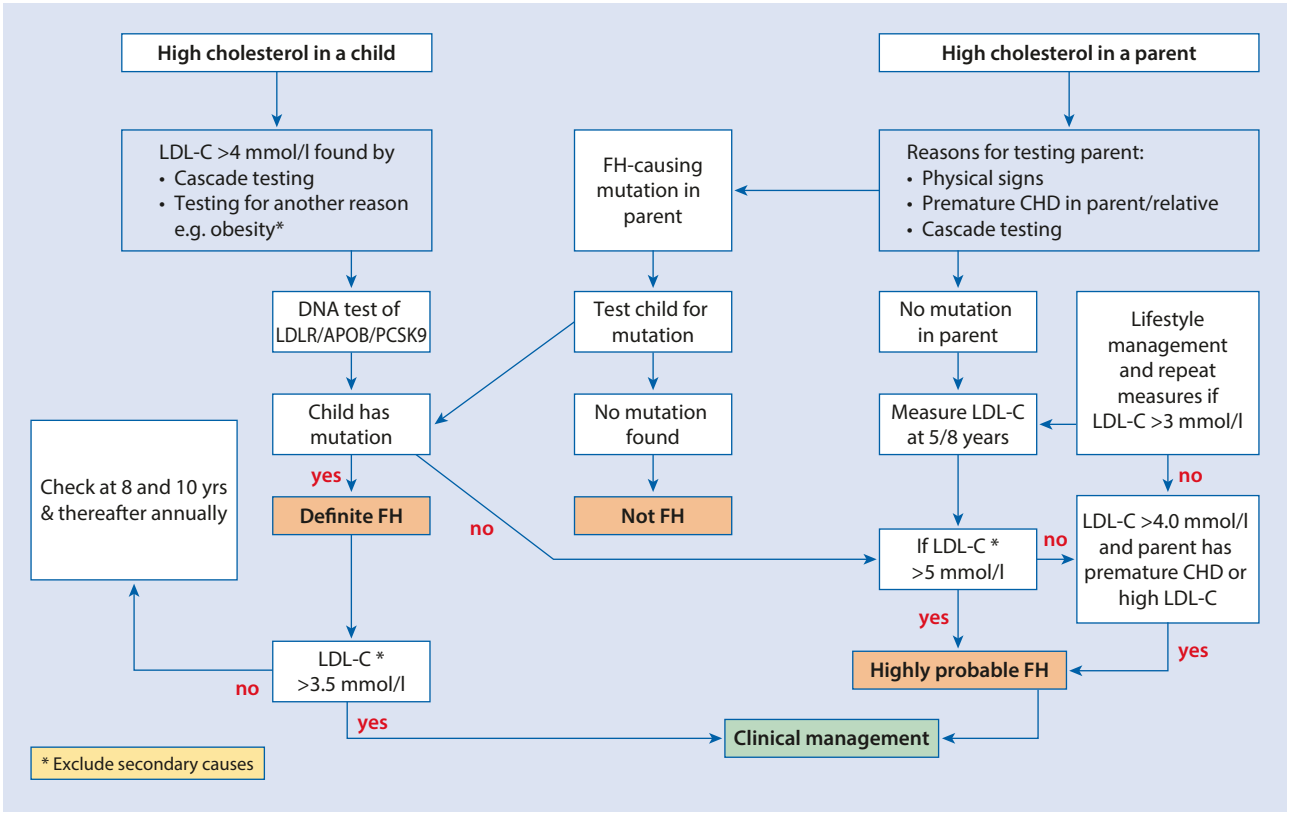

Fig. 36.2 Identification of childhood heterozygous FH

hepatosplenomegaly and life threatening pancreatitis. Severe elevation in TG (>500 mg/dL) is rare in childhood and is usually associated with genetically based recessive metabolic defects, including defects in lipoprotein lipase (LPL) and apoCII (the activator of LPL). With LPL and apoCII deficiency, massive increases in chylomicrons and VLDL-C can occur, producing TG >1000 mg/dL and as high as 5000 to10,000 mg/dL. Such profound increases in TG can produce pancreatitis and eruptive xanthomas, but are not associated with premature atherosclerosis because the TG-enriched particles are too large to enter the vascular wall.

These children require a very low-fat diet (<10% fat). To ensure adequate intake of essential fatty acids, supplements are required. Medium-chain TG, which are absorbed directly into the portal system and do not require chylomicrons for transport to the liver, can have a significant effect on TG, especially in those with an LPL defect. Neither LPL nor ApoCII deficiency responds to lipid-altering medications [39].

Management of these disorders is often a combination of dietary restrictions and drugs with variable success.

The first commercially approved human gene therapy is Glybera (alipogene tiparvovec), an adeno associated viral vector encoding the lipoprotein lipase gene. Glybera is designed to restore the LPL enzyme activity required to enable the processing, or clearance, of fat-carrying chylomicron particles formed in the intestine after a fat-containing meal. In October 2012, the European Commission granted marketing authorisation for Glybera under exceptional circumstances as a treatment for adult patients diagnosed with familial lipoprotein lipase deficiency (LPLD) confirmed by genetic testing, and suffering from severe or multiple pancreatitis attacks despite dietary fat restrictions. However, this subsequently expired following the marketing-authorisation holder's decision not to apply for a renewal [38].

Prompt referral to a metabolic/lipid specialist is necessary to prevent pancreatitis (Table 36.2).

36.3 Disorders of High Density Lipoprotein Metabolism

HDLs are highly heterogeneous and a dynamic group of the smallest and densest lipoproteins present in the circulation. Disorders of HDL are very rare with only three Mendelian autosomal recessive inherited disorders described: Apolipoprotein A-1 deficiency; familial hypoalphalipoproteinaemia (Tangier's disease) and lecithin:cholesterol acyltranferase (LCAT) deficiency.

Patients typically have decreased HDL and Apo A-I and raised cholesterol and triglycerides. The disorders cause premature atherosclerosis. LCAT presents in childhood with a wide range of manifestations (Table 36.2). Elevation of HDL is noted in cholesterol ester transfer protein (CETP) deficiency but these patients are mostly asymptomatic.

36.4 Disorders of Sterol Storage

Intracellular accumulation of cholesterol in certain tissues may occur either due to excessive amounts of cholesterol rich lipids or lipoproteins in plasma; for example cholesterol deposits in xanthomata in familial hypercholesterolaemia.

Intracellular accumulation of cholesterol can also occur when there is an intrinsic abnormality in metabolism of lipids in the cells in which the abnormal storage occurs and are typically seen in inherited deficiencies of lysosomal enzymes catalyzing the breakdown of a complex lipids, for example Nieman Pick Disease (► Chap. 40).

Lysosomal Acid Lipase (LAL) Deficiency, a lysosomal storage disorder encompasses the acute infantile onset form, Wolman disease, and the cholesteryl ester storage disease (CESD) presenting in childhood/adulthood [25]. In both Wolman disease and CESD, the accumulation of cholesteryl ester in the lysosomes is secondary to a deficiency of an esterase that is responsible for hydrolysis of esterified cholesterol in the normal lysosome. Wolman disease presents with extreme faltering growth, malabsorption, hepatosplenomegaly, adrenal calcification and death in early infancy. CESD has a relatively slow course of disease progression with hepatosplenomegaly and microvesicular cirrhosis, premature atherosclerosis and hypercholesterolaemia (elevated LDL-C and decreased HDL-C, with absence of hypertriglyceridaemia) are characteristic feature [48]. Sebelipase alfa (recombinant human lysosomal acid lipase (LAL) enzyme) is licensed for both Wolman disease and CESD, with the potential to improve significantly the life expectancy in Wolman disease and alter the natural history of CESD disease [49].

36.5 Conclusion

Inborn errors of lipoprotein metabolism in childhood are mostly rare and cause significant morbidity and mortality. Heterozygous FH is the most common of these disorders with potential benefit on cardiovascular outcomes when a healthy lifestyle is adopted and lipid lowering therapies are started in a timely manner. Most inborn errors of lipoprotein metabolism require specialist input from metabolic dietitians and physicians. There are several emerging therapies including gene therapy that is likely to alter the natural history of these disorders.

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Disorders of Isoprenoid/ Cholesterol Synthesis

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Contents

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Isoprenoid/Cholesterol Synthesis

Isoprenoids comprise a diverse class of biomolecules with pivotal functions in a variety of cellular processes including cell growth and differentiation, protein glycosylation, signal transduction, etc. [1]. Isoprenoid synthesis starts from acetyl-CoA, which in six sequential enzyme reactions is converted into isopentenyl-PP, the basic 5-carbon unit used for the synthesis of all isoprenoids (◘ Fig. 37.1). The first committed intermediate in sterol isoprenoid synthesis is squalene, which after cyclisation is converted into lanosterol. Conversion of lanosterol into cholesterol may occur via two major routes involving the same enzymes which, depending on the timing of reduction of the Δ^{24} double bond, postulate either 7-dehydrocholesterol or desmosterol as the ultimate precursor of cholesterol.

Introduction

Ten different enzyme defects in the isoprenoid/cholesterol synthetic pathway have been linked with different genetic disorders [2, 3]. Of these, mevalonate kinase deficiency affects the synthesis of all isoprenoids, is associated characteristically with recurrent episodes of high fever and inflammation, and may present with congenital anomalies. Most of the remaining enzyme defects affect the synthesis of cholesterol only. Patients with these defects may present with various multiple congenital and morphogenic anomalies, including internal organ, skeletal and/or skin abnormalities, and/or a marked delay in psychomotor development.

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37.1 Mevalonate Kinase Deficiency

Clinical Presentation

Mevalonate Kinase Deficiency (MKD) is an autoinflammatory disorder characterized by a chronic proinflammatory state of monocytes and lifelong recurring episodes of fever and inflammation [4, 5]. These episodes last 3–7 days, recur on average every 4–6 weeks and are associated with abdominal pain, vomiting and diarrhoea, cervical lymphadenopathy, hepatosplenomegaly, arthralgia and skin rash [5]. Disease onset occurs mostly in the first year of life; episodes often occur without clear cause but may be provoked by vaccinations, physical and emotional stress and minor trauma [5]. MKD represents a clinical and biochemical continuum with classic mevalonic aciduria (MKD-MA) at the severe and hyper-IgD and periodic fever syndrome (MKD-HIDS) at the mild end [4, 6]. MKD-MA patients can also present with developmental delay, ataxia, cerebellar atrophy, hypotonia, severe failure to thrive and dysmorphic features, and may die in early infancy [6, 7].

Metabolic Derangement

MK catalyses the phosphorylation of mevalonate to 5-phosphomevalonate. In cells from MKD-MA patients, MK activity is usually below detection limit, while in cells from MKD-HIDS patients, residual MK activity is 1–10% of the activity in control cells [8–10]. Consequently, high and moderately elevated levels of mevalonic acid are found in plasma and urine of MKD-MA and MKD-HIDS patients, respectively [9, 11]. The synthesis of all isoprenoids is affected to a degree. The inflammatory manifestations are thought to arise from a transient shortage of geranylgeranyl-PP [4, 12, 13]. A relative shortage of sterol isoprenoids during embryonic development may contribute to the congenital anomalies observed in MKD-MA.

Genetics

MKD is caused by biallelic pathogenic variants in *MVK* [8–10]. Most MKD-HIDS patients are compound heterozygotes for a hypomorphic MVK-V377I allele, found exclusively in MKD-HIDS patients, and a second severe allele which can be found also in MKD-MA patients [10]. Relatively few patients homozygous for MVK-V377I are known. The MVK-V377I allele codes for an active enzyme, the correct assembly/maturation of which is disturbed and appears temperature sensitive, which explains the residual MK enzyme activity associated with the MKD-HIDS phenotype [4, 9, 10]. Currently, over 186 different pathogenic variants are known (► http://www.hgmd.cf.ac.uk/).

Diagnostic Tests

MKD can be diagnosed through analysis of mevalonic acid levels in body fluids, preferably by stable isotope dilution gas chromatography-mass spectrometry (GC-MS) [11]. This readily identifies MKD-MA patients, who have high levels of mevalonic acid (1-56 mol/mol creatinine in urine), but may not identify MKD-HIDS patients, who have lower levels even during fever (urinary concentration 0.005–0.040 mol/mol creatinine). The best diagnostic tests remain measurement of MK activities in white blood cells or primary skin fibroblasts [14] and genetic testing of *MVK* [10], both of which are also used for prenatal diagnosis. Carrier detection is done by genetic testing.

Treatment and Prognosis

There is currently no efficacious treatment for MKD. Many MKD-MA patients die in infancy with respiratory failure [6, 7]. HLA-identical allogeneic bone marrow transplantation for MKD-MA may result in remission of the febrile attacks and inflammation [15]. Corticosteroid, colchicine, or cyclosporin treatment may result in clinical improvement in some MKD-HIDS patients, but not for the majority [5]. Simvastatin treatment led to a small decrease in the number of days of

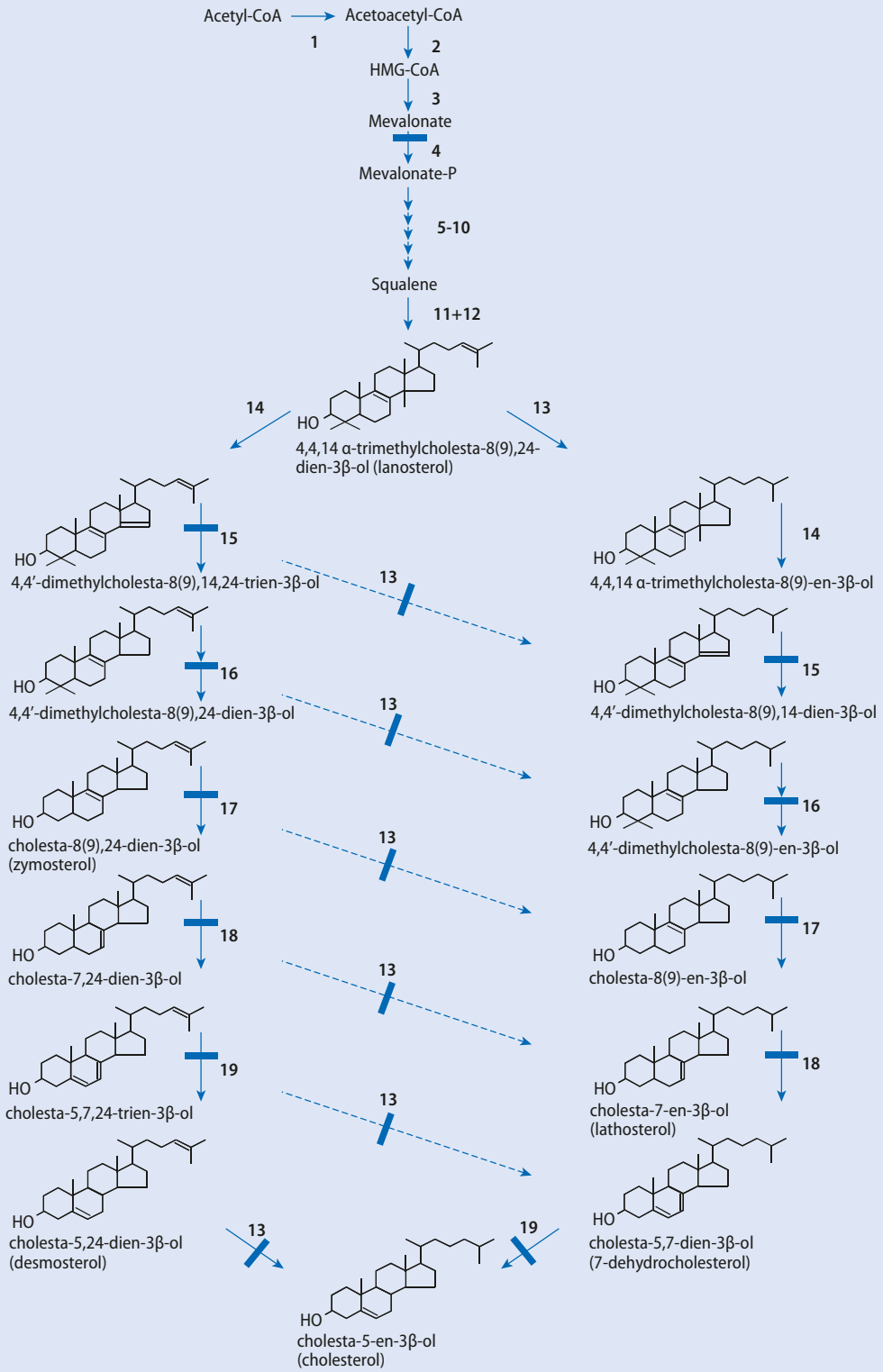

Fig. 37.1 Isoprenoid/cholesterol synthesis pathway. CoA coenzyme A, HMG 3-hydroxy-3-methylglutaryl, P phosphate, PP pyrophosphate. *1* acetyl-CoA acetyltransferase, *2* HMG-CoA synthase, *3* HMG-CoA reductase, *4* mevalonate kinase, *5* mevalonate-P kinase, *6* mevalonate-PP decarboxylase, *7* isopentenyl-PP isomerase, *8* geranyl-PP synthase, *9* farnesyl-PP synthase, *10* squalene synthase, *11* squalene epoxidase, *12* 2,3-oxidosqualene sterol cyclase, *13* sterol Δ24-reductase, *14* sterol C-14 demethylase, *15* sterol Δ14-reductase, *16* sterol C-4 demethylase complex, *17* sterol Δ8-Δ7 isomerase, *18* sterol Δ5-desaturase, *19* sterol Δ7-reductase. Enzyme deficiencies are indicated by **solid bars** across the arrows

illness in a small group of MKD-HIDS patients [16], but in MKD-MA patients statin treatment worsened clinical symptoms. Treatment with etanercept, a soluble p75 TNF alpha receptor-Fc fusion protein, may lead to a reduction in frequency and severity of symptoms in some MKD-HIDS patients [17]. Most promising results have been obtained by treatment with interleukin-1 (IL-1) receptor antagonists such as Anakinra, which blocks the activity of IL-1beta, that becomes elevated in MKD-HIDS [5, 17]. Long-term outcome for MKD-HIDS patients is relatively benign as the clinical symptoms tend to decrease with age [5].

37.2 Porokeratosis

Porokeratosis is a rare skin disorder of keratinization characterized by dry atrophic skin lesions with a hyperkeratotic margin (cornoid lamella). Genetic studies in China revealed different heterozygous (germline) pathogenic variants in the *MVK* gene associated with multiple types of porokeratosis [18]. Subsequent analysis of 134 Chinese probands with porokeratosis [19] revealed multiple additional heterozygous pathogenic variants in the *MVK* gene (encoding mevalonate kinase), the *PMVK* gene (encoding phosphomevalonate kinase, enzyme 5 in ◘ Fig. 37.1), the *MVD* gene (encoding mevalonate-PP carboxylase, enzyme 6), and the *FDPS* gene (encoding farnesyl-PP synthetase, enzyme 9).

Because heterozygous parents or relatives of patients with autosomal recessive MKD generally don't have porokeratosis, the involvement of additional genetic and/or environmental factors in disease manifestation had been suggested. A recent report indeed showed second-hit postzygotic somatic pathogenic variants in the *PMVK* and *MVD* genes in affected tissues of 3 individuals with linear porokeratosis, who were heterozygous for germline pathogenic variants in the respective genes [20]. This strongly suggests that porokeratosis is not an autosomal dominant treat but probably caused by somatic biallelic pathogenic variants in the *MVK*, *PMVK*, *MVD* and *FDPS* genes.

37.3 Squalene Synthase Deficiency

Clinical Presentation

Squalene synthase deficiency has been reported in 3 patients from 2 families [21]. Patients showed profound global developmental delay, structural brain malformations, 2/3 syndactyly of the toes, and facial dysmorphisms.

Metabolic Derangement

Squalene synthase (enzyme 10 in ◘ Fig. 37.1) catalyzes the condensation of 2 farnesyl-PP moieties to form squalene, which is the first isoprenoid committed to sterol synthesis. The deficiency results in low total and LDL-cholesterol and elevated farnesol in plasma and a unique urine metabolic profile with increased saturated and unsaturated branched-chain dicarboxylic acids and glucuronides derived from farnesol [21].

Genetics

Squalene synthase deficiency is caused by biallelic pathogenic variants in the *FDFT1* gene. Two patients were compound heterozygous for a 120-kb deletion and an intronic variant resulting in aberrant splicing and the third patient homozygous for a 16-bp intronic deletion [21].

Diagnostic Tests

Laboratory diagnosis may involve GC-MS analysis of plasma sterols and organic acid analysis of urine. genetic testing of *FDFT1* is the preferred diagnostic test.

Treatment and Prognosis

Treatment is symptomatic.

37.4 Desmosterol Reductase Deficiency (Desmosterolosis)

Clinical Presentation

Currently nine patients with desmosterolosis have been reported. The first reported female infant died shortly after birth and suffered from multiple congenital malformations, including macrocephaly, hypoplastic nasal bridge, thick alveolar ridges, gingival nodules, cleft palate, total anomalous pulmonary venous drainage, ambiguous genitalia, short limbs, arthrogryposis and generalised osteosclerosis [22]. The second reported infant was a boy, who exhibited a far less severe phenotype. At 3 years of age, his clinical presentation included dysmorphic facial features, microcephaly, limb anomalies, and profound developmental delay [23]. Seven additional patients have been reported [24]; one died at 5 days, but all others were alive at 4 months to 15 years. All nine patients had major CNS involvement and agenesis of the corpus callosum and 8 patients had dilated ventricles (but head size varied from microcephalic to macrocephalic). Surviving patients showed learning difficulties and some patients suffered from epilepsy. Other common features include facial dysmorphic features, arthrogryposis and severe failure to thrive [24].

Metabolic Derangement

Desmosterolosis is due to a deficiency of sterol Δ^{24}-reductase (enzyme 13 in Fig. 37.1), which catalyses the reduction of the Δ^{24} double bond of sterol intermediates (including desmosterol) [25]. The deficiency results in elevated levels of desmosterol in plasma, tissue and cultured patient cells.

Genetics

Desmosterolosis is caused by biallelic pathogenic variants in *DHCR24* encoding 3β-hydroxysterol Δ^{24}-reductase. Currently, 10 different pathogenic variants have been identified (► http://www.hgmd.cf.ac.uk/).

Diagnostic Tests

Laboratory diagnosis includes sterol analysis of plasma, tissues or cultured cells by GC-MS (detection of desmosterol) and genetic testing of *DHCR24* (first choice for prenatal diagnosis and carrier detection) [25].

Treatment and Prognosis

At the severe end of the spectrum, patients die in the neonatal period. Surviving patients show moderate to severe developmental delay.

37.5 Lanosterol C14-Demethylase Deficiency

Clinical Presentation

Programmes for detection of causes of cataract by genome sequencing identified a few individuals with biallelic pathogenic variants in *CYP51A1* encoding lanosterol C14-demethylase. Among these were individuals with cataracts only [26], one individual with cataracts, developmental delay, spastic diplegia, and cryptogenic neonatal liver cirrhosis [27] and an infant with cataracts and fulminant liver disease [28].

Metabolic Derangement

Lanosterol C14-Demethylase (enzyme 14 in Fig. 37.1) converts lanosterol into 4,4-dimethylcholesta-8(9),14,24-trien-3β-ol. Sterol profiling may reveal elevated lanosterol and 7-dehydrocholesterol levels [27].

Genetics

Lanosterol C14-Demethylase is due to biallelic pathogenic variants in *CYP51A1*. Currently, 4 different pathogenic variants have been identified (► http://www.hgmd.cf.ac.uk/).

Diagnostic Tests

Laboratory diagnosis may include sterol analysis of plasma, tissues or cultured cells by GC-MS (detection of lanosterol) and genetic testing of *CYP51A1*.

Treatment and Prognosis

Cataracts can be surgically removed.

37.6 Sterol β14-Reductase Deficiency (Hydrops – Ectopic Calcification – Moth-Eaten (HEM) Skeletal Dysplasia or Greenberg Skeletal Dysplasia)

Clinical Presentation

HEM – or Greenberg skeletal dysplasia is characterized by early in utero lethality. Affected fetuses typically present with severe fetal hydrops, short-limb dwarfism, an unusual 'moth-eaten' appearance of the markedly shortened long bones, bizarre ectopic ossification centres and a marked disorganization of chondro-osseous histology, and may present with polydactyly and additional non-skeletal malformations [29, 30].

HEM skeletal dysplasia is allelic to Pelger-Huet anomaly (PHA) [29–32], a rare benign autosomal dominant disorder of leukocyte development characterized by hypolobulated nuclei and abnormal chromatin structure in granulocytes of heterozygotes. Heterozygotes with PHA do not show clinical symptoms, but homozygotes may have variable minor skeletal abnormalities and developmental delay [31, 33]. Two cases of anadysplasia-like spondylometaphyseal dysplasia due to pathogenic variants in *LBR* have been reported [31, 34]. Thus, defects of LBR can create a continuum of clinical manifestations ranging from isolated PHA through PHA with mild skeletal dysplasia to Greenberg skeletal dysplasia.

Metabolic Derangement

HEM skeletal dysplasia is due to a deficiency of sterol Δ^{14}-reductase (enzyme 15 in Fig. 37.1), which catalyses the reduction of the Δ^{14} double bond in early sterol intermediates [29, 30]. Consequently, elevated levels of Δ^{14}-sterol intermediates can be detected in tissues and cells of fetuses with HEM skeletal dysplasia. Heterozygous individuals with PHA do not show aberrant sterol precursors.

Genetics

HEM skeletal dysplasia is caused by biallelic pathogenic variants in *LBR* encoding lamin B receptor [29, 30]. Currently, more than 33 pathogenic variants have been reported (► http://www.hgmd.cf.ac.uk/). PHA may

occur in carriers of sterol Δ^{14}-reductase deficiency [30], but was not observed in a parent carrying a missense variant affecting only the sterol Δ^{14}-reductase region of *LBR* [32].

Diagnostic Tests

Affected fetuses are often detected by fetal ultrasound. Laboratory diagnosis includes sterol analysis of tissues or cells by GC-MS and genetic testing of *LBR* (the first choice for prenatal and carrier detection).

Treatment and Prognosis

Most cases terminate in early embryonic stage (10–20 weeks of gestation). One adult individual diagnosed with PHA and homozygous for a splice-site variant in *LBR* showed developmental delay, macrocephaly and a ventricular septal defect. No information is available on the effect of the variant on cholesterol biosynthesis in this individual. Two patients showed milder skeletal dysplasia [31, 34].

37.7 Deficiency of the C4-Demethylase Complex

The C4-demethylase complex (enzyme complex 16 in Fig. 37.1) catalyzes the sequential removal of the two methyl groups at the C4 position of early sterol precursors and includes 3 components: (i) a 3β-hydroxysteroid C4-methyl oxidase (encoded by *MSMO1*) [35]; (ii) a 3β-hydroxysteroid dehydrogenase (also referred to as a sterol 4α-carboxylate 3-dehydrogenase) that removes a C4 carboxyl group while oxidizing the 3β-hydroxyl group to a 3-keto group (encoded by *NSDHL*) [36], and (iii) a 3-ketosteroid reductase that converts the 3-keto group back to a 3β-hydroxyl group (encoded by *HSD17B7*) [37].

37.7.1 C4-Methyl Sterol Oxidase Deficiency (SMO Deficiency)

Clinical Presentation, Diagnosis and Treatment

The first described patient with pathogenic variants in *MSMO1* presented with severe psoriasiform dermatitis, arthralgias, immune dysfunction, congenital cataracts, failure to thrive, short stature, microcephaly and developmental delay [35]. GC-MS analysis revealed accumulation of C4-methyl sterols in plasma and cultured skin fibroblasts. The patient was treated with oral statin plus cholesterol and bile acid supplementation, which caused improvement in growth, weight gain and joint pains. Topical application of a statin and cholesterol led to an improvement of the skin disease. Four additional patients have been reported: all 4 had cataracts, 3/4 had microcephaly, 2/4 had severe skin disease [38].

37.7.2 Sterol 4α-Carboxylate 3-Dehydrogenase Deficiency

37.7.2.1 CHILD Syndrome in Females

Clinical Presentation

X-linked dominant CHILD syndrome (congenital hemidysplasia with ichthyosiform erythroderma and limb defects) mostly affects females. Patients have similar skin and skeletal abnormalities as observed in CDPX2 patients (see ▶ Sect. 37.8), but with a striking unilateral distribution affecting the right side of the body more often than the left, in contrast to the bilateral distribution in CDPX2 patients [39]. Ichthyosiform skin lesions are usually present at birth and often involve large regions of one side of the body with a sharp line of demarcation in the midline. Alopecia, nail involvement and limb reduction defects with calcific stippling of the epiphysis are common on the affected side. CHILD syndrome is mostly lethal in hemizygous males, but few males with hypomorphic variants have been diagnosed.

Metabolic Derangement

CHILD syndrome is caused by a deficient activity of sterol 4α-carboxylate 3-dehydrogenase [36], which is part of the C4-demethylase complex. Theoretically, the deficiency should lead to the accumulation of 4-methyl sterol precursors; however, these precursors are hardly or not detectable in plasma of patients. Cholesterol levels are normal.

Genetics

CHILD syndrome is inherited as an X-linked dominant trait and due to heterozygous pathogenic variants in *NSDHL* encoding 3β-hydroxysteroid dehydrogenase [36]. Currently, over 33 different pathogenic variants have been identified in *NSDHL* (▶ http://www.hgmd.cf.ac.uk/). In one patient diagnosed with CHILD syndrome, a heterozygous variant was identified in *EBP* [40].

Diagnostic Tests

CHILD syndrome can be diagnosed through genetic testing of *NSDHL* [36]. When negative, one should consider *EBP* [40].

Treatment and Prognosis

In a child with severe limb defects, the long-term outcome is usually poor. Surgical corrections of skeletal abnormalities may be required. The skin lesions respond to treatment with topical cholesterol and a

statin [41]. Penetration of these is improved by glycolic acid [42].

37.7.2.2 CK Syndrome in Males

- **Clinical Presentation, Diagnosis and Treatment**

Most variants causing CHILD syndrome in females are lethal in males. However, hemizygous hypomorphic variants in *NSDHL* have been reported to cause CK syndrome (CKS) in males [43], including thirteen hemizygous males from 2 families who all survived into adult life. All had mild to severe intellectual disability, microcephaly, asthenic habitus with hyperextensible joints, spinal abnormalities and developed seizures during infancy. MRI scans of three patients showed evidence of cerebral cortical malformation, including polymicrogyria or pachygyria. All patients had a distinctive appearance with a long thin face, almond-shaped eyes with epicanthic folds, up slanting palpebral fissures, posteriorly rotated ears, a high forehead, a high palate with dental crowding and micrognathia in childhood. Most were hypotonic and had minimal speech development. Behavioral problems included attention deficit hyperactivity disorder, irritability and aggression.

Heterozygous females of the families did not show features of CHILD syndrome, but exhibited abnormal behavior and problems with working memory.

As with CHILD syndrome, plasma sterol levels are not informative rendering genetic testing of *NSDHL* as the only diagnostic test.

Seizures can be treated with anticonvulsants.

37.8 Sterol Δ8-Δ7 Isomerase Deficiency

37.8.1 X-Linked Dominant Chondrodysplasia Punctata 2 or Conradi-Hünermann Syndrome in Females

- **Clinical Presentation**

X-linked dominant chondrodysplasia punctata 2 (CDPX2), also known as Conradi-Hünermann or Happle syndrome mostly affects females. Patients display skin defects ranging from ichthyosiform erythroderma in the neonate, through linear or whorled atrophic and pigmentary lesions in childhood to striated hyperkeratosis, coarse lusterless hair and alopecia in adults. Additional features are cataracts and skeletal abnormalities including short stature, asymmetric rhizomelic shortening of limbs, calcific stippling of the epiphyseal regions and craniofacial defects. The pattern of the skin defects and variability in severity and asymmetry of the bone and eye abnormalities are consistent with functional X-chromosomal mosaicism. The expression of these skin and skeletal abnormalities can be bilateral and is often asymmetric [39]. The majority of patients show completely normal psychomotor development.

- **Metabolic Derangement**

CDPX2 is caused by a deficiency of sterol Δ^8-Δ^7 isomerase (enzyme 17 in ◘ Fig. 37.1) [44, 45], which results in elevated plasma and cellular levels of cholesta-8(9)-en-3β-ol and 8-dehydrocholesterol; plasma cholesterol levels are often (low) normal [46].

- **Genetics**

CDPX2 is inherited as an X-linked dominant trait and due to pathogenic variants in *EBP* encoding sterol Δ^8-Δ^7 isomerase [44, 45]. Currently, over 94 different disease-causing variants have been identified in predominantly females (► http://www.hgmd.cf.ac.uk/). Variants mostly arise de novo in patients, but gonadal and somatic mosaicism have been observed (e.g. [47]). Inheritance of a variant from an affected mother usually results in a more severe disease in offspring [48].

- **Diagnostic Tests**

Laboratory diagnosis involves GC-MS analysis of plasma sterols [46]. Primary skin fibroblasts or lymphoblasts of patients can be cultured in lipoprotein-depleted medium to induce cholesterol biosynthesis; the enzyme defect is detected by sterol analysis using GC-MS. Finally, genetic testing can be performed (also first choice for prenatal diagnosis).

- **Treatment and Prognosis**

Long-term outcome of CDPX2 patients depends on the severity of clinical symptoms. Many need surgery for cataracts or scoliosis. Correction of scoliosis associated with hemidysplasia of vertebrae requires a special anterior strut graft and a posterior fusion.

37.8.2 Hemizygous EBP Deficiency in Males

- **Clinical Presentation, Diagnosis and Prognosis**

Most pathogenic variants causing CDPX2 in females are lethal in hemizygous males. However, several affected males with aberrant karyotypes, somatic mosaicism and hemizygous, hypomorphic variants have been described (e.g. [49, 50]). Heterozygous mothers of male patients with hemizygous hypomorphic variants are usually asymptomatic.

Males with a 47XXY karyotype or with somatic mosaicism for an *EBP* variant show typical features of CDPX2, including (asymmetrical) bone shortening, stippled epiphyses, ichthytosis, alopecia and mild facial dysmorphism. However, males with true hemizygous pathogenic variants may have different phenotypes ranging from (i) dysmorphic features similar to those

seen in SLOS; (ii) brain abnormalities including Dandy-Walker malformation and agenesis of the corpus callosum; (iii) skin abnormalities including collodion baby and diffuse congenital ichthyosis to (iv) developmental delay and behavioral difficulties [49–51]. Skeletal abnormalities may be totally absent. There is a high mortality rate among affected males, many dying between 1 day and 4.5 years. Survivors have learning difficulties and may show severe developmental delay [49–51].

Increased levels of 8-dehydrocholesterol and 8(9)-cholestenol can be detected in plasma and cultured cells. Genetic testing of *EBP* can be performed to confirm the diagnosis.

37.9 Sterol Δ5-Desaturase Deficiency (Lathosterolosis)

Clinical Presentation

Currently, four patients and one fetus with lathosterolosis have been reported [52–55]. One female presented at birth with severe microcephaly, receding forehead, anteverted nares, micrognathia, prominent upper lip, high-arched palate, postaxial hexadactyly of the left foot and syndactyly between the second to fourth toes and between the fifth toe and the extra digit. From early infancy she suffered from cholestatic liver disease and, during infancy, severe psychomotor delay became apparent [52, 56]. By 6 years, she had developed bilateral cataracts and osteoporosis leading to fractures. The cholestatic liver disease had progressed to cirrhosis with portal hypertension. At age 8 she received a liver transplant; 5-years later she had normal liver functions. The second patient, a boy, had SLOS-like features (see ► Sect. 37.10), including growth failure, microcephaly, ptosis, cataracts, short nose, micrognathia, prominent alveolar ridges, ambiguous genitalia, bilateral syndactyly of the second and third toes, and bilateral postaxial hexadactyly of the feet. His clinical course was marked by failure to thrive, severe delay, increasing hepatosplenomegaly and increased gingival hypertrophy, with death at the age of 18 weeks [53]. The third patient was diagnosed at 22 months and had micrognathia, postaxial hexadactyly of the feet, syndactyly between the second and third toes, and developmental delay [54]. The fourth patient had cataracts, developmental delay, a head circumference <2nd centile, mild hypotonia and subtle dysmorphism (a high-arched palate, anteverted nostrils, long philtrum and clinodactyly of toes) [54].

Metabolic Derangement

Lathosterolosis is due to a deficiency of sterol Δ^5-desaturase (enzyme 18 in ◘ Fig. 37.1), which introduces the C5-C6 double bond in lathosterol to produce 7-dehydrocholesterol [53, 54]. Consequently, elevated levels of lathosterol can be detected in plasma, (tissue) and cultured cells of patients. Cholesterol levels can be normal.

Genetics

Lathosterolosis is caused by biallelic pathogenic variants in *SC5D* encoding 3β-hydroxysterol Δ^5-desaturase. So far 7 different pathogenic variants have been reported (► http://www.hgmd.cf.ac.uk/).

Diagnostic Tests

Laboratory diagnosis includes sterol analysis of plasma, tissues or cultured cells by GC-MS (detection of lathosterol) and genetic testing of *SC5D* [53, 54]. The latter is the first choice for prenatal diagnosis and carrier detection.

Treatment and Prognosis

The first surviving patient had cholestatic liver disease for which she received a liver transplant at the age of 8, which resulted in normal liver functions in 5-year follow up [56]. At the age of 6 she had cataract surgery [57]. One patient received simvastatin, which resulted in normalisation of lathosterol levels and a small rise in IQ (from 55 to 60) [52].

37.10 Smith-Lemli-Opitz Syndrome (7-Dehydrocholesterol Reductase Deficiency)

Clinical Presentation

Patients with Smith-Lemli-Opitz Syndrome (SLOS) can present with a large variety of morphogenic and congenital anomalies constituting a clinical continuum ranging from hardly recognizable through mild to very severe. Most patients have a characteristic craniofacial appearance with microcephaly, a short nose with broad nasal bridge and anteverted nares, a long philtrum, micro-/retrognathia and often blepharoptosis, low-set, posteriorly rotated ears, cleft or high-arched palate, pale hair and broad or irregular alveolar ridges. Common limb abnormalities include cutaneous syndactyly of the second and third toes (>97% of cases), short proximally placed thumbs and, in more severe cases, postaxial polydactyly. Genital abnormalities may include hypospadias, cryptorchidism and ambiguous or even female external genitalia in affected boys. Also common are congenital heart defects, and renal, adrenal, lung and gastrointestinal anomalies. Additional major features are profound prenatal and postnatal growth retardation, neonatal ascites, cholestatic jaundice, mental retardation, feeding difficulties, behavioral problems, sleeping disorder and sunlight sensitivity [58].

Metabolic Derangement

SLOS is caused by a deficiency of 7-dehydrocholesterol reductase (enzyme 19 in Fig. 37.1), which catalyses the predominant final step in cholesterol biosynthesis, i.e. the reduction of the C7-C8 double bond of 7-dehydrocholesterol to produce cholesterol. Consequently, low cholesterol and increased 7-dehydrocholesterol levels are detected in plasma, cells and tissues of most SLOS patients. In addition, increased 8-dehydrocholesterol plasma levels can be detected, probably synthesized from 7-dehydrocholesterol by the enzyme sterol Δ^8-Δ^7 isomerase functioning in reverse. Clinical severity in SLOS correlates best either with absolute cholesterol levels or with the sum of 7-dehydrocholesterol plus 8-dehydrocholesterol expressed as fraction of total sterol (e.g. [59]). The efficiency of cholesterol transfer from mother to fetus may also play a role in determining severity [60]. 7-Dehydrocholesterol and 8-dehydrocholesterol can be further metabolized by both the bile acid and steroid pathways and can undergo free radical oxidation reactions [61]. The resulting downstream products may contribute to the pathogenesis of the disease.

Genetics

SLOS is the most common defect of cholesterol biosynthesis and inherited as an autosomal recessive trait. Incidences range from 1:15,000 to 1:60,000 in Europe [58], with higher incidences in some East-European countries reflecting founder effects.

Currently, over 223 different pathogenic variants have been reported in *DHCR7* encoding 7-dehydrocholesterol reductase (► http://www.hgmd.cf.ac.uk/).

Diagnostic Tests

Laboratory diagnosis includes sterol analysis of plasma or tissues of patients by GC-MS with elevated levels of 7-dehydrocholesterol (and 8-dehydrocholesterol) being diagnostic. Plasma cholesterol is usually low or low normal. Primary skin fibroblasts or lymphoblasts of patients can be cultured in lipoprotein-depleted medium to induce cholesterol biosynthesis, whereupon the accumulation of 7-dehydrocholesterol can be detected by sterol analysis using GC-MS. Finally, genetic testing of *DHCR7* can be performed, also used for prenatal diagnosis and carrier detection.

Treatment and Prognosis

Since most anomalies in SLOS arise during (early) embryonic development [58], it will most probably not be feasible to entirely cure the patients. Most treatments aim to replenish the lowered cholesterol levels through dietary supplementation of cholesterol (at doses ranging from 25 to 300 mg/kg/d in various formulations) with or without bile acids [62]. Biochemically, this may lead to a substantial elevation of plasma cholesterol concentrations, but plasma concentrations of 7-dehydrocholesterol and 8-dehydrocholesterol are often only marginally reduced. Moreover, this treatment does not significantly change the sterol levels in brain, which rely on de novo cholesterol synthesis due to the limited ability of cholesterol to cross the blood-brain barrier. The clinical effects of cholesterol supplementation have been rather disappointing, with hardly any effect on developmental progress [63], and no short-term improvements in behavior [64]. Simvastatin, an oral HMG-CoA reductase inhibitor, has been used with the aim to lower 7-dehydrocholesterol and 8-dehydrocholesterol levels, but no beneficial effects on either anthropometric measures or behavior were seen [65].

In severe SLOS cases, reduced synthesis of cholesterol probably leads to reduced synthesis of adrenal steroids. An ACTH stimulation should be undertaken and, if abnormal, severe stresses (such as major surgery) should be covered by corticosteroid therapy in doses similar to those used for congenital adrenal hyperplasia.

Malformations in SLOS often require surgery, but the disorder poses anaesthetic challenges. The airway may be compromised by micrognathia, prominent incisors, and a cleft palate, which could be managed by mask ventilation or fiber-optic tracheal intubation.

Feeding problems, structural and functional gastrointestinal problems are common. To maximize weight gain, nasogastric or gastrostomy tube feeding may be necessary. Behavioral problems present a major challenge in the care of patients and antipsychotropic medications, have been used with some effectiveness [62].

37.11 Ichthyosis Follicularis with Atrichia and Photophobia (IFAP) Syndrome

The isoprenoid/cholesterol synthesis pathway is regulated by several feedback regulatory mechanisms through which end products of the pathway determine expression of the different genes (transcriptional regulation) and/or the levels of the encoded enzymes (post-translational regulation) [1]. All defects discussed above involve defects in genes encoding biosynthetic enzymes of the pathway. However, defects also have been reported in the *MPTPS2* [66] and *SREBF1* [67] genes in patients with X-linked and autosomal-dominant IFAP syndrome, respectively. The encoded proteins are involved in the SREBP pathway, which is responsible for the transcriptional feedback regulation in response to sterol levels. Although no changes in the serum lipid profiles were noted, the *MPTPS2* variants result in impaired cellular signalling [66] and the *SREBF1* variants in lowered transcription of genes involved in isoprenoid/cholesterol synthesis and keratin synthesis [67].

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Disorders of Bile Acid Synthesis

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Contents

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Bile Acid Synthesis

Bile acids are biological detergents that are synthesised from cholesterol in the liver by modifications of the sterol nucleus and oxidation of the side chain. Synthesis of bile acids can occur by a number of pathways (◘ Fig. 38.1); the most important in adults starts with conversion of cholesterol to 7α-hydroxycholesterol ("neutral pathway"). In infancy, other pathways are more important; one of these starts with the conversion of cholesterol to 27-hydroxycholesterol ("acidic pathway"). The neutral pathway is regulated by feedback inhibition by bile acids on cholesterol 7α-hydroxylase by several mechanisms including direct enzyme inhibition, activation of the farnesoid X receptor (FXR) in the liver and activation of FXR in the intestine leading to production of fibroblast growth factor 19 (FGF19) which travels to the liver to inhibit cholesterol 7α-hydroxylase. The two FXR pathways lead to repression of cholesterol 7α-hydroxylase gene expression. The regulation of the acidic pathway has not been fully elucidated.

Two inborn errors of metabolism affect the modifications of the cholesterol nucleus in both major pathways for bile acid synthesis: 3β-hydroxy-Δ5-C27-steroid dehydrogenase (3β-dehydrogenase) deficiency and Δ4-3-oxosteroid 5β-reductase (5β-reductase) deficiency. These disorders produce cholestatic liver disease and malabsorption of fat and fat-soluble vitamins. Onset of symptoms is usually in the first year of life and, if left untreated, the liver disease can progress to cirrhosis and liver failure. In contrast to many other causes of cholestasis in infancy, plasma γ-glutamyl transpeptidase is normal and an enzyme assay of total 3α-hydroxy bile acids in plasma also yields a normal or low result. Treatment with chenodeoxycholic acid and cholic acid can lead to dramatic improvement in the liver disease and the malabsorption. Neonatal cholestatic liver disease can also be the presenting feature of two disorders affecting oxidation of the cholesterol side chain – sterol 27-hydroxylase deficiency (cerebrotendinous xanthomatosis [CTX]) and α-methylacyl-CoA racemase deficiency. However, these disorders more commonly present later with neurological disease. Chenodeoxycholic acid has been shown to halt or even reverse neurological dysfunction in CTX. Oxysterol 7α-hydroxylase deficiency can present with rapidly progressive liver disease in infancy or later onset hereditary spastic paraparesis. These 3 disorders can be placed in a growing list of defects that may present with transient neonatal cholestatic jaundice followed by a late onset neurodegenerative disorder. It seems likely that the 27-hydroxycholesterol pathway is important in fuelling bile flow in infancy and in the production and metabolism of important oxysterols in the brain later in life.

Other inborn errors of bile acid synthesis include two bile acid amidation defects (cholestatic liver disease and fat-soluble vitamin malabsorption) and cholesterol 7α-hydroxylase deficiency (adults with hyperlipidaemia and gallstones). In disorders of peroxisome biogenesis and peroxisomal β-oxidation, neurological disease usually predominates; these are considered in ► Chap. 40. Three disorders affecting the peroxisomal import of CoA esters of DHCA and THCA and their β-oxidation (ABCD3 deficiency, α-methylacyl-CoA racemase deficiency, and acyl-CoA oxidase 2 deficiency) can present with liver disease and are included here.

Historically individuals with bile acid synthesis defects were discovered and subsequent cases diagnosed by the detection of abnormal metabolites (particularly unusual bile acids and bile alcohols in the urine), however, disorders are now often diagnosed by sequencing of gene panels or by exome/genome sequencing.

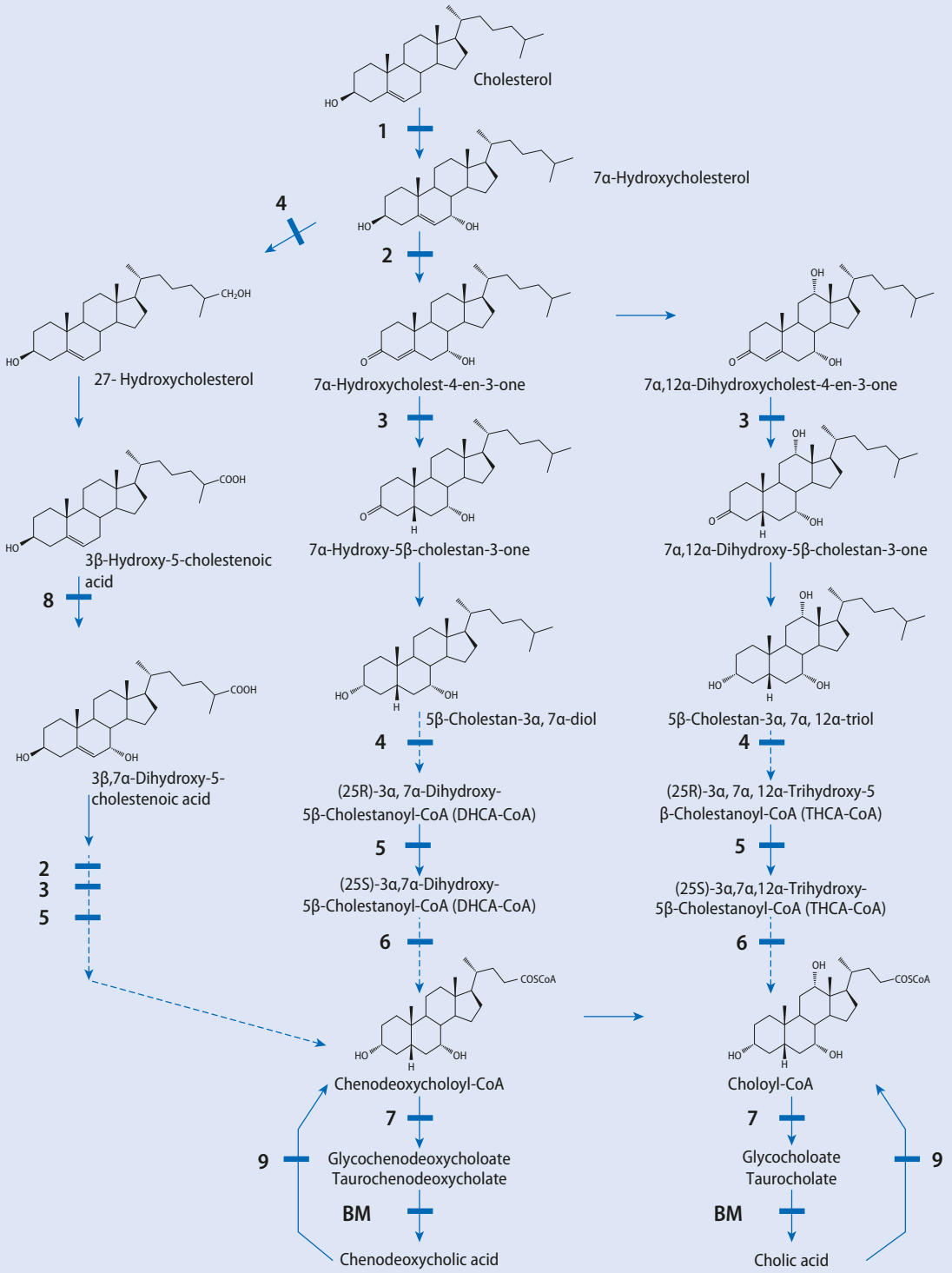

Fig. 38.1 Major reactions involved in the synthesis of bile acids from cholesterol. *1* Cholesterol 7α-hydroxylase, *2* 3β-hydroxy-Δ5-C27-steroid dehydrogenase/isomerase, *3* Δ4-3-oxosteroid-5β-reductase, *4* sterol 27-hydroxylase, *5* α-methylacyl-CoA racemase, *6* proteins needed for peroxisome biogenesis, peroxisomal import of THCA-CoA and DHCA-CoA and their β-oxidation, *7* bile acid-CoA: amino acid N-acyl transferase, *8* oxysterol 7α-hydroxylase, *9* bile acid CoA ligase. Enzyme defects are depicted by **solid bars** across the arrows. BM indicates bacterial metabolism (in the gut)

Introduction

Most of the known enzyme deficiencies of bile acid synthesis affect both the 27-hydroxycholesterol ("acidic") and the 7α-hydroxycholesterol ("neutral") pathways; the exceptions are cholesterol 7α-hydroxylase deficiency and oxysterol 7α-hydroxylase deficiency [1]. Because of the broad specificity of many of the enzymes, the major metabolites are often not those immediately proximal to the block. For instance, in 3β-hydroxy-Δ^5-C_{27}-steroid dehydrogenase deficiency (enzyme 2 in Fig. 38.1) the major metabolite is not 7α-hydroxycholesterol but a series of unsaturated bile acids that have the normal bile acid side chain but persistence of the 3β, 7α-dihydroxy-Δ^5 structure of the nucleus.

38.1 3β-Hydroxy-Δ5-C27-Steroid Dehydrogenase Deficiency

Clinical Presentation

3β-Dehydrogenase deficiency was first described in 1987 [2]. Reviews of 38 children diagnosed in Cincinnati and 18 cases diagnosed in London were published in 2007 and 2010, respectively [3, 4]. Patients presented with neonatal conjugated hyperbilirubinaemia (11/18), rickets (8/18, including 1 with hypocalcaemic tetany and seizures but normal liver function tests), hepatomegaly (7/18), pruritus (3/18), steatorrhoea and failure to thrive (3/18) [4]. Many had documented biochemical evidence of fat-soluble vitamin malabsorption (low 25-OH vitamin D in 10/18, of whom 8 also had low vitamin E levels and 6, low vitamin A levels and 1 had a prolonged prothrombin time responsive to vitamin K). The liver biopsy showed giant cell change and hepatocyte disarray in all cases, with added features of cholestasis in the majority; many had bridging fibrosis. Jacquemin et al. have described a group of patients with 3β-dehydrogenase deficiency who presented with jaundice, hepatosplenomegaly and steatorrhoea (a clinical picture resembling progressive familial intrahepatic cholestasis) between the ages of 4 months and 46 months [5]. Pruritus was absent in these children, in contrast to other children with severe cholestasis. The authors noted normal γ-glutamyl-transpeptidase activities in plasma, low serum cholesterol concentrations and low vitamin E concentrations. Presentation of 3β-dehydrogenase deficiency with chronic hepatitis / cirrhosis in adolescence/adulthood has also been described as have asymptomatic adults [6]. A European survey of paediatric centres in 2017 identified 55 patients suggesting an incidence of 0.99 per 10 million but many of the centres surveyed indicated lack of ready access to specialist laboratory facilities able to make the diagnosis [7]. Cases are now being increasingly identified by exome / genome sequencing.

Metabolic Derangement

3β-Dehydrogenase catalyses the second reaction in the major pathway of synthesis of bile acids: the conversion of 7α-hydroxycholesterol to 7α-hydroxycholest-4-en-3-one (enzyme 2 in Fig. 38.1). When the enzyme is deficient, the accumulating 7α-hydroxycholesterol can undergo side-chain oxidation with or without 12α-hydroxylation to produce 3β,7α-dihydroxy-5-cholenoic acid and 3β,7α,12α-trihydroxy-5-cholenoic acid, respectively. These unsaturated C_{24} bile acids are sulphated in the C3 position; a proportion is conjugated to glycine, and they can be found in high concentrations in the urine. Concentrations of bile acids in the bile are low [8]. It is probable that the sulphated Δ^5 bile acids cannot be secreted into the bile canaliculi and fuel bile flow in the same way as occurs with the normal bile acids; indeed they probably inhibit bile acid-dependent bile flow. There are at least two possible ways in which this sequence of events might lead to damage to hepatocytes and, ultimately, to cirrhosis:

- The abnormal metabolites produced from 7α-hydroxycholesterol may be hepatotoxic as well as cholestatic.
- Failure of bile acid-dependent bile flow may lead to hepatocyte damage, perhaps as a result of the accumulation of toxic compounds normally eliminated in the bile.

Genetics

3β-Dehydrogenase deficiency is an autosomal-recessive trait caused by mutations in *HSD3B7*. In 2000, Schwarz et al. showed that the original patient described by Clayton et al. in 1987 was homozygous for a 2-bp deletion in exon 6 (Δ1057–1058) [9]. In 2003, Cheng et al. reported mutations in 15 additional patients from 13 kindreds with 3β-dehydrogenase deficiency [10]. In patients with neonatal cholestasis, they identified deletions (310delC, 63delAG), a splice site mutation (340+1 G>T) and a missense mutation (E147K).

Diagnostic Tests

The diagnosis is established by demonstrating the presence of the characteristic bile acids (with a Δ^5 double bond, 3β-hydroxyl/sulphate group and 7α-hydroxyl group) in plasma or urine. It is important to remember that bile acids with a Δ^5 double bond and a 7-hydroxy group are acid labile. Analysis by fast-atom-bombardment mass spectrometry (FAB-MS) or electrospray ionisation tandem mass spectrometry (ESI-MS/

MS) overcomes this problem [11, 12]. The diagnosis is confirmed by demonstrating damaging bi-allelic mutations in *HSD3B7*.

Plasma

If plasma bile acids are analysed using a GC-MS method that does not include a solvolysis step, the profile of non-sulphated bile acids that is obtained shows concentrations of cholic and chenodeoxycholic acid, which are extremely low for an infant with cholestasis. The concentration of 3β,7α-dihydroxy-5-cholestenoic acid is increased. Inclusion of a solvolysis step reveals the presence of high concentrations of 3β,7α-dihydroxy-5-cholenoic acid (3-sulphate) and 3β,7α,12α-trihydroxy-5-cholenoic acid (3-sulphate). These can also be detected when plasma is analysed by FAB-MS or when a neonatal blood spot is analysed by ESI-MS [12].

Urine

Urine analysed by negative ion FAB-MS or ESI-MS shows the characteristic ions of the diagnostic unsaturated bile acids: mass/charge ratios (m/z) = 469, 485, 526 and 542. Using ESI-MS/MS, the sulphated Δ^5 bile acids (m/z 469 and 485) are detected as parents of m/z 97; glycine conjugates of sulphated Δ^5 bile acids (m/z 526 and 542) are additionally detected as parents of m/z 74. Some patients excrete the di- and trihydroxy 5-cholenoic acids largely in non-sulphated forms (unconjugated [m/z 405] and conjugated with glycine [m/z 446,462]).

Fibroblasts

3β-Dehydrogenase can be assayed in cultured skin fibroblasts using tritiated 7α-hydroxycholesterol [13]. Patients show very low activity.

Treatment and Prognosis

Emergency treatment of coagulopathy with parenteral vitamin K may be required [4]. Vitamin D deficiency may be severe enough to require intravenous calcium as well as vitamin D therapy. However, long-term treatment with fat-soluble vitamins is not required because bile acid replacement therapy corrects all the fat-soluble vitamin deficiencies. Untreated 3β-dehydrogenase deficiency has led to death from complications of cirrhosis before the age of 5 years; patients with milder forms of the disorder may survive, with a chronic hepatitis or even remain asymptomatic, into their second decade or beyond. The response to treatment depends upon the severity of the liver disease at the time of starting treatment. In patients with a bilirubin level less than 120 μM and an AST level less than 260 U/l, chenodeoxycholic acid therapy has led to a dramatic improvement in symptoms and in liver function tests within 4 weeks, and to an improvement in the liver biopsy appearances within 4 months. The dose of chenodeoxycholic acid that has been used is 12–18 mg/kg/day initially (for 2 months), followed by 9–12 mg/kg/day maintenance. In one infant with severe disease, chenodeoxycholic acid (15 mg/kg/day) led to a rise in bilirubin and AST. Her treatment regimen was changed to 7 mg chenodeoxycholic acid/kg/day plus 7 mg cholic acid/kg/day. Over the course of 15 months, her bilirubin and transaminases returned to normal, and a repeat liver biopsy showed a more normal parenchyma and less inflammation. Follow-up of patients treated with chenodeoxycholic acid has shown that, after a median follow-up of 5.5 years (range 1–17 years) 12 out of 13 treated children had no signs of liver disease or of fat-soluble vitamin deficiency [14]. Gonzalez et al. have reported treatment with cholic acid alone in 15 patients with 3β-dehydrogenase deficiency. They described normalisation of physical examination findings, laboratory test results and liver ultrasound, and improvement in liver biopsy appearances [14]. Continuing follow-up to a median of 24.3 years indicated that the patients remained well on a mean dose of cholic acid of 6.9 mg/kg/d with 5 women having 10 uneventful pregnancies on treatment [15]. Successful pregnancies have also been recorded in patients on combined treatment with chenodeoxycholic acid and cholic acid [16]. Treatment can be monitored by suppression of urinary excretion of unsaturated bile acids [8, 17].

Bile-acid-replacement therapy may work in one of two ways:

- By fuelling bile acid-dependent flow (hence directly relieving cholestasis).
- By suppressing the activity of cholesterol 7α-hydroxylase (thereby reducing the accumulation of potentially toxic metabolites of 7α-hydroxycholesterol).

38.2 Δ4-3-Oxosteroid 5β-Reductase Deficiency

Clinical Presentation

Patients who excrete 3-oxo-Δ^4 bile acids as the major urinary bile acids can be divided into four groups – those who have proven mutations in both alleles of *SRD5B1* (*AKR1D1*, the gene encoding the 5β-reductase enzyme) [18–23]; those in whom this has been excluded [24]; those in whom only one mutation has been found [21, 22], and those in whom the results of gene analysis have not been published [25, 26]. In the last three groups, the cause of excretion of 3-oxo-Δ^4 bile acids remains uncertain and, since this pattern of urinary metabolite excretion can be a nonspecific consequence of severe liver disease [27,

28], the description in this chapter will focus on the eight patients with proven 5β-reductase mutations on both alleles.

In three of the seven families first described, the parents were consanguineous [19–22]. Seven of the 8 patients presented with cholestatic jaundice in the neonatal period, the eighth at 3 months. All 8 had raised transaminases which were associated with normal γ-GT in 6 and mildly elevated γ-GT in 2. Low vitamin E was documented in 3 and markedly prolonged clotting times, which improved with parenteral vitamin K, were recorded in one. Liver biopsies showed giant cell transformation, canalicular and hepatocellular cholestasis, portal inflammation, septal fibrosis, occasional necrotic foci and, in some cases, increased extramedullary haemopoiesis. Two patients had significant steatosis. Without treatment, cholestasis persisted in all cases.

Metabolic Derangement

Mutations in *SRD5B1* lead to reduced activity of the hepatic enzyme, 5β-reductase (enzyme 3 in Fig. 38.1), that brings about the 5β(H) saturation of the C4 double bonds of bile acid precursors such as 7α-hydroxy-cholest-4-en-3-one and 7α,12α-dihydroxy-cholest-4-en-3-one. These intermediates can then undergo side-chain oxidation to produce the corresponding C24 bile acids. The mechanism of hepatocyte damage and cholestasis in 5β-reductase deficiency is unknown; as with 3β-dehydrogenase deficiency, toxicity of unsaturated intermediates and unsaturated bile acids and loss of / inhibition of bile acid-dependent bile flow have been postulated. Deficiency of the 5β-reductase enzyme also prevents 5β(H) saturation of the Δ^4 double bond of 3-oxo-Δ^4 steroid hormones; this affects urinary steroid profiles but does not appear to have any obvious physiological effects [29].

Genetics

Primary 5β-reductase deficiency is an autosomal recessive disorder caused by mutations in *SRD5B1* [18–23]. The homozygous mutations that have been described include c.385C>T (p.L106F), c.850C>T (p.R261C), c.511delT (frameshift, premature stop codon), c.662C>T (p.P198L), and p.Q285X. Documented compound heterozygous mutations include c.467C>G (p.P133R) with c.850C>T (p.R261C), c.396C>A with c.722A>T, p.G223E with p.R261C and p.R50X with p.R266Q. Some mutant proteins appear to have markedly reduced stability [30].

Diagnostic Tests

Plasma

GC-MS analysis of plasma bile acids reveals low or low normal concentrations of chenodeoxycholic acid (normal concentration 0.2–12.7 μM) and cholic acid (normal concentration 0.4–6.7 μM). In contrast, the plasma concentrations of 3-oxo-Δ^4 bile acids are markedly elevated, i.e. to 7α-hydroxy-3-oxo-4-cholenoic acid >1.5 μM and 7α,12α-dihydroxy-3-oxo-4-cholenoic acid >2.0 μM. Analysis of plasma bile acids by ESI-MS/MS shows taurine-conjugated (parents of m/z 80) and glycine-conjugated (parents of m/z 74) 3-oxo-Δ^4 bile acids present at concentrations similar to those of their saturated analogues [18].

Urine

Analysis of urine by FAB-MS or ESI-MS/MS shows the presence of major ions attributable to the glycine conjugates of 7α-hydroxy-3-oxo-4-cholenoic acid and 7α,12α-dihydroxy-4-cholenoic acid (m/z = 444 and 460; parents of m/z 74) and their taurine conjugates (m/z = 494 and 510; parents of m/z 80) and sometimes the taurine conjugate of 7α,12α-dihydroxy-3-oxo-4-cholestenoic acid (m/z 552; parents of 80). The normal saturated bile acids (m/z 448, 464, 498, 514) are at background level.

The identities and relative amounts of urinary bile acids can be confirmed by GC-MS analysis following enzymatic deconjugation. In patients shown to have primary 5β-reductase deficiency, the 3-oxo-Δ^4 bile acids have comprised more than 90% of the total urinary bile acids; a lower percentage is found in most children whose excretion of 3-oxo-Δ^4 bile acids is secondary to liver damage of other aetiology.

Treatment and Prognosis

Emergency treatment for vitamin K deficiency may be required. Vitamin D may be needed for rickets. 5β-Reductase deficiency can progress rapidly to liver failure. However, treatment with bile acid replacement therapy can lead to normalisation of liver function and long-term (at least 14 years) good health. Successful regimens include; (i) chenodeoxycholic acid plus cholic acid (e.g. 8 mg/kg/day of each) [18–20]; (ii) cholic acid alone (e.g. initially 13 mg/kg/day, subsequently 6 mg/kg/day) [17, 23]; (iii) ursodeoxycholic acid (40 mg/kg/d) for 4 months followed by chenodeoxycholic acid (25 mg/kg/d) [21]; and (iv) ursodeoxycholic acid (7 mg/kg/d) with chenodeoxycholic acid (10 mg/kg/d reducing to 5 mg/kg/d) [22]. The patient who responded to chenodeoxycholic acid plus cholic acid had previously failed to respond to ursodeoxycholic acid alone and logic dictates that treatment probably requires a primary bile acid that can feed back to reduce synthesis of bile acid precursors. Infants with liver failure (prothrombin ratio >1.3) at the time of starting treatment did not respond adequately and were transplanted or died.

Two patients with 5β-reductase deficiency followed up for 20 years were well with normal liver function tests on continuing cholic acid therapy (7.5 mg/kg/d) [23]. However, one patient who had stopped taking bile acid replacement therapy was also well with normal liver

function at 13 years [29] and we are aware of one individual who is homozygous for a pathogenic mutation in *AKR1D1*/*SRD5B1* (c.580-1G>A) and who is 37 and has never had liver dysfunction [31]. So there is some doubt as to whether all individuals with biallelic pathogenic mutations in *AKR1D1* need to be treated and whether lifelong treatment with bile acids is always indicated.

38.3 Cerebrotendinous Xanthomatosis (Sterol 27-Hydroxylase Deficiency)

Clinical Presentation

The average age of diagnosis of cerebrotendinous xanthomatosis (CTX) is 35 years with a diagnostic delay of 16 years; the disorder has been described as a paediatric disease diagnosed in adulthood [32]. Clinical signs and symptoms include adult-onset progressive neurological dysfunction and non-neurologic manifestations i.e. tendon xanthomas, premature atherosclerosis, osteoporosis, and respiratory insufficiency [33]. Sometimes, cholestatic jaundice in infancy is the first manifestation of CTX [34–36], however, it usually improves spontaneously. Chronologically, the next (or the first) symptom of CTX is often mental retardation detected during the first decade of life. Cataracts may also be present as early as 5 years of age and in a study in the USA of individuals with idiopathic bilateral cataracts from ages 2 to 21 years, Freedman et al. found 1.8% had CTX [37]. Wevers et al. [38] documented four Dutch patients in whom persistent diarrhoea was present from early childhood. Motor dysfunction (spastic paresis, ataxia, expressive dysphasia) develops in approximately 60% of patients in the second or third decade of life. Tendon xanthomata may be detectable during the second decade of life but usually appear in the third or fourth decade. The Achilles tendon is the most common site; other sites include the tibial tuberosities and the extensor tendons of the fingers and the triceps. Premature atherosclerosis leading to death from myocardial infarction occurs in some patients. In others, death is caused by progression of the neurological disease with increasing spasticity, tremor and ataxia and pseudobulbar palsy. It is important to recognise that the neurological deterioration is very variable [39] (▶ Chap. 2). For example, some patients are normal intellectually but suffer from a neuropathy or mild spastic paresis; others have no neurological signs but present with psychiatric symptoms resembling schizophrenia. The most serious consequences of the disease are the development of xanthomas in the brain and the neurological symptoms caused by these. The preferential site of the brain xanthomas is in the white matter of the cerebellum. Magnetic resonance imaging (MRI) of the brain in CTX may show diffuse cerebral atrophy and increased signal intensity in the cerebellar white matter on T_2-weighted scans [40]. Osteoporosis is common in CTX and may produce pathologic fractures; it is associated with low plasma concentrations of 25-hydroxy-vitamin D and 24,25-dihydroxy-vitamin D [41]. Patients with untreated CTX usually die from progressive neurological dysfunction or myocardial infarction between the ages of 30 years and 60 years.

Metabolic Derangement

CTX is caused by a defect in the gene for sterol 27-hydroxylase (enzyme 4 in ◘ Fig. 38.1), the mitochondrial enzyme that catalyses the first step in the process of side-chain oxidation, which is required to convert a C27 sterol into a C24 bile acid [42]. 5β-Cholestane-3α,7α,12α-triol cannot be hydroxylated in the C27 position and accumulates in the liver. As a result, it is metabolised by an alternative pathway, starting with hydroxylation in the C25 position (in the endoplasmic reticulum). Further hydroxylations, e.g. in the C22 or C23 position, result in the synthesis of the characteristic bile alcohols that are found (as glucuronides) in the urine. Bile acid precursors other than 5β-cholestane-3α,7α,12α-triol also accumulate. Some of these (e.g. 7α-hydroxy-cholest-4-en-3-one) are probably converted to cholestanol by a pathway involving 7α-dehydroxylation. Because patients with CTX have a reduced rate of bile-acid synthesis, the normal feedback inhibition of cholesterol 7α-hydroxylase by bile acids is disrupted. This further enhances the production of bile alcohols and cholestanol from bile acid precursors. The symptoms of CTX are produced in part by accumulation of cholestanol (and cholesterol) in almost every tissue of the body, particularly in the nervous system, atherosclerotic plaques and tendon xanthomata. Lack of 3β,7α-dihydroxy-5-cholestenoic acid may contribute to motor neuron damage [43].

Sterol 27-hydroxylase is active in extrahepatic tissues, where it converts cholesterol into 27-hydroxycholesterol, which can be further metabolised and eliminated from cells. This pathway provides a route for the elimination of cholesterol; this route acts as an alternative to the high-density lipoprotein-mediated reverse cholesterol transport [44]. Disruption of this pathway in CTX provides a further explanation for the accumulation of cholesterol in the tissues.

Genetics

CTX is inherited as an autosomal recessive trait caused by biallelic mutations in *CYP27A1* [45–47].

Diagnostic Tests

Plasma

The concentration of cholestanol in plasma can be determined by GC or high-performance liquid chromatography (HPLC). Patients with CTX have plasma con-

centrations in the range of 30–400 μM (normal range = 2.6–16 μM). The plasma cholestanol / cholesterol ratio may be a better discriminant than the absolute cholestanol concentration. Plasma 27-hydroxycholesterol is below the normal range. The following bile acid precursors have been detected at increased concentrations in plasma: 7α-hydroxycholesterol, 7α-hydroxy-cholest-4-en-3-one, 7α,12α-dihydroxy-cholest-4-en-3-one. Plasma concentrations of bile acids are low; plasma concentrations of bile alcohol glucuronides are elevated. Newborn screening using mass spectrometric analysis of dried blood spots has been investigated. Flow injection analysis measuring the ratio of cholestanetetrol glucuronides to taurochenodeoxycholate is a good initial screen [48, 49] and specificity is further improved by a second tier using LC-MS/MS.

Urine

Negative ion FAB-MS or ESI-MS/MS indicate that major cholanoids in the urine are cholestanepentol glucuronides, giving rise to an ion with m/z ratio 627 [50]. GC-MS analysis shows that the major alcohols are 3,7,12,23,25-pentols and 3,7,12,22,25-pentols in adults. Increased urinary bile-alcohol concentrations can be detected using an enzyme assay (7α-hydroxysteroid dehydrogenase) [51]. The urinary bile-alcohol excretion following cholestyramine administration has been used as a test for carriers of CTX [52].

Fibroblasts

27-Hydroxylation of C27 sterols can be measured in cultured skin fibroblasts, and the enzyme activity is virtually absent in fibroblasts from patients with CTX [53].

DNA

CTX can be diagnosed by sequencing the exons and exon-intron boundaries of *CYP27A1* and can also be detected by whole genome/exome sequencing.

38

■ Treatment and Prognosis

The results of treatment with chenodeoxycholic acid were first reported in 1984 [54]. The rates of synthesis of cholestanol and cholesterol were reduced, and plasma cholestanol concentrations fell. A significant number of patients showed reversal of their neurological disability, with clearing of the dementia, improved orientation, a rise in intelligence quotient and enhanced strength and independence. The MRI appearances did not, however, show obvious improvement [55]. Urinary excretion of bile-alcohol glucuronides is markedly suppressed. Chenodeoxycholic acid almost certainly works by suppressing cholesterol 7α-hydroxylase activity; ursodeoxycholic acid, which does not inhibit the enzyme, is ineffective [56]. Adults have usually been treated with a dose of 750 mg/day chenodeoxycholic acid.

More recent trials of chenodeoxycholic acid [57] at a dose of 750 mg/d for adults and 5-15 mg/kg/d for children, confirm the reduction of plasma cholestanol and urine bile alcohol excretion and also show a reduction in plasma 7α-hydroxy-cholest-4-en-3-one. Neurological disability improved in 85% on the Rankin score and in 85% on the EDSS score. Diarrhoea, epilepsy and peripheral neuropathy, when present, resolved in all patients. Pyramidal dysfunction improved or stabilised in 60% (10/15) and cerebellar dysfunction in 89% (12/14). Psychiatric impairment resolved, improved or stabilised in 86% (6/7) of patients. However, parkinsonian symptoms, a rare disease manifestation/association that occurred in only 2 patients during the course of the study, did not respond.

A trial of chenodeoxycholic acid in a cohort of 14 individuals showed normalization of plasma cholestanol within a few months [58]. There was significant clinical improvement in patients up to 25 years of age whose treatment was initiated less than 15 years after the onset of neurological symptoms but patients whose treatment was initiated more than 25 years after the onset of neurological disease continued their clinical deterioration. MRI brain scans showed no volume loss during chenodeoxycholic acid treatment and diffusion weighted imaging showed improved fibre integrity of the pontocerebellar tract and internal capsule.

A trial of cholic acid treatment has been undertaken in 12 adults with CTX [59]. Cholic acid was able to significantly reduce plasma cholestanol levels in treatment naïve patients and keep cholestanol levels low in patients switched from chenodeoxycholic acid to cholic acid. Ten of the 12 remained stable clinically or improved but two deteriorated. In view of the limited experience with cholic acid, chenodeoxycholic acid remains the bile acid of choice for treatment of the neurological manifestations of CTX.

Other treatments that have been used in CTX include 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors e.g. lovastatin [60] (but one study found lovastatin ineffective [56]) and low-density lipoprotein apheresis [61]. Cholestatic liver disease in infancy can be self-limiting, but in those children in whom it is not, bile acid treatment has been successful; cholic acid is probably preferable to chenodoexycholic acid [34, 35].

38.4 Oxysterol 7α-Hydroxylase Deficiency

■ Clinical Presentation

Oxysterol 7α-hydroxylase was first described in a 10-week-old male infant with severe cholestasis, cirrhosis and liver synthetic failure [62]. A second patient was also jaundiced from early infancy and died of liver failure at 11 months [63]. We have diagnosed an infant who presented with liver failure and hypoglycaemia at 3 months but recovered completely with chenodeoxycholic acid treatment [64]. Oxysterol 7α-hydroxylase deficiency has also been identified

as a cause of a recessive form of hereditary spastic paraplegia (HSP5/SPG5) [65, 66]. Patients can present with neurological dysfunction at any age from 1 to 41 years. Weakness of the lower limbs with hypertonia and hyperreflexia is associated with posterior column sensory impairment as evidenced by diminished vibration sensation and proprioception, and some degree of bladder dysfunction. Schöls et al. studied the natural history of a cohort of 34 genetically confirmed cases of SPG5 [67]. Clinically SPG5 manifested in childhood or adolescence (median 13 years). Gait ataxia was a common feature. Individuals with SPG5 lost the ability to walk independently after a median disease duration of 23 years and became wheelchair dependent after a median 33 years.

Metabolic Derangement

This recessive disorder is due to mutations in the gene encoding microsomal oxysterol 7-hydroxylase (enzyme 8 in Fig. 38.1), leading to inactivity of this enzyme and accumulation of 27-hydroxycholesterol, 3β-hydroxy-5-cholestenoic acid and 3β-hydroxy-5-cholenoic acid. The pathway of bile acid synthesis via 27-hydroxycholesterol (which is thought to be very important in infancy) is completely disrupted, and the monohydroxy bile acids that accumulate are particularly hepatotoxic. Accumulation of 3β-hydroxy-5-cholestenoic acid and deficiency of 3β,7α-dihydroxy-5-cholestenoic acid probably contribute to motor neuron damage [43].

Genetics

The two originally reported children with liver disease were homozygous for nonsense mutations, R388X and R112X, respectively, in *CYP7B1*. Three further patients with homozygous R112X mutations presenting with liver disease in infancy, have been described from Taiwan [23]. The first patient shown to respond to chenodeoxycholic acid treatment had homozygous p.R417C mutations. The patients with HSP were mostly homozygous for missense mutations p.S363F, p.F216S, p.G57R and p.R417H. However, one individual with HSP had the p.R388X nonsense mutation.

Diagnostic Tests

In infants with liver disease, analysis of urine by FAB-MS has revealed major peaks of m/z ratio 453 and 510 attributable to 3β-hydroxy-5-cholenoic acid 3-sulphate and its glycine conjugate. However, in our patient, ESI-MS revealed taurine-conjugated 3β-hydroxy-5-cholenoic (m/z 480) as the major abnormal bile acid. GC-MS analysis of plasma indicated that the main bile acids were 3β-hydroxy-5-cholenoic acid and 3β-hydroxy-5-cholestenoic acid. 27-hydroxycholesterol was also markedly elevated in plasma [62–64, 66–68]. In cohorts of individuals with SPG5, measurements of plasma 27-hydroxycholesterol and 25-hydroxycholesterol have been shown to be reliable diagnostic markers [66–68].

Treatment and Prognosis

The first patient presenting with liver disease in infancy showed a deterioration with ursodeoxycholic acid and no improvement with cholic acid and required a liver transplant for hepatic failure at the age of 4 months. The second also failed to respond to ursodeoxycholic acid and died of liver failure at 11 months. Our patient responded rapidly to treatment with chenodeoxycholic acid (initially 25 mg/kg/d) and is well at 6 years of age. Successful treatment with CDCA has also been reported by Chen et al. in an infant with homozygous R112X mutations [23]. Obviously, some patients must never develop significant liver disease and present later with HSP.

Clinical trials of treatments for SPG5 are underway. Schöls et al. reported that 9 weeks treatment with atorvastatin (40 mg/d) in 14 SPG5 patients led to a significant fall in plasma 27-hydroxycholesterol [67]. Marelli et al. reported similar results with statin treatment and also found that treatment with chenodeoxycholic acid corrected an abnormal serum bile acid profile in SPG5 patients [68]. They suggested that the combination of atorvastatin and chenodeoxycholic acid treatment could be considered. However, no data is yet available on any possible effect of either of these treatments on the neurological disease.

38.5 Bile Acid Amidation Defect 1: Bile Acid CoA: Amino Acid N-Acyl Transferase Deficiency

Clinical Presentation

Bile acid CoA: amino acid *N*-acyl transferase (BAAT) deficiency is found amongst the Amish, in whom presentation takes the form of failure to thrive, with pruritus in some cases, and occasionally coagulopathy, but without jaundice [69]. Two out of four affected patients suffered chronic upper respiratory infection. We have diagnosed BAAT deficiency in a 3-month-old infant with cholestatic jaundice, vitamin D deficiency and mild portal and focal lobular hepatitis seen on liver biopsy [70]. Setchell et al. described 8 patients with homozygous mutations in *BAAT* presenting with fat-soluble vitamin deficiency, some with growth failure or transient neonatal cholestatic hepatitis [71].

Metabolic Derangement

Without the enzyme bile acid coenzyme A: amino acid *N*-acyl transferase (enzyme 7 in Fig. 38.1), encoded by *BAAT*, the CoA esters of chenodeoxycholic acid and cholic acid cannot be converted to their glycine and taurine conjugates. The unconjugated bile acids are secreted into the bile but are inefficient at solubilising lipid in the gut. Hence the failure to thrive and fat-soluble vitamin malabsorption.

Genetics

Defective amidation of bile acids in the Amish is caused by homozygosity for a missense mutation (c.226A>G; p.M76V) in *BAAT*. Our patient was homozygous for a nonsense mutation (p.R139X). The cohort described by Setchell also had homozygous mutations – c.1156C>A (p.G386R), c.206A>T (p.D69V), c.58C>T (p.R20X) and c.250C>A (p.P84T).

Diagnostic Tests

Analysis of urine by negative ion FAB-MS or ESI-MS shows that the major urinary bile acid is an unconjugated trihydroxy-cholanoic acid (m/z 407); GC-MS shows that it is unconjugated cholic acid. Other bile acids that may be detected include sulphated dihydroxycholanoic acid (s) (m/z 471) and trihydroxycholanoic acids (m/z 487) and glucuronidated dihydroxycholanoic acid (s) and trihydroxycholanoic acid(s) (m/z 567 and 583).

Treatment and Prognosis

Treatment of vitamin K deficiency may be life-saving, treatment of rickets may require 1α-hydroxycholecalciferol or 1,25-dihydroxycholecalciferol. The Amish patients probably had improvement in symptoms with ursodeoxycholic acid as did our patient. Treatment with glycocholic acid (15 mg/kg/d) led to an improvement in growth in prepubertal patients with growth delay and improved vitamin D and vitamin E absorption as judged by loading tests [72].

38.6 Bile Acid Amidation Defect 2: Bile Acid CoA Ligase Deficiency

Clinical Presentation

Mutations in the bile acid-CoA ligase encoded by *SLC27A5* lead to a urine bile acid profile dominated by unconjugated cholic acid (very similar to the profile seen in patients with *BAAT* mutations). However, whether there is a similar phenotype is currently uncertain. Two sisters born to consanguineous Pakistani parents who share the same genotype have been described. One was asymptomatic; the other had cholestatic liver disease but had two other possible causes for cholestasis – a prolonged period of parenteral nutrition as a premature neonate, and a homozygous missense mutation (c.1772A > G) in *ABCB11*, predicted to alter a highly conserved amino-acid residue (p.N591S) in bile salt export pump (BSEP) [73].

Metabolic Derangement

In the gut, taurine- and glycine-conjugated bile acids are hydrolysed by bacteria, producing free cholic acid and chenodeoxycholic acid. These bile acids return to the liver in the enterohepatic circulation and must be converted to their CoA esters prior to reconjugation with taurine and glycine; this is thought to be the main role of the bile acid CoA ligase (enzyme 9 in Fig. 38.1). Deficiency leads to a build-up of unconjugated bile acids in the enterohepatic circulation and, as they are less efficient detergents than the conjugated bile acids, there is malabsorption of fat and fat-soluble vitamins.

Genetics

Analysis of *SLC27A5* showed that the sisters were homozygous for a mutation in this gene – p.His338Tyr; c.1012c>t, which is in a highly conserved area of the gene, and which is probably important for protein activity.

Diagnostic Tests

The urine bile acid profile, shows the following compounds: nonamidated chenodeoxycholic acid (391) and cholic acid (407; major peak), their glucuronides (567 and 583) and chenodexocholic acid sulphate (471). Plasma bile acids were 89% unamidated (normal <20%). Screening of *SLC27A5* shows mutations.

Treatment and Prognosis

Treatment with oral glycocholic acid may be considered in view of the success reported for the BAAT defect [72].

38.7 Cholesterol 7α-Hydroxylase Deficiency

Clinical Presentation

Homozygous cholesterol 7α-hydroxylase deficiency has been detected in three adults with hypercholesterolaemia, hypertriglyceridaemia and premature gallstone disease [74]. One had premature coronary and peripheral vascular disease. Their LDL cholesterol levels were noticeably resistant to treatment with HMG-CoA reductase inhibitors (statins). A study of the kindred revealed that individuals heterozygous for the mutation were also hyperlipidaemic, indicating that this is a codominant disorder We have observed a heterozygous deletion of exons 1 to 6 of *CYP7A1* in an adult with

hyperlipidaemia, gallstone disease and small duct cholangiopathy [75].

Metabolic Derangement

Cholesterol 7α-hydroxylase (enzyme 1 in Fig. 38.1) is the first step in the major pathway for bile acid synthesis (and therefore for cholesterol catabolism) in adults. Reduced activity of the enzyme leads to accumulation of cholesterol in the liver, leading to down-regulation of LDL receptors and hypercholesterolaemia.

Genetics

Cholesterol 7α-hydroxylase deficiency is caused by mutations in *CYP7A1*. The only mutation published to date is a frameshift mutation (p.L413fsX414) that results in loss of the active site and enzyme function. Heterozygous loss of exons 1 to 6 appears to produce a similar phenotype. Other variants of *CYP7A1* are associated with total cholesterol and LDL-C levels as well as with changes in the risk of ischaemic heart disease [76].

Diagnostic Tests

In one homozygote the cholesterol content of a liver biopsy was shown to be increased. Faecal bile acid output was reduced, and the ratio chenodeoxycholic acid-derived faecal bile acids/cholic acid-derived faecal bile acids was increased, suggesting increased activity of the alternative 27-hydroxylase pathway for bile acid (predominantly chenodeoxycholic acid) synthesis.

Treatment and Prognosis

Treatment with a powerful HMG-CoA reductase inhibitor (atorvastatin) and niacin is required to bring plasma levels of cholesterol and triglycerides under control. The variability of the disorder and the long-term prognosis are not known.

38.8 Disorders of Peroxisome Biogenesis, Peroxisomal Import and Peroxisomal β-Oxidation

These conditions are described in ▶ Chap. 42. Neurological disease usually dominates the clinical picture, but some children with Zellweger syndrome or infantile Refsum's disease have quite marked cholestatic liver disease. In 3 disorders affecting peroxisomal β-oxidation of THCA, liver disease can be the presenting feature:

38.8.1 PMP70 Deficiency: *ABCD3* Mutations

Mutations in *ABCD3* cause a defect involving peroxisomal import of THCA-CoA, DHCA-CoA and branched-chain fatty acyl-CoAs which was associated with liver disease with onset in infancy and needing liver transplantation by 4 years [77].

38.8.2 α-Methylacyl-CoA Racemase Deficiency

α-Methylacyl-CoA racemase (AMACR) deficiency was first described in 2000 [78]. Neurological problems can start at any age from childhood to late adult life. They include developmental delay, epilepsy, acute encephalopathy, tremor, pigmentary retinopathy, hemiparesis, spastic paraparesis, peripheral neuropathy, depression, headache, cognitive decline and ataxia [78–81]. In 2001, presentation with neonatal cholestatic liver disease was documented: Van Veldhoven et al. described an infant with AMACR deficiency who presented with a coagulopathy due to vitamin K deficiency; a sibling had died of a major bleed with the same cause [82, 83]. The infant had mild cholestatic jaundice with raised aspartate aminotransferase and, in contrast to 3β-dehydrogenase deficiency, 5β-reductase deficiency and CTX, a raised γ-GT. Liver biopsy showed a mild nonspecific lymphocytic portal infiltrate and abundant giant cell transformation.

Metabolic Derangement

Side-chain oxidation of cholesterol produces the 25*R* isomer of 3α,7α,12α-trihydroxycholestanoyl-CoA [(25*R*)-THC-CoA], and α-oxidation of dietary phytanic acid produces (some) (2*R*)-pristanoyl-CoA. Before these substrates can undergo peroxisomal β-oxidation they need to be converted to the *S*-isomers by AMACR (enzyme 5 in Fig. 38.1). It is likely that decreased production of cholic acid and chenodeoxycholic acid contributes to cholestatic liver disease and fat-soluble vitamin malabsorption. The pathogenesis of the neurological disease is not understood. AMACR is also involved in the metabolism of ibuprofen and related drugs [84].

Genetics

AMACR deficiency is caused by mutations in *AMACR*. Pathogenic mutations in the adults with neurological disease included a common mutation, p.S52P, and p.L107P [78–81]. The p.S52P mutation was also found in the siblings who presented with neonatal coagulopathy [82, 83].

Diagnostic Tests

Analysis of plasma bile acids by GC-MS reveals increased concentrations of DHCA and THCA; HPLC-ESI-MS/MS can be used to show that it is the (25R) isomer of THCA that is accumulating. GC-MS analysis of

fatty acids in plasma shows an elevated concentration of pristanic acid with mildly elevated/normal plasma phytanic acid concentration and normal very long chain fatty acids. Studies on cultured skin fibroblasts show very low activity of AMACR [82]. AMACR gene testing is available in several clinical panels.

■ Treatment and Prognosis

Parenteral vitamin K may be life-saving. Cholic acid therapy was important in preventing continuing fat-soluble vitamin malabsorption in the cholestatic neonate described by van Veldhoven et al. and Setchell et al. [82, 83]. Its role in improving the liver disease is less certain as, given that adults with the disorder do not show signs of liver disease, there may be spontaneous resolution (as in CTX). The role of a low phytanic acid diet is uncertain; it appeared to prevent further deterioration in at least one of the adults with neurological disease. Plasma exchange has also been used to lower plasma phytanic acid but without obvious clinical benefit [63]. The influence of bile acid therapy on the development and progression of neurological disease is also unknown at present.

38.8.3 Acyl-CoA Oxidase 2 Deficiency

There are three case reports of ACOX2 deficiency. In 2016 Vilarinho et al. described an 8 year old boy with intermittently elevated transaminase levels, liver fibrosis, mild ataxia and cognitive impairment [85]. In 2017 Monte et al. described an adolescent with persistent hypertransaminasemia following an episode of severe liver damage upon exposure to non-steroidal anti-inflammatory drugs [86]. In 2018, Ferdinandusse et al. described a severely affected infant who presented at birth with arthrogryposis, a fractured forearm, scoliosis, and persistent pulmonary hypertension (probably secondary to diaphragmatic eventration and lung hypoplasia). She developed seizures with hypomagnesaemia, hypocalcaemia and hypokalaemia and had a neurogenic myopathy. Conjugated hyperbilirubinaemia was present in the first week of life and she also had hypoalbuminaemia and persistent elevation of alanine aminotransferase. She had continuing cardiorespiratory problems and died at the age of 6 months [87].

All 3 affected individuals had elevated concentrations of taurine-conjugated THCA and other C27 bile acids in plasma. All had reduced ACOX2 protein level or reduced expressed enzyme activity. Mutations (homozygous) were, respectively, a premature stop codon (p.Y69*) [85], a missense mutation (p.R225W) [86], and a 4 nucleotide deletion (c.461-464delTCTG) leading to a premature stop (p.Thr154Serfs*25) [87].

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Disorders of Nucleic Acid Metabolism, tRNA Metabolism and Ribosomal Biogenesis

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Contents

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Nucleotide Metabolism

The central dogma codifies the three-quarters of a century-old recognition that genetic information is vectoral, with transfer from DNA to protein through an RNA intermediate. The RNA component of this process includes three major classifications of molecules: messenger RNA (mRNA), ribosomal RNA (rRNA), and transfer RNA (tRNA). Each of these molecules in turn undergoes processing from primary transcripts to mature species, with each step mediated by enzymatic reactions. Finally, it must be noted that the process for proteins encoded on the mitochondrial chromosome differ from those on the nuclear chromosomes both in cellular location and the enzymes involved. Genetic defects of these enzymes lead to inborn errors of RNA production or processing. ◘ Figure 39.1 summarizes the interplay of the various pathways, including ectonucleotide metabolism (*purple*), nucleic acid metabolism (*green*), tRNA metabolism (*blue*) and ribosomal biogenesis (*red*).

Introduction

This chapter focuses on disorders of extracellular nucleotide (ectonucleotide) and nucleic acid metabolism, disorders of tRNA metabolism, and ribosomal biogenesis disorders. All these defects belong to the group of complex molecules from the simplified classification of IEM (► Chap. 1). Defects involving the biosynthesis, salvage and catabolism of mononucleotides (purines and pyrimidines) are discussed in ► Chap. 32.

◘ Table 39.1 summarizes only some disorders with specific clinical symptoms, according to the specific pathway involved. Representative disorders within each class are discussed below.

39.1 Nucleotide and Nucleic Acid metabolism

See ◘ Fig. 39.1, purple and green pathways.

Ectonucleotides modulate signaling via purinergic receptors, such as ATP receptors (P2X receptors representing ligand-gated ion channels, P2Y receptors representing G protein-coupled receptors), or adenosine receptors (A1 and A3 receptors stimulate adenylyl cyclase, while A2A and A2B receptors inhibit it). Disorders are related either due to insufficient product synthesis, or impaired signaling.

Nucleic acids (DNA and RNA) are polymers of nucleotides. Disorders occur when failure to process endogenous nucleic acids leads to their incorrect recognition as exogenous nucleic acids (such as viral particles), which elicits an immune response. Disorders of nucleic acid metabolism thus lead to an autoinflammatory phenotype.

39.1.1 Disorders of Ectonucleotide Metabolism – Prototype: Ectopic Calcification

Ectonucleotide pyrophosphatase 1 (ENPP1) cleaves ATP into AMP and pyrophosphate (PPi). PPi represents the main inhibitor of hydroxyapatite deposition, and a deficiency of PPi leads to ectopic calcification. Infants with ENPP1 deficiency can thus present with widespread arterial calcification (Generalized Arterial Calcification of Infancy; GACI), leading to early demise in a significant number of cases [1]. Arterial stenosis is common, and is thought to be a consequence of AMP or adenosine deficiency [2]. Those who survive the disease are likely to develop hypophosphatemic rickets in later life. Diagnosis is suspected based on imaging findings; treatment is supportive, although enzyme replacement therapy is currently under development.

ABCC6 (ATP-binding cassette, subfamily C, member 6) deficiency also manifests with arterial calcification, whether in earlier life (GACI), or in adulthood (Pseudoxanthoma Elasticum; PXE). ABCC6 is known to mediate the export of ATP and other nucleoside triphosphates [3]; although ATP has been proposed as the substrate of ABCC6 transport [4], the evidence is not conclusive, and the actual substrate remains elusive.

CD73, encoded by the *NT5E* gene, cleaves AMP to adenosine, and the enzyme deficiency is associated with Arterial Calcification due to CD73 Deficiency (ACDC) [5], as well as with calcification around small (hand) joints.

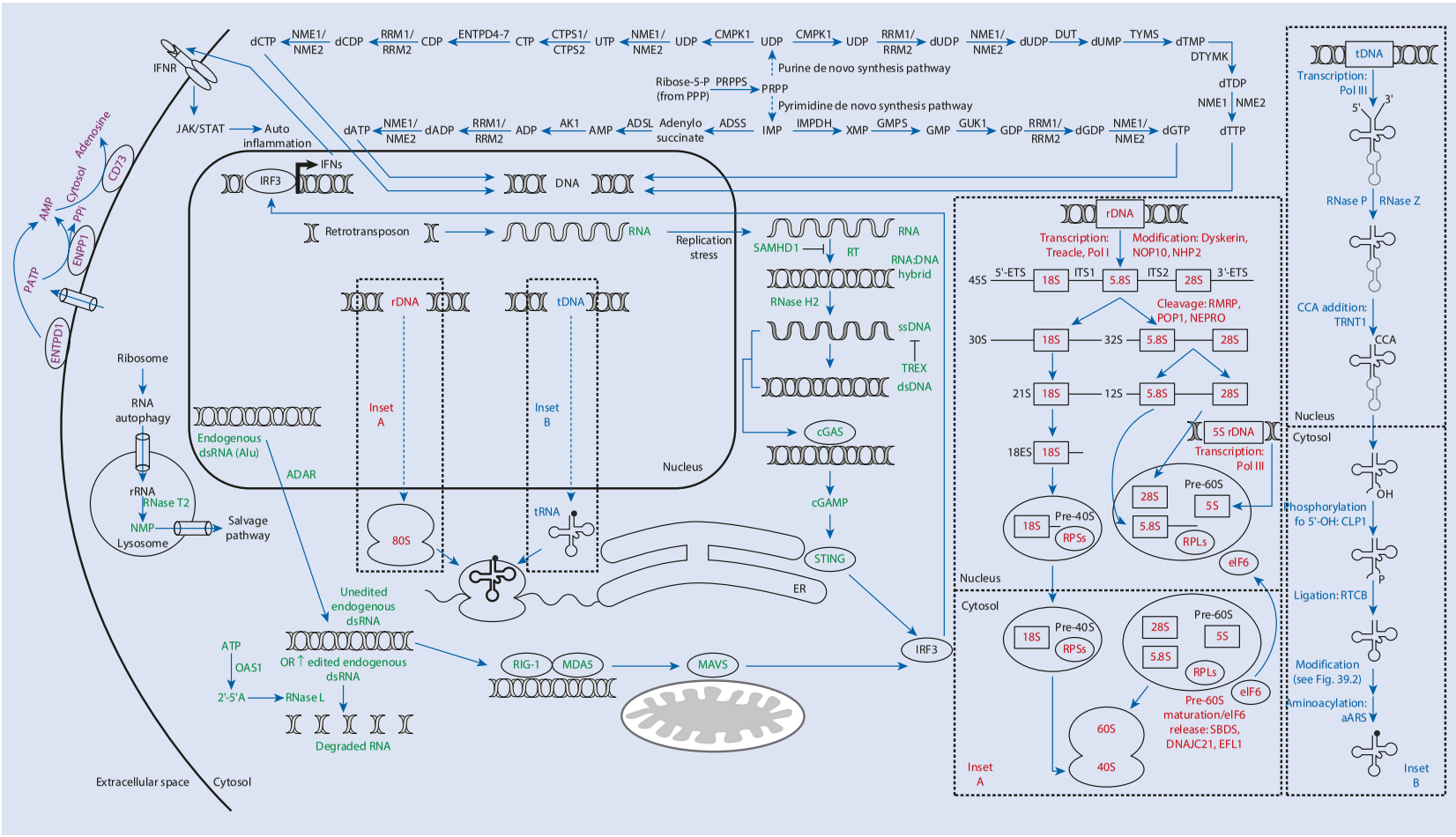

Fig. 39.1 Overview of nucleotide metabolism, ectonucleotide metabolism (purple), nucleic acid metabolism (green), tRNA processing and modification (blue), and ribosomal biogenesis (red). The steps in nucleic acid metabolism catalyzed by RT, SAMHD1, RNase H2 and TREX1 also take place inside the nucleus (not shown). The protein synthetic machinery consists of mRNA, aminoacyl-tRNAs, and the ribosomes. Ribosomes are composed of two large protein complexes (the 40 and 60S subunits containing 33 and 49 proteins respectively), and four ribosomal RNAs, all encoded in the nuclear genome for cytoplasmic ribosomes. Additional maturation factors and accessory proteins are required for ribosomal assembly and function. Transfer RNAs (tRNAs) serve as the informational link between mRNA and protein. They are encoded by nuclear genes, but the mitochondrial t-RNA genes are part of the mitochondrial chromosome. After transcription and processing (removal of 5′-leader and 3′-trailer, CCA addition, splicing and modification), are charged with specific amino acids by tRNA synthetases, and can then interact with mRNA within ribosomal binding sites which allow attachment of the amino acid to a growing peptide chain. There are two sets of tRNAs, one for cytoplasmic protein synthesis and one serving a similar function in the mitochondria. Abbreviations (only for terms not already mentioned in Table 39.1): 2′-5'A 2′-5′-oligoadenylate, ADSS adenylosuccinate synthetase, ADSL adenylosuccinate lyase, AK1 adenylate kinase 1, cGAMP cyclic GMP-AMP, cGAS cGAMP synthase, CMPK1 cytosolic UMP/CMP kinase, CTPS1/CTPS2 CTP synthetase, dsDNA double-stranded DNA, dsRNA double-stranded RNA, DTYMK deoxythymidylate kinase, DUT deoxyuridine triphosphatase (located in mitochondria), ETS external transcribed spacer, GMPS GMP synthetase, GUK1 guanylate kinase 1, IFNs interferons, IFNR interferon receptor, IRF3 interferon regulatory factor 3, ITS internal transcribed spacer, MAVS mitochondrial antiviral-signaling protein, NME1/NME2 nucleoside diphosphate kinase, NMP nucleoside monophosphate, PPP pentose phosphate pathway, PRPP phosphoribosylpyrophosphate, PRPPS PRPP synthetase, RRM1/RRM2 ribonucleotide reductase, M1 and M2 subunits, RT reverse transcriptase, RTCB RNA 2′,3′-cyclic phosphate and 5'-OH ligase, ssDNA single-stranded DNA, TRNT1 tRNA nucleotidyltransferase, CCA-adding, TYMS thymidylate synthetase

Table 39.1 Some disorders with specific clinical symptoms, according to the specific pathway involved

Main clinical features		Genes involved
Phenotypes with predominant neurological manifestations		
Early-onset encephalopathies Brain image alterations Frequent extra-neurologic signs	**Nucleotide and nucleic acid metabolism disorders**	
	Aicardi-Goutières syndrome: encephalopathy, acquired microcephaly, spasticity, leukodystrophy, basal ganglia calcification, increased CSF lymphocytes, sterile pyrexia, thrombocytopenia, elevated LFTs. Other manifestations: Chilblain lupus, retinal vasculopathy.	*TREX1*, *RNASEH2A*, *RNASEH2B*, *RNASEH2C*, *SAMHD1*, *ADAR1* and *IFIH1* (AGS1-7 respectively) *RNASET2* (mimic of AGS)
	Aminoacyl-tRNA synthetases and tRNA processing metabolism disorders	
	Pontocerebellar hypoplasia type 2B, 2F, 2C, 2A, 4, 5 and 10. PCH type 2 and 10: Respiratory and feeding difficulties at birth. PCH type 4: polyhydramnios followed severe congenital encephalopathy (see text)	*TSEN2, TSEN15, TSEN34, TSEN54, CLP1*
	Psychomotor delay, severe spasticity, axial hypotonia, leukoencephalopathy with hypomyelination	*DARS1* (with brainstem and spinal cord involvement), *QARS1* (microcephaly with cerebral and cerebellar atrophy), *EPRS* (hypomyelinating leukodystrophy type 15), *AIMP1* (with nystagmus and join contractures), *AIMP2*
	Ribosomal biogenesis	
	Hypomyelinating leukodystrophy with hypodontia, hypogonadotropic hypogonadism, ataxia. Cerebellar atrophy and hypoplastic corpus callosum (variable).	*POLR1C* (hypomyelinating leukodystrophy type 11), *POLR3A,* (type 7 with progressive spastic-ataxia; adolescence onset), *POLR3B* (type 8)
Intellectual disability Associated with other neurological manifestations mainly **microcephaly and seizures**	**Aminoacyl-tRNA synthetases and tRNA processing metabolism disorders**	
	Dysmorphic features	*TRMT1, NSUN2, ADAT3*
	Short stature, early-onset diabetes mellitus	*TRMT10A*
	Severe myoclonic seizures, hypotonia, dystonia	*DALRD3*
	X-linked ID, abnormal behavior	*FTSJ1*
	Hypotonia, movement disorder	*ELP2*
	Autism, hypotonia, truncal ataxia, marfanoid habitus	*MOCS3*
	Galloway-Mowat syndrome: ID, microcephaly, hypotonia, seizures, cerebellar atrophy, early-onset steroid-resistant nephrotic syndrome	*YRDC, GON7, LAGE3, OSGEP, TP53RK, TPRKB, WDR4*
	Hypotonia	*PUS3*
	Developmental delay, brittle hair and nails	*CARS1*
	Seizures, ataxia	*SARS1*
	ID, growth failure, cholestasis, chronic pulmonary disease, sensorineural hearing loss (AR)	*YARS1*
	Seizures, cortical atrophy	*VARS1*
	ID, hypotonia, growth failure, liver dysfunction, zinc deficiency	*IARS1*
	Ribosomal biogenesis	
	X-linked ID, microcephaly, hypotonia. Cerebellar hypoplasia. Spondyloepiphyseal dysplasia.	*RPL10*
	MacInnes syndrome. Developmental delay, brachycephaly, trichomegaly.	*RPS23*

Table 39.1 (continued)

Main clinical features		Genes involved
Motor disorders Ataxia and Spastic paraparesis	**Nucleotide and nucleic acid metabolism disorders**	
	Complicated form of hereditary spastic paraplegia (type 64)	*ENTPD1*
	Aminoacyl-tRNA synthetases and tRNA processing metabolism disorders	
	Hypomyelinating leukodystrophy (type 9). Psychomotor delay, cerebellar dysfunction.	*RARS1*
	Pontocerebellar hypoplasia. Psychomotor delay, cerebellar dysfunction.	*RARS2*
	Hypomyelination with brainstem and spinal cord involvement and leg spasticity	*DARS1*
	Progressive cerebellar ataxia, LD with brainstem and spinal cord involvement; lactic acidosis	*DARS2*
	Ribosomal biogenesis	
	Autosomal recessive adolescent-onset progressive spastic ataxia	*POLR3A*
Motor disorders Neuropathy	**Aminoacyl-tRNA synthetases and tRNA processing metabolism disorders**	
	Familial dysautonomia (hereditary sensory and autonomic neuropathy type 3) with gastrointestinal dysfunction, orthostatic hypotension, hypertensive crises, alacrima, absent fungiform papillae of tongue.	*POLR3A*
	Axonal Charcot-Marie-tooth disease	*AARS1* (AD); epileptic encephalopathy with hypomyelination (AR), *GARS1, HARS1* (AD); Usher syndrome (AR), *YARS1* (AD), *KARS1* (adulthood, hearing loss in childhood), *MARS1* (AD, adulthood)

(continued)

Table 39.1 (continued)

Main clinical features		Genes involved
Other complex encephalopathies with neurodegeneration "ARS2 genes": associated with combined oxidative phosphorylation deficiency	**Aminoacyl-tRNA synthetases and tRNA processing metabolism disorders**	
	Delayed psychomotor development, regression, ataxia, spasticity, dystonia. Hypomyelinating leukodystrophy (type 15).	*EPRS1*
	Combined oxidative phosphorylation deficiency; Severe neurodevelopmental delay and movement disorder, +/− seizures; lactic acidosis	*WARS2, MARS2* (Spastic ataxia in French Canadian cohort), *EARS2* (leukodystrophy), *CARS2* (with progressive neurodegeneration and seizures), *VARS2, NARS2* (variable phenotype with myopathy, neurodegeneration with seizures, cerebral atrophy; ragged red fibers), *TARS2* (severe psychomotor delay and seizures), *FARS2* (infantile seizures, encephalopathy, psychomotor), *AARS2* (Cardiomyopathy, Progressive leukodystrophy with ovarian failure),
	Perrault syndrome (sensorineural hearing loss and ovarian failure)	*LARS2* (with non-immune hydrops with lactic acidosis and sideroblastic anemia), *HARS2*
	Cataracts, sensorineural hearing loss, neuropathy, skeletal dysplasia in a French-Canadian family; growth hormone deficiency	*IARS2*
	Prematurity, progressive renal failure, metabolic alkalosis, and pulmonary hypertension	*SARS2*
	Leukodystrophy with brainstem and spinal cord involvement; lactic acidosis; slowly progressive cerebellar ataxia	*DARS2*
	Myopathy, lactic acidosis, sideroblastic anemia	*YARS2*
	Pontocerebellar hypoplasia	*RARS2*
	Early infantile encephalopathy with seizures	*PARS2*
	Ribosomal biogenesis	
	Cognitive regression after a period of normal development; eventual severe ID	*UBTF1*
	Progressive cerebral degeneration, angiomatous-like blood vessels, spasticity, dystonia, seizures, cognitive decline. Leukoencephalopathy with brain calcifications and cysts.	*SNORD118*

Table 39.1 (continued)

Main clinical features				Genes involved
Neurological disorders with diverse organ involvement	**Aminoacyl-tRNA synthetases and tRNA processing metabolism disorders**			
	Growth delay, hypotonia, brain calcifications with cysts, interstitial lung disease with cholesterol pneumonitis, liver dysfunction			*FARSA, FARSB*
	Intellectual disability, hypotonia, growth failure, liver dysfunction, zinc deficiency			*IARS1*
	Ribosomal biogenesis			
	Wiedemann-Rautenstrauch syndrome: extreme growth failure, progeroid features, lipodystrophy, ID.			*POLR3A*
Brain imaging abnormalities				
Leukodystrophy	**Calcifications**	**Pontocerebellar hypoplasia**	**Cerebellar atrophy +/− cerebral atrophy**	**Leukodystrophy with hypomyelination**
TREX1, RNASEH2A, RNASEH2B, RNASEH2C, SAMHD1, ADAR1 and *IFIH1* (AGS1-7 respectively) *RNASET2* (mimic of AGS): LD with calcifications *DARS2* (+brainstem and spinal cord), *EARS2, AARS2* *SNORD118* (LD with brain calcifications and cysts)	*FARSA, FARSB* (calcifications with cysts) *SNORD118* (LD with calcifications and cysts)	*TSEN2, TSEN15, TSEN34, TSEN54, CLP1RARS2*	*QARS1* (cerebral and cerebellar atrophy and hypomyelination) *AIMP1, AIMP2* (+ hypomyelination, BG signal abnormality) *UBTF1* *YRDC, GON7, LAGE3, OSGEP, TP53RK, TPRKB, WDR4:* Galloway-Mowat, cerebellar atrophy	*RARS1* (with thin corpus callosum) *DARS1* (with white matter lesions: supratentorial, brainstem, cerebellum and spinal cord) *EPRS1, AARS1* *AIMP1, AIMP2* (+brain atrophy) *POLR1C, POLR3A, POLR3B*
Phenotypes with predominant extra-neurological features				
Hematological and immunological manifestations	**Nucleotide and nucleic acid metabolism disorders**			
	Immunodeficiency with hyper-IgM syndrome (type 2 and 5)			*AICDA, UNG*
	Ribosomal biogenesis			
	Diamond-Blackfan anemia type 1, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 respectively. Normochromic macrocytic anemia, high erythrocyte deaminase, dysmorphic features (Cathie facies), congenital malformations (thumb, heart, genitourinary), increased risk of leukemia and osteosarcoma			*RPS19, RPS24, RPS17, RPL35A, RPL5, RPL11, RPS7, RPS10, RPS26, RPL26, RPL15, RPS29, TSR2, RPS28, RPL27, RPS27, RPL18, RPL35, RPS15A*
	Familial isolated congenital asplenia. Isolated asplenia with predisposition to infections from encapsulated bacteria			*RPSA*
Cardiological and vascular manifestations	**Nucleotide and nucleic acid metabolism disorders**			
	Singleton-Merten syndrome (SMS) type 1 and 2 (vascular calcification, arrhythmias, skin and other manifestations)			*IFIH1, DDX58*
	Generalized arterial calcification of infancy (GACI); pseudoxanthoma elasticum (PXE), joint calcification, rickets, hearing loss.			*ABCC6, ENPP1*
	Arterial calcification due to deficiency of CD73 (ACDC)			*NT5E*
	Ribosomal biogenesis			
	Dilated cardiomyopathy			*TAF1A*

(continued)

Table 39.1 (continued)

Main clinical features		Genes involved
Skin and connective tissue prominent manifestations Some disorders associated with osteoarticular and hematological manifestations	**Nucleotide and nucleic acid metabolism disorders**	
	Singleton-Merten syndrome (SMS) type 1 and 2 (vascular calcification, arrhythmias, nail hypoplasia, psoriasis, early-onset glaucoma, early loss of dentition, osteoporosis)	*IFIH1, DDX58*
	STING-associated vasculopathy (SAVI) with severe skin lesions of face and digits leading to ulceration or amputation; interstitial lung disease	*TMEM173*
	Periarticular calcification + Augustine-null blood type	*SLC29A1*
	Aminoacyl-tRNA synthetases and tRNA processing metabolism disorders	
	Nonphotosensitivity trichothiodystrophy (type 7)	*TARS1*
	Ribosomal biogenesis	
	Abnormal skin pigmentation, nail dystrophy, leukoplakia of oral mucosa, solid tumors, progressive bone marrow failure, premature hair graying, atresia of lacrimal ducts	*DKC1, NOLA3, NOLA2*
	Dyskeratosis congenita: Bone marrow failure, short stature, radial ray defects, skin pigmentation abnormalities, nail dystrophy	*NPM1*
	Cartilage-hair hypoplasia (more severe form: anauxetic dysplasia type 1). Metaphyseal dysplasia, short stature, joint laxity, hair hypoplasia, anemia, increased risk for malignancy, T-cell and B-cell immunodeficiency	*RMRP*
	AD non-syndromic aplasia cutis congenita (of the scalp)	*BMS1*
	AD hypotrichosis simplex	*RPL21*
Skeletal abnormalities	**Ribosomal biogenesis**	
	Treacher Collins syndrome type 1,2,3,4, and acrofacial dysostosis, Cincinnati type respectively. Bilateral malar and mandibular hypoplasia, downslanted palpebral fissures, eyelid coloboma, conductive hearing loss, cleft palate	*TCOF1, POLR1D, POLR1C, POLR1B, POLR1A*
	Cartilage-hair hypoplasia (more severe form: anauxetic dysplasia type 1). Metaphyseal dysplasia, short stature, joint laxity, hair hypoplasia, anemia, increased risk for malignancy, T-cell and B-cell immunodeficiency	*RMRP*
	Spondyloepimetaphyseal dysplasia with severe short stature (anauxetic dysplasia type 2), mild intellectual disability	*POP1*
	Severe short stature (anauxetic dysplasia type 3), brachydactyly, joint laxity, developmental delay	*NEPRO*
	Spondyloepimetaphyseal dysplasia with short stature	*RPL13*

Table 39.1 (continued)

Main clinical features		Genes involved
Lung disease	**Nucleotide and nucleic acid metabolism disorders**	
	TMEM173 STING-associated vasculopathy (SAVI): interstitial lung disease	*TMEM173*
	OAS1 Pulmonary alveolar proteinosis with hypogammaglobulinemia	*OAS1*
	Aminoacyl-tRNA synthetases and tRNA processing metabolism disorders	
	Interstitial lung disease with liver dysfunction	*FARSA, FARSB,* (with cholesterol pneumonitis + neurological involvement), *MARS1* (pulmonary alveolar proteinosis)
Renal abnormalities	**Aminoacyl-tRNA synthetases and tRNA processing metabolism disorders**	
	Galloway-Mowat syndrome: early-onset steroid-resistant nephrotic syndrome and other neurological signs (see above)	*YRDC, GON7, LAGE3, OSGEP, TP53RK, TPRKB, WDR4*
Endocrine dysfunction	**Ribosomal biogenesis**	
	POLR3H-related primary ovarian insufficiency. Female infertility	*POLR3H*
Liver dysfunction	**Aminoacyl-tRNA synthetases and tRNA processing metabolism disorders**	
	Chronic and acute intermittent hepatopathy	*LARS1*
	Interstitial lung disease, pulmonary alveolar proteinosis and liver disease (AR)(infancy)	*MARS1*
Other disorders with diverse organ involvement	**Nucleotide and nucleic acid metabolism disorders**	
	Histiocytosis, lymphadenopathy, hypertrichosis, cutaneous hyperpigmentation, hepatomegaly, heart anomalies, hearing loss, hypogonadism, insulin-dependent diabetes, skeletal dysplasia	*SLC29A3*
	Ribosomal biogenesis	
	Prenatal and postnatal growth retardation, prominent nose, joint contractures, rocker-bottom feet, intellectual disability, trisomy 18 phenocopy	*EMG1*
	Poly(A)-specific ribonuclease deficiency. Pulmonary fibrosis and bone marrow failure (dominant); dyskeratosis congenita (recessive)	*PARN*
	Shwachman-Diamond syndrome. Exocrine pancreatic dysfunction with pancreatic lipomatosis and fat malabsorption, metaphyseal dysplasia, growth failure, cytopenias, predisposition to leukemia	*SBDS, DNAJC21, EFL1, EIF6*

AGS Aicardi-Goutières syndrome, *ID* intellectual disability, *AD* autosomal dominant, *AR* autosomal recessive, *LD* leukodystrophy

39.1.2 Disorders of Nucleic Acids: Autoinflammatory Phenotype – Prototype: Aicardi-Goutières Syndrome

Disorders of nucleic acid metabolism are a pleiomorphic group of conditions that can cause characteristic clinical phenotypes, but are also relatively non-specific, leading to delays in diagnosis. Most patients are likely to be seen in neurology clinics first, and come to attention for genetic testing after significant delay. Early suspicion, especially in the face of developmental delay or regression, should trigger application of broad-based next generation sequencing to achieve prompt diagnosis. For the most part, treatment options lag behind diagnosis for most diseases and are focused on amelioration of symptoms.

The prototypical clinical disorder within this group is Aicardi-Goutières syndrome (AGS), defined by the clinical and radiological features of an early onset, severe, neurologic disorder with intracranial calcification, leukoencephalopathy, and cerebral atrophy. AGS is considered to represent an abnormal response to endogenous or self-derived nucleic acids.

Clinical Presentation

The clinical presentation of AGS can be divided in three forms, and mutations in any of the seven AGS genes may result in each phenotype: (1) Prenatal onset mimicking an acquired transplacental infection. At birth, the clinical picture is dominated by neurologic symptoms including irritability, feeding difficulties, jitteriness, microcephaly, abnormal movements and epileptic seizures. Hematological disturbance like thrombocytopenia, anemia and liver dysfunction can be present and can resolve after a few weeks. This presentation is associated with profound developmental defects and increased risk of death in infancy. *TREX1* mutations are most commonly associated with this presentation (2) Infantile onset is the most frequent form of AGS, presenting in the first few months of life. Features are the same as the neonatal form. Parents report an abrupt onset of irritability and sometimes episodes of sterile pyrexia. In some cases, development has been normal prior to this event, and disease onset is associated with a loss of previously acquired skills. The encephalopathic stage typically lasts for several months, coinciding with the neurological regression. Mutations in *RNASEH2B* represent the most frequent genotype. (3) Later onset (beyond the first year of life) presents with an abrupt or subacute onset of neurologic regression after a period of normal development. *ADAR1*, *IFIH1* and *RNASEH2B* mutations have been identified in patients with previous undefined spastic paraparesis. In *SAMHD1* mutations, a milder phenotype has been described, showing normal or mild intellectual disability, microcephaly, spastic paraparesis and inflammatory skin manifestations [6–8].

Most patients are severely intellectually and physically impaired. However, some children with *RNASEH2B* and *SAMHD1* mutations have preserved intellectual function. Most patients exhibit a severe acquired microcephaly, but in those children with preserved intellect the head circumference is normal. Typically, patients develop truncal hypotonia, poor head control, limb spasticity and dystonia. Seizures are reported in around 50% of patients, and some children demonstrate a marked startle reaction. Skin lesions are an important feature of AGS, frequently referred to as chilblains, that are exacerbated by cold temperature and thus more prominent during winter months. Glaucoma is also a recurrent feature. Features of autoimmunity, most commonly thyroiditis and less frequently lupus-like disease, can also occur [6, 7, 9].

The major clue to the diagnosis in neonatal- and infantile-onset AGS usually comes from neuroimaging, showing a characteristic pattern comprising diffusely abnormal white matter, cerebral atrophy, particularly involving anterior temporal lobes, and intracranial calcification. On the other hand, neuroimaging can be normal in late-onset AGS with spastic paraparesis. Other neuroimaging patterns described are bilateral striatal necrosis (*ADAR1*) and intracerebral large vessel involvement with intracranial stenoses, aneurysms and intracerebral hemorrhage (*SAMHD1*) [6, 10, 11].

Genetics

Mutations in any of the following 7 genes account for ~95% of patients with the classical AGS phenotype: *TREX1* (AGS1), *RNASEH2A* (AGS2), *RNASEH2B* (AGS3), *RNASEH2C* (AGS4), *SAMHD1* (AGS5), *ADAR1* (AGS6), and *IFIH1* (AGS7). In most cases, inheritance is autosomal recessive; however, specific heterozygous gain-of-function mutations have been observed in *TREX1* and *ADAR1*, and all AGS-related mutations in *IFIH1* are dominant [6, 12, 13].

Diagnostic Tests

Mutations in any of these genes can result in an induction of type 1 interferon production and an upregulation of interferon-stimulated genes, similar to the type 1 interferon response following exposure to viral DNA or RNA, probably explaining why the clinical features of AGS may resemble those of a viral infection [6]. The use of the term "type I interferonopathies" has been applied to this spectrum of disease [6, 14]. Cerebrospinal fluid shows lymphocytosis and elevated interferon-α. However, the level of both falls to normal over the first

few years of life. CSF interferon-α appears to be a reliable marker early in the disease course, and more recently the "interferon signature" in peripheral blood, a measure of expression of interferon signaling genes, has been considered a biomarker for the disease. Recently, elevated C26:0 lysophosphatidylcholine (C26:0 Lyso-PC), currently used in newborn screening panels for X-linked adrenoleukodystrophy, has been identified at birth in patients with a molecular and clinical diagnosis of AGS. The inflammatory response, demonstrated by interferon activation, was consistent with the elevation of phosphatidylcholines. The authors speculated that the elevation of C26:0 Lyso-PC may be a marker of a downstream inflammatory pathway in AGS pathophysiology [7, 15, 16].

Treatment

A more targeted approach to therapy is currently being explored. A trial of reverse transcriptase inhibitors (RTIs) in AGS has recently been published, including patients that were treated with a combination of abacavir, lamivudine and zidovudine. Recent reports with Janus kinase (JAK1), an essential component of the type I interferon receptor complex, show encouraging results in patients with JAK inhibition and type I interferonopathies. Results with the JAK1/2 inhibitor, ruxolitinib, also are promising but a clinical trial has not yet been completed. Other strategies to block interferon signaling downstream of a nucleic acid stimulus, including anti-interferon antibodies, antibodies against the type I interferon receptor, and molecules targeting components of the pathways transducing a type I interferon response, are under investigation [14, 17].

39.2 tRNA Processing Disorders

See Fig. 39.1, insert B, blue pathway.

39.2.1 Disorders of Pre-tRNA Splicing – Prototype: Pontocerebellar Hypoplasia

Transfer RNAs (tRNAs) reach maturity and functionality after a complex series of processing and modification events. Genes encoding tRNAs are transcribed by RNA polymerase III into precursor tRNAs (pre-tRNAs) which undergo a series of posttranscriptional processing and modification events to mature into functional tRNAs. Neurons are particularly sensitive to defects in tRNA splicing, with reported links between mutations in the tRNA splicing machinery and neurodegenerative disorders [18, 19].

Clinical Presentation

Pontocerebellar hypoplasia (PCH) is a group of prenatal onset neurodegenerative disorders with different underlying genetic causes classified based on clinical, neuroradiological and neuropathological characteristics. Since the original description, the phenotype of PCH has been significantly broadened, including nine different subtypes with great clinical and neuroradiological variability of which several involve tRNA splicing defects [20, 21]. Classification of the PCH2 subtypes was done chronologically based on the order of the identification of respective disease genes.

PCH type 2 is the most frequent form of PCH, and typical clinical features include respiratory and feeding difficulties at birth, dyskinesia, chorea, progressive microcephaly and severe cognitive and motor impairment. PCH type 2A, is the most prevalent and best characterized of all PCH subtypes. Clinically, it is distinguished by generalized clonus and swallowing incoordination in the neonate. The toddler and young child present with spasticity, dystonia, chorea and epilepsy. Motor development is poor and cognitive development is almost absent. Patients often exhibit feeding difficulties requiring gastrostomy. The subtypes classified as PCH2B, PCH2C and PCH2F, are rare and result in a similar phenotype. The *TSEN54*-related PCH spectrum shows severe impairment of cognitive and motor development.

PCH type 4 clinically presents prenatally with polyhydramnios followed by a severe congenital encephalopathy including hypertonia, contractures and primary hypoventilation requiring mechanical ventilation. PCH type 10 was reported in a group of families with a similar phenotype as PCH type 2, and mutations in the *CLP1* gene have been identified. Neuroimaging shows brainstem and cerebellar hypoplasia, with the cerebellar hemispheres more affected than the vermis. Brain MRI shows a "dragonfly" configuration of the cerebellum, resulting from severely affected hemispheres ("wings") and relative sparing of the vermis ("body"). In about 40%, the cerebral cortex shows mild or severe atrophy [20–23].

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The transfer-RNA (tRNA) splicing endonuclease complex (TSEN complex)-consisting of four protein subunits is involved in splicing of intron-containing pre-tRNAs. CLP1 also interacts with the TSEN complex for an efficient tRNA splicing.

Mutations in genes encoding TSEN complex subunits and associated genes (*CLP1*) impair tRNA splicing and subsequently alter protein translation. Mutations in *TSEN54* are responsible for PCH type 2 and 4 in most European cases. The majority of patients with well-defined PCH2 have a homozygous c.919G > T (p.

A307S) transversion in *TSEN54*, a likely founder mutation. PCH2, 4 and 5 are allelic disorders and represent a continuum of clinical phenotypes [19–21].

Treatment

No treatments are available so far.

39.2.2 Disorders of tRNA Modification – Prototype: Non-syndromic Intellectual disability

Transfer RNA undergoes several modifications needed for proper function (Fig. 39.2). Many disorders of tRNA modification are associated with non-syndromic intellectual disability, including TRMT1, FTSJ1, NSUN2, ADAT3, and PUS3 deficiencies. Other defects of cytosolic tRNA modification lead to Galloway-Mowat syndrome, with intellectual disability, cerebellar atrophy, and early-onset steroid-resistant nephrotic syndrome; the latter findings elicit a differential diagnosis of primary CoQ10 biosynthetic defect. Diagnosis is based on molecular grounds.

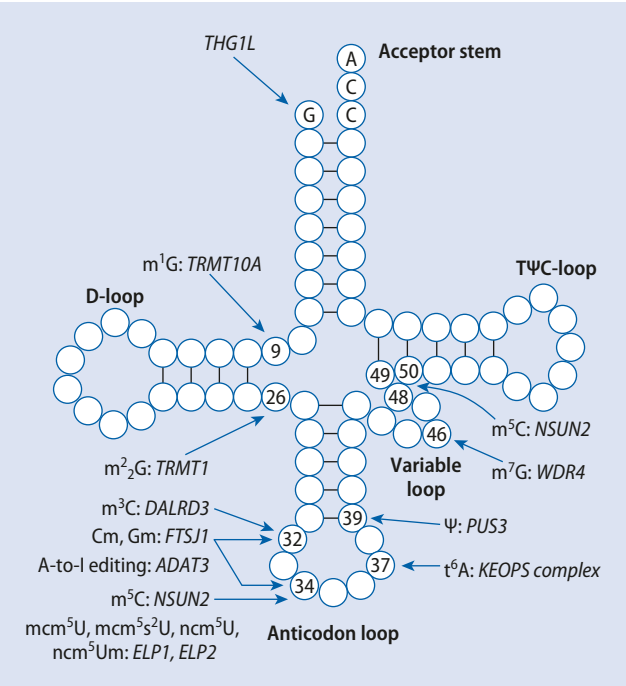

Fig. 39.2 Secondary cloverleaf structure of tRNA showing position and type of modification, as well as protein/complex responsible for such modification. Only modifications of cytosolic tRNA (not mitochondrial tRNA) are shown. Cm 2'-O-methylcytidine, Gm 2'-O-methylguanosine, m1G 1-methylguanosine, m22G N2,N2-dimethylguanosine, m3C 3-methylcytosine m5C, 5-methylcytosine, m7G 7-methylguanosine, mcm5U 5-methoxycarbonylmethyluridine, mcm5s2U 5-methoxycarbonylmethyl-2-thiouridine, ncm5U 5-carbamoylmethyluridine, ncm5Um 5-carbamoylmethyl-2'-O-methyluridine, Ψ pseudouridine, t6A N6-threonylcarbamoyladenosine

39.2.3 Disorders of tRNA Aminoacylation: Neurodegenerative and Systemic Disorders

Mutations in the tRNA synthetase genes fall into two categories. The first are those involved in cytoplasmic protein synthesis [24]. These enzymes synthesize the amino acid-tRNA conjugates in the cytoplasm. The second group of tRNA synthetases are transported to mitochondria, where they synthesize the amino acid-tRNAs used for mitochondrial protein synthesis [25]. With the exception of GARS and KARS, mitochondrial and cytoplasmic aaRSs are encoded by distinct nuclear genes. Each compartment has unique tRNA synthetases that cannot substitute for each other if one has a mutation.

Clinical Manifestation

The phenotype of each group of tRNA synthetases is variable, and the symptoms for each tRNA synthetase defect are different. However, each specific defect tends to have a more consistent phenotype.

The cytoplasmic defects (typically denoted as *XARS1*, where *X* is the amino acid attached by the enzyme to its respective tRNA) can present as neurodegenerative disorders like Charcot-Marie-Tooth disease with progressive weakness and muscle atrophy, Perrault syndrome with sensorineural hearing loss and ovarian dysgenesis, Usher syndrome with congenital deafness and retinitis pigmentosa, infantile leukodystrophy, or visceral presentations like interstitial lung and liver disease. Inheritance is autosomal dominant or recessive. Specific findings with each defect are listed in Table 39.1.

All mitochondrial tRNA synthetases are nuclear encoded and inherited as autosomal recessive conditions designated as *XARS2*. The most common phenotype is that of a combined, usually severe, disorder of oxidative phosphorylation (see also ▶ Chap. 10). However, other phenotypes overlap those seen in defects of cytoplasmic tRNA synthesis including Perrault syndrome, interstitial lung disease with pulmonary hypertension, and leukodystrophy with microcephaly. Renal disease with hyperuricemia and primary metabolic alkalosis is seen in SARS2 deficiency [26]. Specific findings with each defect are listed in Table 39.1.

Diagnostic Tests

Diagnosis should be facilitated by aminoacylation assays as shown recently for patients with LARS2 and

KARS deficiencies [27]. For the most part, however, diagnosis is based on symptoms and most often a rapid move to broad molecular testing as there are no disease-specific biochemical markers.

Treatment

Therapies are limited and usually symptomatic. Infusion of high levels of leucine and/or total protein in patients with LARS1 deficiency has been reported to improve acute liver dysfunction [28]. However, similar treatment with specific amino acids has either not been reported or has not been successful in the other disorders.

39.3 Ribosomal Biogenesis

See Fig. 39.1, insert A, red pathway.

Mitochondrial ribosomal proteins are nuclear encoded, but the mitochondrial ribosomal RNA genes are part of the mitochondrial chromosome. Thus, all told, there is the potential for hundreds of disorders related to ribosomal structure and function. This chapter will only deal with non-mitochondrial ribosomal disorders.

39.3.1 Disorders of Pre-rRNA Transcription: Craniofacial Anomalies

Disorders of pre-rRNA transcription lead to defects of neural crest cell development. Since neural crest cells give rise to craniofacial mesenchyme (including bones and cartilage), these disorders lead to craniofacial anomalies [29]. Acrofacial dysostosis is thus seen in Treacher Collins syndrome and acrofacial dysostosis, Cincinnati type; the latter, caused by mutations in the *POLR1A* gene, can be accompanied by limb anomalies such as bowed femurs. Diagnosis is suspected on clinical grounds, although the specific molecular defect can only be ascertained by sequencing.

39.3.2 Disorders of 5S rRNA and tRNA Transcription: Neurodegeneration, Leukodystrophy and Systemic Disorders

Clinical Presentation

Biallelic loss-of-function variants of RNA polymerases (*POLR3A* and its interactors *POLR3B* and *POLR1C*) are known to cause a series of related but still different entities. *POLR3A* variants can cause 4H leukodystrophy, Wiedemann-Rautenstrauch syndrome (WRS), and progressive spastic ataxia [30–32]. Variants in *POLR1C* can cause hypomyelinating leukodystrophy type 11, and variants in *POLR3B* have been found in individuals with cerebellar hypoplasia-endosteal sclerosis (CHES), that shows overlap with both 4H leukodystrophy and WRS [30, 31].

The exact pathogenesis explaining the various signs and symptoms associated with decreased POLR3A activity, such as myelination, dental, bone and fat tissue abnormalities, remains unclear [30, 32].

POLR3-related leukodystrophy is a well-recognizable clinical entity if all features are present. It is a hypomyelinating leukodystrophy with different combinations of four major clinical findings: (1) neurologic dysfunction; (2) abnormal dentition (delayed dentition, hypodontia, oligodontia, and abnormally placed or shaped teeth); (3) endocrine abnormalities (hypogonadotropic hypogonadism manifesting as delayed or absent puberty or short stature); and (4) ocular abnormality (progressive myopia).

POLR3-related leukodystrophyy and 4H leukodystrophy are the terms for five previously described overlapping clinical phenotypes, described before the molecular basis was delineated, including: hypomyelination, hypodontia, hypogonadotropic hypogonadism (4H syndrome); ataxia, delayed dentition, and hypomyelination; tremor-ataxia with central hypomyelination; leukodystrophy with oligodontia; and hypomyelination with cerebellar atrophy and hypoplasia of the corpus callosum. A large cohort of *POL3A* and *POL3B* mutation-proven cases of 4H syndrome is characterized by a progressive disorder with motor dysfunction due to increasing ataxia, and sometimes with episodes of faster deterioration triggered by minor infections [33]. The main neurological manifestation is developmental delay usually noted between the age of 1–2 years (50%). Most of the patients present with signs of cerebellar dysfunction (tremor, dysmetria and gait ataxia), and some patients are never able to walk independently. Other features include dystonia and oculomotor disorders (abnormal smooth pursuit, nystagmus, vertical gaze limitation). Pyramidal signs are usually absent in children and develop slowly in older patients. Cognition vary widely, from normal cognitive abilities to intellectual disability in most. Among non-neurologic manifestations, dental abnormalities are present in most patients (delayed dentition and hypodontia), as well as delayed puberty or primary amenorrhea, and progressive myopia [31, 33].

WRS is a neonatal progeroid condition characterized by prenatal and early postnatal growth retardation, sparse scalp hair, generalized lipodystrophy with characteristic fatty tissue accumulations, and an unusual face characterized by a triangular shape, apparently low-set eyeballs partly covered by the lower eyelids, small mouth, pointed chin and natal teeth [30].

More recently, hypomorphic biallelic variants in *POLR3A* have been reported as a cause of autosomal recessive adolescent-onset progressive spastic ataxia. The

phenotype additionally includes tremor and involvement of the central sensory tracts, leading to the diagnosis of complicated hereditary spastic paraplegia. Some patients present ataxia with extrapyramidal features and dental problems (hypodontia, periodontal disease) [32, 34].

Neuroimaging abnormalities are a key feature in *POLR3*-related leukodystrophy, with brain MRI showing hypomyelination in combination with relative preservation of myelination of specific brain structures. T2 hypointensity of the dentate nuclei, ventrolateral thalamus, pyramidal tracts within the posterior limb of the internal capsule, globus pallidus and optic radiations is present. Moreover, cerebellar atrophy can be present. Supratentorial atrophy and thin corpus callosum is present in adult patients, reflecting progressive cerebral white matter volume loss [33, 35]. MRI findings differ in progressive spastic ataxia, and the most consistent finding on FLAIR images is bilateral hyperintensities along the superior cerebellar peduncles, combined with a hypointense correlate in T1-weighted images, indicating secondary myelin degradation. In addition, most cases show cervical cord atrophy and slight hypoplasia of the corpus callosum [32, 34].

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RNA polymerase (pol) I synthesizes the large rRNA, pol II synthesizes mRNA, and pol III synthesizes tRNA and 5S rRNA. The nuclear RNA polymerases are complex enzymes, made up of 12 or more subunits. Reduction of POLR3A leads to reduction of the total pool of tRNAs and a deregulated transcription of certain types of noncoding RNAs [36, 37].

Treatment is still symptomatic and individualized, including a multidisciplinary team. Seizures, spasticity or dystonia are managed in a routine manner [31, 33].

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39.3.3 Disorders of Pre-rRNA Processing: Skeletal Dysplasia and Systemic Disorders

Mutation of specific proteins that participate in telomere maintenance and pre-rRNA modification are associated with dyskeratosis congenita, while defects in dyskerin and NOP10 affecting the catalytic pseudouridylation site cause cataracts, enterocolitis, sensorineural hearing loss, and steroid-resistant nephrotic syndrome [38]. Defects in pre-rRNA cleavage lead to skeletal dysplasias, such as cartilage-hair hypoplasia (*RMRP*), *POP1*, and *NEPRO* deficiencies. The diagnosis can be suspected on clinical and radiographic grounds.

39.3.4 Disorders of Maturation of 40S and 60S Ribosomal Subunits – Prototype: Diamond-Blackfan Syndrome

Diamond-Blackfan syndrome(s) is the most common phenotype related to ribosomal dysfunction [39]. It is caused by mutations in at least 20 genes, the most common of which is *RPS19*. Originally recognized as a genetic form of normochromic, macrocytic anemia, congenital anomalies are present in 30–50% of patients, including craniofacial. Malformations, congenital heart defects, and genitourinary abnormalities. Upper extremity (especially thumb) abnormalities are particularly characteristic. Other blood cell lines may be diminished. Low birthweight and infantile failure to thrive are common. Malignancies may develop including acute myelogenous leukemia, myelodysplastic syndrome, and some solid tumors, especially osteosarcoma. The diagnosis is usually first suspected on the basis of hematologic abnormalities or congenital anomalies, Hemoglobin F and red blood cell adenosine deaminase may be elevated. Confirmation is by DNA sequencing [40]. Mutations in *RPS19* account for ~25% of patients with the rest of patients having scattered mutations across the other >20 genes. Therapy is symptomatic and may include RBC transfusions or bone marrow transplant [41].

39.3.5 Disorders of Active 80S Ribosome Assembly: Shwachman-Diamond Syndrome

Shwachman-Diamond syndrome (SDS) is characterized by exocrine pancreatic insufficiency, hematologic abnormalities, and metaphyseal changes [42]. The pancreatic involvement leads to malabsorption, growth failure, fat-soluble vitamin deficiency, low serum concentrations of isoamylase and cationic trypsinogen, and fatty infiltration with a hyperechoic pancreas. The most common hematologic abnormality is neutropenia (intermittent or persistent), but other frequent findings include anemia, thrombocytopenia, myelodysplasia and leukemia [43]. Severe skeletal changes in infancy can lead to short ribs mimicking asphyxiating thoracic dystrophy. SDS is caused by an inability to remove the eukaryotic initiation factor 6 (EIF6) from the pre-60S ribosome subunit, a process needed for the assembly of the active 80S ribosome [44].

The diagnosis is typically suspected on clinical grounds, and is confirmed molecularly.

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Disorders of Sphingolipid Synthesis, Sphingolipidoses, Niemann-Pick Disease Type C and Neuronal Ceroid Lipofuscinoses

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Sphingolipid Structure and Metabolism

Sphingolipids are ubiquitous lipids found in all mammalian cell membranes and in plasma lipoproteins. Many exhibit dual functions, as key structural elements, but also as modulators of numerous biological/physiological functions. Their backbone is a long chain sphingoid base (sphingosine being the prototype) that can be N-acylated by a variety of fatty acids, forming ceramides (Cer) (◘ Fig. 40.1). Depending on the type of hydrophilic head group linked to the 1-OH group of the sphingoid base, two main classes of sphingolipids are distinguished. Phosphosphingolipids contain phosphorylcholine (sphingomyelin), phosphorylethanolamine, or a phosphate group. Glycosphingolipids contain one (glucose or galactose) or several sugar residues and can be overly complex. Sialic acid (N-acetyl-neuraminic acid in humans) containing glycosphingolipids are named gangliosides. Depending on the precise structure (sugar and linkage) of the oligosaccharide moiety, several glycosphingolipid lineages (ganglio-, globo-, etc) have been defined. "Cerebroside" usually refers to the major myelin lipid galactosylceramide; "sulfatide" to its sulfated derivative. Lysosphingolipids (e.g., psychosine) lack the fatty acid of ceramide. The main sphingolipids are depicted in ◘ Figs. 40.1. and 40.2. Sphingolipids are synthesised and degraded in different subcellular compartments. A further aspect of sphingolipid homeostasis not discussed here is the recycling or salvage pathway.

Biosynthesis (◘ Fig. 40.1). The de novo synthesis of ceramide occurs in the endoplasmic reticulum (ER) and starts by the condensation of serine and palmitoyl-CoA, a reaction catalysed by serine palmitoyltransferase. The resulting 3-ketosphinganine is reduced to sphinganine prior to N-acylation by ceramide synthases. Distinct fatty acids, including 2-hydroxylated long chain fatty acids and ω-hydroxylated ultra-long chain fatty acids, can be incorporated (▶ Chap. 42). Then, dihydroceramide is desaturated to produce ceramide. The sphingosine that is released by the action of ceramidases on the ceramide derived from the degradation of complex sphingolipids can also be N-acylated by ceramide synthases. Subsequent steps of sphingolipid synthesis (except galactosylceramide) occur in the Golgi apparatus, where ceramide is transformed to sphingomyelin or to glucosylceramide; stepwise addition of further monosaccharides, catalysed by specific glycosyltransferases, leads to the formation of more complex neutral glycosphingolipids and gangliosides. Sphingolipids are then transported and inserted into various membranes.

Degradation (◘ Fig. 40.2). After transport by the endolysosomal pathway to the lysosome, sphingolipid degradation proceeds by stepwise hydrolysis by specific acid sphingohydrolases, some of which may need cofactors called sphingolipid activator proteins (also lysosomal) for their in vivo action. Specific non-lysosomal degradation reactions also operate at the plasma membrane, Golgi/ER interface, or ER level (◘ Fig. 40.1).

Introduction

This chapter first discusses inherited diseases involving the metabolism of sphingolipids. Sphingolipidoses, i.e., diseases resulting from defects in the lysosomal degradation of sphingolipids, constitute one of the major historical groups among lysosomal storage disorders (LSDs). Defects of sphingolipid biosynthesis or of non-lysosomal sphingolipid degradation, more recently identified, are also briefly described. Niemann-Pick C disease, now reclassified as a disorder of lysosomal trafficking of cholesterol also involving sphingolipids, constitutes a separate category. The chapter finally describes neuronal ceroid lipofuscinoses, now recognised as another important group among LSDs.

40.1 Disorders of Sphingolipid Synthesis

Most of the genes encoding the enzymes, transporters and activators operating in the sphingolipid synthesis pathway have been characterised, and an increasing number of monogenic defects affecting some steps of the biosynthesis of sphingolipids have been delineated in recent years (◘ Table 40.1). A majority of disorders were first described as a component of a genetically heterogeneous clinical syndrome – e.g., hereditary sensory and autonomic neuropathies (HSAN), autosomal recessive hereditary spastic paraplegias,– before the function of the protein encoded by the mutated gene was recognised. Mechanisms underlying the pathophysiology of most sphingolipid synthesis disorders are still enigmatic. Except for HSAN1, the reported mutations result in a loss of function of the corresponding enzyme. Alterations in the sphingolipid profile of the diseased tissues have not been described in all conditions. In general, it is still unknown whether tissue dysfunction and symptoms are due to the lack (or insufficient production) of one or more sphingolipid species, and/or accumulation of a precursor molecule or a potentially toxic metabolite.

Regarding genetic transmission, except for HSAN1 due to a defect in serine palmitoyltransferase 1 or 2, and possibly the defects in ceramide synthase 2 and sphingomyelin synthase 2, enzymatic deficiencies of sphingolipid synthesis are inherited as autosomal recessive traits. So far, their diagnosis relies on DNA analysis

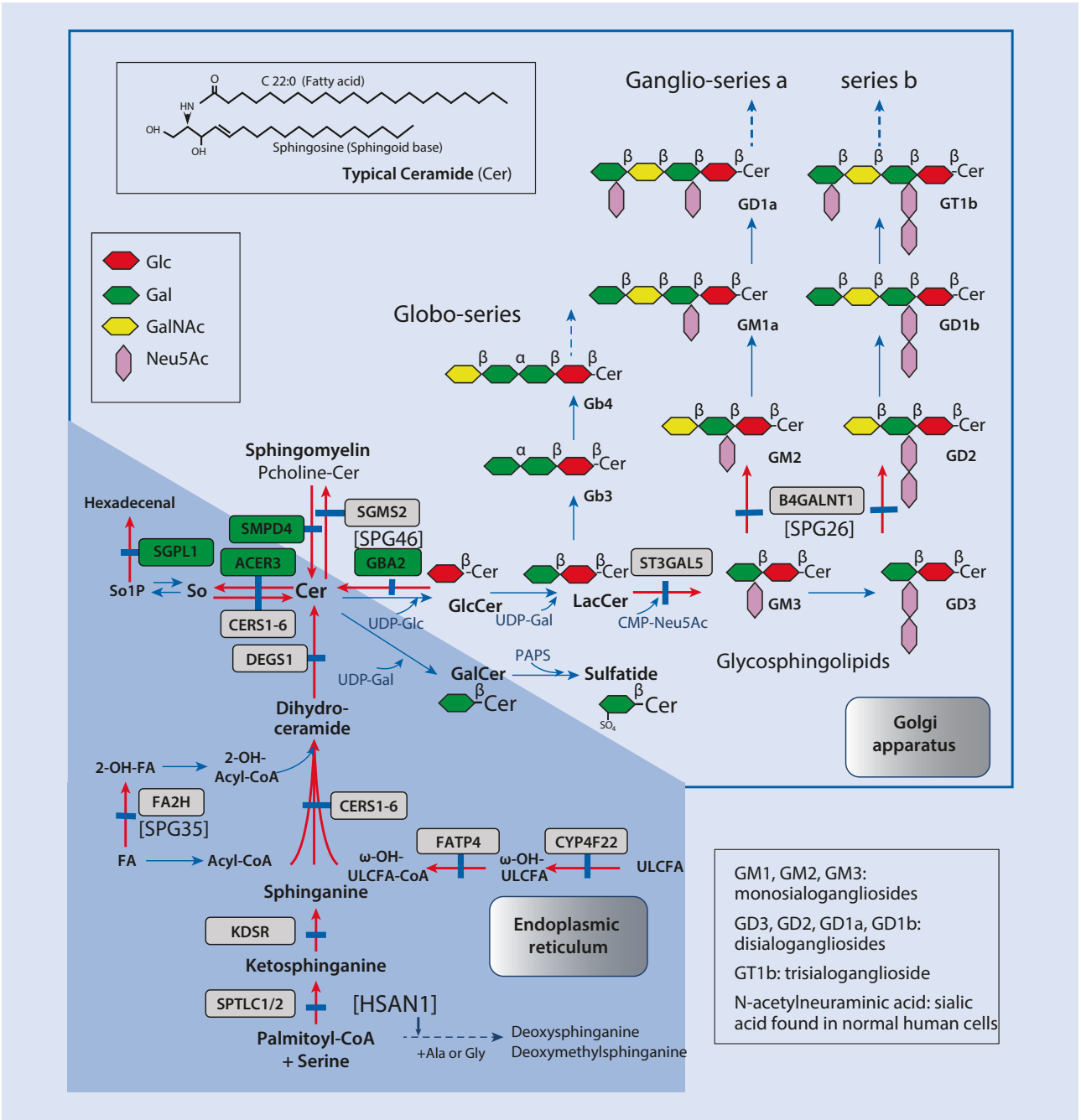

Fig. 40.1 Schematic representation of the structure of the main sphingolipids, depicting pathways of their biosynthesis, and of their non-lysosomal degradation. Red arrows denote the defective pathways that are discussed in this chapter. Solid black bars indicate metabolic blocks. Mutated genes are indicated within grey (biosynthesis) or green (non-lysosomal degradation) boxes. Cer ceramide, FA fatty acid, Gal galactose, GalCer galactosylceramide, GalNAc N-acetyl-galactosamine, Gb3 globotriaosylceramide, Gb4 globotetraosylceramide (globoside), Glc glucose, GlcCer glucosylceramide, LacCer lactosylceramide, Neu5Ac N-acetyl-neuraminic acid, 2-OH-FA 2-hydroxy-fatty acid, ω-OH-ULCFA ω-hydroxy ultra-long chain fatty acid, PAPS 3′-phosphoadenosine-5′-phosphosulfate, Pcholine phosphorylcholine, So sphingosine, So1P sphingosine-1-phosphate, ULCFA ultra-long chain fatty acid

although biochemical testing in plasma is possible for detection of HSAN1. There is currently no effective specific therapy for this type of IEM. Most of these conditions remain extremely rare. Their clinical spectrum is broadening with description of new cases and the field is likely to quickly evolve in the near future. For these reasons, and since comprehensive reviews on the subject with exhaustive referencing have been published quite recently [1, 2], only a brief outline of each disorder will be given in this chapter.

Table 40.1 Sphingolipid biosynthesis disorders

Enzyme	Gene	Metabolic disturbance	Main clinical features
Disorders with primarily nervous system involvement			
Serine palmitoyltransferase, subunit 1 or 2	*SPTLC1* or *SPTLC2*	Accumulation of 1-deoxysphingolipids (in plasma)	[HSAN1] – Peripheral sensory neuropathy, distal sensory loss, ulcerative mutilations Macular telangiectasia type 2
Ceramide synthase 1	*CERS1*	Possibly decreased C18-ceramide levels (in cultured cells)	Progressive myoclonic epilepsy and cognitive decline
Ceramide synthase 2	*CERS2*	Possibly decreased very-long chain ceramide levels (in cultured cells)	Progressive myoclonic epilepsy
Dihydroceramide delta4-desaturase	*DEGS1*	Increased dihydroceramides/ceramides ratio (in cultured fibroblasts) and dihydrosphingolipids in plasma	Hypomyelinating leukodystrophy
Fatty acid 2-hydroxylase	*FA2H*	Possibly decreased hydroxylated sphingomyelin levels (in cultured cells)	Spastic paraplegia [SPG35], dystonia, dysarthria, ataxia
GM3 synthase	*ST3GAL5 (SIAT9)*	Lack of GM3, GD3 and higher gangliosides, and increased lactosylceramide and Gb4 levels (in plasma and cultured cells)	[Amish infantile epilepsy] Epilepsy, intellectual disability, 'salt-and-pepper' syndrome
GM2/GD2 synthase	*B4GALNT1*	Decreased GM2 and increased GM3 levels (in cultured cells)	Spastic paraplegia [SPG26], ataxia
Disorders with primarily skin involvement			
3-Ketosphinganine reductase	*KDSR*	Reduced ceramide content in skin	Symmetric erythrokeratoderma
Ceramide synthase 3	*CERS3*	Lack of ceramides with very-long chain fatty acids (in cultured cells)	Ichthyosis [ARCI9]
(Ultra-long chain) fatty acid ω-hydroxylase	*CYP4F22*	Decreased ultra-long acylceramide levels (in skin and cultured cells)	Ichthyosis [ARCI5]
Patatin-like phospholipase domain-containing 1	*PNPLA1*	Loss of ω-O-acylceramides in skin	Ichthyosis [ARCI10]
Disorders with primarily bone involvement			
Sphingomyelin synthase 2	*SGMS2*	Increased capacity of sphingomyelin synthesis (in cultured cells)	Osteoporosis and skeletal dysplasia

40.1.1 Serine Palmitoyltransferase (Subunit 1 or 2) Deficiency and HSAN1

A defect in the very first step of sphingolipid biosynthesis is the major cause underlying the dominant hereditary sensory and autonomic neuropathy (HSAN1). Other (unrelated) genes that have been linked to HSAN1 are *ATL1*, *RAB7A* and *DNMT1* [1]. This peripheral neuropathy is characterised by a late onset (between the second and fourth decade), a slow disease progression, and primarily sensory deficits (loss of pain and temperature sensation spreading from the distal limbs). Painless ulcerations in the lower limbs are quite frequent, as well as spontaneous lancinating pain attacks. Hypohidrosis is also seen. Some patients exhibit a more severe phenotype, starting in early childhood, with motor involvement, global hypotrophy, and developmental retardation [1]. Macular telangiectasia type 2 is also associated with the same gene defects [3].

Current evidence related to the metabolic derangement points to the accumulation of abnormal sphingoid bases (and their derivatives) as the main pathogenic mechanism. Specific mutations of ***SPTLC1* or *SPTLC2*** encoding subunits 1 or 2 of serine palmitoyltransferase, the first and rate-limiting step in the de novo synthesis of sphingolipids, alter its substrate specificity. Instead

of using L-serine as a substrate (Fig. 40.1), the mutant enzyme preferentially uses L-alanine or L-glycine. The resulting 1-deoxy-sphinganine and 1-deoxymethyl-sphinganine, and 1-deoxy-ceramides (or some other derivatives), which cannot be converted to complex sphingolipids, appear to account for the observed neurotoxicity. Of note is the fact that only several missense mutations in the *SPTLC1* or *SPTLC2* gene cause the autosomal dominant disorder HSAN1. Substitution of Ser331 in the subunit 1 of serine palmitoyltransferase seems to result in an early-onset and more severe phenotype.

When a hereditary sensory neuropathy (or macular telangiectasia type 2) is suspected, elevated plasma levels of 1-deoxy-sphinganine and 1-deoxymethyl-sphinganine, as determined by liquid chromatography coupled to mass spectrometry, provide a strong biochemical argument in favour of a SPTLC1/2 defect. Moreover, plasma 1-deoxy-sphingolipid levels seem to correlate with disease severity.

There is currently no effective specific therapy. However, a 10-week pilot study on patients affected with HSAN1 showed that, as in a mouse model for this disease, L-serine supplementation (200 or 400 mg/kg/day) could reduce the plasma levels of 1-deoxysphingolipids [4, 5]. Whether such a supplementation can ameliorate the sensory deficits still requires further investigation.

A recent study has identified novel, dominantly acting *SPTLC1* variants that cause a childhood-onset form of amyotrophic lateral sclerosis. In contrast to the situation of HSAN1, these variants result in unregulated activity of serine palmitoyltransferase and elevated levels of its canonical sphingolipid products [6].

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40.1.2 Ketosphinganine Reductase Deficiency and Hyperkeratosis

A skin disorder with well-demarcated symmetric scaling, erythema, and keratoderma mostly affecting the face, palms and soles has been described in four probands [7]. Four additional patients with hyperkeratosis or a harlequin ichthyosis-like phenotype were reported, in whom thrombocytopenia was also observed [8]. All carried biallelic mutations in *KDSR*, which encodes the second enzyme of sphingolipid biosynthesis. Deficient activity of 3-ketosphinganine reductase led to a reduction of total ceramide content in the affected skin. Systemic therapy by isotretinoin markedly improved the scaling and erythema in two patients.

40.1.3 Defects in Ceramide Synthases 1 and 2 and Myoclonic Epilepsy

Six human ceramide synthases, encoded by ***CERS*** genes, have been characterised. They display distinct tissue-specificities as well as acyl-CoA substrate specificities, which can explain the neurological (CERS1 and 2) or dermatologic (CERS3, see ▶ Sect. 40.1.8) expression in case of a defect in one of them.

Very recently, a homozygous missense mutation in *CERS1* has been identified in 4 siblings of an Algerian family showing progressive myoclonic epilepsy and cognitive decline/dementia. The mutation was associated with decreased C18-ceramides levels in cultured fibroblasts. It has also been proposed that progressive myoclonic epilepsy since age 10 in an adult patient (associated with ataxia, dysarthria and photosensitivity) was due to a heterozygous deletion of *CERS2* together with decreased very-long chain ceramides in fibroblasts [1].

40.1.4 Dihydroceramide Δ4-Desaturase Deficiency and Leukodystrophy

This autosomal recessive condition has been described in 20 patients, presenting with a progressive tetraspasticity, developmental delay and failure to thrive [9, 10]. Variable disease severity has been observed, including isolated spastic paraparesis. The most severely affected patient died at 18 months of age. Abnormal eye movements were frequently seen before 6 months of age. Seizures were also frequently reported. EMG showed reduced nerve conduction velocities. Brain MRI revealed a general hypomyelination, a thinning of corpus callosum, and progressive cerebellar and supra- and infratentorial atrophy.

Pathogenic mutations in *DEGS1* result in reduced activity of the desaturase that catalyses the last step of ceramide biosynthesis, i.e., the introduction of a 4,5-double bond in the sphingoid base backbone. This leads to increased levels of dihydrosphingolipids (dihydro-ceramides, sphingomyelins and monohexosylceramides), and increased dihydroceramide/ceramide ratio in patients' blood, muscle, and fibroblasts.

40.1.5 Fatty Acid 2-Hydroxylase Deficiency (SPG35/FAHN)

Mutations in *FA2H* encoding fatty acid 2-hydroxylase result in a complex hereditary spastic paraplegia, SPG35, also called fatty acid hydroxylase-associated

neurodegeneration (FAHN). To date, about 70 patients have been reported, with varied ethnicity. Most patients present in childhood and develop slowly progressive lower and then upper limb spasticity, dysarthria, and mild cognitive decline. Dystonia is another common neurologic feature, and a rigid-hypokinetic syndrome may appear over time. MRI shows signs of leukodystrophy and diffuse cortical and pontocerebellar atrophy. Neurodegeneration with brain iron accumulation (NBIA), mostly located in globus pallidus (T2 hypodensity, but no "eye of the tiger" sign), can occur, although not in all patients. The clinical spectrum of SPG35 is widening, with later onset patients and more clinical variability [11].

The underlying abnormality is likely the insufficient production of 2-hydroxy-galactosphingolipids. Indeed, 2-hydroxylated long chain and very-long chain fatty acids are essentially found in galactosylceramides and sulfatides from myelin, and their proportion relative to non-hydroxylated fatty acids is known to increase with brain development and myelin maturation. Not unexpectedly, in *Fa2h*-deficient mice, brain galactosylceramides were found to contain almost exclusively non-hydroxylated fatty acids.

40.1.6 GM3 Synthase Deficiency and Amish Epilepsy Syndrome

The deficiency of GM3 synthase resulting from *ST3GAL5 (SIAT9)* mutations causes an autosomal recessive infantile-onset symptomatic epilepsy, also called Amish epilepsy syndrome. During the first 3 months of life, affected children show irritability and failure to thrive. Then, within the first year of life, generalized tonic-clonic seizures as well as other seizure types develop, along with a profound developmental stagnation and regression. In some patients, brain MRI shows occipital white matter abnormalities and atrophy in the visual cortex. The severity of the disease varies significantly, some patients suffering from visual loss and deafness. Most patients exhibit hyperpigmented macules on the dorsal part of hands and feet, but also in other locations. Some patients also show patches of skin depigmentation. These skin changes are not associated with the severity of the neurologic disease. The combination of hyper and hypo-pigmented skin maculae, facial dysmorphism, scoliosis, intellectual disability, seizures, choreoathetosis, and spasticity has been described under the term "salt-and-pepper" syndrome. Associated biochemical features in plasma and cultured cells are the lack of GM3, GD3 and higher gangliosides, and increased lactosylceramide and Gb4 levels.

40.1.7 GM2/GD2 Synthase Deficiency (SPG26)

Mutations of *B4GALNT1* resulting in a defect of GM2/GD2 synthase are associated with SPG26, a slowly progressive complex hereditary spastic paraplegia with mild to moderate cognitive impairment. A dozen of multiplex families from various ethnic origins have so far been described. The clinical picture is a progressive weakness, with spastic gait and lower limb spasticity. EMG shows an axonal sensorimotor neuropathy in many patients. The disease can be accompanied by cerebellar symptoms, dysarthria, and dysphagia [12], as well as fever-induced ataxia with myokymia. Studies in cultured fibroblasts of patients have shown decreased GM2 levels with an increase of its precursor, GM3.

40.1.8 Defects in Skin Ceramide Synthesis: Autosomal Recessive Congenital Ichthyoses (ARCI)

Autosomal recessive congenital ichthyoses (ARCI) represent a heterogeneous group of disorders of epidermal cornification, in which at least 9 causative genes have been identified. Three of those, *CERS3*, *CYP4F22* and *PNPLA1*, encode proteins involved in ceramide synthesis (◘ Fig. 40.1). Specific ceramides are particularly abundant in the stratum corneum of the skin, where they play a crucial role in maintaining skin barrier homeostasis, preventing water loss, and protecting against microbial infections. These ceramides may in particular contain α- or ω-hydroxylated fatty acids and ultra-long chain fatty acids (ULCFAs; C26 or longer) (▶ Chap. 42). Acylceramides are unique, very hydrophobic ceramide species present in the epidermis, which contain C28-C36 ω-hydroxylated ULCFAs and are further esterified with linoleic acid.

CYP4F22 encodes the fatty acid ω–hydroxylase required for acylceramide synthesis, using ULCFAs as substrates [13]. Ceramide synthase 3 (*CERS3*), which is markedly expressed in the skin, generates epidermis-specific ceramides by N-acylating sphinganine with ULCFA-CoAs (and likely ω-hydroxylated ULCFA-CoAs). Indeed, functional analysis of a skin sample and in vitro differentiated keratinocytes from a patient with a *CERS3* missense mutation severely affecting

enzyme activity demonstrated an impairment in the synthesis of ceramides with non-hydroxylated and ω-hydroxylated ULCFA moieties, disturbing epidermal differentiation and leading to premature keratinisation. On the other hand, *PNPLA1* encodes a protein (patatin-like phospholipase domain containing 1) with transacylase activity, that transfers linoleic acid from triacylglycerols to the ω-hydroxy fatty acid in ceramide [14]. Once these acylceramides are glucosylated at the C1 position, the corresponding glucosylceramides are transported to the so-called lamellar bodies, possibly by ABCA12, the mutations of which cause harlequin ichthyosis (ARCI4).

Acylceramides in the stratum corneum have been shown to play a key role in the formation and stabilisation of cornified envelopes through covalent binding to corneocyte proteins, and ultimately in skin permeability barrier. It is therefore logical that defective synthesis of these lipids will manifest as severe skin disorders. For the above defects, the ARCI clinical phenotype has been quite variable in different patients, often including lamellar ichthyosis and palmo-plantar hyperlinearity, but also in some cases collodion membrane at birth (that may be self-improving) and more or less severe congenital ichthyosiform erythroderma. For ichthyotic manifestations due to these defects, topical application of some specific ceramides could be envisioned.

40.1.9 Sphingomyelin Synthase 2 Mutations and Osteoporosis

Heterozygous pathogenic variants in *SGMS2* have been reported in 6 families affected by early-onset osteoporosis [15]. Whereas patients carrying the p.R50* variant had a normal development, low-bone mineral density, a history of multiple vertebral and peripheral fractures, and transient peripheral nerve palsies, patients with a missense variant presented with a more severe phenotype, including short stature, more severe cranial sclerosis and spondylometaphyseal dysplasia. The mechanisms that underlie the consequences of mutations in the sphingomyelin synthase 2 isoform on skeletal homeostasis and bone mineralization still require investigation.

40.1.10 Mutations in Ceramide Kinase-Like (CERKL) Gene and Retinal Dystrophy

Mutations in *CERKL* have been associated with a group of inherited retinal dystrophies presenting as retinitis pigmentosa or cone rod dystrophy. The name ceramide kinase-like was given because of 29% identity and 50% similarity with the human ceramide kinase that converts ceramide into ceramide 1-phosphate, but neither the substrate nor the function of the CERKL protein are yet known. At the current stage of knowledge, this disorder thus does not belong to sphingolipid synthesis disorders. It has been listed here by default due to its name, as it cannot yet be classified from a metabolic viewpoint (see [1] for more details).

40.2 Disorders of Lysosomal Sphingolipid Degradation: Sphingolipidoses

Sphingolipidoses are a subgroup of lysosomal storage disorders in which sphingolipids accumulate in one or several organs as the result of a primary deficiency in enzymes or activator proteins involved in their degradative pathway. Except for Fabry disease (X-linked recessive), the mode of inheritance is autosomal recessive. The clinical presentation and course of the classic forms are often typical. Late-onset forms, often less typical, have been overlooked in the past. No global biochemical screening procedure is available to date, but multiplex assay of lyso-sphingolipids can be of help. In nearly all sphingolipidoses (but not activator deficiencies) the diagnosis, oriented by clinical findings and anamnesis, is easily made by demonstration of the enzymatic defect, generally expressed in most cells and tissues (leukocytes represent the most widely used enzyme source, followed by dried blood spots). All efforts should then be made to identify the causal mutations in every patient. Conversely, whenever possible, diagnoses made in a proband by first-line gene analysis should be completed by a functional assay. Some specific therapies are well established (e.g., non-neuronopathic Gaucher and Fabry diseases), and more have been approved or are in late stage of clinical trials. However, despite recent advances, progress towards therapy of the neurological forms remains limited.

Lysosomal Degradation of Sphingolipids and Sphingolipidoses

Only the main sphingolipids implicated in sphingolipidoses are depicted in ◘ Fig. 40.2. The scheme illustrates their degradation by stepwise hydrolysis, and shows enzymatic blocks leading to a disease. It also indicates which sphingohydrolase or sphingolipid activator protein (▶ Sects. 40.2.4, 40.2.5, 40.2.6, and 40.2.9) is implicated, as well as the name commonly used to designate the various disorders.

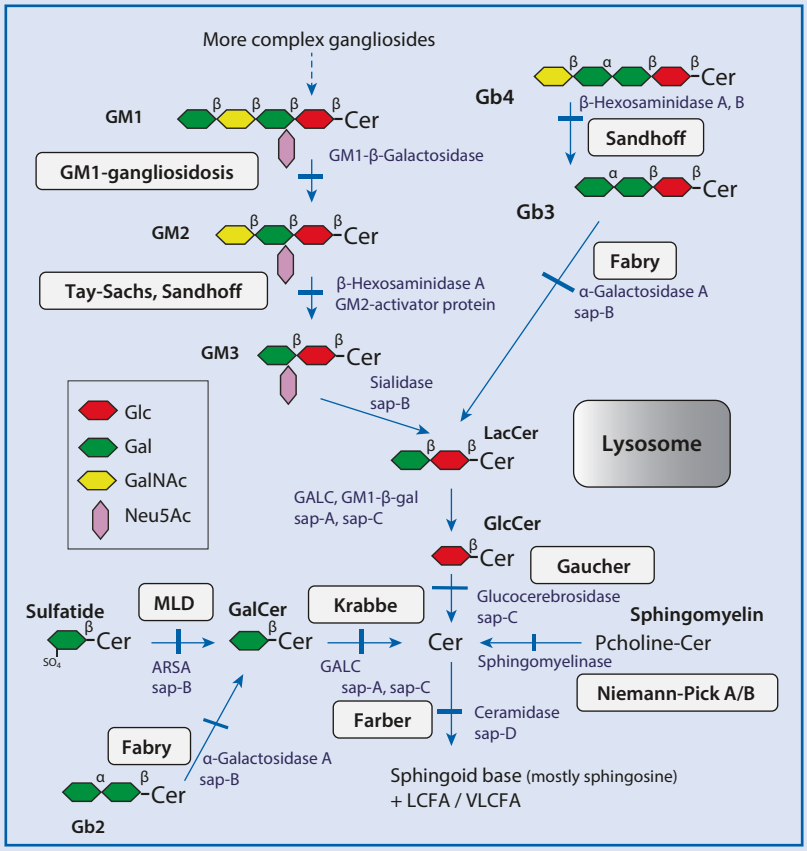

Fig. 40.2 Lysosomal degradation of sphingolipids. ARSA arylsulfatase A, GALC galactocerebrosidase, GalCer galactosylceramide (or galactocerebroside), GalNAc N-acetyl-galactosamine, Gb2 galactobiosylceramide, Gb3 globotriaosylceramide, Gb4 globotetraosylceramide (globoside), GlcCer glucosylceramide (or glucocerebroside), GM1 GM1 ganglioside, GM2 GM2 ganglioside, GM3 GM3 ganglioside, LacCer lactosylceramide, LCFA long chain fatty acids, MLD metachromatic leukodystrophy, Neu5Ac N-acetyl-neuraminic acid (sialic acid), Pcholine, phosphorylcholine, sap saposin, VLCFA, very long chain fatty acids. Enzyme defects are indicated by solid bars across the arrows

40.2.1 Gaucher Disease

Clinical Presentation

Historically, three clinical phenotypes of Gaucher disease (GD) are recognised, but the full disease spectrum is a continuum. All types are panethnic, but type 1 has a particularly high prevalence in the Ashkenazi Jewish population (carrier frequency 1:13). The overall incidence is about 1:40,000–1:50,000 live births. For a comprehensive review, see [16].

Type 1 It is defined by the lack of neurological symptoms, and accounts for about 90% of all cases in the Western world. It can present at any age, but manifests in childhood in more than half of patients. There is a wide variability in the pattern and severity of the symptoms, from extremely handicapping to asymptomatic forms, with most symptomatic patients having visceral, haematological and (more frequently in adults) skeletal disease. Children often show severe splenomegaly, generally associated with hepatomegaly. The degree of visceromegaly is highly variable, in both children and adults. Hypersplenism may lead to anaemia, thrombocytopenia and, thus, a bleeding tendency. Leukopenia is less frequent. Children may show delayed growth and menarche. Subcapsular splenic infarctions may cause attacks of acute abdominal pain and medullary infarction of long bones, excruciating pain referred to as bone crises. Essentially in adult patients, bone involvement represents a major cause of morbidity. Aseptic necrosis of the femoral head and spontaneous fractures due to osteopenia are other common complications. Lung involvement with diffuse infiltration may occur. In adults, pulmonary hypertension has been described in rare, usually splenectomised, patients. Co-morbidities with close association to GD have been identified, particularly non-Hodgkin's B-cell lymphoma and multiple myeloma, and Parkinson's disease [17–19]. Peripheral polyneuropathy was also reported more frequently than in a control population.

Type 2 (acute neuronopathic GD) Classically, patients present early in infancy with brain stem dysfunction and pyramidal signs. Retroflexion of the neck, opisthotonos, feeding difficulties and squint are major early signs, apnoeas appear later, and trismus and stridor are less frequent. Splenomegaly is constant but may not be present initially. The downhill clinical course is rapid, with pronounced spasticity, failure to thrive and cachexia, and few of these patients survive beyond the age of 2 years. Some other patients show strabismus, paucity of facial movements, less sign or none at all of pyramidal involvement, irritability or cognitive impairment and a slower course (some survive up to 5 years) [16, 20].

The **perinatal lethal form** is associated with hepatosplenomegaly, pancytopenia, and skin changes. Many of these cases are associated with hydrops fetalis, and some have been described as "collodion babies". Arthrogryposis is seen in 40% of cases [20].

Type 3 (subacute or chronic neuronopathic GD) This type is heterogeneous. It is the predominant form in Far-East countries, India, Pakistan, and Egypt. The mean age at onset is 5 years (between 5 months and 46 years), with a mean age of neurological onset around 8 years. The most common form consists in severe systemic involvement and supranuclear saccadic horizontal gaze palsy, with or without developmental delay, hearing impairment and other brain stem deficits. The second most common phenotype shows a relatively mild systemic disease but progressive myoclonic encephalopathy, with seizures, dementia, and death. There are also patients with severe systemic involvement and supranuclear gaze palsy who develop a progressive myoclonic encephalopathy [16, 21]. Brain stem auditory evoked response (BAER) testing may reveal abnormal wave forms (III and IV). A particular presentation with cardiac involvement (heart valve and aortic calcification), supranuclear gaze palsy, mild hepatosplenomegaly, and bone disease, has been associated with homozygosity for the D409H mutation. In neurological GD, extrapyramidal involvement has also been observed. In view of future clinical trials, consensus criteria for inclusion of a patient within neuronopathic GD (especially gaze palsy) have been defined [22].

Metabolic Derangement

The primary metabolic defect resides in a block of the lysosomal degradation of (β-)glucosylceramide (glucocerebroside, glucosylceramide, GlcCer, Gb1) and glucosylsphingosine. In the vast majority of cases this is due to the deficient activity of acid β-glucosidase (glucocerebrosidase, glucosylceramidase) (◘ Fig. 40.2). Exceedingly rare cases, presenting as type 3 or 1 (reviewed in [23]) are due to a deficiency of the saposin sap-C (▶ Sect. 40.2.9). GlcCer accumulates in macrophages, inducing their transformation into Gaucher cells. GlcCer storage is massive in liver and spleen of patients in all types. Although elevated in cerebral grey matter of type 2 and type 3 patients, its concentration in brain remains low. Glucosylsphingosine (or lysoGb1) also accumulates (in much smaller amounts) in tissues (but not in brain of GD type 1 patients), and in plasma for all types. This compound, formed by slow deacylation of GlcCer, also plays a role in pathophysiology of the disease, directly (immunogenicity [24], promotion of aggregation of α-synuclein, disruption of cellular Ca^{2+} homeostasis) or indirectly, through an alternate pathway involving the neutral glucosylceramidase GBA2 (◘ Fig. 40.1) [16]. The pathophysiology of the disease remains poorly understood, including the link between mutated GBA1 protein and Parkinson disease, and the relationship between Gaucher cells in tissues developed from M2 macrophages and blood monocytes with an inflammatory M1 phenotype [16].

Genetics

The disease (except for sap-C deficiency) is caused by mutations in *GBA1* (>500 known). The most common variant in Ashkenazim, c.1226A>G (p.Asn409Ser) (former N370S), is also very frequent in Caucasian populations. The finding of one single such allele in a patient is predictive of a non-neuronopathic phenotype. The severity can vary widely in Gaucher patients with the same genotype, including N370S homozygotes [25]. The second most frequent mutation, c.1448 T>C (p.Leu483Pro) (former L444P), first described in Norbottnian type 3, is usually associated with types 3 or 2 when homozygous. Complex alleles due to genetic rearrangements are more frequently observed in severe forms, including perinatal lethal forms. A number of genotype-phenotype correlations have been made [26].

Diagnostic Tests

Bone marrow examination (not mandatory) may have revealed Gaucher cells. Several plasma biomarkers, e.g. chitotriosidase, the chemokine CCL18/PARK, and lysoGb1 are typically very elevated, but they are above all used to monitor treated patients (below). The assay of glucocerebrosidase activity in peripheral blood lymphocytes/ leukocytes or dried blood spots (DBS) (using fluorogenic or short-chain glucosylceramide substrates) constitutes the primary diagnostic test. DNA testing, complicated by the existence of a highly homologous pseudogene, is required for carrier detection, and improves diagnostic accuracy for patients with high residual enzyme activity. In sap-C deficiency, glucocerebrosidase activity is normal; the findings of Gaucher cells and elevated levels of lysoGb1 (or, less specific, chitotriosidase), should lead to *PSAP* sequencing.

Treatment and Prognosis

Two approaches are currently available for the specific treatment of GD type 1 (and visceral manifestations of type 3 [27]: enzyme replacement therapy (ERT) and sub-

strate reduction therapy (SRT). Splenectomy enhances the risk of progression of the disease at other sites, especially bone and lung, and should be avoided. Pregnancy is not contraindicated in untreated patients, but bleeding may become critical before and after birth; there is a good experience of ERT throughout pregnancy [28]. Conducted with slow infusions of a recombinant enzyme exposing mannose groups (optimal uptake by macrophages), ERT has largely proved safe and effective. Imiglucerase has been used worldwide for over 25 years; velaglucerase alfa [29] was approved 10 years ago, taliglucerase alfa 8 years ago [30]. The natural history of GD type1 can be dramatically improved. ERT prevents progressive manifestations and ameliorates GD-associated anaemia, thrombocytopenia, organomegaly, bone pain, fatigue, and bone crises. However, the enzyme does not cross the blood-brain barrier, and this treatment has no effect on the neurological manifestations of types 2/3. While ERT aims at restoring the degradation rate of the accumulated substrate, SRT tends to reduce the cell burden by slowing down the rate of synthesis of the substrate to a level where it can be slowly cleared by a deficient enzyme with some residual activity. This may be achieved by small molecules that can be administered orally. Two inhibitors of GlcCer synthase are currently approved for treatment of GD type 1: miglustat [31], and eliglustat [32]. Thus far, no specific treatment is approved for neuronopathic forms. A trial is planned with venglustat (another substrate inhibitor able to cross the blood brain barrier), in combination with imiglucerase.

40.2.2 Acid Sphingomyelinase-Deficient Niemann-Pick Disease (Type A, Type B and Intermediate Forms)

Since the early 1980s, the heterogeneous group of "Niemann-Pick disease" has been divided in two separate entities: "acid sphingomyelinase-deficient Niemann-Pick disease" or "acid sphingomyelinase deficiency" (ASMD) [33], and Niemann-Pick disease type C (below).

■ Clinical Presentation

ASMD has historically been categorised into a severe, acute neuronopathic form, or type A, and a non-neuronopathic form, or type B, but there appears to be a continuum ranging from mild to severe type B, and then from late-onset neurological forms toward severe classic type A. Type A has its highest prevalence in Ashkenazim and is rare in other ethnic groups. Type B does not have an Ashkenazi Jewish predilection, and appears more frequent in southern Europe, North Africa, Turkey, the Arabian Peninsula, and in Chile than in northern Europe.

Classic Niemann-Pick Disease Type A The neonatal period is usually normal, with vomiting or diarrhoea, or both, appearing in the first weeks of life. Failure to thrive often motivates the first consultation, leading to the discovery of a prominent and progressive hepatosplenomegaly and lymphadenopathy, in most cases before 3-4 months of age and sometimes earlier. Hypotrophy is observed in 70% of the cases [34, 35]. Neurological examination is essentially normal until the age of 5-10 months, when the child shows hypotonia, progressive loss of acquired motor skills, lack of interest in the surroundings and reduction of spontaneous movements. Psychomotor retardation may at first be overlooked owing to the poor general condition. Initial axial hypotonia is later combined with bilateral pyramidal signs. A decrease of nerve conduction velocities is generally present. A cherry-red spot in the retina is a typical feature but is often not present until an advanced stage. Loss of motor function and intellectual deterioration continue to the point where patients become spastic and rigid. Seizures are rare. Brownish-yellow discoloration and xanthomas may be detected in the skin. Death usually occurs between 1.5 and 3 years. Cases with a milder systemic involvement, slightly protracted onset of neurological symptoms and slower course are also seen [34].

Niemann-Pick Disease Type B Type B is a chronic, non-neuronopathic disease, with a highly variable degree of systemic involvement. Most typically, the presenting sign is splenomegaly or hepatosplenomegaly in late infancy or childhood [35, 36], but discovery may occur at any age from birth until late adulthood. Bruising and epistaxis are frequent. Hypersplenism occurs in a small proportion of patients. Splenectomy, seldom necessary, should be avoided. The most constant associated signs are radiographic abnormalities of the lung (diffuse, reticulonodular infiltrations) and interstitial lung disease with variable impairment of pulmonary function (low DLCO) [37]. In adults with a long follow-up, pulmonary involvement is often the main complaint, ranging from dyspnoea on exertion (frequent) to oxygen dependency. In children, retarded body growth is a common finding between the ages of 6 and 16 years. Skeletal age and puberty are often delayed [36]. Alterations of liver function are in general mild, but possibly underestimated; a few cases have been described with liver cirrhosis and liver failure. Hypercholesterolaemia with markedly decreased HDL-cholesterol is common even in children. Other features associated with the disease are joint/limb pain, bruising, headache, abdominal pain, and diarrhoea. True type B patients do not have neurological involvement and are intellectually intact, but ophthalmoscopic examination may reveal a retinal macular halo or cherry red maculae [36]. Although there are severe forms, a common phenotype is that of a moderately serious disorder compatible with an essentially normal lifespan [38]. In a longitudinal study the disease was characterised by hepatosplenomegaly, worsening athero-

genic lipid profile, gradual deterioration in pulmonary function and stable liver dysfunction.

Intermediate Forms of ASMD This is a heterogeneous category. Some patients are closer to type A with a late infantile, juvenile, or adult neurological onset and a slowly progressive disease that may include cerebellar ataxia, extrapyramidal involvement, or psychiatric disorders [39, 40]. Some others are closer to type B, with minimal nervous system involvement (often peripheral neuropathy) and/or mild mental retardation [41].

Metabolic Derangement

A primary deficiency of the lysosomal (or acid) sphingomyelinase (ASM) (Fig. 40.2) resulting from mutations in *SMPD1* leads to the progressive accumulation of sphingomyelin in systemic organs in all types of the disease, and in brain in the neuronopathic forms [33]. Sphingomyelin storage is massive in liver and spleen in type A, slightly less so in type B. A significant increase of unesterified cholesterol occurs secondarily [42]. By in vitro measurements, a marked ASM deficiency is observed in all patients, but hydrolysis of sphingomyelin in live cells demonstrates a significant level of residual activity in typical type B patients, suggesting this could be enough to protect the brain. Sphingosylphosphocholine (lysosphingomyelin) (increased in systemic organs of all types and in type A brain) likely participates in the pathogenic cascade.

Genetics

More than 250 disease-causing mutations of *SMPD1* are known [43]. In Ashkenazi Jewish type A patients, 3 variants collectively account for >90% of alleles. In type B, the globally most common (albeit with large regional differences) mutation, p.Arg610del (R608del), has always been correlated with a non-neuronopathic phenotype even in heteroallelic status. *SMPD1* appears paternally imprinted.

Diagnostic Tests

Bone marrow usually reveals the presence of (nonspecific) foamy and/or sea-blue histiocytes. Among plasma biomarkers, only a striking elevation of lysosphingomyelin is specific of ASMD, since abnormal levels of the oxysterols cholestane-3β,5α,6β-triol and 7-ketocholesterol, and of "lysosphingomyelin-509" (now more properly renamed N-palmitoyl-O-phosphocholine-serine or PPCS), are also elevated in Niemann-Pick C (▶ Sect. 40.4), and for oxysterols, in acid lipase deficiencies and some other conditions (reviewed in [44]). Chitotriosidase is moderately elevated. The diagnosis is made by demonstration of a deficiency in ASM activity in leukocytes/lymphocytes, DBS, or cultured cells (much higher level of activity) [45]. The choice of a specific substrate is critical. Radioactively labelled native sphingomyelin or a short-chain analogue with LC-MS/MS measurement are best. The fluorogenic substrate should be used with caution. The in vitro assay does not reliably distinguish A from B phenotypes.

Treatment and Prognosis

Recommendations for clinical monitoring of ASMD patients have been published [46]. In type A patients, bone marrow transplantation (BMT) has not improved symptoms. In type B, splenectomy may have a deleterious effect on the lung disease. Pregnancy is not contraindicated, although monitoring for bleeding is advisable. Morbidity/mortality of types B and intermediate has been studied [47]. No specific therapy is yet approved, but interim results at 30 months of an ERT phase 1b trial in 5 type B adults treated with olipudase alfa have shown safety and efficacy [48], and a multicentric phase 2–3 trial is underway in adults and children.

40.2.3 GM1 Gangliosidosis

Clinical Presentation

Three main clinical phenotypes are described, based on age at first symptom and severity of disease progression.

In the typical early infantile form (or type 1) [49, 50] infants are often hypotonic in the first days or weeks of life, with poor head control. The arrest in neurological development is observed at 3–6 months of age. Feeding difficulties and failure to thrive are common. Many patients show facial and peripheral oedema. Dysmorphic features (87% of cases), with coarse facies, moderate macroglossia, hypertrophic gums, depressed nasal bridge) are present very early or develop with time. Cardiomyopathy is seen in 1/3 of cases. Hepatomegaly and later splenomegaly are almost always present. Dorsolumbar kyphoscoliosis is common. The few patients showing no dysmorphic expression and possibly no hepatosplenomegaly overlap with type 2a (below). After a few months, signs of visual failure appear, often with a pendular nystagmus. A macular cherry-red spot is found in about 50% of cases, but seldom before 6 months of age. As time passes, hypotonia gives way to spasticity. Rapid neurological regression is usual after the first year of life. Seizures occur in10% of cases. Most patients die before age 3. Radiological signs in the long bones and spine are constant in typical patients but can be minimal in cases with only psychomotor deterioration. Subperiosteal bone formation can be present at birth. Widening of the diaphyses and tapering of the extremities appear later. At the age of 6 months, striking Hurler-like bone changes are seen, with vertebral beaking in the thoracolumbar zone, broadening of the shafts of the long

bones with distal tapering and widening of the metacarpal shafts with proximal pinching of the four lateral metacarpals. Prenatal symptoms have also been described, and GM1 gangliosidosis is a cause of non-immune foetal hydrops.

Type 2 is characterized by more variability of neurological signs, the frequent absence of dysmorphia and hepatosplenomegaly, less severe skeletal changes, and a progression slower than type 1. Type 2 is now subdivided into a late infantile variant (type 2a), with onset between 7 months and 2-3 years of age, and a juvenile variant (type 2b) with onset between 3 and 10 years of age [50, 51] . First signs can be unsteadiness in sitting or standing, muscle hypotonia, often gait abnormalities, but also pyramidal signs or dysphagia (late infantile form), ataxia, dysarthria, dystonia. Seizures are frequent. Cognitive decline is not always present in the juvenile form. Vision is generally normal. Radiography of the spine reveals moderate but constant changes, with mild anterosuperior hypoplasia of the vertebral bodies at the thoracolumbar junction.

The adult form or chronic late-onset variant (or type 3) has a less severe phenotype, with onset in late childhood, adolescence or adulthood, Dysarthria, dysphagia, and extrapyramidal signs, especially dystonia, are the most common signs. Cognitive impairment is absent to moderate, and there are no ocular abnormalities. Bone changes are inconstant. The course of the disease is very slow [52–54].

Metabolic Derangement

GM1 gangliosidosis is due to a deficient activity of lysosomal acid β-galactosidase (Fig. 40.2), which cleaves glycoconjugates containing a terminal β-galactosidic linkage and is necessary for the degradation of GM1 ganglioside, other galactose-containing glycosphingolipids or oligosaccharides, and keratan sulfates (► Chap. 41). Consequently, the most severe forms of the disease combine features of a neuronal lipidosis, a mucopolysaccharidosis and an oligosaccharidosis. Acid β-galactosidase functions in a multienzyme lysosomal complex with neuraminidase, the protective protein/cathepsin A (PPCA) and N-acetyl-galactosamine-6-sulfate sulfatase. This explains the quite similar clinical phenotype of galactosialidosis, a distinct condition due to the deficiency of PPCA, which causes a combined secondary deficiency of acid β-galactosidase and acid sialidase (neuraminidase) (► Chap. 41). Finally, β-galactosidase deficiency can be associated with two clinically different diseases, GM1 gangliosidosis, with prominent features of a sphingolipidosis, and Morquio B disease (mucopolysaccharidosis type IVB), in which abnormalities of mucopolysaccharide metabolism prevail. In tissues from patients with GM1 gangliosidosis, three main groups of accumulated compounds have been identified: the sphingolipid GM1 ganglioside, glycoprotein-derived oligosaccharides and keratan sulfate. Massive storage of GM1 occurs in brain tissue. Increased levels of its lysocompound, potentially of pathogenetic significance, have been reported. Galactose-containing oligosaccharides have been found in liver and urine. Keratan sulfate and other mucopolysaccharides accumulate in liver and spleen. Keratan sulfate excretion in urine is lesser in GM1 gangliosidosis than in Morquio B disease.

Genetics

More than 160 disease-causing mutations of *GLB1* have been described. Neither the type nor location of the mutation correlates well with a specific phenotype.

Diagnostic Tests

Vacuolated lymphocytes may be found in peripheral blood, and foamy histiocytes in the bone marrow. Radiographic bone examination showing Hurler-like abnormalities (above) may suggest the diagnosis. In the infantile form, brain computerised tomography (CT) and magnetic resonance imaging (MRI) usually give nonspecific results, with diffuse atrophy of the central nervous system (CNS) and features of myelin loss in the cerebral white matter. Lesions in the basal ganglia may be present in the adult form. Analysis of urinary oligosaccharides is a good orientation test. In the classic early infantile form, excretion is massive, with a pathognomonic profile. Excretion can be much lower in forms with predominant neurodegenerative disease. Mucopolysaccharide analysis in urine usually shows increased levels of keratan sulfate. The diagnosis is established by demonstration of a deficient activity of acid β-galactosidase, which can be measured on leukocytes or DBS using an artificial fluorogenic substrate. A subsequent study of neuraminidase should be performed to exclude galactosialidosis.

Treatment and Prognosis

There is currently no approved specific treatment. A combined miglustat/ketogenic diet (Syner-G) is in trial in infantile forms [49]. Two gene therapy phase 1-2 trials are ongoing or planned.

40.2.4 GM2 Gangliosidoses

GM2 gangliosidoses are divided into three genetic and biochemical subtypes: Tay-Sachs disease (B or B1 variant) (TSD), Sandhoff disease (0 variant) (SD), and GM2 activator deficiency (AB variant). All are characterised by impaired lysosomal catabolism of GM2 ganglioside (Fig. 40.2), which requires three gene products: the β-hexosaminidase α- and β-subunits and

the GM2 activator protein. TSD corresponds to a deficiency of the α-subunit and thus of β-hexosaminidase A (αβ-heterodimer), SD, to a deficiency of the β-subunit and thus of both β-hexosaminidases A and B (ββ-homodimer). Classic TSD has a remarkably high carrier rate (estimated to ~1:27) in the Ashkenazi Jewish population, and in subjects of French-Canadian descent. Infantile forms are most common, but juvenile and adult forms are also recognised. A particular enzymatic/molecular variant of TSD (B1 variant) has a high incidence in Northern Portugal and is globally more frequent in southern Europe. Variant AB is exceedingly rare, albeit probably underdiagnosed.

Clinical Presentation

The infantile forms of the three subtypes have a remarkably similar presentation and evolution [49, 55, 56]. Around 4-6 months of age, motor weakness and hypotonia are the usual earliest signs, almost constantly associated with a typical startle response to sounds with extension of the arms (hyperacusis). Hypotonia progresses, with loss of acquired milestones. Loss of visual attentiveness is also seen early, and ophthalmoscopic examination almost invariably reveals a typical macular cherry-red spot in the retina. Blindness follows, and spasticity, swallowing disorder, and seizures develop. Macrocephaly begins by 18 months of age. By year 3 the child is demented and decerebrate. Death often occurs, due to aspiration pneumonia. In SD, despite an additional accumulation of glycolipids and oligosaccharides in visceral organs, organomegaly and bony abnormalities are rarely observed.

Late infantile and juvenile forms [55, 57] are mostly due to a deficiency of β-hexosaminidase A (often B1 variant). The onset of symptoms is usually between 2 and 10 years of age, with ataxia, incoordination, and dysarthria, followed by progressive psychomotor deterioration, spasticity, and seizures. Myoclonus can be prominent. Cherry red-spots are inconstant.

Chronic or adult forms [58–60] can show 4 typical presentations: lower motor neuron disorder, cerebellar ataxia, psychosis often with mood disorder (30–50% of adult-onset patients, particularly TSD), or a complex phenotype mixing these manifestations, e.g. a syndrome of lower motor neuron and spinocerebellar dysfunction with supranuclear ophthalmoplegia. Some patients show autonomic dysfunction.

Metabolic Derangement

The catabolism of GM2 ganglioside requires the GM2 activator protein to extract GM2 from the plasma membrane before presenting it to hexosaminidase A (αβ-heterodimer). Hexosaminidase B (ββ-homodimer) hydrolyses other substrates with a terminal hexosamine (glycoproteins and glycolipids), but not GM2 ganglioside. In TSD (affecting the α-subunit), hexosaminidase A only is deficient. In SD (affecting the β-subunit) both hexosaminidases are inactive. In GM2 activator deficiency, the substrate is not made available to the otherwise normally functioning enzyme. All types are characterised by storage of GM2 ganglioside in neurons. This results in meganeurites, with aberrant neurite formation that may play a role in the pathophysiological mechanisms. GM2 storage is very pronounced in infantile forms, less so in juvenile forms, and even less in adult forms. Increased levels of lyso-GM2 have also been reported in brain tissue of infantile forms. In SD, asialo-GM2 also accumulates in brain, while other compounds - such as globoside and oligosaccharides - accumulate in liver and other visceral organs.

Genetics

More than 130 mutations of *HEXA* have been identified. Three of them account for >95% of the Ashkenazi Jewish alleles. A carrier screening programme initiated in the 1970s was successful to decrease incidence of the disease in this population. A 7.6 kb deletion is common in French Canadian patients. Mutations at codon 178 result in the enzymatic B1 variant. Relatively good genotype-phenotype correlations have been reported. More than 40 mutations (including a common 16 kb deletion) in *HEXB*, and 6 in the GM2-activator *GM2A* gene have been described.

Diagnostic Tests

The clinical diagnosis can easily be confirmed by enzyme testing on leukocytes, serum, DBS, or cultured fibroblasts. The assay for total hexosaminidases (A + B) using a synthetic fluorogenic substrate is straightforward and allows the diagnosis of SD. For hexosaminidase A (deficient in TSD), the sulfated synthetic substrate specific for the α-subunit is the method of choice (B and B1 variant); a high residual activity is found in SD, owing to excess of hexosaminidase S (αα-dimer). In GM2 activator deficiency, hexosaminidase A activity measured in vitro is normal; electron microscopic examination of a skin or conjunctival biopsy may provide strong evidence in favour of the diagnosis by demonstrating concentric lamellated bodies in nerve endings. The CSF shows increased levels of GM2. The definitive diagnosis requires *GM2A* sequencing.

Treatment and Prognosis

Seizures are generally responsive to standard treatment. No effective curative treatment is currently available. Neither miglustat nor chaperone therapy (pyrimethamine) trials led to measurable clinical improvement. A combined miglustat/ketogenic diet (Syner-G) is still in trial in infantile forms [49]. Trials using venglustat (SRT,

see ► Sect. 40.2.1) in late-onset forms, and N-acetyl-L-leucine, are recruiting.

40.2.5 Krabbe Disease

Clinical Presentation

Krabbe disease (or globoid cell leukodystrophy) leads to demyelination of the central and peripheral nervous system. Its estimated overall incidence is between 0.75 and 1 in 100,000 live births. It is more frequent in Scandinavia (but not in Finland). The classic early infantile form accounts for about 65% of diagnosed cases. The incidence of late onset cases (more common in Japan, Italy, Sicily) has been underestimated.

Infantile forms Clinical presentation is quite uniform, usually very suggestive of the diagnosis.

In the early infantile form, the onset is from birth to 6 months of age (often 3–4 months) [61]. Initial symptoms include increasing irritability, crying, vomiting and other feeding problems, hyperesthesia, tonic spasms on light or noise stimulation, and signs of peripheral neuropathy. Bouts of unexplained fever are also common. This stage with hypertonic episodes is followed by permanent opisthotonic posturing with characteristic flexed upper extremities and extended lower extremities. Seizures may appear. Hyperpyrexia and hypersalivation are frequent. As the disease progresses blindness occurs, followed by loss of bulbar functions and hypotonia. Death occurs from hyperpyrexia, respiratory complications, or aspiration, classically before the age of 2 years but in current practice not so rarely later.

In the late infantile phenotype (onset between 7 and 12 months, about 10% of cases), patients typically present with a loss of developmental milestones and poor feeding, crying and irritability being later signs [62].

Later onset forms Clinical recognition of these forms is more difficult.

The juvenile form [63] starts between the ages of 13 months and 10 years (mostly <5 years). The first signs are often gait disturbances (spastic paraparesis or ataxia or both, sometimes spastic hemiplegia) in a previously normal or mildly retarded child. Visual failure with optic atrophy is also a common symptom in younger children [63]. Peripheral neuropathy is only present in approximately half of the cases. Time of onset and severity of mental deterioration are variable. Seizures are infrequent but can be a major therapeutic problem. The disease course is quite variable and unpredictable, even in siblings. Many patients show initial rapid deterioration followed by gradual progression lasting for years.

Most adult patients (reviewed in [64]) present with a gait disorder, showing a pyramidal syndrome with spastic paraparesis, with or without peripheral neuropathy. One third have additional cerebellar ataxia. Usually they do not show cognitive dysfunction. At MRI, hyperintensities along the pyramidal tracts are a characteristic and nearly constant sign.

Metabolic Derangement

Krabbe disease (◘ Fig. 40.2) results from β-galactosylceramidase (or galactocerebrosidase, cerebroside β-galactosidase, GALC) deficiency, a lysosomal enzyme that catabolises (β-)galactosylceramide – a major lipid component of myelin – as well as lactosylceramide and galactosylsphingosine (psychosine). In vivo, galactosylceramide degradation further requires the saposin sap-A. Four cases due to sap-A deficiency are known [65]. GALC deficiency leads to an accumulation of galactosylceramide in the pathognomonic "globoid cells" (multinucleated macrophages) seen in the demyelinating lesions of the white matter. Recent work has shown that psychosine - a toxic metabolite which accumulates in the oligodendrocytes and the Schwann cells - is formed by slow deacylation of galactosylceramide [66]. The study also confirmed the "psychosine hypothesis" [67], in which this highly apoptotic compound is thought to play a major role in the pathogenesis of the disease and, more specifically, to underlie the early destruction of oligodendrocytes characteristic of the infantile form.

Genetics

More than 250 *GALC* mutations are known. The most frequent mutant allele (never found in Japanese patients) combines a large (30-kb) deletion and the polymorphism c.550C>T (historical 502C>T); [64, 68, 69]. Some common polymorphisms influence enzyme activity and may be responsible for a pseudodeficiency state, particularly when in compound heterozygosity with a disease-causing allele [68]. Four infantile cases were assigned to mutations in the sap-A domain of *PSAP*.

Diagnostic Tests

MRI shows areas of hyperintensity on T2-weighted images (► Sect. 1.5.6) that correlate well with areas of demyelination and globoid cell accumulation [70]. In late-onset cases, T2-weighted images may show more localised areas of hyperintensity with less involvement of cerebellum and deep grey matter [71]. In adult-onset cases, typical T2 hyperintensities along the pyramidal tracts involving optic radiations and corticospinal tracts are nearly constant [64, 72]. In typical infantile cases, CT shows diffuse cerebral atrophy with hypodensity of the white matter. Calcifications may be observed in the thala-

mus, basal ganglia, and periventricular white matter. Motor nerve conduction velocities are consistently low in infantile and most late infantile cases, but only about 60% of juvenile or adult patients display signs of peripheral neuropathy. Brain stem evoked potentials have also been studied [73]. Protein in CSF is usually elevated in infantile cases, but inconstantly in late-onset cases. The ultimate diagnosis is made by studying GALC activity in leukocytes, DBS, or cultured fibroblasts. This assay is subject to pitfalls of either technical (substrate) or biological (pseudodeficiency) nature. Use of a natural radiolabelled substrate (historical gold standard) is currently challenged by LC-MS/MS techniques using short-chain analogues [74, 75]. The less sensitive fluorogenic substrate should be used with caution. In Krabbe disease, like in metachromatic leukodystrophy (▶ Sect. 40.2.6), a pseudodeficiency state is relatively common and can lead to misinterpretation of correct data. Genotyping is essential as prenatal diagnosis is currently done using molecular genetics. In sap-A deficiency, GALC activity was found deficient in leukocytes but not in cultured fibroblasts (sap-A may stabilise GALC). A recent observation is the usefulness of psychosine measurement (in plasma, DBS, or red blood cells) for screening and diagnosis, and differentiation of patients with an infantile vs late onset form [76].

Treatment and Prognosis

Detailed management guidelines have been published [77]. In advanced disease, supportive analgesic treatment of the often-severe pain that can result from radiculopathy is important, as is treatment of spasticity. Allogenic BMT or cord blood transplantation may be effective in preventing onset or halting progression of the disease in late-onset cases. In symptomatic infantile cases HSCT has given poor results, but umbilical cord blood transplantation to asymptomatic 12- to 44-day-old babies appeared promising [78], leading to newborn screening in several states in the USA [79]. However, long-term follow-up indicated that over time most children developed slowly progressive motor and language deterioration along with somatic growth failure and persistent cognitive deficits [80]. A recent study in mouse models indicates that HSCT likely exerts its therapeutic benefit by restoring phagocyte function rather than cross-correcting myelin cells of GALC [81]. Promising preclinical attempts to gene therapy have been published.

40.2.6 Metachromatic Leukodystrophy

Clinical Presentation

Metachromatic leukodystrophy (MLD) is panethnic, with reported incidences ranging between 1:40,000 and 1:170,000, with higher frequencies in specific ethnic groups.

The late infantile form [82] is the most common. First symptoms appear before 30 months of age (usually between 1 and 2 years, with a median onset of 18 months): walking delay, progressive difficulty in locomotion around 14-16 months (weaker lower limbs and falls); 15% of children never walk independently. Examination usually shows hypotonia, reduced or absent deep tendon reflexes and extensor plantar responses. Walking and then standing soon become impossible. The child develops spastic quadriplegia, speech deterioration, gradual mental regression and optic atrophy leading to blindness, followed by a vegetative state and death. Gallbladder abnormalities are quite frequent [83].

The early-juvenile form, with age of onset between 30 months and 4–6 years. Has usually a similar, but less rapid evolution. In the late-juvenile form the onset ranges between 6 and 14 years. Failure in school, behavioural problems or disturbance of cognitive function may precede motor abnormalities. Progressive difficulties in walking, with pyramidal signs and peripheral neuropathy, together with cerebellar ataxia constitute the most common presentation, but various other symptoms can occur, such as hemiplegia, dystonia and choreoathetosis. Spasticity may become prominent, and seizures may also develop [82].

A severity scoring based on a gross motor function classification ("GMFM") has been developed for late infantile and juvenile forms [84]. Age of entry into the different stages and dynamics of decline of gross motor function [85], natural course of language and cognition [86] have been reported.

Two distinct types of adult MLD have been identified. In the first group, patients have predominant motor disease, with pyramidal and cerebellar signs, dystonia and peripheral neuropathy, or isolated peripheral neuropathy. In the more frequent second group, behavioural and psychiatric problems (often confused with schizophrenia) are the presenting symptoms, followed by dementia and spastic paresis [87].

Metabolic Derangement

The primary metabolic defect is a block in lysosomal degradation of sulfatide (or galactosylceramide-sulfate) and other sulfated glycolipids (◘ Fig. 40.2). In vivo, sulfatide is presented to the enzyme arylsulfatase A (ARSA) as a 1:1 complex with sap-B (▶ Sect. 40.2.9). A deficiency of either ARSA or sap-B can cause MLD. A few cases with sap-B deficiency have been documented, most with a late infantile form. Sulfatide is a prominent lipid component of the myelin sheath. Its ratio to galactocerebroside plays a role in the stability and physiological properties of this membrane. Progressive accumulation of sulfatides (and of lysosulfatide) in the central and peripheral nervous system will soon lead to disruption of the newly formed myelin and intense demyelination. In MLD, sulfatides also accumulate in the kidney

(reflected by abnormal excretion in urine sediment), and the gallbladder.

Genetics

About 200 different *ARSA* mutations are known [88]. There is a relatively good genotype-phenotype correlation [82]. Two very frequent *ARSA* polymorphisms (leading to the loss of an N-glycosylation site or of a polyadenylation signal) result in reduction of the amount of enzyme and constitute the molecular basis of ARSA pseudodeficiency [88]. They often occur jointly but can also be found independently. In some countries, as many as 15% of the general population carry one pseudodeficiency (*pd*) allele [82]. MLD due to sap-B deficiency is panethnic, but seems more frequent in Saudi Arabia, Turkey, and North Africa. These patients have mutations in *PSAP*.

Diagnostic Tests

MRI shows similar fairly characteristic symmetrical changes of the central white matter in all forms. A sheet-like area of abnormal T2 signal hyperintensity initially envelops the frontal and parietal periventricular and central white matter regions, faint in mild disease and denser in moderate to severe disease. As severe disease develops, the sheet of white matter signal intensity abnormality also involves the inner half of the subcortical white matter, and a tigroid pattern emerges [89, 90]. The late infantile form also involves cerebral atrophy. Abnormalities are also described by diffusion tensor imaging (DTI) [91] and proton magnetic resonance spectroscopy (MRS). In most patients, motor nerve conduction velocities of peripheral nerves are decreased, and sensory nerve action potentials have a diminished amplitude with a prolonged peak latency. Decreased nerve conduction is not always present in adult MLD. The CSF protein content is usually elevated in late infantile patients (although not at an early stage), inconstantly in the juvenile form and rarely in the adult form.

Determination of ARSA activity in leukocytes (or cultured fibroblasts) using a p-nitrocatechol-sulfate substrate constitutes the first biochemical test. Development of LC-MS/MS methods is in progress [92]. Pseudodeficiency is a major pitfall [82]. Individuals homozygous for a pd. allele (1–2% of the European population), or subjects compound heterozygotes for a disease-causing mld and a pd. allele, have about 5–15% of normal ARSA activity but no detectable clinical abnormality or pathology. Deficient ARSA activity is therefore not enough to conclude to the diagnosis of MLD. The study of sulfatides in the urinary sediment circumvents the problem. MLD (but also multiple sulfatase deficiency, see below) patients excrete massive (late infantile and juvenile patients) or significant (adult-onset type) amounts of sulfatides, while subjects with an ARSA pseudodeficiency have levels within or slightly above the normal range. ARSA pseudodeficiency also poses problems in genetic counselling. In a newly diagnosed family, it is important to measure enzyme activity in both parents. Full genotyping of the index case and study of parental DNA are highly recommended. Prenatal testing of MLD by DNA analysis is the preferred strategy.

Another cause of erroneous interpretation of an ARSA deficiency is multiple sulfatase deficiency (MSD), due to a deficiency in the formylglycine-generating enzyme encoded by *SUMF1*. Whenever a deficiency of one sulfatase is found, it is mandatory to systematically measure the activity of another one (here, arylsulfatase B or iduronate-2-sulfatase) to exclude MSD, as the clinical picture can be misleading, and urinary excretion of sulfatides (but also of glycosaminoglycans) is abnormal.

In MLD patients with sap-B deficiency, the in vitro ARSA assay will not show a deficiency. Studies of sulfatides and globotriaosylceramide (Gb3) excretion in urine are essential. Both lipids are elevated (combined MLD and Fabry pattern). The definitive diagnosis requires *PSAP* sequencing.

Treatment and Prognosis

Symptomatic treatment of spasticity and of pain resulting from radiculopathy is important. Allogenic HSCT has been performed in a number of cases. It is generally considered that adult-onset and juvenile-onset patients benefit, with slowing of the disease progression and improvement of cognitive functions, but challenging reports have appeared [82, 93]. Whether HSCT is indicated in the late infantile form remains controversial [82]. Symptomatic patients are not candidates; presymptomatic affected siblings who received HSCT showed significant difference in survival and CNS involvement compared with untransplanted siblings, with no effect on the peripheral neuropathy. A recent study indicates that donor macrophages can digest accumulated sulfatides and indirectly enable remyelination, albeit without evidence of cross-correction of oligo-and astro-glia [94]. Nevertheless, preliminary evidence of safety and therapeutic benefit has been reported in a phase I/II trial with lentiviral haematopoietic stem cell gene therapy in presymptomatic or very-early symptomatic late infantile/early-juvenile patients [95]. A phase 1-2 trial with intrathecal delivery of recombinant ARSA is also ongoing.

40.2.7 Fabry Disease

Clinical Presentation

Fabry disease, the only X-linked sphingolipidosis, is associated with severe multiorgan dysfunction [96–98]. From data from a recent newborn screening study [99] and after correction for non-pathogenic variants, an incidence of

1:8500 males is obtained, much higher than historical estimations. This suggests a considerable underdiagnosis of atypical phenotypes. Of note, many heterozygous females are symptomatic. Patients are typically divided into a classic form and non-classic (variant or late-onset) forms, which correlate with residual enzyme activity and mutations [100]. Hemizygous males with the classic form have a disease onset during the first decade, typically with crises of severe pain in the extremities (acroparaesthesias) provoked by exertion or temperature changes, that may last hours to days. Unexplained bouts of fever and hypohidrosis, heat, cold and exercise intolerance, gastrointestinal problems, and corneal dystrophy (cornea verticillata) not affecting vision, are other manifestations. At this stage, renal function, urinary protein excretion and cardiac function and structure are generally still normal. Characteristic skin lesions, angiokeratomas, appear on the lower part of the abdomen, buttocks, and scrotum in 80% of patients. Progressive renal involvement, which may result in end-stage renal disease and require dialysis or transplantation, occurs in adulthood. Cardiac manifestations include left ventricular hypertrophy, valvular disease (mitral insufficiency), ascending aortic dilatation, coronary artery disease and conduction abnormalities leading to congestive heart failure, arrhythmias, and myocardial infarction. Cerebrovascular manifestations include early stroke, transient ischaemic attacks, white matter lesions, hemiparesis, vertigo or dizziness, and complications of vascular disease, in particular hearing loss. Depressive symptoms are also frequent. Acroparaesthesias, neuropathic pain, gastrointestinal problems can occur even in early childhood (before 5 years of age) [98]. Patients belonging to the second group show atypical cardiac, renal, or cerebrovascular manifestations with a milder, later onset phenotype, or a single organ involvement. Clinical manifestations in heterozygous females range from asymptomatic to full-blown disease, as severe as in affected males but with globally a later onset and slower progression.

Metabolic Derangement

The primary defect is a deficient activity of the lysosomal enzyme α-galactosidase A, which releases galactose from ceramide trihexoside (globotriaosylceramide, Gb3) and related glycosphingolipids (especially galabiosylceramide, Gb2), due to mutations of *GLA* (Fig. 40.2). This results in progressive accumulation of Gb3 in many tissues. The striking elevation of lysoGb3 observed in plasma of patients and tissues of Fabry mice suggests that this compound is also an important player in pathological events of the disease [101]. In vascular endothelial cells, perithelial and smooth muscle cells, this leads to ischaemia and infarction especially in the kidney, heart, and brain. Early and substantial deposition of Gb3 occurs in podocytes, leading to proteinuria, and with age, in cardiomyocytes, causing cardiac hypertrophy and conduction abnormalities. Small-fibre polyneuropathy is the cause of pain and anhidrosis. Lysosomal storage and cellular dysfunction are believed to trigger a cascade of events resulting in tissue ischaemia and development of irreversible cardiac and renal tissue fibrosis [97].

Genetics

Fabry disease has an X-linked recessive transmission. Adequate genetic counselling in the family, including female carrier detection, is therefore essential. Nearly 1000 variants of *GLA* are known, and defining their pathogenicity remains a crucial problem, especially in screening programmes [102]. For key mutations associated with the classic or non-classic phenotypes, see [103]. De novo mutations are rare. In females, the X-chromosome inactivation pattern seems more contributive to disease expression than the mutation itself [104].

Diagnostic Tests

In affected males with the classic phenotype, the disease is readily diagnosed by showing a profoundly deficient α-galactosidase A activity in leukocytes. DBS are better suited to large-scale screening, but subsequent confirmation in leukocytes is essential. In hemizygous patients with a variant form, interpretation may sometimes be difficult due to a high residual activity. In heterozygous females, the enzyme assay is not reliable since it shows normal to low levels of activity. Measuring lysoGb3 in plasma or DBS has proved a valuable complementary tool for the diagnosis of patients with variant forms and female heterozygotes [102, 105]; this biomarker correlates with the disease phenotype, and it can also be used for monitoring of therapy [106]. In urinary sediment, Gb3 and Gb2 are excreted in large amounts by untreated hemizygous males (except those with a renal graft or with a cardiac variant), and in smaller amounts by 90% of heterozygote females, symptomatic or not. The diagnosis must in all cases be confirmed by *GLA* analysis. Knowing the pathogenic mutation conditions further family screening. Results of *GLA* sequencing alone can often be difficult to interpret in cases of suspected Fabry disease [107], and definite diagnosis should therefore combine several biological and clinical criteria. In atypical – particularly cardiac – variants, electron microscopic study of the target organ may be necessary [107].

Treatment and Prognosis

The disease results in a significant reduction in life expectancy due to renal disease and cardio- or cerebrovascular complications [97]. There is also the psychosocial burden of a rare, chronic, and progressive disease. Management and treatment recommendations have been published for adult patients [103] and children [108]. Alleviation of pain and treatment of the renal and cardiac disease are important issues. Dialysis or renal

transplantation may be necessary for patients with end-stage renal failure. There is a more than 15 year-experience of ERT with recombinant α-galactosidase A products (agalsidase alpha or agalsidase beta). Long-term studies have shown a small but significant effect on cardiovascular and renal complication rates, above all loss of renal function, provided treatment is started early enough. It does not prevent strokes, nor progression of the disease. Several factors may influence effectiveness [106, 109]. The efficacy of ERT in adult female patients has been reviewed [110]. The oral pharmacological chaperone migalastat has also been approved for treatment of patients aged 16 years or older with an amenable mutation [111, 112]. Treatments under evaluation are second-generation ERT (pegunigalsidase-alfa, with a much longer plasma half-life; moss-alphaGal), and recent SRT compounds (venglustat and lucerastat), as well as gene-based therapy; (see [106] for review).

40.2.8 Farber Disease/Acid Ceramidase Deficiency

Clinical Presentation

The very rare "Farber lipogranulomatosis"is clinically heterogeneous. It often presents during infancy causing death within the first year, but later onset cases (up to an adult age) have been described, as well as foetal forms [113]. The most frequent signs are painful joint swelling, deformation and contractures, periarticular subcutaneous nodules, and hoarseness due to laryngeal involvement. The presentation of some patients mimics juvenile idiopathic arthritis [114]. Hepatomegaly and a macular cherry-red spot may be present. Neurological manifestations are of variable severity (from mild to psychomotor deterioration and epilepsy); juvenile-onset patients may show neurological involvement only. A distinct form of acid ceramidase deficiency showing spinal muscular atrophy and progressive myoclonic epilepsy (SMA-PME) has been delineated [115], with more cases being recently described [116].

Metabolic Derangement and Genetics

The deficiency of acid ceramidase activity leads to the storage of ceramides in various organs [117]. More than 60 mutations of *ASAH1* have already been described [118], including a large deletion.

Diagnostic Tests

Electron microscopy of an excised nodule or of a skin biopsy may reveal inclusions with typical curvilinear bodies in histiocytes, and "banana bodies" in Schwann cells. In vitro measurement of ceramidase activity requires a specific substrate available in only few laboratories [119]; so are ceramide precursors loading tests in living fibroblasts or ceramide levels determinations. It is therefore often easier and quicker to directly sequence *ASAH1*.

Treatment and Prognosis

Currently there is no specific therapy. Good results of BMT have been reported only in patients without CNS involvement [120]. Development of ERT and gene therapy is being facilitated by the availability of a suitable mouse model.

40.2.9 Prosaposin Deficiency

Clinical Presentation

The nine published cases have shown almost the same course, with severe neurovisceral storage disease manifesting immediately after birth with rapidly fatal course and death between 4 and 30 weeks of age. The patients have hepatosplenomegaly, hypotonia, massive myoclonic bursts, abnormal ocular movements, dystonia, and seizures [121].

Metabolic Derangement and Genetics

Sphingolipid activator proteins are small glycoproteins that are required as cofactors for the lysosomal degradation of sphingoglycolipids with short hydrophilic head groups and ceramide. They act either by solubilising the substrate or by mediating enzyme binding to the membrane or modifying the enzyme conformation. *PSAP* encodes the prosaposin protein, which is transported to the lysosome where it is processed to four homologous proteins. Sap-A is a cofactor for degradation of galactosyl- and lactosylceramide; its deficiency causes a Krabbe disease variant (4 cases known); sap-B is involved in the in vivo degradation of sulfatides and Gb3, and its deficiency causes an MLD variant (>25 cases known); sap-C is necessary for hydrolysis of glucosylceramide, and its deficiency causes a Gaucher disease variant (5 cases known). Although no patient has been described with sap-D deficiency, this factor is implicated in ceramide degradation. Prosaposin deficiency is due to the combined lack of all four sap- factors, explaining tissue storage of all the lipids cited above. Lipid studies in liver tissue revealed a combined increase of glucosylceramide, lactosylceramide and ceramide. The disorder is autosomal recessive. Mutations identified in patients explain abolished production of the prosaposin precursor and thus of all four factors.

Diagnostic Tests

Gaucher-like cells are found in bone marrow. Study of glycolipids in urine sediment shows a pattern close to that described for sap-B deficiency. Galactocerebrosidase activity has been reported to be deficient in leukocytes and fibroblasts. Loading tests

in living fibroblasts have shown a severe block in ceramide hydrolysis. In practice, the finding of a concomitant elevation of lysoGb3 and lysoGb1 in plasma [121], or of sulfatides and Gb3 in urine, should lead to complete *PSAP* sequencing.

40.3 Disorders of Non-Lysosomal Sphingolipid Degradation

40.3.1 Non-lysosomal β-Glucosidase (GBA2) Deficiency: SPG46 and Ataxia

GBA2 is a membrane-associated protein localised at the endoplasmic reticulum (ER) and Golgi, most likely facing the cytosol. This enzyme can hydrolyse glucosylceramide to ceramide and glucose. While acting on the same substrate but in a different subcellular location, GBA2 is distinct from the lysosomal acid β-glucosidase GBA1 deficient in Gaucher disease (▶ Sect. 40.2.1). The formed ceramide re-enters the biosynthetic pathway (◘ Fig. 40.1) or could play a role as a bioactive lipid in case of excessive formation.

Since 2013, several studies have shown that mutations in *GBA2* should be added to the heterogeneous group of ARCAs (autosomal recessive cerebellar ataxias), and also underlie the hereditary (complex) spastic paraplegia locus SPG46. Most patients with GBA2 deficiency develop in childhood a marked spasticity in lower extremities with progressive gait disturbances and later, ataxia and other cerebellar signs. Variable additional symptoms have been reported, such as hearing loss or cognitive impairment, or Marinesco-Sjögren syndrome. Some patients presented testicular hypotrophy associated with spermatozoid head abnormalities [1]. Besides DNA sequencing, diagnosis can be achieved by determination of enzyme activity using a specific method.

Potential interactions between GBA1 and GBA2 may play a role in Gaucher disease. The paradoxical clinical amelioration reported in mouse models of Gaucher and Niemann-Pick C (▶ Sects. 40.2 and 40.4) diseases after GBA2 inhibition remains an intriguing observation [122, 123].

40.3.2 Neutral Sphingomyelinase-3 Deficiency

Affected children from 12 unrelated families have just been described to harbour biallelic variants in *SMPD4*, which encodes a putative neutral sphingomyelinase, neutral sphingomyelinase-3, a type of enzyme able to hydrolyse sphingomyelin at neutral pH. Sixteen different *SMPD4* variants were identified, all likely resulting in loss of function of the corresponding protein [124]. Most patients shared a severe neonatal presentation including intra-uterine growth retardation, microcephaly, neonatal respiratory distress, congenital arthrogryposis, abnormal muscular tone, and seizures. One third of children died before 1 year of age. MRI showed microcephaly with a simplified gyral pattern, cerebellar hypoplasia and hypomyelination. Whether this phenotype and the ER stress and disturbed autophagy observed in fibroblasts from affected children is linked to abnormal sphingomyelin degradation requires further investigation.

40.3.3 Alkaline Ceramidase 3 (ACER3) Deficiency: Infantile Leukodystrophy

Whether the deficiency of ACER3, which is not a lysosomal storage disease, represents a true sphingolipid degradation or a remodelling defect remains to be determined. A homozygous missense mutation in *ACER3*, coding for alkaline ceramidase 3, localised to both the Golgi complex and the ER, has recently been described in two siblings with leukodystrophy. They presented with neurological regression at 6–13 months of age, truncal hypotonia, appendicular spasticity, dystonia, optic disc pallor, peripheral neuropathy, and neurogenic bladder. The *ACER3* mutation was associated with undetectable ACER3 catalytic activity towards natural and synthetic ACER3-specific substrates, and an accumulation in plasma of ACER3 substrates, C18:1- and C20:1-ceramides and dihydroceramides, as well as some complex sphingolipids, including monohexosylceramides and lactosylceramides [125].

40.3.4 Sphingosine-1-phosphate Lyase (SGPL1) Deficiency: A Multisystemic Disorder

Loss of function of sphingosine 1-phosphate (S1P) lyase, the last enzyme which irreversibly cleaves S1P and releases a fatty aldehyde, thus connecting sphingolipid and glycerophospholipid metabolisms, results in various clinical phenotypes. The most severe, early-onset forms include foetal hydrops with congenital brain malformations, congenital steroid-resistant nephrotic syndrome, primary adrenal insufficiency, adrenal calcifications, ichthyosis, primary hypothyroidism, neurological defects, and lymphopenia [126–129]. A juvenile form characterised by an axonal peripheral neuropathy has also been reported [130]. The plasma levels of S1P as well as the sphingosine/sphinganine ratio were increased in the patients.

40.4 Niemann-Pick Disease Type C

Clinical Presentation

Niemann-Pick disease type C (NP-C) is panethnic, with an estimated incidence at birth around 1 in 100,000 [131, 132]. The clinical course is extremely heterogeneous and age at presentation varies from the perinatal period to late adulthood. Visceral involvement (liver, spleen, and lung) and neurological or psychiatric manifestations arise at different times, and they follow an independent course. Systemic disease, when present, always precedes the onset of neurological symptoms; the systemic component may decrease with time, be minimal, or absent. Apart from a small subset of patients who die in the perinatal period and exceptional adult cases, all patients ultimately develop a progressive and fatal neurological disease. For periods other than perinatal, some patients may show only systemic signs, while others start to show neurological symptoms. A classification by neurological form (rather than by age at disease onset) is justified, because a correlation between age at neurological onset and following course of disease and lifespan has been established [131].

Perinatal Presentations

Foetal period

Foetal hydrops or **foetal ascites** (often with splenomegaly) can occur.

Neonatal period

In early life, liver involvement is often present. About one third of NP-C patients show a prolonged neonatal cholestatic icterus with hepatosplenomegaly. In most patients, the cholestasis resolves spontaneously and only hepatosplenomegaly remains. Such patients will later develop neurological symptoms, although rarely with an adult onset [131, 133]. In a few infants, the liver disease worsens, and they die from hepatic failure before 6 months of age, defining a neonatal, cholestatic rapidly fatal form. Affected siblings may present differently and develop a neurological (often early infantile) form. A few neonates (more often with *NPC2* mutations) develop a severe respiratory insufficiency with pulmonary alveolar proteinosis and die in their first months, or after onset of neurological symptoms in their first years. Isolated (hepato)splenomegaly can also start at the neonatal period [131].

Period with Isolated Systemic Symptoms Isolated splenomegaly or hepatosplenomegaly can be the first sign of NP-C and be detected at any age (differential diagnosis with NP-B and Gaucher type 1), with a highly variable delay before onset of neurological symptoms. A handful of adults (aged up to 60 years) have been described with systemic disease only [131].

Neurological Forms

Early infantile form

In the severe early infantile neurological onset form, infants with a pre-existing hepatosplenomegaly (often with a history of neonatal cholestatic jaundice) show hypotonia and an early delay in motor milestones that becomes evident between the ages of 9 months and 2 years. Most never learn to walk. The mental status is less severely affected. A loss of acquired motor skills is followed by spasticity with pyramidal tract involvement and mental regression. Signs of white matter involvement are present. Survival rarely exceeds 6 years [131, 134].

Late-infantile- and juvenile-onset neurological forms (classic NP-C, 50–60% of incident cases)

In the late infantile form, hepatosplenomegaly has generally been present for a varying period, but may be absent. Language delay is frequent. At the age of 3–5 years, the first obvious neurological signs are gait problems and clumsiness, due to ataxia. The motor problems worsen, cognitive dysfunction appears. In the juvenile form, onset of neurological disease is between 5–6 and 12 years, with more insidious and variable symptoms. Splenomegaly is variable. School problems, with difficulty in writing and impaired attention, are common and may lead to misdiagnosis. The child becomes clumsier with increasing learning disabilities, and obvious ataxia. In both forms, vertical supranuclear saccades palsy, with an increased latency of initiation of vertical saccades, is almost constant when correctly assessed, and an early sign. Vertical supranuclear gaze palsy develops later. Gelastic cataplexy occurs in about 20% of patients and can be the presenting symptom. As ataxia progresses, dysphagia, dysarthria and dementia develop. Action dystonia is also frequent. About half of the patients develop seizures, which may become difficult to treat. In a later stage, the patients develop pyramidal signs and spasticity and severe swallowing problems. Most require gastrostomy. Death usually occurs between 7 and 12–14 years of age in late-infantile-onset patients, and is very variable in the juvenile form, some patients being still alive by age 30 or more [131, 133].

Adolescent/adult onset form

Age at diagnosis varies between 15 and 60 years or more. In adult-onset patients, presentation is even more insidious, and diagnosis seldom made at an early stage. Atypical signs may in retrospect have been present since adolescence. Major signs are ataxia, dystonia and dysarthria, movement disorders, with variable cognitive dys-

function; psychiatric symptoms and dementia are dominant in certain patients [133, 135]. In recent cohorts, supranuclear vertical saccades palsy was a nearly constant sign. Epilepsy is rare in adult NP-C. Splenomegaly is inconstant.

Metabolic Derangement

The disease is due to a defect of NPC1 (in most cases) or NPC2. NPC1 is a large late endosomal/lysosomal protein with 13 transmembrane helices [of which 5 form a sterol-sensing domain (SSD)], and 3 luminal loops. NPC2 is a small, single-domain luminal lysosomal protein. Although the full function of NPC1 may be more complex, it is established that the two proteins work in sequence, in a "hand-off" model, to regulate the export of endocytosed cholesterol from the late endosomal/lysosomal (LE/Ly) compartment. (see also ▶ Chap. 44) Loss of function of either protein thus results in accumulation and sequestration of unesterified cholesterol in LE/Ly (hence a delay in subsequent homeostatic reactions). The transport mechanism is in large part elucidated. Facilitated by lysobisphosphatidic acid (LBPA), NPC2 binds cholesterol from intraluminal vesicles, docks into NPC1 middle loop and transfers the sterol to the N-terminal NPC1 loop. The latter rotates to form a tunnel pathway between the other loops, allowing cholesterol to pass through the glycocalyx and reach the SSD [136, 137]. Final steps of egress are still unknown. The defect of NPC1 or NPC2 results in a similar complex lipid storage profile. In liver and spleen, besides unesterified cholesterol, sphingomyelin, several glycolipids, sphingosine and LBPA, accumulate, with no prevailing compound. In brain, despite clearly abnormal filipin staining in neurons, there is no global increase of cholesterol (nor of sphingomyelin) in grey matter, and storage of GM2 and GM3 gangliosides is the dominant abnormality [42]. Sphingolipid accumulation appears to be secondary to cholesterol storage [42, 138]. Main pathologic changes in brain, besides neuronal storage, are a prominent loss of Purkinje cells, neuroaxonal dystrophy, neurofibrillary tangles, meganeurite formation and ectopic dendritogenesis. Signs of myelination delay and severe myelin loss are only prominent in the early infantile neurological form [42]. Other described abnormalities such as Impairment in Ca^{2+} release from acidic compartments and defects in autophagy likely play a significant role in the pathogenic cascade.

Genetics

Approximately 95% of patients harbour mutations in *NPC1*, the remainder in *NPC2*. More than 500 disease-causing *NPC1* mutations are known. The most frequent ones in patients of western European descent are p.Ile1061Thr, followed by p.Pro1007Ala, albeit with marked geographical differences. Some 60 families are known with *NPC2* mutations. Studies in multiplex families indicate that mutations correlate with the global neurological form rather than with the systemic manifestations. Certain mutations (e.g., p.Pro1007Ala) are associated with a milder block in cholesterol trafficking ('variant' filipin test, see below) [131, 132].

Diagnostic Tests

Neuroimaging is generally not contributive to the diagnosis. Foamy and sea-blue histiocytes may (not always) be found in bone marrow aspirates. Until recently, the "filipin test" (i.e. demonstration in cultured cells of an accumulation of unesterified cholesterol in perinuclear vesicles, visualised by fluorescence microscopy after staining with filipin) was considered as the first-line diagnostic assay, followed by genetic testing [139]. This incurred a skin biopsy and fibroblast culture, and expert interpretation in the 15% of cases with milder accumulation ("variant profile") [44]. Several plasma metabolites have emerged as sensitive NP-C biomarkers [44], and their measurement (in plasma, or DBS for some of them), has now replaced the filipin test as the recommended first-line assay [132]. The oxysterols cholestane-3β, 5α, 6β-triol and 7-ketocholesterol, the bile acid 3β,5α,6β-trihydroxy-cholanoyl-glycine, and *N*-palmitoyl-*O*-phosphocholine-serine [PPCS] (correct structure and name of "lysosphingomyelin-509"), are elevated in nearly all patients with NP-C, but also in acid sphingomyelinase deficiency (ASMD) (and for oxysterols, in some other diseases) (▶ Sect. 40.2.2) [44]. Distinction can be made by the concomitant study of lysosphingomyelin, strikingly elevated only in ASMD. Elevated values for one or several biomarkers are a strong argument, but the tests have limitations, and the definitive diagnosis of NP-C requires molecular analysis of *NPC1* and *NPC2*. In the not so rare cases in which genetic testing remains inconclusive, the filipin test regains ground as the best functional approach. Only molecular genetics testing is now used for prenatal diagnosis [131, 139].

Treatment and Prognosis

Clinical management guidelines have been published [132, 139]. Cataplectic attacks can be treated by clomipramine or CNS stimulants. Management of epilepsy, when present, is essential. With progression, most patients will require tube feeding or gastrostomy. To date, miglustat is the only treatment specifically approved for neurological manifestations of NP-C, in the EU and other countries (not in the USA). Indications, clinical utility, and monitoring have been discussed [139], and studies prior to 2018 reviewed [140]. Initial data indicating effect on swallowing impairment, stabilisation of patients for 1 year or more and a slower rate of progression of the disease after treatment have been confirmed. Patients with later onset forms appear as better responders [141, 142]. Effect on survival has also been studied.

Encouraging results were obtained in a phase 1-2 trial with intrathecal administration of 2-hydroxypropyl-β-cyclodextrin (HPBCD) [143], but data from the phase 2b-3 trial have not been published. Promising results have been reported for a phase 2/3 trial with oral administration of arimoclomol, a heat shock (hsp70 and hsp40) proteins enhancer [144]. Other trials with IV administration of HPBCD, or oral administration of N-acetyl-L-leucine are ongoing. At variance with NP-C1, there is a rationale for HSCT in NP-C2 patients, but neurological follow-up at 9 years of age in the only patient known to survive the procedure is rather poor [134].

40.5 Neuronal Ceroid Lipofuscinoses

Neuronal ceroid lipofuscinoses (NCLs) are a group of inherited progressive neurodegenerative diseases, among the most frequent in childhood. The term NCL is widely used in Europe, but the generic name "Batten disease" is common in the USA. The past 25 years have seen major advances in the field and the clinical diversity has now been linked to a wide genetic heterogeneity, with 13 different genes identified to date. Five of them encode soluble proteins, the others encode transmembrane or cytosolic proteins whose function still remain incompletely understood. NCLs have been linked to lysosomal storage diseases, due to the lysosomal accumulation of lipopigments, and localisation of several NCL proteins to the lysosome, even though other NCL proteins are localised to non-lysosomal cellular compartments.

Clinical Presentation

NCLs are usually characterised by progressive psychomotor retardation, seizures, visual loss, and early death. Four main clinical forms have been described according to the age of onset and the order of appearance of clinical signs: infantile, late infantile (the most common in South Europe), juvenile (common in Anglo-Saxon countries), and adult (rare) [145]. However, numerous other clinical variants have been reported. This clinical heterogeneity is related to the diversity of the genes involved and to the variable severity of mutations. Therefore, the first classification based on the clinical forms has now been replaced by a new one using the genetic loci and including various forms with different ages of onset although one form is usually predominant for each gene [146] (◘ Table 40.2).

Classic Infantile Neuronal Ceroid Lipofuscinosis (Santavuori-Haltia Disease) Linked to *PPT1* (CLN1 Disease) Its incidence is high in Finland (1 in 20,000). Children with infantile NCL are normal at birth. Symptoms usually begin between 6 and 24 months. They include delayed development, hypotonia, deceleration of head growth, seizures, and jerks. Sleep disturbances are seen in most children. Rapid visual impairment occurs due to optic atrophy and macular degeneration. Stereotyped hand movements may be present. Death takes place in the first decade of life. Whereas mutations in *PPT1* are mainly responsible for this classic infantile NCL, later-onset forms (juvenile, adult) have also been described, probably due to less severe mutations.

Variants due to another gene

Mutations have occasionally been found in *KCTD7* (CLN14 disease) in patients with infantile-onset progressive myoclonic epilepsy (PME), vision loss, cognitive and motor regression, and premature death. *KCTD7* mutations have also been involved in non NCL-phenotypes such as opsoclonus myoclonus ataxia syndrome associating acute onset of myoclonus and ataxia with abnormal opsoclonus-like eye movements [147, 148].

Classic Late Infantile Neuronal Ceroid Lipofuscinosis (Jansky-Bielschowsky Disease) Linked to TPP1 (CLN2 Disease) Children may be initially referred for delayed speech. Seizures, which may be of any type (partial, generalised tonic-clonic, absences) occur between 2 and 4 years of age. Ataxia, myoclonus, and developmental regression become apparent, followed by a gradual decline of visual ability culminating in blindness by 5 or 6 years. Death happens in middle childhood after a bedridden stage. Besides this classic late-infantile NCL, mutations in *TPP1* have also been involved in atypical phenotypes with delayed onset and slower progression. Moreover, mutations in *TPP1* have been reported in autosomal recessive spinocerebellar ataxia 7 (SCAR7). Patients showed ataxia, but neither visual abnormalities nor epilepsy, and the disease is slowly progressive until old age.

Variants due to other genes

Variants with similar or later onset, or delayed evolution compared to the classic late infantile form have been described. The Northern epilepsy or progressive epilepsy with mental retardation (EPMR) linked to *CLN8* is characterised by tonic-clonic seizures occurring between 5 and 10 years. Mental deterioration is observed 2–5 years after the onset of epilepsy. Vision problems are rare. Some patients are surviving well over 40 years. Mutations in *CLN8* have also been reported in a subset of late-infantile patients from Turkish consanguineous families. Variants in *CLN5* have been identified in Finland as well as other countries, such as Italy [149]. This form usually begins around 4.5–6 years of age by clumsiness and difficulties in concentration. Visual impairment, ataxia and epilepsy appear a few years later. Life expectancy is between 13 and 35 years. Two additional genes are commonly involved in late-infantile variants

Table 40.2 Classification of NCLs. The different loci are organized according to the age of onset of the main clinical form (indicated in bold in the right column). The non-NCL phenotypes associated to the same genes are given in italics. *ATP13A2* and *KCTD7* have rarely been linked with NCL phenotypes

Gene (disease name)	Protein name (abbreviation)	Protein location and type	Clinical forms
CTSD (CLN10 disease)	Cathepsin D	Lysosomal enzyme	**Congenital** Late infantile, juvenile, adult
PPT1 (CLN1 disease)	Palmitoyl protein thioesterase 1 (PPT1)	Lysosomal enzyme	**Classic infantile** Late infantile, juvenile, adult
KCTD7 (CLN14 disease)	Potassium channel tetramerization domain-containing protein 7 (KCTD7)	Cytosolic protein, associated with plasma membrane	Infantile, late infantile (rare) ***Progressive myoclonic epilepsy*** ***Opsoclonus-myoclonus ataxia-like syndrome***
TPPI (CLN2 disease)	Tripeptidyl peptidase 1 (TPP1)	Lysosomal enzyme	**Classic late infantile** Juvenile, protracted, *spinocerebellar ataxia recessive type 7 (SCAR7)*
CLN5 (CLN5 disease)	CLN5 protein	Soluble lysosomal protein	**Late infantile** Juvenile, adult
CLN6 (CLN6 disease)	CLN6 protein	Endoplasmic reticulum membrane protein	**Late infantile** **Adult type A Kufs** *Juvenile cerebellar ataxia*
MFSD8 (CLN7 disease)	MFSD8 Major facilitator domain-containing protein 8	Lysosomal membrane protein	**Late infantile** Juvenile, protracted *Adult macular or cone-rod dystrophy*
CLN8 (CLN8 disease)	CLN8 protein	Endoplasmic reticulum membrane protein	**Late infantile** Protracted **Northern epilepsy (EPMR)**
CLN3 (CLN3 disease)	CLN3 protein	Lysosomal membrane protein	**Classic juvenile** Protracted *Autophagic vacuolar myopathy, retinitis pigmentosa, adult cone-rod dystrophy*
ATP13A2 (CLN12 disease)	ATP13A2	Lysosomal membrane protein	Juvenile (rare) ***Kufor-Rakeb syndrome, hereditary spastic paraplegia (SPG78), juvenile onset amyotrophic lateral sclerosis-like***
DNAJC5 (CLN4 disease)	Cysteine-string protein alpha (CSPα)	Cytosolic protein, associated with vesicular membranes	**Adult type A Kufs (dominant)**
CTSF (CLN13 disease)	Cathepsin F	Lysosomal enzyme	**Adult type B Kufs**
GRN (CLN11 disease)	Progranulin	Soluble lysosomal protein	**Adult** *Frontotemporal lobar dementia (heterozygous)*

presenting a clinical pattern close to the CLN2 disease. Mutations in *CLN6* are mainly seen in patients originating from South Europe, the Indian subcontinent, and South America. *CLN7* (*MFSD8*) has been initially involved in Turkish patients with late-infantile NCL, but abnormalities in this gene have now been reported in patients from different countries [147].

Classic Juvenile Neuronal Ceroid Lipofuscinosis (Batten or Spielmeyer-Vogt Disease) Linked to CLN3 (CLN3 Disease) The onset is between 4 and 10 years of age. Visual failure is usually the first clinical sign and it results in total blindness in 2–3 years. Seizures appear between 5 and 18 years. They are tonic-clonic at onset, but multifocal motor seizures become more frequent with age.

Speech becomes dysarthric and echolalia is frequent. Many patients develop signs of parkinsonism. Mental capacity is progressively altered, and dementia becomes evident in several years. Behavioural problems with aggressiveness may occur. Most patients live until the late teens or early/late 20s. Cardiac signs have been reported in adolescent and adult CLN3 patients, such as left ventricular hypertrophy and bradycardia leading to a risk of death [150]. A protracted atypical phenotype has recently been reported in patients showing a rapid visual failure followed 20 years later by seizures, hypertrophic cardiomyopathy, the presence of autophagic vacuoles in muscle biopsy and only mild cognitive impairment after 40 years of evolution.

▪ Variant due to another gene

Mutations in *ATP13A2* (now CLN12 disease) have rarely been associated with a juvenile NCL variant showing learning difficulties around 8 years, followed by unsteady gait, myoclonus, mood disturbance, and extrapyramidal signs such as akinesia, rigidity and dysarthric speech. *ATP13A2* is mainly involved in non-NCL disorders such as Kufor-Rakeb syndrome (rare parkinsonian syndrome with juvenile onset), but also autosomal recessive spastic paraplegia (SPG78) and juvenile-onset amyotrophic lateral sclerosis-like [148].

Adult Neuronal Ceroid Lipofuscinosis (Kufs Disease)

Symptoms usually start around age 30 years, but onset during adolescence or late adulthood has been reported. Kufs disease is usually inherited as an autosomal recessive trait, but a rare dominant form (called Parry disease) also exists. Classically, two major forms of Kufs disease have been delineated. Type A is characterised by PME while type B is marked by dementia and a diversity of motor signs. Retinal vision is generally preserved. Previously the genes involved in these forms had remained uncharacterised although *PPT1* mutations had been found in some patients.

CLN6 is now considered as a major gene in recessive type A Kufs disease and the dominant form (called CLN4 disease) has been linked to *DNAJC5* (*CLN4*) encoding cysteine-string protein alpha (CSPα). Causal abnormalities have also been found in *CTSF* (*CLN13*) encoding cathepsin F in patients with type B Kufs disease. Moreover, mutations have been reported in *GRN* (*CLN11*) encoding progranulin in siblings with rapidly progressive visual failure around 20 years, myoclonic seizures, cerebellar ataxia, and early cognitive deterioration. Unexpectedly, these patients were homozygous for a *GRN* mutation, while heterozygous mutations in the same gene are a major cause of frontotemporal lobar dementia. These two diseases significantly differ by their age of onset and neuropathology [147].

▪ Congenital form

This rare form presents with microcephaly and seizures at birth, resulting in death within the first days of life. Mutations in *CTSD* (or *CLN10*) have been found in some patients, but other causative genes probably remain to be identified [147].

▪ Metabolic Derangement

Ceroid lipofuscinoses are characterised by the accumulation of autofluorescent ceroid lipopigments, mainly in neural tissues. They show different ultrastructural patterns, such as granular, curvilinear or fingerprint profiles [151]. The main components of this storage material are either saposins A and D in infantile forms, or subunit c of mitochondrial ATP synthase in late infantile and juvenile forms. They are probably not disease-specific substrates, but secondary markers. NCL proteins are mainly localised in the lysosome (CLN1, CLN2, CLN3, CLN5, CLN7, CLN10, CLN12, CLN13), but also in the ER (CLN6, CLN8) or in the cytosol in association with vesicular membranes (CLN4, CLN14). Some of them are soluble proteins: palmitoyl protein thioesterase 1 (CLN1), tripeptidyl peptidase 1 (CLN2), cathepsin D (CTSD), cathepsin F (CTSF) and CLN5. Others are transmembrane proteins (CLN3, CLN7, CLN12), the function of which is still incompletely understood; for a review, see [148].

Briefly, CLN1 is involved in the degradation of S-fatty acylated proteins (depalmitoylation) and in the maintenance of the synaptic pool by regulating exo- and endocytosis and synaptic vesicle recycling. (see also ► Chap. 44) It is linked to CSPα (CLN4) which is likely its substrate. CLN2 is a serine protease which removes tripeptides from proteins facilitating their degradation in lysosomes, but its substrates are not yet clearly defined. It is possibly involved in macroautophagy and TNFα-induced apoptosis. Cathepsin D is an aspartyl protease important for apoptosis and autophagy; it is probably implicated in the biogenesis and synaptic transmission in GABAergic neurons. Cathepsin F is a lysosomal cysteine protease. It is involved in endosomal and lysosomal trafficking regulation via its newly discovered role in the cleavage of LIMP-2 (a mannose-6-phosphate independent receptor). The function of CLN5 is still debated, but it has been reported to interact with different other NCL proteins, especially CLN8 and CLN3. Progranulin (CLN11) plays important roles in inflammation, tumorigenesis, and lysosomal function (lipid homeostasis). CLN3 function has not been elucidated, but this protein was shown to impact ionic balance, endolysosomal trafficking and autophagy. CLN6 and CLN8 localize to the ER. It has recently been demonstrated that they form a complex recruiting lysosomal enzymes at the ER to promote their transfer to the Golgi

[152]. MFSD8 (CLN7) belongs to the major facilitator superfamily and has been reported to be an endolysosomal chloride channel. Loss of CLN7 leads to alterations in mTORC1 signalling, as well as lysosomal size and exocytosis. ATP13A2 (CLN12) is a lysosomal P5-type ATPase with a neuroprotective activity during oxidative stress, metal exposure or α-synuclein toxicity. KCTD7 (CLN14) has been implicated in the regulation of neural signalling and transmission (K^+ conductance) and of autophagy-lysosomal pathways. CSPα, altered in the rare dominant CLN4 adult form, is a chaperone protein abundant in neurons essential for short and long-term synaptic maintenance.

Genetics

NCLs are usually inherited in an autosomal recessive manner (except the CLN4 adult form which is dominantly transmitted). They result from mutations in the 13 known genes encoding the various NCL proteins [147] (■ Table 40.2). Numerous *PPT1* (*CLN1*) mutations have been reported, but p.Arg122Trp and p.Arg151* are common in Finnish and non-Finnish patients, respectively. Two mutations are common in *TPPI* (CLN2): c.509-1G > C and p.Arg208*, but around 150 variants, mainly private, have now been described [153]. For *CLN3,* a 1 kb deletion (c.461-280_677 + 382del966) is particularly frequent (80–90% of alleles). Concerning *CLN5*, p.Tyr392* is frequent in the Finnish population, but different mutations have been found in other countries. Northern epilepsy is mainly due to the p.Arg24Gly variant, but other *CLN8* abnormalities have been described in patients presenting with late infantile forms. Numerous variants have been reported in the other NCL genes characterised to date; details are given in the NCL Mutation Database (► http://www.ucl.ac.uk/ncl-disease/mutation-and-patient-database).

Diagnostic Tests

Electrophysiological studies are helpful to establish the diagnosis of NCLs. Electroretinogram (ERG) is generally diminished at presentation and it becomes rapidly extinguished. In infantile NCL, the first abnormality in the electroencephalogram (EEG) is the disappearance of eye opening/closing reaction, followed by a loss of sleep spindles. Then, EEG becomes rapidly flat. In CLN2 disease, an occipital photosensitive response to photic stimulation at 1–2 Hz with eyes open is present. MRI shows progressive brain atrophy, particularly severe in CLN1 disease, sometimes beginning on cerebellum in other forms. Vacuolated lymphocytes are a common feature of CLN3 disease. Electron microscopy (EM) on skin biopsies shows the presence of pathological inclusions. Granular osmiophilic deposits (GROD) are mainly found in early-onset forms (CLN1 and CLN10). Curvilinear (CV) profiles are present in the classic CLN2 disease and in the variant CLN7, while fingerprints (FP) are common in CLN3. Mixed inclusions diversely associating GROD, CV and FP are found in other clinical forms. EM remains useful to confirm the diagnosis of atypical forms of NCL [151].

For the CLN1 and CLN2 diseases, diagnosis is rapidly established by measuring the activity of palmitoyl protein thioesterase 1 and of tripeptidyl peptidase 1, respectively. These enzymatic tests can be performed on leukocytes, DBS, or cultured fibroblasts. For CLN3 disease, diagnostic testing can be firstly targeted to the common 1 kb deletion. For all the NCL genes, complete sequencing is performed either using Sanger sequencing or more frequently next generation sequencing (NGS) based on gene panels focused on lysosomal storage disorders or other conditions (myoclonic epilepsies, retinitis pigmentosa, ...) sharing clinical features with NCLs. Prenatal diagnosis is available for NCL families using the specific enzymatic test and/or detection of the previously characterised mutations. Preimplantation diagnosis can also be offered to parents in some countries.

Treatment and Prognosis

Among symptomatic treatments, antiepileptic drugs need to be selected with caution (lamotrigine is usually efficient, but carbamazepine and phenytoin can worsen the symptoms). Diazepines should be useful on seizures, anxiety, and sleep disturbances. Gastrostomy is used to maintain nutritional status in the late stages of the disease. Specific therapies are in development for NCL and some of them have already entered the clinic [154]. In CLN2, enzyme replacement therapy (ERT) based on intracerebroventricular infusion of recombinant TPP1 (Cerliponase alfa) has demonstrated its capacity to slow or stabilise the disease progression [155]. It has now been approved for the treatment of CLN2 disease. Other ERTs might be suitable for NCLs involving soluble lysosomal proteins. Gene transfer approaches have been largely investigated in different animal models and clinical trials are now ongoing or planned, using either direct intracerebral (CLN2) or intrathecal (CLN6, CLN3) administration of AAV vectors (serotype 9 or 10). Gene therapy will probably be used for other NCLs in the future. In addition, different pharmacological therapies focused on potential targets to modulate disease have been explored. In CLN1, a treatment combining cysteamine bitartrate and N-acetylcysteine did not significantly change the course of the disease. A non-steroidal immunosuppressant, mycophenolate mofetil, was tested on CLN3 patients with no clear clinical benefit. Other candidate drugs will likely be found based on further advances

on the molecular pathways involved in NCLs [156]. Neural stem cell transplantation is another option tested in patients, but without meaningful benefits to date.

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Glycosaminoglycans and Oligosaccharides Disorders: Glycosaminoglycans Synthesis Defects, Mucopolysaccharidoses, Oligosaccharidoses and Sialic Acid Disorders

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Contents

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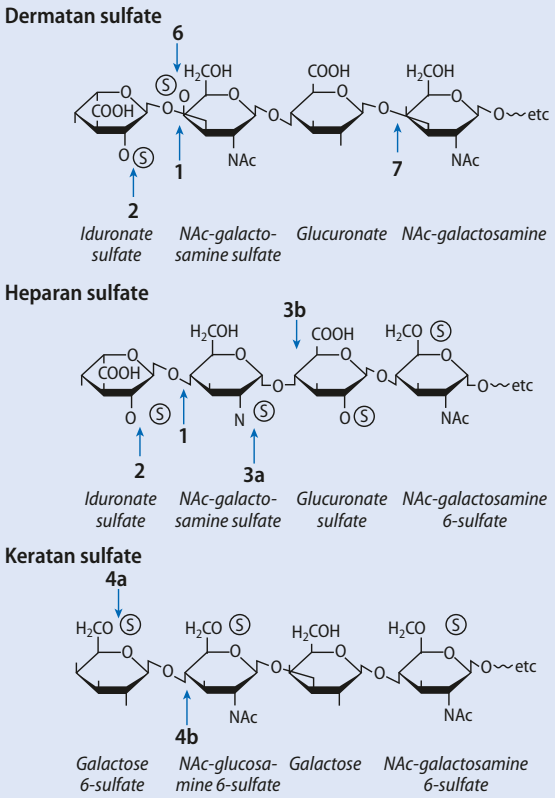

Fig. 41.1 Main repeating units in mucopolysaccharides and location of the enzyme defects in the mucopolysaccharidoses. *NAc* N-acetyl, *S* sulfate, *1*, α-iduronidase (MPS I: Hurler and Scheie disease), *2*, iduronate sulfatase (MPS II: Hunter disease); *3a*, heparan N-sulfatase (MPS IIIA: Sanfilippo A disease); *3b*, α-N-actylglucosamindase (MPS IIIB: Sanfilippo B disease); *4a*, N-acetyl-galactosamine-6-sulfatase (MPS IVA: Morquio A disease); *4b*, β-galactosidase (MPS IVB: Morquio B disease); *6*, NAc-galactosamine-4 sulfatase (MPS VI: Maroteaux-Lamy disease); *7*, β-glucuronidase (MPS VII: Sly disease)

Glycosaminoglyans

Glycosaminoglycans, (GAGs, mucopolysaccharides) are essential constituents of connective tissue, including cartilage and vessel walls. They are composed of long sugar chains, containing highly sulfated, alternating uronic acid and hexosamine residues, assembled into repeating units. The polysaccharide chains are bound to specific core proteins within complex macromolecules called proteoglycans (PG). GAGs are grouped in two families: sulfated GAGs, mainly represented by chondroitin sulfate (CS), dermatan sulfate (DS), keratan sulfate (KS), heparan sulfate (HS) and heparin (Fig. 41.1) and nonsulfated GAGs including mainly hyaluronan (HA). PG biosynthesis involves several enzymes and transporters in four main steps: core protein synthesis, GAG synthesis (including the linker tetrasaccharide and subsequent chain elongation), GAG sulfation and PG secretion. Core protein synthesis occurs in the rough endoplasmic reticulum where some early modifications, such as N-glycosylation, take place. The core protein then moves to the Golgi apparatus for GAG biosynthesis (see also ▶ Chaps. 43 and 44). Degradation of GAGs takes place inside the lysosomes and requires several acid hydrolases. Deficiencies of specific degradative enzymes are the cause of a variety of eponymous disorders, collectively termed mucopolysaccharidoses (MPSs).

41

Introduction

Genetic defects in enzymes that are involved in the lysosomal degradation of the GAGs, collectively termed mucopolysaccharidoses (MPSs), (Fig. 41.1) and the oligosaccharide chains of glycoproteins (Fig. 41.6), collectively termed oligosaccharidosis lead to chronic and invariably progressive disorders. Although these disorders share many clinical features, the presentation can be highly variable and the spectrum of phenotypic severity is extremely broad. Signs and symptoms include bone

dysplasia (dysostosis multiplex), hepatosplenomegaly, neurological abnormalities, cardiac disease and, in some of the disorders, developmental regression. Life expectancy is generally reduced at the severe end of the clinical spectrum. MPSs and oligosaccharidoses are transmitted in an autosomal recessive manner, except for the X-linked MPS II (Hunter syndrome). Diagnosis of these disorders is usually by detecting increased concentrations of (partially degraded) GAGs or oligosaccharides in urine, confirmed by specific enzyme assays in serum, leukocytes or skin fibroblasts followed by mutational analysis.

Over recent years important advances have been made in the disease modifying treatment of a number of the MPSs, including enzyme replacement therapy (ERT) and hematopoietic stem cell transplantation (HSCT) and many more treatment options, including gene therapy, are currently under study. While current treatments may result in improvement of a number of clinically relevant symptoms there is generally significant residual disease, especially involving the skeletal tissue, growth, the heart and the cervical spine. Prenatal diagnosis is possible for all the MPSs and oligosaccharidoses.

Disorders of GAGs and oligosaccharides synthesis may not present primarily with neurological symptoms but rather should be suspected in patients with a combination of characteristic clinical features in more than one connective tissue compartment: bone and cartilage, ligaments, and subepithelial (skin, sclerae). Some produce distinct clinical syndromes mostly presenting with bone dysplasias (▶ Sect. 1.6.12). Disorders involving synthesis of N-Glycans and O-Glycans are generally classified as congenital disorders of glycosylation (CDGs) (▶ Chap. 43). GAG synthesis defects can also affect protein homoeostasis, intracellular trafficking, assembly of matrix proteins and cell signalling. (▶ Chap. 44).

41.1 Glycosaminoglycans Synthesis Defects

A number of genetic disorders of bone and connective tissue caused by mutations in genes encoding for glycosyltransferases, sulfotransferases and transporters that are responsible for the synthesis of sulfated GAGs have been described [1]. Twenty-eight defects that are currently known are summarized in ◘ Table 41.1 after the recent review by Paganini et al. [2].

41.2 Mucopolysaccharidoses

41.2.1 Clinical Presentation

Seven separate types of MPSs are distinguished: Mucopolysaccharidosis (MPS) I, II, III, IV, VI, VII, IX and X (◘ Table 41.2). In addition, differences in phenotypic severity has led to separation of MPS I in different subtypes (Hurler, Hurler/Scheie and Scheie phenotypes; MPS IH, IH-S and IS respectively) and in MPS II in the neuronopathic and non-neuronopathic phenotypes. Furthermore, MPS III is subdivided into 4 types (A to D), depending on the deficient enzyme and MPS IV into 2 subtypes (A and B), also depending on the specific enzyme deficiency. Both MPS III, IV and VI may result in a severe (often called 'classical') phenotype as well as a (much) more attenuated phenotype. In MPS III this is also referred to as rapid progressing (RP) and slow progressing (SP) phenotypes.

All MPSs are rare and information about their birth prevalence is relatively limited. Although there are differences between regions, MPS I and MPS III are the most prevalent disorders, except for the United Arab Emirates and Turkey where MPS VI is most frequent.

MPS are all chronic, progressive and multisystem disorders. Although patients generally appear normal at birth, accumulation of GAGs already starts before birth and may lead to very early symptoms such as hydrops fetalis and intrauterine death (MPS VII) or the presence of skeletal deformities such as thoracolumbar kyphosis at birth (MPS IH). In general, MPSs present with one or more of the following characteristic symptoms:

1. Dysmorphic features from an early age in combination with growth failure, umbilical and/or inguinal hernia, protruding abdomen and musculoskeletal disease; all frequent in MPS IH and MPS IH-S, MPS II, MPS VI and MPS VII.
2. Primarily skeletal disease (skeletal dysplasia) with growth failure and relatively minor dysmorphic features (MPS IV).
3. Slowing of cognitive development (especially of language development) and behavioural problems followed by cognitive decline in combination with (mild) coarse facial features (MPS III).

It is important to note that the wide phenotypic spectrum observed in all MPS subtypes can lead to an atypical, pauci-symptomatic, presentation which may easily lead to misdiagnosis, for instance, of a skeletal dysplasia e.g. in MPS IVA and MPS VI, or a stable cognitive impairment in MPS III, with an increasing number of MPS diagnoses being made in adults.

Important signs and symptoms in MPS include:

- Dysostosis multiplex is a term used to describe the skeletal disease associated with MPS and consists of a collection of radiographic abnormalities resulting from defective endochondral and membranous growth throughout the body. Typically, the growth of the long bones is stunted, vertebral bodies are hypoplastic and abnormally shaped, which may result in kyphosis with or without scoliosis (◘ Fig. 41.2), and the knees are in the valgus position. Hip abnor-

Table 41.1 Glycosaminoglycans (GAGs) synthesis defects (after [2])

Causative gene	Clinical entities	Skeletal phenotype
Defects in the synthesis of the linker region 'linkeropathies'. Chondroitin sulfate, DS, HS and heparin are attached to serine residues of the core protein through a tetrasaccharide linkage region, composed of one xylose (Xyl), two gal and one GlcUA (see ► Chap. 43)		
XYLT1	Desbuquois dysplasia type 2	Disproportionate short stature, microretrognathia, congenital dislocations
XYLT2	Spondyloocular syndrome	Short trunk,osteopenia with bone fragility, eye defects, hearing impairment, cardiac septal defects
FAM20B	Neonatal short limb dysplasia	Midface hypoplasia, thoracic hypoplasia, Very short stature, multiple dislocations
B4GALT7	Ehlers–Danlos syndrome (EDS) Spondylodysplastic type 1 including Larsen	DD, short stature,osteopenia, radioulnar synostosis, hypermobile joints, loose but elastic skin
B3GALT6	EDS spondylodysplastic type 2 syndrome,	Severe kyphoscoliosis, epimetaphyseal dysplasia, craniofacial disproportion, osteopenia, joint laxity, defective wound healing, loose skin,hypotonia.
B3GAT3	Larsen-like syndrome	DD, multiple dislocations, bone fragility, heart valves anomalies, cutis laxa
Defects in GAG chain elongation including substrate synthesis. After the synthesis of the linkage region, GAG chain elongation continues with the polymerization of specific repeated disaccharide units		
CSGALNACT1	Joint dislocations and skeletal Dysplasia, Desbuquois-like	Nonproportioned stature, hyperlordosis, Mild facial dysmorphism and joint laxity
CHSY1	Temtamy preaxial brachydactyly syndrome	DD, growth retardation, joint dislocations, abnormal bone patterns in hand and feet, deafness
EXTL3	Immunoskeletal dysplasia with neurodevelopmental abnormalities	Skeletal dysplasia with short stature, Craniosynostosis, vertebral anomalies, kyphosis, brachydactyly, DD and immunodeficiency
EXT1/EXT2	Hereditary multiple exostosis Type 1 and 2 (multiple osteochondromas)	Cartilaginous/bony tumours in long bones, ribs and other skeletal elements.
SLC35D1	Schneckenbecken dysplasia	Neonatal lethal chondrodysplasia, oval vertebral bodies, extremely short long bones and small ilia
SLC35A3	Multiple congenital Malformation syndrome	Limb deformities, knee and hip dislocation, scoliosis, hand and foot anomalies
SLC10A7	Skeletal dysplasia, osteoporosis, multiple Dislocations and amelogenesis imperfecta syndrome	Severe disproportionate short stature, microretrognathia, congenital dislocations, advanced ossification, amelogenesis imperfecta

Table 41.1 (continued)

Causative gene	Clinical entities	Skeletal phenotype
CANT1	Desbuquois dysplasia type 1	Joint dislocations, facial dysmorphism, advanced bone age,extra ossification Centre, phalangeal dislocation
TGDS	Catel–Manzke syndrome	Cleft palate, glossoptosis, micrognathia, Bilateral hyperphalangy
DSE	Ehlers–Danlos syndrome Musculocontractural type 2	Joint dislocations, skin hyperextensibility, Bruisability and fragility, muscle hypoplasia
GORAB	Gerodermia osteodysplastica	Lax and wrinkled skin, osteoporosis, reduced bone mass, susceptibility to fracture
Defects in GAG chain sulfation. Sulfation is an important step in PG biosynthesis; in the Golgi apparatus GAGs are sulfated by specific sulfotransferases that catalyse the transfer of sulfate groups from 30-phosphoadenosine 50-phosphosulfate (PAPS), the universal sulfate donor, to the sugar moiety of GAG chains. The intracellular sulfate pool rely mainly on extracellular uptake by the SLC26A2 transporter and only a small amount may come from the catabolism of sulfur-containing amino acids (► Chap. 20)		
SLC26A2	Achondrogenesis type 1B	Foetal or perinatal lethality, micromelia, Short extremities and trunk
	Atelosteogenesis type 2	Perinatal lethality, shortened limbs, cervical kyphosis, flattened vertebrae with coronal clefts, cleft palate,'hitchhiker' thumb
	Diastrophic dysplasia	Joint dysplasia, joint pain and contractures, scoliosis, cleft palate,'hitchhiker' thumb, cystic swelling of the external ear
	Recessive multiple epiphyseal dysplasia	Scoliosis, clubfoot and double-layered patella
PAPSS2	Spondyloepimetaphyseal dysplasia, Pakistani-type	Short stature, short and bowed lower limbs, enlarged knee joints, kyphoscoliosis, mild brachydactyly, osteoarthritis
	Brachyolmia type 1	Short trunk, platyspondyly with irregular and narrow intervertebral spaces, scoliosis, corneal opacities in some cases
CHST3	Recessive Larsen syndrome	Short stature, knee dislocation, hip dislocation, clubfoot, kyphoscoliosis, intervertebral disc degeneration, rarely cardiac involvement
CHST14	Ehlers–Danlos syndrome Musculocontractural type 1	Contractures of thumbs and fingers, clubfoot, kyphoscoliosis, hypotonia, hyperextensible thin skin, easy bruisability, joint hypermobility, atrial septal defect, ocular involvement
IMPAD1	Chondrodysplasia with joint dislocations, gPAPP type	Short stature, brachydactyly, joint dislocations, micrognatia, cleft palate, facial dysmorphism

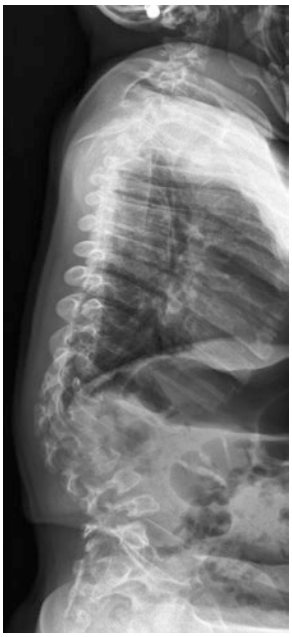

Fig. 41.2 Lateral X-ray of the spinal column of a 12 year old MPS I Hurler patient who had a successful hematopoietic stem cell transplantation at the age of 1 year. Abnormally shaped dysplastic vertebral bodies with thoracolumbar kyphosis. In addition, broad oar shaped ribs can be observed

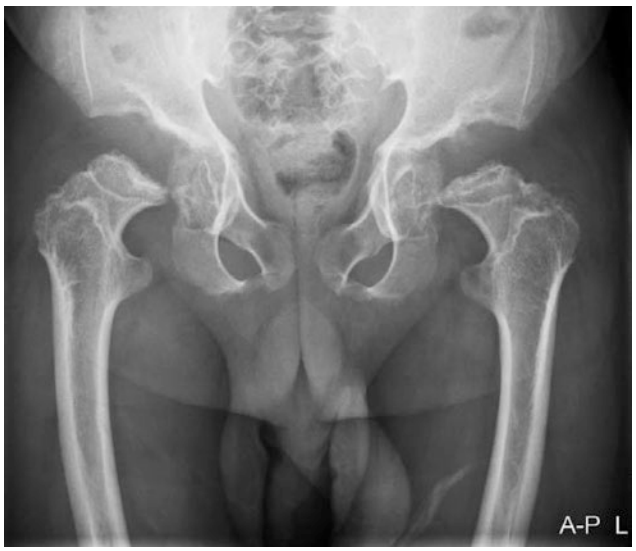

Fig. 41.3 Femoral and acetabular pathology due to dysostosis multiplex in a 15 year old boy with MPS IVA

malities, due to failure of ossification of the lateral acetabular roof, medial proximal epiphyseal growth failure of the femur and coxa valga, lead to a form of hip dysplasia often accompanied by deformation leading to subluxation or dislocation of the femoral head (Fig. 41.3).

Other radiologic findings include bullet-shaped metacarpals and phalanges, an enlarged and thickened skull, broad clavicles and broad oar-shaped ribs (Fig. 41.2). The pathophysiology of the dysostosis multiplex is complex and not fully understood. Intra- and extracellular deposition of GAGs and GAG fragments leads to impaired cell-to-cell signalling, altered biomechanical properties and upregulated inflammatory pathways, all of which are believed to affect the growth plate, osteoclasts and osteoblasts, thus contributing to the skeletal dysplasia. Dysostosis multiplex can be observed in all MPSs, but is most pronounced in MPS I, II, IVA, VI and VII (Table 41.2).

Facial dysmorphism, with progressive coarsening of facial features with flat face, depressed nasal bridge, bulging forehead, thickening of the tongue and lips, thick and often abundant hair (hirsutism), is a feature in MPS I, VI and VII and to a lesser extent in MPS II, III and IVA (Figs. 41.4 and 41.5).

- The cause of the dysmorphism is the combination of dysostosis multiplex in facial and cranial bones and subcutaneous storage of GAGs.
- Corneal clouding, probably as a result of accumulation of GAGs in keratocytes is a common feature in MPS I, VI and VII but can also be detected in patients with III and IVA. In addition, glaucoma, retinopathy and optic nerve disease are common in MPS [3].
- Cardiac valve disease with thickening of valves leading to dysfunction (insufficiency and/or stenosis) is most common in MPS with accumulation of dermatan sulfate (MPS I, II, VI and VII), which is the most abundant GAG in heart valves [4]. A generally milder valvular disease also occurs in MPS IVA. In addition, cardiomyopathy and coronary artery stenosis has been reported in a number of MPS.
- Hepatosplenomegaly, which is often and erroneously, considered as a clinical hallmark of lysosomal storage disorders, is a common symptom in MPS I, II, VI and VII but can be minimal or absent in the more attenuated, slowly progressive phenotypes. In addition, hepatosplenomegaly is usually absent in MPS IVA (and probably IX) and often less prominent in MPS III.
- Central nervous system (CNS) disease with progressive cognitive impairment occurs in the severe, rapid progressive, phenotypes of MPS I and II and in MPS VII, and is a key feature of MPS III; all MPS in which heparan sulfate is one of the accumulating GAGs. Other disorders of the CNS include compression of the spinal cord due to stenosis of the spinal canal (MPS I, II, VI and VII), atlanto-occipital instability (MPS IVA) and communicating hydrocephalus (MPS I, II and VI). Compression of the

Table 41.2 The 7 different MPS, with the most important clinical signs and symptoms

MPS type	Disease name	Dysostosis multiplex	Valvular heart disease	Progressive cognitive impairment	Spinal cord compression	Enzyme deficiency	Gene	Main GAG accumulating (urine screening)	Diagnostic enzyme assay
MPS IH	Hurler	+++	+++	+++	++	Alpha-L-iduronidase	*IDUA*	HS, DS	WBC
MPS IH-S	Hurler-Scheie	++	++	++	++				
MPS IS	Scheie	++	++	–	++				
MPS II	Hunter, neuronopathic phenotype	++	++	+++	++	Iduronate-2-sulfatase	*IDS*	HS, DS	Plasma
	Hunter, attenuated phenotype	++	++	–/+	++				
MPS IIIA	Sanfilippo A	+	+/–	+++	–	Heparan-*N*-sulfatase	*SGSH*	HS	WBC
MPS IIIB	Sanfilippo B	+	+/–	+++	–	*N*-acetyl glucosamindase	*NAGLU*		Plasma
MPS IIIC	Sanfilippo C	+	+/–	+++	–	Acetyl CoA glucosamine *N*-acetyltransferase	*HGSNAT*		WBC
MPS IIID	Sanfilippo D	?	?	+++	–	*N*-acetyl-glucosamine-6-sulfatase	*GNS*		WBC
MPS IVA	Morquio A	+++	+	–	+++	*N*-acetylgalactosamine-6-sulfatase	*GALNS*	KS, CS	WBC
MPS IVB	Morquio B	+++	+	–	+++	B-galactosidase	*GLB1*	KS	WBC
MPS VI	Maroteaux-Lamy	+++	+++	–	+++	*N*-acetylgalactosamine-4-sulfatase	*ARSB*	DS	WBC
MPS VII	Sly	+++	++	+++	+	B-glucuronidase	*GUSB*	DS, HS, CS	WBC
MPS IX	–	?	?	?	?	Hyaluronidase	*HYALI*	HA	Cultured cells
MPS X		++	++	?	–	Arylsulfatase K (ARSK)	*ARSK*	+/– DS	
Multiple sulfatase deficiency	Austin	++	+	+++	?	Formylglycine-generating enzyme	*SUMF1*	HS, DS	WBC

Accumulating GAGs: *HS* heparan sulfate, *DS* dermatan sulfate, ***KS*** keratan sulfate, *CS* chondroitin sulfate, *HA* hyaluronic acid
Presence of signs and symptoms: − never reported, +/− vary rare, + can be present, ++ often present, +++ almost always present,? not known (only very few patients reported)

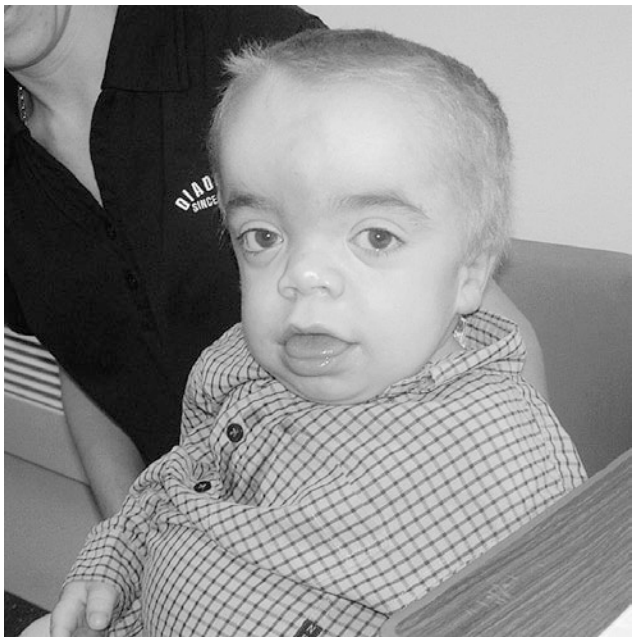

Fig. 41.4 Facial features of Hurler syndrome (MPS IH)

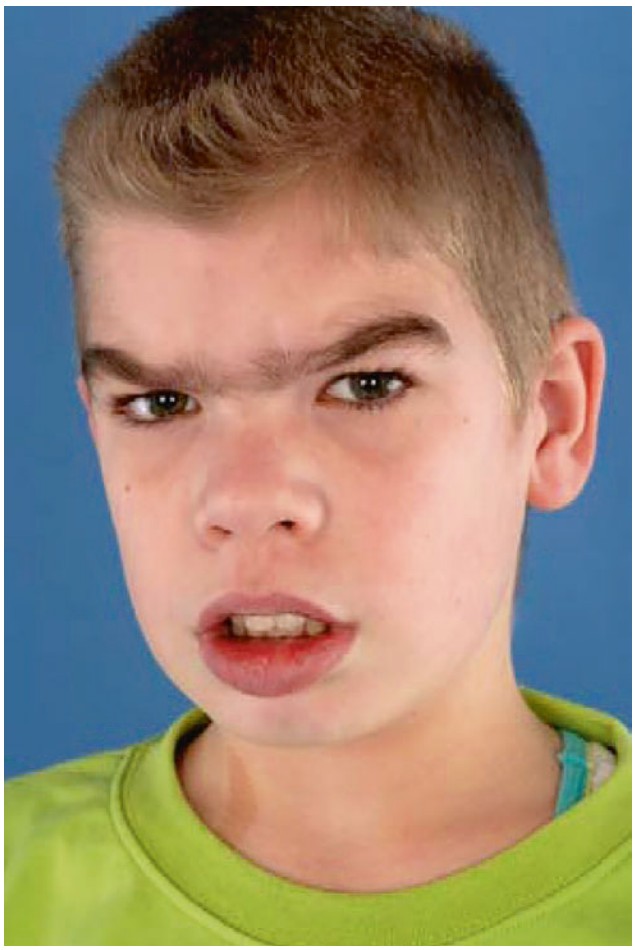

Fig. 41.5 Classical facial features in a 10 year old boy with Sanfilippo syndrome

spinal cord in MPS I, II and VI is caused by dural thickening (pachymeningitis cervicalis) and thickening of the transverse ligaments at the level of the the craniocervical junction. This often presents insidiously with loss of endurance before more obvious signs of ascending paralysis become apparent.

- Recurrent inguinal and umbilical hernia are a frequent finding in MPS I, II and VI and are probably related to abnormalities in connective tissue due to accumulation of dermatan sulfate in combination with increased intra-abdominal pressure as a result of hepatosplenomegaly.

MPS I: Hurler Syndrome (MPS IH), Hurler-Scheie Syndrome (MPS IH-S) and Scheie Syndrome (MPS IS)

Patients with MPS I have deficiency of the enzyme α-l-iduronidase (Fig. 41.2) and accumulate the GAGs dermatan and heparan sulfate (DS, HS). Infants with severe disease (MPS 1H, Hurler syndrome) are usually diagnosed in the first year of life [5]. Upper airway obstruction and frequent ear, nose and throat infections dominate the clinical picture at an early stage. The full clinical picture of short stature, hepatosplenomegaly, increasing facial dysmorphism (Fig. 41.4), cardiac disease, progressive learning difficulties and corneal clouding generally evolves over the second and third years of life but may also be present early in the first year of life. Signs and symptoms of dysostosis multiplex, leading to severe spinal and hip disease, are particularly pronounced in MPS IH. If left untreated, patients with severe MPS I usually die before the age of 10 years as a result of cardiorespiratory disease. The severe MPS IH phenotype appears to be much more prevalent than the more attenuated forms of the disease although under- or misdiagnosis may be involved. At the other end of this clinical spectrum patients with Scheie syndrome (MPS IS) are intellectually normal, often reach an almost normal height and can live a normal life span, although many patients become disabled as a result of progressive joint disease, corneal opacity and cardiac valve lesions. The symptoms of patients between these two extremes can be extremely variable (Hurler-Scheie syndrome, MPS I H-S) and can include short stature, coarse facies, corneal clouding, joint stiffness, deafness and valvular heart disease. The onset of symptoms in MPS IH-S is observed between ages 3 and 8 years, and there is usually variable intellectual dysfunction. Untreated, the condition usually leads to death from cardiac or respiratory disease during the second or third decade of life. As separating the MPS I H-S phenotype from the MPS IH and IS phenotypes can be difficult or sometimes not possible, classification of MPS I in a neuronopathic phenotype (including MPS IH and the more severe MPS I H-S patients) and a non-neuronopathic phenotype (including the more attenu-

ated MPS I H-S and the MPS IS patients) seems to be a more realistic approach.

Hunter Syndrome (MPS II)

MPS II (Hunter syndrome) differs from other MPS in that its inheritance is X-linked recessive and manifesting female heterozygotes are exceptionally rare. Like MPS I, this disorder is a spectrum with severely affected patients sharing many of the clinical signs and symptoms of patients with the severe form of MPS I, with the exception that the cornea remains clear in MPS II [6]. Differentiating MPS II from MPS I on clinical signs and symptoms is thus often difficult or not feasible. Severe patients (neuronopathic phenotype) appear to be more prevalent than attenuated, non-neuronopathic patients. More recently, an intermediate phenotype group has been reported for MPS II. These patients may have a stable cognitive impairment of many decades [7].

Prominent Mongolian blue spots and a characteristic papular rash are other features that are prominent in severe MPS II. Patients with the more attenuated form of MPS II can live well into adult life, and a number have gone on to have their own families.

Sanfilippo Syndrome (MPS III)

There is a defect in the degradation of heparan sulfate in all of the four subtypes of MPS III (A, B, C and D, Sanfilippo syndrome). This results in a disorder which primarily affects the central nervous system, whereas somatic abnormalities are relatively mild [8], often leading to a considerable delay in diagnosis. The condition has three phases. After a symptom free interval of one to two years, the first phase, usually before diagnosis, consists of slowing of cognitive development, often primarily affecting speech. Some patients have ear disease and will fail hearing tests, which is the usual reason given, initially, for the speech delay. In the second phase (age 3–10 years), cognitive development stops and the illness is dominated by a severe behavioural disturbance, characterized by hyperactivity, challenging behaviour, and profound sleep disturbances, gradually followed by a decline in cognition. Abundant and thick scalp hair and hirsutism and progressive coarse facial features are frequently noted (◘ Fig. 41.5). The third phase of the illness (usually starting at the end of the first decade) is associated with continuing loss of skills and motor functions, epilepsy and slow deterioration into a vegetative state, death usually occurring early in the third decade. There are no absolute differences in clinical signs and symptoms between the MPS III subtypes. As in all the other MPSs there is considerable heterogeneity, and not all patients will follow the same rapid progression of neurocognitive deterioration and patients with attenuated phenotypes have been reported [9–11]. Somatic manifestations in MPS III include mild dysostosis multiplex, ENT problems and, sometimes, hepatomegaly.

Morquio Disease (MPS IV)

MPS IV (Morquio disease) is caused by a defect in the degradation of keratan sulfate. In classic Morquio type A (MPS IVA, galactose-6-sulfatase deficiency) the patients are affected by a very severe skeletal dysplasia characterised by vertebral platyspondyly, hip dysplasia (◘ Fig. 41.3) and genu valgum [12]. Intellectual impairment does not occur in MPS IVA, but the height prognosis is very poor, with affected adults ranging from 95 to 105 cm when fully grown. Odontoid hypoplasia is often associated with a high risk for atlanto-occipital subluxation which renders the patients vulnerable to acute or chronic cervical cord compression. However, patients with a more attenuated phenotype may show only moderate to (almost) no growth retardation with, during childhood years, only few skeletal manifestations easily misdiagnosed as for instance bilateral Perthes disease. In Morquio B (MPS IVB, β-galactosidase deficiency) the skeletal involvement is similar, but often not as pronounced, but patients may have central nervous system disease and a slowly progressive neurodegenerative course (β-galactosidase deficiency also causes GM1-gangliosidosis see ► Sect. 40.2.3).

Maroteaux-Lamy Syndrome (MPS VI)

Patients with MPS VI (Maroteaux-Lamy syndrome) have somatic features resembling MPS I, but without neurological impairment [13]. As in all MPSs, MPS VI shows a wide phenotypic spectrum of symptoms. The characteristic skeletal dysplasia includes short stature, dysostosis multiplex and degenerative joint disease. In addition, patients may have cardiac valve disease, hearing loss, obstructive sleep apnoea, corneal clouding, carpal tunnel disease, and inguinal or umbilical hernia. Cervical cord compression, communicating hydrocephalus, optic nerve atrophy and blindness may occur, often closely resembling MPS I.

Sly Syndrome (MPS VII)

MPS VII (β-glucuronidase deficiency, Sly syndrome) is a very rare and variable disorder, which probably has non-immune hydrops fetalis as its most common presentation. Patients who survive pregnancy have a clinical disease similar to MPS I, including the same degree of clinical heterogeneity. In patients who survive the prenatal and neonatal presentation with hydrops, the hydrops may resolve followed by the typical MPS I like presentation. However, as in all other MPS disorders, patients with a much more attenuated phenotype are now increasingly reported. In these patients clinical signs and symptoms may resemble those of MPS I, II and VI [14].

- **MPS IX**

MPS IX (hyaluronidase deficiency) appears to be extremely rare. This disorder was first reported by Natowicz et al. [15] and has only been reported in 4 patients from 2 families and is clinically characterized by short stature and periarticular soft masses and symptoms resembling familial juvenal idiopathic arthritis.

- **Multiple Sulfatase Deficiency (Austin Disease)**

Multiple sulfatase deficiency is caused by mutations in the sulfatase modifying factor 1 gene (*SUMF1*), leading to a deficiency of the FGE (formylglycine-generating enzyme) protein. As FGE is involved in the posttranslational activation of all sulfatases in the endoplasmatic reticulum, its deficiency leads to a deficiency of 17 different sulfatases, including all lysosomal sulfatases. Multiple sulfatase deficiency is very rare and clinical signs and symptoms are variable, with progressive psychomotor retardation invariably present [16]. However, the course of the disease varies from rapid progressive to a slower evolution [17]. Urinary GAG concentrations can be in the normal range, necessitating direct enzymatic assay of multiple sulfatases in the diagnostic workup or *SUMF1* mutation analysis.

41.2.2 Metabolic Derangement

MPS comprise a group of lysosomal storage disorders caused by a deficiency in one (or more in the case of multiple sulfatase deficiency) of the lysosomal enzymes (hydrolases) involved in the degradation of GAGs (Table 41.1). GAGs are linear polysaccharides ubiquitously distributed in extracellular matrices and on cell surfaces throughout the body and have many structural and complex biological functions. The classification of these polysaccharides is based on the repeating structural units in the molecule (Fig. 41.1). GAGs display a very high degree of heterogeneity with regards to molecular mass, disaccharide construction, and the degree of sulfation, all important for their biological functions.

The GAGs dermatan sulfate, heparan sulfate, chondroitin sulfate and keratan sulfate (DS, HS, CS and KS) are long polysaccharides, generally covalently attached to specific core proteins that form proteoglycans. The GAG hyaluronan (HA) is not sulfated or protein linked. There are important differences in the abundance of the different GAGs between different tissues, which appear to be partially related to the signs and symptoms of the different MPSs.

41.2.3 Genetics

All MPSs are inherited as autosomal recessive traits except for MPS II, which is X-linked. All genes have been located and sequenced and genetic testing is available for all disorders for confirmation and for carrier detection, allowing genetic counselling and family planning. Genotype - phenotype correlation is generally poor. However, several mutations, including two nonsense mutations, can predict the severe Hurler phenotype in MPS I [18]. With the exception of only a few predictive mutations in MPS II, IIIA, IV and VI, there is only poor predictive value of genotyping in the other MPSs.

41.2.4 Diagnostic Tests

Diagnosis of MPS used to rely on quantification of urinary GAGs by a dimethylmethylene blue dye binding assay (DMB) [18], followed by two-dimensional electrophoresis for qualification of the type of excreted GAGs. However, MS/MS based methods proved to have a superior sensitivity and are now considered gold standard for urinary GAG screening [19–21]. A positive screening is followed by analysis of the relevant enzyme activity in leucocytes or cultured skin fibroblasts.

Enzymatic studies in leucocytes and/or plasma will establish a final diagnosis. Subsequent mutational analysis will identify the mutations, which can sometimes be predictive of the phenotype in some MPSs, and can be used for genetic counselling of involved families. In case of a sulfatase deficiency, it is necessary to measure at least one other sulfatase, in order to exclude multiple sulfatase deficiency as the cause of the disease.

As early initiation of treatment may lead to improved outcomes, several countries or regions have introduced newborn screening (NBS) for a number of mucopolysaccharidoses, predominantly MPS I, in their national screening program, including the US, Taiwan and Northern Italy [22–26]. High-throughput enzyme assays by either MS/MS or fluorimetric analysis allow for sensitive multiplexed screening of MPS I, II and VI.

41.2.5 Treatment and Prognosis

Multi-disciplinary symptomatic care remains the most important aspect of the management of patients with MPS. As MPSs are all multi-systemic disorders, multidisciplinary teams preferably should involve at least

orthopaedic surgeons, neurologists, neurosurgeons, ear, nose and throat surgeons, cardiologists, physical therapists, rehabilitation specialists and ophthalmologists. Metabolic paediatricians, internists and clinical geneticists are often essential in such teams to guarantee the necessary holistic approach. Expert opinion based guidelines for the management of MPS I, II, IVA and VI have been published [27–31]. In addition, guidelines on the management of several clinical symptoms and procedures, including spinal cord compression in MPS IVA [30] and MPS VI [31, 32]; orthopaedic management of extremities in MPS IVA [33]; thoracolumbar kyphosis in MPS I [34, 35]; hip dysplasia in MSP I [36] and MPS VI [37]; anaesthesia and airway management, including OSAS, in MPSs [38, 39]; cardiac disease [4] and eye disorders [3, 40, 41] may help to optimize treatment. Pain is a very common symptom in MPS [42] and this should be addressed directly and separately. Severe behavioural problems and sleep disturbances, generally present in patients with MPS III, often require a tailored psychological and pharmacological treatment plan.

It is important to note that anaesthesia in patients with MPS needs special attention as it carries a high risk due to the upper airway obstruction resulting from anatomical changes due to the dysostosis multiplex and GAG deposition in soft tissues, restrictive pulmonary disease, cardiovascular disease and potential instability of the atlanto-occipital joint [34]. Therefore, anaesthesia should be performed experienced team and after full information about the clinical signs and symptoms of the individual patient has been acquired.

In addition, patients with MPSs may have an increased risk for peri- or post-surgical development of spinal cord lesions, remote from the site of surgery, leading to paraplegia [43–45]. This may be caused by a combination of low mean arterial pressure in conjunction with potentially compromised arterial spinal circulation, the duration of the surgery and the position on the operating table. Stringent preoperative evaluation, careful positioning on the operation table, perioperative monitoring of motor-evoked potentials and somatosensory-evoked potentials and prevention of low blood pressure during the operation are probably essential to prevent these complications. As a result of the complexity of the disorders, necessitating the presence for a dedicated multi-disciplinary team, patients with MPS are best managed at specialized treatment centres.

Disease modifying treatment options are available for several of the MPS and consist of hematopoietic stem cell transplantation (HSCT) and intravenous enzyme replacement therapy (ERT). Clinical studies on the effectiveness of intra-cerebroventricular enzyme therapy, intrathecal enzyme therapy and gene therapy are currently ongoing, and may lead to a significant improvement of the clinical outcome of MPS over the next years.

Hematopoietic Stem Cell Transplantation

HSCT for an MPS was first performed in the UK over 30 years ago in a patient with MPS I Hurler phenotype and this procedure is now the preferred treatment strategy for these patients, if diagnosed before the age of approximately 2.5 years [46] (Table 41.3). The success of HSCT has dramatically improved over the last decades, with a marked decrease in morbidity and mortality and an improved rate of engraftment, due to changes in chemotherapeutic conditioning, supportive care and stem cell source [62]. A recent large multi-centre study showed that the long-term outcome of Hurler patients after HSCT improves after early referral for HSCT, using noncarrier donors and regimens designed to achieve full-donor chimerism [62]. However, there is still considerable residual disease burden in many patients, often related to the musculoskeletal system which apparently responds less well to HSCT. Early diagnosis of MPS I Hurler patients through NBS may lead to early transplantation, thus improving the outcome of HSCT. Indeed, early HSCT leads to improved cognition in MPS I [63]. Long-term effectiveness of early HSCT in MPS I on important late complications such as cardiac valve disease and skeletal disease still remains to be established. Nevertheless, older age at HSCT is associated with a poorer physical quality of life during long-term follow-up of these children, early transplantation will also improve the somatic outcome [64].

HSCT is also performed in patients with the severe neuropathic phenotype of MPS II [48] and in patients with MPS IV [50], MPS VI [51] and MPS VII [52], and beneficial effects have been reported. However, as only small patient series have been studied, the exact place of HSCT in the treatment of these MPS needs to be further delineated (Table 41.3). HSCT has been shown to be largely ineffective in MPS III [49].

Enzyme Replacement Therapy

Intravenous ERT for MPSs was first approved for clinical use in MPS I [65, 66] and later for MPS II [67], IVA [68], VI [69] and VII [70]. These pivotal trials, in combination with long-term follow up studies, have demonstrated that ERT can effectively treat a number of symptoms resulting in improvement on the 6 minute walk test, improved joint mobility and pulmonary function, a reduction of liver and spleen size and improved growth, all of which may lead to improved survival [70–72]. However, all studies show that there is generally still significant residual disease burden despite long-term ERT. Studies comparing the effects of ERT in sibships, which include older siblings treated with ERT after the development of significant clinical symptoms, and younger siblings treated before the onset of significant symptomatology, have demonstrated that an early start

Table 41.3 HSCT indication in Mucopolysaccharidoses and Oligosaccharidosis

Disease	Numbers reported	Indication	Other therapeutic options	Key refs
MPSI	>200	Standard of care in neuronopathic phenotype	–	[47]
MPSII	>25	In young, neuronopathic cases with a good donor	ERT	[48]
MPSIII	>10	Not indicated (not effective)	–	[49]
MPSIVA	>5	Experimental	ERT	[50]
MPSVI	>45	Usually offered in cases where ERT not available	ERT	[51]
MPSVII	1	Experimental	ERT	[52]
Alpha mannosidosis	20–30	In young, neuronopathic cases with a good donor	ERT	[53, 54]
Beta mannosidosis	<5	Experimental (poor results in the single, symptomatic patient reported)		[55]
Fucosidosis	<10	Experimental (better outcomes in the very young with unrelated donors)		[56]
Galactosialidosis	0	Supportive animal data, no human experience		[57]
ISSD/Salla disease	0	Not indicated (membrane protein)		
Aspartylglucosaminuria	<10	Experimental (better outcomes in the very young with unrelated donors)		[58]
Schindler disease	0	Not indicated		
Sialidosis (ML type I)	<5	Experimental (no benefit in early onset phenotypes but no experience in later onset cases)		[59]
Mucolipidosis type II/III	30–40	Not indicated		[60]
Mucolipidosis type IV		Supportive animal data, no human experience		[61]
Pycnodysostosis		Experimental		

of ERT, i.e. before irreversible changes have occurred, leads to a much more favourable outcome [73–76]. This again highlights the importance of an early diagnosis, allowing early start of treatment. As intravenously administered enzyme does not cross the blood–brain barrier, at least not in sufficient amounts at the labelled doses, intravenous ERT will not result in neurocognitive benefits, which limits its effectiveness in MPS I Hurler and Hurler-Scheie, as well as in the severe, neuronopathic, form of MPS II.

The formation of alloantibodies against the recombinant enzyme has been demonstrated to occur in most patients during ERT and these antibodies may interfere with the enzymatic activity and/or with cellular uptake. Studies in MPS I and MPS II indeed demonstrated interference of antibodies with biochemical and clinical effects of the infused enzyme [77–79]. However, in other MPSs such an interference has not been established.

The extremely high costs involved with long-term ERT has led to discussions with reimbursing authorities on criteria for cost-effectiveness. In some countries, decisions on reimbursement of drugs depend on the incremental costs per quality-adjusted life year (QALY). It is clear, however, that for ultra-rare diseases such as MPSs other economic models need to be used [80, 81]. Evaluation of long-term efficacy of ERT, using robust and clinically relevant outcome measures, is essential to demonstrate the effectiveness of these treatments in the 'real world', i.e. outside the domain of trials, and this will only be feasible through international cooperation. Furthermore, long-term independent post marketing studies including core outcome sets for orphan medical products, enforced by marketing and/or reimbursing authorities, are needed to fully reveal the effectiveness of ERT in MPS [82].

A novel subtype of mucopolysaccharidosis caused by arylsulfatase K (ARSK) deficiency has recently been described affecting 4 individuals from two unrelated consanguineous families with homozygous mutations in *ARSK* [83]. ARSK is a recently characterised lysosomal hydrolase involved in GAG degradation that removes the 2-O-sulfate group from 2-sulfoglucuronate. The clinical phenotypes included short stature, coarse facial features

and dysostosis multiplex. Two of the four individuals had additional cardiac and ophthalmological abnormalities. Mild elevation of dermatan sulfate was detected in two. It is suggested that the disorder is designated MPS X.

41.3 Oligosaccharidoses and Mucolipidoses

Oligosaccharides/Glycoproteins

Almost all the secreted and membrane-associated proteins of the body are glycosylated, as well as numerous intracellular proteins, including the lysosomal acid hydrolases. A great variety of oligosaccharide chains are attached to the protein backbone via the hydroxyl group of serine or threonine (O-linked), or via the amide group of asparagine (N-linked), to form tree-like structures (Fig. 41.6). The chains usually have a core composed of N-acetylglucosamine and mannose, often contain galactose, fucose and N-acetylgalactosamine, and frequently possess terminal sialic acids (N-acetylneuraminic acid). Oligosaccharide chains with a terminal mannose or mannose-6-phosphate are involved in the targeting of lysosomal enzymes to lysosomes. This recognition marker is synthesised in two steps from UDP-N-acetylglucosamine (Fig. 41.7). Deficiencies of the enzymes required for the degradation of the oligosaccharide chains cause oligosaccharidoses (glycoprotein storage diseases). Defects of the synthesis of the mannose-6-phosphate recognition marker result in the mislocalisation of lysosomal enzymes. Defects of the synthesis of the oligosaccharide chains are discussed in ► Chap. 40.

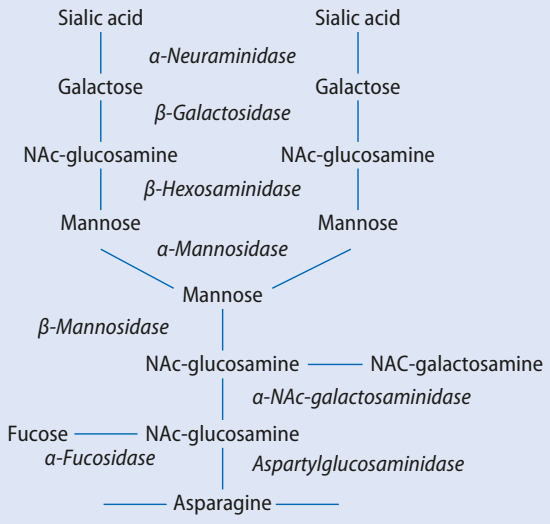

Fig. 41.6 General composite example of a glycoprotein oligosaccharide chain. NAc N-acetyl. Degradative enzymes are listed in *italics*

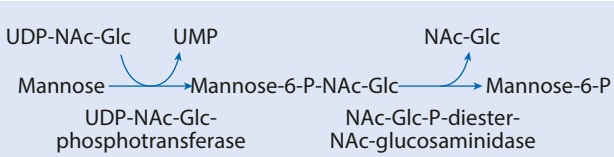

Fig. 41.7 Synthesis of mannose-6-phosphate recognition marker. *NAc-Glc*, N-acetylglucosamine; *UDP*, uridine diphosphate; *UMD*, uridine monophosphate. Enzymes are listed in *italics*

Oligosaccharidoses or glycoprotein storage disorders share many features in common with MPSs, but the urine GAG screen (by many screening assays) is normal or only shows nonspecific abnormalities. For convenience, the mucolipidoses (MLs), disorders that combine clinical features of MPS and sphingolipidoses, are also considered here. These include sialidosis I (ML I), which is caused by α-neuraminidase deficiency, and mucolipidosis II (ML II) and its milder allelic variant mucolipidosis III (ML III), both of which are caused by the deficiency of UDP-N-acetylglucosamine-1-phosphotransferase, an enzyme not involved in lysosomal degradation but in the synthesis of a recognition marker.

41.3.1 Clinical Presentation

Mannosidosis

A deficiency of α-mannosidase gives rise to the extremely variable disorder α-mannosidosis. Most patients exhibit a mild MPS IH (Hurler) phenotype, associated with variable learning difficulties, hepatosplenomegaly, deafness and a progressive skeletal dysplasia. α-Mannosidosis can be complicated by an immune deficiency which can dominate the clinical progression of the disease [84]. Survival to adulthood is common but not universal and later years may be dominated by psychiatric manifestations. β-Mannosidosis, which is due to a deficiency of β-mannosidase, is much less prevalent than α-mannosidosis and is very variable, but severe learning difficulties, challenging behaviour, deafness and frequent infections are relatively common [85].

Fucosidosis

Patients with fucosidosis lack the typical facial dysmorphism seen in the other disorders described in this chapter. Deficiency of α-fucosidase activity leads to a variable neurodegenerative disorder, often with seizures and mild dysostosis. Affected patients often exhibit prominent and widespread angiokeratomas, which often progress with age [86].

Galactosialidosis

Galactosialidosis is caused by the combined deficiency of the lysosomal enzymes β-galactosidase and α-neuraminidase. The combined deficiency has been

found to result from a defect in protective protein/cathepsin A (PPCA), an intralysosomal protein which protects these enzymes from premature proteolytic processing. The clinical features of affected patients include hydrops fetalis as well as a more slowly progressive disorder associated with learning difficulties, dysostosis multiplex and corneal opacity [87].

Transport Defects

The allelic disorders Salla disease (Finnish type sialuria) and infantile free sialic acid storage disease (ISSD) result from mutations in *SLC17A5* coding for sialin, a lysosomal membrane protein that transports sialic acid out of lysosomes. ISSD has a severe phenotype with infantile onset (including severe visceral involvement, cardiomyopathy, skeletal dysplasia and learning difficulties), while the more slowly progressive Salla disease, has a milder phenotype with later onset. Both disorders cause learning difficulties, but ISSD is generally fatal in early childhood whilst patients with Salla disease often survive into middle age [88]. Mutations in *SLC17A5* have also been reported in patients with mental retardation and hypomyelination [89] but while free sialic acid was increased in CSF, the urinary excretion of free sialic acid was normal. Finally, cerebellar ataxia with elevated CSF free sialic acid (CAFSA) has been reported in five patients who in addition to the ataxia had peripheral neuropathy and cognitive decline [90]. The molecular basis of this new disease is yet to be established.

Sialuria (French Type)

Sialuria is caused by mutations in *GNE*, encoding uridinediphosphate-N-acetylglucosamine 2-epimerase (UDP-GlcNAc 2-epimerase). As a result of a failure of feedback inhibition there is excessive free sialic acid synthesised. Only 9 patients with the disorder have been described; the inheritance is thought to be autosomal dominant. Clinical features include hepatosplenomegaly, coarse facial features, and varying degrees of developmental delay. The disorder differs from sialidoses in that there is accumulation and excretion of free sialic acid and neuraminidase activity is normal or increased [91, 92].

N-Acetylneuraminate Pyruvate Lyase Deficiency

This defect in sialic acid catabolism has only been described thus far in 2 siblings with skeletal and cardiac disease in addition to sialuria [93]. Further patients have exhibited alternative phenotypes and it remains to be seen whether further families will expand or verify this phenotype (unpublished observations).

Aspartylglucosaminuria

This disorder is due to a deficiency of aspartylglucosaminidase and has a higher prevalence in Finland but is rare in other countries. A characteristic facial dysmorphism has been described and there is slowly progressive psychomotor retardation, with death in middle age [94, 95].

Schindler Disease

This disease, due to α-N-acetylgalactosaminidase deficiency, is a rare, clinically heterogeneous disorder with a wide spectrum, including an early-onset neuroaxonal dystrophy and a late-onset form characterised by abundant angiokeratoma. It is unclear how many of the manifestations seen in the reported early onset patients are related to α-N-acetylgalactosaminidase [96] as many of the most severely affected individuals may also have a PLA2G6 defect known to be responsible for severe neuroaxonal dystrophy itself. A number of asymptomatic individuals have also been demonstrated.

Sialidosis (α-Neuraminidase Deficiency, ML I)

This disorder is characterised by the progressive lysosomal storage of sialic acid-rich glycopeptides and oligosaccharides caused by a deficiency of the enzyme α-neuraminidase. It has also been termed Mucolipidosis type 1 (ML I) or, in the attenuated form, cherry red spot myoclonus syndrome. The sialidoses are distinct from the sialurias (infantile sialic acid storage disease, ISSD and Salla disease), in which there is storage and excretion of free sialic acid rather than bound sialic acid. The clinical spectrum in sialidosis ranges widely, from a multi-system presentation with hydrops fetalis to slowly progressive seizure presentations with myoclonus. The cherry red spot seems common throughout.

I-Cell Disease (ML II) and Pseudo-Hurler (ML III)

Patients with ML II have a profound storage phenotype often presenting in the newborn period or even prenatally with fractures. This seems to be related to a transient secondary hyperparathyroidism and may be due to deficient calcium transport to the foetus [97].

There is often a very severe skeletal dysplasia, and patients often have a small head circumference. Cardiomyopathy, valve disease and severe coronary artery disease can also be present. Most children die primarily of respiratory failure in the first decade [98]. Cognitive attainment appears less than normal however is difficult to assess.

ML III is extremely variable, and many patients survive into adult life with little or no learning difficulty. Carpal tunnel syndrome is almost universal and cardiac

valve disease is common. Skeletal dysplasia, including an erosive arthropathy affecting ball-and-socket joints (shoulder and hips), can be extremely disabling in adults with ML III.

- **Mucolipidosis Type IV (ML IV)**

ML IV is caused by mutations in MCOLN1 coding for mucolipin-1, a protein found in the membrane of lysosomes and endosomes and which is involved in trafficking of lipids and proteins. The disorder is characterised by developmental delay and progressive visual impairment. Of patients described with ML IV, most have been Ashkenazi in origin [99, 100]. Delay is moderate to severe and usually becomes apparent during the first year of life. Affected individuals have intellectual disability, limited or absent speech, difficulty chewing and swallowing, hypotonia that gradually turns into spasticity, and problems controlling hand movements. Most patients are unable to walk independently. In about 15 percent of affected individuals, the psychomotor problems worsen over time. Vision may be normal at birth in people with typical ML IV, but it becomes increasingly impaired during the first decade of life with corneal clouding and progressive retinal dystrophy. By their early teens, affected individuals have severe vision loss or blindness.

- **Pycnodysostosis**

Cathepsin K is a lysosomal cysteine proteinase abundant in osteoclasts, where it plays a vital role in the resorption and remodelling of bone. A deficiency of this enzyme was shown to be associated with the skeletal dysplasia pycnodysostosis, the disorder thought to be the cause of Toulouse-Lautrec's disability [101]. In addition to short stature (150–160 cm), affected individuals have a generalised increase in bone density and wormian bones of the skull with open fontanelles, partial absence of the distal phalanges and bone fragility [102]. Dental abnormalities, communicating hydrocephalus and life threatening upper airway obstruction are also common.

41.3.2 Metabolic Derangements

The vast majority of disorders are defects of single enzymes involved in the degradation of oligosaccharides (Fig. 41.6). The exceptions are ML II (and III), the transport defects (ISSD and Salla disease) and galactosialidosis. ML II and III share the same post-translational modification defect due to the absence of UDP-N-acetylglucosamine-1-phosphotransferase (Fig. 41.7), the enzyme necessary for synthesis of the marker required to target newly formed lysosomal enzymes to the lysosomes. As a result, the enzymes are mistrafficked to the extracellular space. In the transport defects the gene encoding the lysosomal membrane protein sialin is defective. Urinary excretion of free sialic acid is considerably elevated in these conditions. The combined defect of neuraminidase and β-galactosidase (galactosialidosis) is caused by a lack of the protective protein cathepsin A (PPCA), which is responsible for stabilisation of the enzyme complex within the lysosomes and their protection from rapid proteolytic degradation. PPCA may also have a role in the protection of elastin-binding protein (EBP) at the cell surface [103].

What is unclear is how the metabolic derangement leads to the clinical and functional defects seen in the patients, especially those affecting the central nervous system. The clinical phenotype partially depends on the type and amount of storage substance, but the pathogenic cascades leading to disease in the brain remain poorly understood.

41.3.3 Genetics

The disorders in this group are all inherited in an autosomal recessive manner. Genetic diagnosis is possible for all the oligosaccharidoses and in some cases (eg pycnodysostosis) is the only means to conclusively diagnose these cases. All families should be referred for genetic counselling and for carrier detection where available and necessary, and also given information on the availability of prenatal diagnosis and preimplantation genetic diagnosis.

41.3.4 Diagnostic Tests

The diagnosis of most oligosaccharidoses is based on clinical suspicion, supported by appropriate clinical and radiological examinations followed by urinary examination for oligosaccharide excretion and then specific enzyme assay, usually on white blood cells (see Table 41.2). Thin-layer chromatography is traditionally used to detect abnormal urinary excretion of oligosaccharides and sialic acid however is not always available and may miss some cases. Tandem MS/MS technology offers improved methods to ideally miss fewer patients [104]. Pycnodysostosis can only currently be diagnosed by mutation analysis. Patients with ML II and III are often missed on urinary testing, and therefore the definitive diagnosis should be confirmed by the

finding of significant elevations in a number of plasma lysosomal enzyme levels.

41.3.5 Treatment and Prognosis

Palliative and supportive care remains an important aspect of the holistic management of patients with these disorders. For most conditions it is the only available therapy. All the disorders described in this section (perhaps with the exception of some patients with Schindler disease) have a progressive course, some very life-limiting. Multidisciplinary management is essential, and patients are best managed in specialist centres with access to a comprehensive range of clinical and supporting services. Ear, nose and throat surgery, orthopaedic and neurosurgical intervention may all be indicated at some stage in affected patients. The anaesthetic considerations must not be forgotten, as the facial dysmorphism, skeletal dysplasia and upper airways obstruction present in many of these patients can be a challenge, although usually less severe than in MPSs.

Attempts at disease modifying treatment for this group of disorders currently include HSCT and ERT. HSCT has been performed in a number of cases of alpha mannosidosis, aspartylglucosaminuria and in fucosidosis, with evidence of at least partial correction of the biochemical defect, and some evidence in mannosidosis for neurodevelopmental benefit (especially if transplanted early [6]) (Table 41.2). The role of HSCT in the rest of the group still remains unclear. Intravenous ERT is now approved for alpha mannosidosis, but as so many of these disorders involve neurological manifestations, it is unclear how successful ERT can be for the others of this group of disorders. Bisphosphonate treatment has been proposed for management of the bone pain in MLIII with variable success.

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Inborn Errors of Non-Mitochondrial Fatty Acid Metabolism Including Peroxisomal Disorders

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Contents

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Fatty Acid Homeostasis in Humans

Fatty acids are hydrocarbon chains which terminate in a carboxylic acid group (R-COOH) and which may vary in length, degree of unsaturation (mono/polyunsaturated FA), and number and types of side-chain substitutions (methyl branched-chain FA, hydroxylated FA, etc.). Fatty acid homeostasis in humans involves many different enzymes and transport proteins localized in different compartments within the cell. FA may be derived from exogenous dietary sources but can also be synthesized from acetyl-CoA via the Fatty Acid Synthase (FAS) complex in the cytosol. The virtually exclusive product of the FAS complex is palmitic acid. With a few exceptions as detailed below, palmitic acid as well as all fatty acids derived from dietary sources must be activated to the corresponding fatty acyl-CoA ester via one of many acyl-CoA synthetases (ACS). In humans the ACS-family includes 27 members which all differ in terms of their catalytic properties, subcellular localization, tissue distribution and otherwise. Important fatty acids which cannot be synthesized endogenously, are the essential fatty acids linoleic acid (C18:2ω6) and linolenic acid (C18:3ω3). Of these 27 ACS-enzymes, FALC4 activates arachidonic acid (C20:4ω6) and eicosapentaenoic acid (C20:5ω3) preferentially whereas FATP4 plays an indispensable role in the formation of the CoA-ester of ultra-long chain fatty acids (ULCFA) required for the production of ω-hydroxy-acylceramides. FA present in simple as well as complex lipids are widely different in terms of their length, degree of unsaturation, types of side-chain modification, among others. These modifications include: (1.) chain elongation as mediated by the endoplasmic reticulum (ER) FA chain elongation complex; (2.) reduction of acyl-CoAs by FAR1 and FAR2 to the corresponding fatty alcohols for incorporation into ether lipids as part of the so-called Fatty Alcohol Cycle (◘ Fig. 42.1, lower left); (3.) desaturation by two different sets of enzymes localized in the ER belonging to the stearoyl-CoA desaturase (SCD) and fatty acid desaturase (FADS) families, respectively; (4.) 2-hydroxylation of fatty acids as mediated by the enzyme FA 2-hydroxylase (FA2H) also localized in the ER; and (5.) FA ω-hydroxylation as mediated by the ER enzyme CYP4F22 required for the synthesis of ω-O-acylceramides. Chain elongation involves a four-step cycle of events which starts off with a condensation reaction in which a certain acyl-CoA reacts with malonyl-CoA to generate a 3-ketoacyl-CoA which is now two carbon atoms longer in size. This key reaction is catalyzed by one of 7 different enzymes named ELOVL1–7 which differ in substrate preference, tissue distribution and regulation. The 3-ketoacyl-CoA formed in this way, is then reduced to the corresponding 3-OH-acyl-CoA by a single 3-ketoacyl-CoA reductase (KAR) in a reaction driven by NADPH. Subsequently, the 3-OH-acyl-CoAs are dehydrated by one of 4 different 3-hydroxyacyl-CoA dehydratases (HACD1–4) and the enoyl-CoA esters produced are finally converted into an acyl-CoA ester elongated by two carbon atoms by a single enzyme TER (◘ Fig. 42.1, blue area top left). The chain elongation system can handle a large variety of acyl-CoAs which includes activated fatty acids derived from endogenous synthesis such as palmitic acid and oleic acid which is generated from palmitic acid via one cycle of chain elongation and the Δ9-desaturase SCD1. All the synthesised acyl-CoA esters can then be used for the synthesis of all the thousands of different lipid species encountered in human beings. These lipid species serve multiple different physiological roles ranging from their barrier function in the plasma and organellar membranes to their complex role in signal transduction. Furthermore, phospholipids are also important storage sites of bioactive molecules including the eicosanoids which are released from membrane-bound lipid species by means of the enzyme cPLA2 thus producing arachidonic acid (C20:4ω6) (◘ Fig. 42.1, upper part and ▶ Chap. 35). Proper homeostasis dictates that all the different lipid species are not only synthesized but are also subjected to degradation. The end result of this degradative process is the release of the multiple fatty acid species present in simple and complex lipid species which can either be recycled or degraded. The peroxisomal β-oxidation system plays a key role in this degradative process since peroxisomes are able to oxidize a large variety of different FA which for a large part (◘ Fig. 42.1, right panel) cannot be handled by the mitochondrial β-oxidation system. Furthermore, peroxisomes are the sole site of FA α-oxidation required for the oxidation of 3-methyl branched-chain FA like phytanic acid (3,7,11,15-tetra methylhexadecanoic acid). This system includes the enzymes phytanoyl-CoA 2-hydroxylase (PHYH), 2-hydroxyacyl-CoA lyase 1 (HACL1), and pristanal dehydrogenase as mediated by an ill-defined peroxisomal aldehyde dehydrogenase (ALDH). Oxidation of 2-hydroxy FA derived from 3-methyl branched-chain FA is catalysed by the peroxisomal enzyme HACL1 whereas oxidation of other 2-hydroxy FA as derived from sphingosine catabolism for instance is catalysed by the ER enzyme HACL2 (◘ Fig. 42.1, upper right). In addition, oxidation of ω-hydroxy fatty acids containing >16 carbon atoms, is also primarily if not exclusively catalysed by peroxisomes and the same is true for the oxidation of VLCFA and ULCFA as well as eicosanoids (▶ Fig. 41.1, upper right). The peroxisomal β-oxidation system consists of three different

acyl-CoA oxidases (ACOX 1–3) each with their own substrate specificity and coupled to catalase to reduce H_2O_2 back to O_2; two bifunctional enzymes named LBP and DBP; and finally two thiolases of which the second (SCPx) is the thiolase reactive with 3-ketoacyl-CoAs with a 2-methyl group as in pristanic acid (2,6,10,14-tetramethylpentadecanoic acid) and the two bile acid synthesis intermediates di-and trihydroxycholestanoic acid. In contract to the mitochondrial β-oxidation system which involves fatty acylcarnitines as the species to be transported (▶ Chap. 12), peroxisomes import fatty acids in their acyl-CoA form as mediated by three different half-ABC-transporters named ABCD1, 2, and 3. Peroxisomes lack a citric acid cycle and an oxidative phosphorylation system which implies that they require the intimate interaction notably with the mitochondrion to degrade FA all the way to CO_2 and H_2O as indicated in ◘ Fig. 42.1 (bottom right). Other products of peroxisomal β-oxidation are not metabolized further and are excreted in bile, and/or urine. This includes the breakdown products of eicosanoids which upon β-oxidation generate the dinor-, tetranor-, and/or hexanor derivatives.

■■ Introduction

Fatty acids (FAs) play multiple physiological roles in humans as building blocks of (phospho)lipids, precursor of other biomolecules including long-chain alcohols and eicosanoids, oxidizable substrate, and signaling molecules, and occur in multiple different forms which vary widely in terms of their length, degree of unsaturation, and number and types of side-chain modifications. Biosynthesis of all these different FAs may start from palmitic acid synthesized de novo by the FASN-complex or from exogenous, dietary FAs including the essential FAs linoleic acid and linolenic acid for the synthesis of FAs from the (omega-3) and (omega-6) series, respectively. Proper homeostasis of FA metabolism dictates that the FAs synthesized and incorporated into other lipid species also undergo degradation and/or recycling. In this Chapter we will describe the essential features of FA metabolism in human cells with on the one hand the biosynthesis of all the different FAs to be incorporated into lipid species and the different disorders involved, and on the other hand the degradation of the FAs released from these lipid species and the different disorders involved. Altogether, these include the disorders of ether lipid biosynthesis, and peroxisomal α- and β-oxidation, the disorders of fatty acid chain elongation and fatty acid/fatty alcohol and fatty aldehyde metabolism and the disorders of eicosanoid metabolism. We will begin with the disorders of ether lipid biosynthesis.

42.1 Disorders of Ether Lipid Biosynthesis

Ether lipid (EL) synthesis involves the coordinated interaction between the peroxisome and ER with the first enzymatic steps taking place in peroxisomes after which the product synthesized in the peroxisome i.e. alkyl-glycerone-3-phosphate (alkylGNP, also known as alkylDHAP) moves to the endoplasmic reticulum to be converted into the different ether lipids including plasmalogens (◘ Fig. 42.1, bottom left). Deficiencies have been described in all of the three peroxisomal enzymes involved in ether lipid biosynthesis which includes: (1) glycerone-3-phosphate acyltransferase (GNPAT) deficiency; (2) alkyl glycerone-3-phosphate synthase (AGPS) deficiency; and (3) peroxisomal fatty acyl-CoA reductase (FAR1 deficiency) (◘ Fig. 42.1, bottom left). Furthermore, we recently described an autosomal dominant neurological disorder caused by de novo mutations in *FAR1* resulting in uncontrolled synthesis of ether lipids [1]. Together GNPAT, AGPS and FAR1 deficiency constitute the primary ether lipid biosynthesis deficiencies. The clinical phenotype is that of Rhizomelic Chondrodysplasia Punctata (RCDP). Ether lipid biosynthesis can also be deficient as the secondary consequence of a defect in peroxisome biogenesis either generalized as in the Zellweger Spectrum Disorders (to be discussed later in ▶ 42.2.9.) or partial, affecting the import of a small subset of peroxisomal proteins equipped with an N-terminal peroxisomal targeting signal called PTS2. AGPS is such a PTS2-protein and its uptake into peroxisomes is mediated by the PTS2-receptor protein PEX7 (◘ Fig. 42.2 plus legend) which if deficient causes RCDP type 1. PEX7 requires the interaction with another protein PEX5L in order to execute its function and a deficiency of PEX5L has been described as RCDP type 5.

■ Clinical Presentation

The classical phenotype associated with a defect in the biosynthesis of ether lipid biosynthesis is Rhizomelic Chondrodysplasia Punctata (RCDP) [2]. Patients with classical RCDP have a disproportionally short stature primarily affecting the proximal parts of the extremities (rhizomelia), typical facial appearance including a broad nasal bridge, epicanthus, high-arched palate, dysplastic external ears and micrognathia, congenital contractures, characteristic ocular involvement, dwarfism and severe mental retardation with spasticity. X-ray studies usually show symmetrical shortening of femur and humerus with irregular and broad metaphyses, calcific stippling of the epiphyses, absent capital femur epiphyses, coronal clefts of the vertebrae, increased intravertebral disc spaces, cupping of dorsal ribs, and a barrel-formed thorax. Neurological impairments include truncal hypertonia, spastic tetraplegia, and epilepsy. Most classical patients do not reach any developmental milestones and

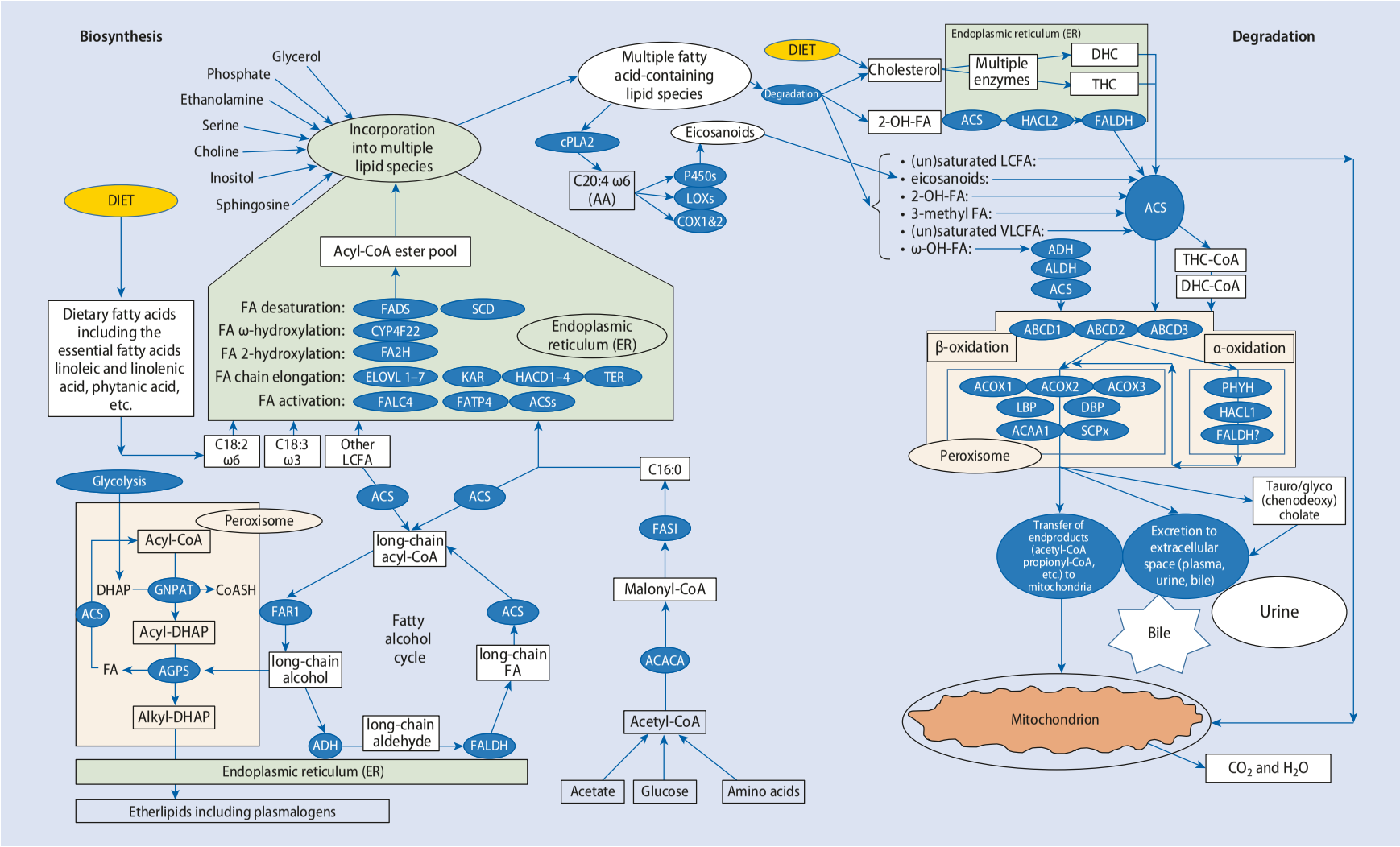

Fig. 42.1 Schematic diagram of fatty acid homeostasis in humans. Fatty acid homeostasis in humans involves the complicated interaction between multiple enzymes and transport proteins in different compartments within the cell with a dominant role played by the endoplasmic reticulum, the cytosol, and peroxisomes as described in the text. (Abbreviations used: AGPS alkyl glycerone 3-phosphate synthase, also known as alkyl dihydroxyacetone 3-phosphate synthase (ADHAPS), GNPAT glycerone 3-phosphate acyltransferase, also known as dihydroxyacetone 3-phosphate acyltransferase (DHAPAT), ACS acyl-CoA synthetase, FAR1 fatty acyl-CoA reductase 1, ELOVL Elongation of Very Long Chain Fatty Acids protein, KAR 3-ketoacyl-CoA reductase, HACD 3-hydroxyacyl-CoA dehydratase, TER 2,3-trans-enoyl-CoA reductase, FACL4 fatty acid-CoA ligase 4, FATP4 fatty acid transport protein 4, FA2H fatty acid 2-hydroxylase, CYP4F22 cytochrome P450 enzyme, family 4F, member 22, FADS fatty acid desaturase, SCD stearoyl-CoA desaturase, cPLA2 cytosolic Phospholipase A2, LOX lipoxygenase, COX cyclo-oxygenase, ADH alcohol dehydrogenase, ALDH aldehyde dehydrogenase, ABCD ATP binding cassette protein, subfamily D, ACOX acyl-CoA oxidase, LBP L-specific bifunctional protein, DBP D-specific bifunctional protein, ACAA1 peroxisomal acetyl-CoA: acetoacetyl-CoA thiolase 1, SCPx sterol carrier protein X, PHYH phytanoyl-CoA 2-hydroxylase HACL1 hydroxyacyl-CoA lyase 1, HACL2 hydroxyacyl-CoA lyase 2, DHCA dihydroxycholestanoic acid, THCA trihydroxycholestanoic acid)

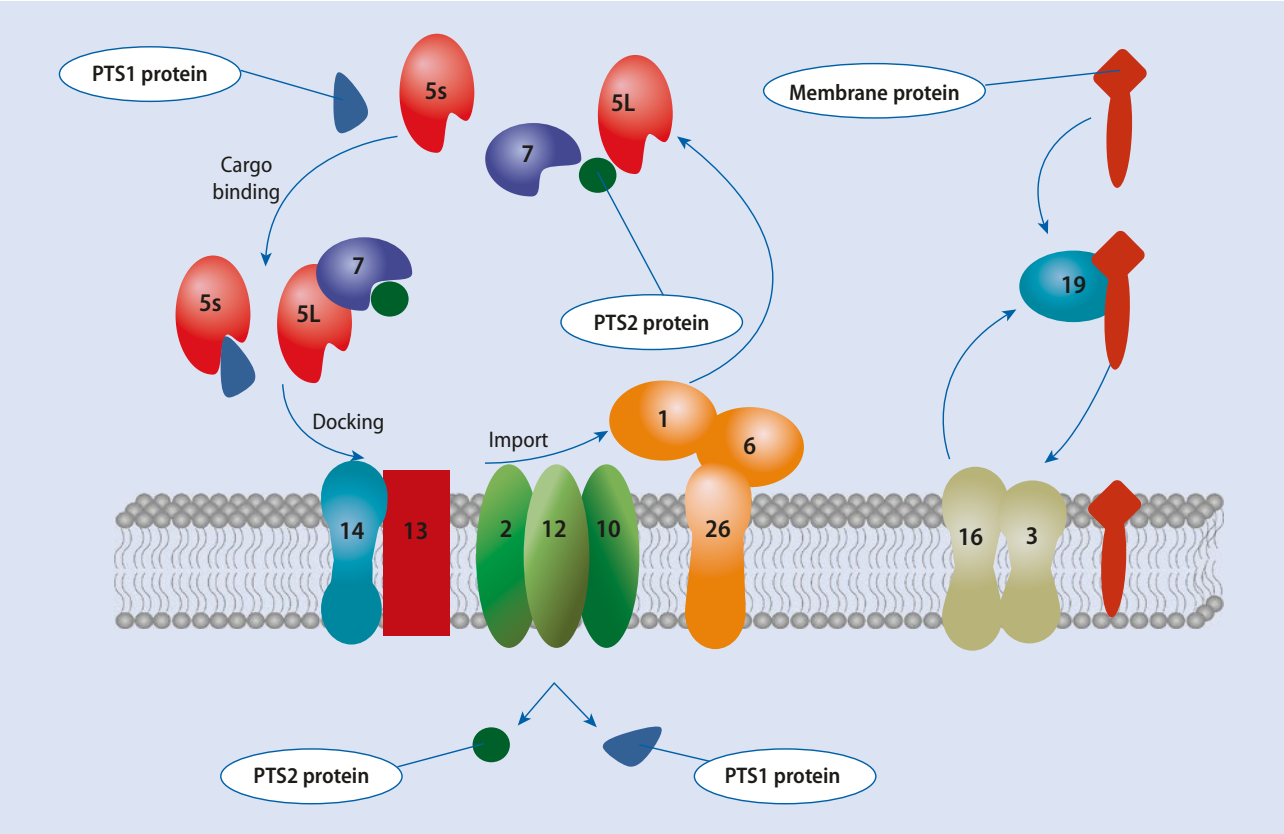

Fig. 42.2 Schematic diagram showing the biogenesis of peroxisomes in humans. Peroxisomes can either originate from a specific subdomain of the endoplasmic reticulum or may form by growth and division of pre-existing peroxisomes. In both cases peroxisomes need to take up peroxisomal proteins from the cytosol since all peroxisomal proteins except from a few, are synthesised on free polyribosomes. Specific targeting of peroxisomal matrix proteins to peroxisomes followed by their import, is mediated by two different import pathways. The first is the PTS1-protein import pathway in which the PTS1 receptor PEX5S (indicated as 5S) recognizes the PTS1 signal on peroxisomal proteins in the cytosol, binds the protein and escorts the PTS1 protein to the docking part of the peroxisomal protein import machinery consisting of PEX13 and PEX14. The next step involves the translocation of the PTS1 protein across the peroxisomal membrane mediated by PEX2, PEX10, and PEX12 after which the PTS1 receptor PEX5S is recycled back into the cytosol for another round of import via the concerted action of PEX1, PEX6, and PEX26. A subset of peroxisomal matrix enzymes are equipped with a different targeting signal called PTS2 as present in the enzymes AGPS (Fig. 42.1) and phytanoyl-CoA 2-hydroxylase. Import of PTS2 proteins requires PEX7 as the PTS2-protein receptor in close collaboration with PEX5L which provides stability to the PEX7 protein. The actual import mechanism for PTS2 proteins is identical to that of PTS1 proteins. Import of peroxisomal membrane proteins like PEX3 and PEX16 proceeds via a different mechanism with PEX19 serving the role as receptor which binds membrane proteins in the cytosol

do not survive beyond 12 years of age with death occurring from respiratory complications (see [3] for review). The majority of patients exhibit cardiac malformations [4], recurrent respiratory infections, and have feeding difficulties. Apart from the classical form of RCDP, a small number of patients with milder phenotypes have been described including those lacking the characteristic rhizomelia with only bone dysplasia and mild intellectual disability and others with a Refsum-like presentation [5]. The clinical signs and symptoms of these milder forms have recently been described in detail in a series of 16 patients [6]. Most had cataract, growth retardation, joint contractures, and developmental delay. Other features were: learning disability (87%), behavioural issues (56%), seizures (43%), and cardiac defects (31%).

In all forms of RCDP, ether lipid synthesis is impaired resulting in a deficiency in erythrocytes (using plasmalogens as readout) and other tissues. The molecular basis is heterogeneous with 5 causative genes identified so far, including *PEX7, GNPAT, AGPS, FAR1* and *PEX5L*.

42.1.1 Peroxin 7 (PEX7) Deficiency (RCDP Type 1)

RCDP type 1 as caused by bi-allelic mutations in PEX7 is most frequent among RCDP patients [7, 8]. PEX7 is responsible for the import of all peroxisomal proteins equipped with a PTS2-signal which includes the enzyme AGPS but also phytanoyl-CoA 2-hydroxylase (PHYH) which, if deficient, causes the impaired oxidation of phytanic acid. Accordingly, PEX7 deficiency causes the combined deficiency of both AGPS and phytanoyl-CoA

2-hydroxylase as reflected in decreased ether lipids including reduced erythrocyte plasmalogen levels and the accumulation of phytanic acid in plasma.

42.1.2 Glycerone-3-Phosphate Acyltransferase (GNPAT) deficiency (RCDP Type 2)

RCDP type 2 is caused by mutations in *GNPAT*, formerly known as *DHAPAT* [9]. Only about ten patients with this subtype have been described. Clinical features were indistinguishable from type 1.

42.1.3 Alkylglycerone-3-Phosphate Synthase (AGPS) Deficiency (RCDP Type 3)

RCDP type 3 is caused by mutations in *AGPS*. The enzyme AGPS is responsible for the formation of the ether-bond which is so characteristic for ether lipids [9]. AGPS deficiency has only been described in a few patients whose clinical signs and symptoms resemble those of RCDP type 1.

42.1.4 FAR1 Deficiency (RCDP Type 4)

RCDP type 4 is caused by mutations in the gene (*FAR1*) coding for the peroxisomal enzyme acyl-CoA reductase which is responsible for the formation of the long-chain alcohol from the corresponding acyl-CoA ester required for the AGPS reaction (Fig. 42.1) [10]. Although the three patients described with RCDP type 4 demonstrated several of the characteristic features of RCDP including profound growth retardation, developmental delay, pyramidal track dysfunction and seizures as in the other types of RCDP, all patients lacked the characteristic rhizomelic shortening of the long bones despite the markedly reduced plasmalogen levels in erythrocytes. Whether this has to do with the fact that there is a second acyl-CoA reductase (FAR2) with a different tissue expression than FAR1, remains to be established.

Heterozygous *de novo* variants affecting the Arg480 residue of FAR1 lead to an autosomal dominant disorder where a loss of feedback regulation of FAR1 leads to increased FAR1 protein levels and an enhanced catalytic activity of FAR1 thus resulting in an increased rate of ether lipid biosynthesis as reflected in marked lipidomic changes including increased plasmalogens. Patients presented in early infancy with spastic paraparesis and bilateral cataracts. *FAR1* should be considered as a candidate gene and added to the list for hereditary spastic paraplegia, cerebral palsy, and juvenile cataracts [1].

42.1.5 PEX5L Deficiency (RCDP Type 5)

RCDP type 5 has so far been described in four patients from two independent families [11]. The defect is caused by a frame shift mutation located in the PEX5L-specific exon 9 thus interfering with the proper synthesis of PEX5L while leaving the synthesis of PEX4S and thus the import of PTS1-proteins intact (Fig. 42.2).

Metabolic Derangement

In all five types of RCDP ether lipid biosynthesis is deficient which leads to a deficiency of ether lipids and ether phospholipids, including plasmalogens in tissues and erythrocytes. In types 1 and 5 but not in types 2, 3, and 4 there is also accumulation of phytanic acid which is age and diet dependent since phytanic acid is only derived from exogenous, dietary sources. Erythrocyte plasmalogen levels have been found to be deficient in all RCDP patients although this may only be partial with levels reaching up to 40% of control in those most mildly affected.

Genetics

All types of RCDP are autosomal recessive disorders and bi-allelic mutations in the five genes involved with each of the five forms of RCDP have been described in literature which includes two large series of type 1 patients [7, 8].

Diagnostic Tests

Erythrocyte plasmalogen analysis is a highly reliable diagnostic test which if abnormal immediately points to a deficiency of PEX7, GNPAT, AGPS, FAR1, or PEX5L. Although each of the different forms of RCDP can be worked out in fibroblasts, at least in our own laboratory, the finding of reduced plasmalogen levels is usually directly followed up by molecular analysis.

Treatment and Prognosis

At present, there is no realistic curative therapy for either form of RCDP. In mouse mutant models ether phospholipid and plasmalogen levels can be corrected in all tissues except the brain, by dietary supplementation of alkylglycerol [6, 12].

42.2 Disorders of Peroxisomal Fatty Acid β-Oxidation

Peroxisomes are the main site of non-mitochondrial fatty acid β-oxidation (FAO) (Fig. 42.1). They do not play a significant role in the oxidation of medium-and long-chain FA which are predominantly oxidized in mitochondria, but are indispensable for the oxidation of a host of different FA including: (1) VLCFA, notably C26:0 and ULCFA; (2) pristanic acid as derived from

exogenous sources either as pristanic acid itself or as phytanic acid which generates pristanic acid upon α-oxidation in peroxisomes (◘ Fig. 42.1); (3) the bile acid intermediates di-and trihydroxycholestanoic acid; (4) long-chain dicarboxylic acids derived from ω-hydroxy FA; and (5) a range of eicosanoids including PGF2-α, 8-iso-PGF2-α, thromboxane B2, 12-HETE, 15-HETE, LTB4, LTE4, and 11,12-EET. In patients with a defect in peroxisome biogenesis as in ZSD patients, the oxidation of all these FA is impaired whereas in the different single peroxisomal β-oxidation enzyme deficiencies abnormalities are restricted to a subset of FA. Oxidation is mediated by three different acyl-CoA oxidases (ACOX1–3), two bifunctional proteins with both enoyl-CoA hydratase activity and 3-hydroxyacyl-CoA dehydrogenase activity (LBP and DBP) and two thiolases (ACAA1 and SCPx) [13–15] (◘ Fig. 42.1). Genetic deficiencies at the level of *ACOX 1*, *ACOX2*, and *ACOX3*, *HSD17B4* (DBP), and *SCP2* have been identified as detailed below. Peroxisomes can only degrade FA partially after which the end products of peroxisomal β-oxidation including acetyl-CoA, propionyl-CoA, and various medium-chain acyl-CoAs are shuttled to mitochondria either as carnitine-ester or as free acid for full oxidation to CO_2 and H_2O [16]. The number of β-oxidation cycles catalysed by peroxisomes differs per FA. Indeed, di-and trihydroxycholestanoic acid (DHCA and THCA) only undergo one cycle of β-oxidation in peroxisomes, pristanic acid three cycles of β-oxidation whereas eicosanoids may undergo one, two, or three cycles of β-oxidation to produce the corresponding dinor, tetranor, or hexanor derivatives. Apart from the enzymes listed above, peroxisomes contain a range of other enzymes required for the oxidation of unsaturated and (2R)-methyl branched chain FA. So far only a single defect in the oxidation of the latter FA has been described which is 2-methylacyl-CoA racemase (AMACR) deficiency. The peroxisomal FAO disorders can be subdivided into two groups including: (1) the primary peroxisomal β-oxidation deficiencies: 42.2.1–42.2.8 and (2) the secondary deficiencies due to peroxisome biogenesis defects (▶ Sect. 42.2.9).

42

42.2.1 X-Linked Adrenoleukodystrophy (ALD)

▪ Clinical Presentation

ALD is the most common peroxisomal disorder with a 1/17.000 birth incidence for males and females combined [17, 18]. Patients are normal at birth but eventually all become symptomatic. ALD used to be considered a disease with distinct phenotypes but it is perhaps more appropriate to consider it as a progressive neurodegenerative disease that affects the cerebral white matter, the spinal cord, and peripheral nerves, the adrenal glands, as well as the testes [19]. However, there are large and unpredictable differences in age of onset and severity of symptoms [19].

A progressive leukodystrophy (cerebral ALD) can occur in male patients with a lifetime prevalence of about 60% with roughly 40% presenting before the age of 18 (peak incidence between 4–12 years of age) [20]. MRI lesions develop (long) before symptoms become apparent. Initially these are non-specific and include behavioural changes and cognitive deficits eventually causing difficulties at school or at work. Auditory agnosia is a relatively frequent sign that is often overlooked. As the disease progresses, overt neurological deficits develop including visual impairment, motor dysfunction, and sometimes epileptic seizures. Eventually, on average 2–3 years after onset of symptoms, patients are quadriplegic and require complete care including tube feeding [21]. MRI scans of the brain show pathognomonic confluent white matter lesions with gadolinium enhancement just beyond the leading edge of the lesions [22]. Lifetime prevalence of adrenal insufficiency is up to 80% in male patients, and is often the presenting clinical syndrome in childhood [20]. Adrenal insufficiency can be difficult to diagnose with initial symptoms such as fatigue, (frequent) hospitalizations with dehydration after minor illness, with more specific symptoms such as skin hyperpigmentation, clear Addisonian crisis, with hypotension, and hypoglycaemia appearing much later. In contrast to auto-immune adrenal insufficiency patients with ALD are often only glucocorticoid insufficient [20]. All adult patients eventually develop spinal cord disease characterized by degeneration of mainly the dorsal columns and corticospinal tracts [24]. The main symptoms include a disordered gait due to spasticity and sensory ataxia, as well as bowel and bladder dysfunction. Symptoms are slowly progressive typically worsening over the years [23]. Disease presentation and severity differ markedly between patients even within the same family. There appears to be no apparent phenotype/ genotype correlation nor do plasma VLCFA levels correlate with disease severity [19].

Women with ALD develop spinal cord disease and peripheral neuropathy with about 90% of women having symptoms and/or signs of spinal cord disease by the age of 60. Symptoms occur at a later age, however, when compared to males and rarely start before 40 years. Furthermore, adrenal insufficiency is extremely rare in women (<1%) and the occurrence of cerebral ALD has only been reported occasionally.

▪ Metabolic Derangement

In ALD, the oxidation of VLCFA is deficient due to the absence or functional deficiency of the peroxisomal ABC half-transporter ALDP/ABCD1 (◘ Fig. 42.1). This leads to elevated VLCFA-CoA levels in the cytosol and their subsequent incorporation into a variety of different lipid species including cholesterol esters, phospholipids, and sphingolipids. As a consequence, VLCFA accumulate in virtually all tissues including erythrocytes, white blood cells and plasma.

Genetics

ALD is caused by mutations in *ABCD1*. Hundreds of different mutations have been identified including point mutations, deletions, insertions, splice site mutations (see ► https://adrenoleukodystrophy.info/). The percentage of de novo mutations is less than 4% and, consequently, it is important to undertake genetic counselling and screening in ALD families in order to detect individuals at risk including heterozygous women, boys, and adults with adrenal insufficiency and those who are asymptomatic neurologically with normal neuroimaging.

Diagnostic Tests

The most frequently used test for ALD has long been the analysis of VLCFA including C22:0, C24:0, and C26:0 in plasma after alkaline and acid hydrolysis to release the VLCFA from all lipid species. False-positive results have been reported in patients on a ketogenic diet [25] and especially upon consumption of peanut-containing food [26]. Plasma VLCFA levels have been reported to be normal in 5–15% of women with ALD. Importantly, measurement of C26:0-lysophosphatidylcholine (C26:0-lysoPC) in plasma or bloodspots identifies all males and females with ALD [27]. Neonatal screening based on the analysis of C26:0-lysoPC in bloodspots is currently performed in several US states and is now on its way to be implemented in the Netherlands [28]. Also, with trio whole exome sequencing now rapidly becoming a routine diagnostic test, at least in some countries, identification of patients via this route is not uncommon.

Treatment and Prognosis

Cerebral ALD can be halted by allogeneic haematopoietic cell transplantation (HCT) in boys and adult males, but only at a very early stage of the onset of disease, in practice when patients start to develop cerebral demyelination on brain MRI but have no or minimal neurologic symptoms. This will arrest the cerebral demyelination [29]. For cerebral ALD an MRI severity score, known as the Loes score, has been devised ranging from 0 (no disease) to 34 [22]. HCT is not recommended for patients with a score of 9 or more since outcome is poor [30] whereas patients with a score between 4.5–9 show considerable cognitive decline [31]. HCT is associated with very high mortality in adult patients with advanced spinal cord disease who are no longer ambulatory and have an indwelling catheter for bladder dysfunction. Accordingly, this can be considered an additional eligibility criterion [29]. Although there have been major improvements in HCT, morbidity and mortality remain high [29]. Graft versus host disease (GvHD) remains another major problem.

Cartier and co-workers described ex vivo lentiviral gene therapy that allows patients to be treated with cells derived from autologous bone marrow [32] and recently, a phase III trial was completed [33]. Although HCT using this approach is much safer compared to HCT with allogeneic bone marrow, and the treatment is efficacious in the short term, long term results are not yet available. Unfortunately, it is likely that HCT has no beneficial effects on the other manifestations of the disease including the spinal cord disease. For this reason, patients are only transplanted when radiological signs of cerebral ALD occur [34]. This implies MRI surveillance for male patients since the onset of cerebral ALD cannot be predicted [35]. A guideline for follow-up has been published [35]. Unfortunately, if a boy or man has no family history of ALD and presents with signs and symptoms of cerebral ALD, HCT is usually no longer an option. In those cases, cerebral ALD generally progresses relentlessly causing severe disability and death on average 2–3 years after the start of symptoms. This explains why ALD is now being added to newborn screening programs, at least in some parts of the world. In male patients follow-up of adrenal function is recommended, and hormone therapy should be started when adrenal insufficiency develops [35]. For the spinal cord disease occurring in adulthood in males and females, there is currently no disease modifying therapy available. Rehabilitation programs may provide supportive care. Treatment of bladder dysfunction and neuropathic pain also improves the quality of life. 4-Aminopyridine (Fampridine) improves the speed of walking without rest in 70% of men or women with spinal cord disease. Finally, Lorenzo's oil which is a mixture of oleic acid C18:1) and erucic acid (C22:1), allows the normalisation of plasma VLCFA but unfortunately has no curative or preventive effects and should no longer be prescribed [36]. At present several clinical trials are underway with new potential drugs to stabilize disease.

42.2.2 D-Bifunctional Protein (DBP) Deficiency

The clinical signs and symptoms of DBP deficiency are in general very severe and resemble those observed in ZSD. Moreover, the MRI-features in DBP deficiency resemble those observed in ZSD. DBP deficiency is one of the more frequent peroxisomal β-oxidation disorders second to ALD. In 2006 we reported the clinical, biochemical and genetic findings in 126 patients [37]. Infants are usually born full-term with no evidence of intrauterine growth retardation. The clinical presentation is dominated by neonatal hypotonia (98%), combined with seizures within the first months of life (93%) and failure to thrive (43%). Dysmorphic features including macrocephaly, high forehead, flat nasal bridge, low-set ears, large anterior fontanelle, and micrognathia were found in most patients. Virtually none were found to acquire any signifi-

cant milestones whereas the few that did improve, subsequently showed progressive loss of motor achievements. DBP deficiency can be subdivided into three different groups but the clinical features, however, associated with either of the three different subtypes are indistinguishable and the same applies to the prognosis [37]. More recently, patients have been identified by whole exome sequencing with much milder phenotypes. This includes four patients presenting with a juvenile-onset condition comprising cerebellar ataxia, hearing loss, peripheral neuropathy and premature ovarian failure suggestive of Perrault syndrome [38] and three adult siblings with a slowly progressive, juvenile-onset phenotype comprised of cerebellar atrophy, ataxia, intellectual decline, hearing loss, sensory motor neuropathy, and supratentorial white matter changes in two of the three probands [39].

Metabolic Derangement

DBP plays a crucial role in the peroxisomal β-oxidation of most FA handled by peroxisomes which explains the accumulation of VLCFA, pristanic acid, as well as the bile acid precursors di-and trihydroxycholestanoic acid.

Genetics

DBP deficiency is an autosomal recessive disorder caused by bi-allelic mutations in *HSD17B4* which codes for DBP. A large number of private mutations have been identified as well as one more frequent mutation [37].

Diagnostic Tests

VLCFA analysis in plasma is the method of choice as initial biochemical test which is often combined with the analysis of pristanic and phytanic acid in the same analysis [40]. More recently, it has become clear that analysis of C26:0-lysoPC as first developed for ALD [41] provides superior sensitivity as a marker for VLCFA accumulation [42]. In order to obtain a complete picture of the peroxisomal metabolome in the patient's blood, we prefer to measure the bile acid intermediates di-and trihydroxycholestanoic acid [43] and determine the plasmalogen levels in erythrocytes [44]. These analyses should be followed up by detailed studies in fibroblasts (see [45] for detailed discussion).

Treatment and Prognosis

At present there are no realistic options for curative treatment. Furthermore, the prognosis is poor.

42.2.3 Acyl-CoA Oxidase 1 (ACOX1) Deficiency

Acyl-CoA oxidase 1 (ACOX1) deficiency was first reported in 1988 as pseudo-neonatal adrenoleukodystrophy. Plasma VLCFA were abnormal. However, liver biopsies revealed the normal presence of peroxisomes albeit of enlarged size which argued against a defect in peroxisome biogenesis [46]. Since then, ACOX1 deficiency has been described in many patients [47]. Most exhibited neonatal-onset hypotonia, seizures, failure to thrive, psychomotor retardation, sensorineural hearing loss, hepatomegaly, and visual loss with retinopathy. In 50% there were dysmorphic features resembling those observed in ZSD. Patients may show some early motor development but they typically regress by 2–3 years of age. In more recent years, later-onset forms have been described, including two adult patients with normal early developmental milestones who first developed progressive neurologic symptoms in later childhood.

Metabolic Derangement

VLCFA are increased in ACOX1 deficiency whereas all other peroxisomal biomarkers are normal.

Genetics

ACOX1 deficiency is an autosomal recessive disorder caused by bi-allelic mutations in *ACOX1* which are mostly private [47].

Diagnostic Tests

The laboratory diagnosis of ACOX1 deficiency usually starts with the analysis of the different peroxisomal biomarkers notably the VLCFA in plasma. C26:0-lysoPC is now the analyte of choice. If abnormal, subsequent analyses in blood and fibroblasts are required to pinpoint the defect [46].

Treatment and Prognosis

No treatment options have been described other than supportive measures. The prognosis is usually poor with early death at a median age of 5 years of age (range: 4–10) [47].

42.2.4 2-Methylacyl-CoA Racemase (AMACR) Deficiency

Clinical Presentation

Following its first description in 2000 [48] in patients characterized by an adult-onset motor neuropathy, AMACR deficiency has been described in some 10 patients with markedly different signs and symptoms ranging from seizures, ataxia, visual impairment with pigmentary retinopathy to fulminant liver failure very early in life [49]. More recently, we identified a number of additional patients with very mild signs and symptoms restricted to mild liver dysfunction (► See also Sect. 38.8.2).

Metabolic Derangement

The enzyme AMACR is localised in both mitochondria and peroxisomes and plays a crucial role in the oxidation of FA with a methyl group at the 2-position, namely pristanic acid (and its breakdown products) and the bile acid precursors di-and trihydroxycholestanoic acid. 50% of pristanic acid and 100% of the bile acid precursors have the (2R)-configuration and these enantiomers need to be racemised by AMACR to the (2S)-configuration before β-oxidation can take place. If AMACR is deficient, (2R)-fatty acids, most prominently (2R)-pristanic acid and di-and trihydroxycholestanoic acid, accumulate.

Diagnostic Tests

Analysis of di-and trihydroxycholestanoic acid in plasma is the method of choice. Preferably a method should be used which allows discrimination between the two diastereomers of di-and trihydroxycholestanoic acid [50]. The finding of elevated di-and trihydroxycholestanoic acid should be followed up directly by molecular analyses rather than biochemical studies in fibroblasts.

Genetics

Bi-allelic mutations in *AMACR* have been described in all patients.

Treatment and Prognosis

At this moment there is no proven effective therapy other than supportive measures which may include supplementation of fat-soluble vitamins. Patients may well benefit from dietary supplementation with cholic acid not only to correct for a possible shortage of cholic acid as one of the end products of bile acid synthesis but also to inhibit formation of the two bile acid synthesis intermediates, di-and trihydroxycholestanoic acid (► Sect. 38.4).

42.2.5 Sterol Carrier Protein 2 Deficiency

SCPx deficiency has only been described in a few cases [51–53]. The first patient presented with torticollis and dystonic head tremor, slight cerebellar signs with intention tremor, nystagmus, hyposmia, and azoospermia. The patient carried homozygous mutations in *SCP2* which generates two transcripts giving rise to two different proteins named SCP2 (20 kDa) and SCPx (58 kDa) [51]. SCPx catalyzes the last step in the peroxisomal oxidation of 2-methyl branched chain fatty acids, the thiolytic cleavage and is also known as the peroxisomal branched-chain thiolase. If deficient, pristanic acid and the bile acid intermediates di-and trihydroxycholestanoic acid and especially the bile alcohols derived from them accumulate [51]. The second patient showed neurodegeneration with iron accumulation in the brain [52] whereas the third patient exhibited an unusual retinal phenotype [53].

42.2.6 PMP70 (ABCD3) Deficiency

PMP70 deficiency due to mutations in ABCD3 has only been identified in a single patient who presented with hepatosplenomegaly and severe liver disease and showed the striking accumulation of the two bile acid intermediates di-and trihydroxycholestanoic acid. The liver disease progressed to the extent that liver transplantation was required but the patient expired shortly thereafter [54] (► Sect. 38.8.1).

42.2.7 Acyl-CoA Oxidase 2 (ACOX2) Deficiency

ACOX2 is one of three different acyl-CoA oxidases and the only one which reacts with the CoA-esters of di-and trihydroxycholestanoic acid [55] (► Sect. 38.8.3). The first reported patient was an 8-year old male with intermittently elevated transaminase levels, liver fibrosis, mild ataxia, and cognitive impairment [56]. A second patient was an adolescent boy with elevated transaminases of unknown origin which had already persisted for more than 2 years [57]. Bile acid analysis revealed the presence of di-and trihydroxycholestanoic acid. Molecular analysis revealed bi-allelic homozygous mutations in *ACOX2*. We recently identified a third patient [58].

42.2.8 Contiguous ABCD1, DXS1357A-Deletion Syndrome (CADDS)

In 2002 three newborns were described with clinical signs and symptoms suggestive of ZSD [59]. The patients, all male, suffered from motor and intellectual disabilities, dystonia, sensory neural deafness, and white matter changes. Plasma VLCFA were elevated in all three patients. Detailed studies in fibroblasts, however, revealed that peroxisome biogenesis was normal and that the defect in these patients was confined to the peroxisomal degradation of VLCFA, since all other peroxisomal functions were normal. These puzzling findings were resolved by the identification of a small deletion in the Xq28 region spanning the 5′ ends of *ABCD1* and *DXS1357E*/*BCAP31* which codes for BAP31, an abundant ER protein of unresolved function. *ABCD1* and *BCAP31* are located head-to-head at Xq28. Only a few

additional patients have since been identified which includes a patient with a larger deletion extending to *SLC6A8* coding for the creatine transporter [60]. Interestingly, isolated BAP31 deficiency due to mutations in *DXS1357E*/*BCAP31* has been described and the affected patients had severe global developmental delays, dystonia, sensory neural deafness, failure to thrive, microcephaly, dysmorphic facial features, and hypomyelination [61].

42.2.9 Generalized Peroxisomal Fatty Acid Oxidation Deficiencies: Zellweger Spectrum Disorders

Clinical Presentation

Zellweger Syndrome (ZS) originally described as cerebro-hepato-renal syndrome, is the prototypical ZSD. Patients usually present shortly after birth with severe hypotonia, seizures, cranial facial dysmorphia including a high forehead, large anterior fontanelle, hypertelorism, epicanthal folds, a high arched palate, and micrognathia. Microgyria, pachygyria, and heterotopia can be seen upon magnetic resonance imaging (MRI). Ocular abnormalities are frequent and include retinitis pigmentosa, cataract, glaucoma, and corneal clouding. Cortical renal cysts are usually identifiable on ultrasound. Cardiovascular malformations and pulmonary hypoplasia have also been documented. The absence of peroxisomes in ZS patients was first reported by Goldfischer et al. in 1973 based on a simple catalase staining test [62]. Peroxisomes were subsequently found to be deficient in two other disorders including a neonatal neurologic syndrome, called neonatal adrenoleukodystrophy (NALD) so named because of the accumulation of VLCFA, and an early infantile syndrome named infantile Refsum disease (IRD) in which the accumulation of phytanic acid was identified first. It has since become clear that the phenotypic variability extends beyond the categories ZS, NALD, and IRD and this has led to the introduction of the term Zellweger Spectrum Disorder (ZSD). Although part of a wider phenotypic spectrum, ZSD can roughly be divided into 3 groups which include: (1) a neonatal-infantile form; (2) a childhood form; and (3) an adolescent-adult form as detailed below (see [63] for details) but like many metabolic disorders these are part of a phenotypic spectrum.

Neonatal-Infantile Presentation This is a severe phenotype that closely resembles classic ZS. Patients typically present after birth with severe hypotonia, seizures, typical dysmorphic features, hepatic dysfunction, and other abnormalities. Most patients do not reach any developmental milestones and usually die in the first year of life.

Childhood Presentation Although partially overlapping with the neonatal-infantile presentation, this form is more variable with onset usually within the first to second year of life with patients presenting with delayed developmental milestones, failure to thrive, osteoporosis, liver dysfunction and fat malabsorption mimicking gastrointestinal disorders. Usually, there is progressive bilateral visual and sensory neural hearing impairment. Ocular abnormalities include retinitis pigmentosa, cataract, optic nerve atrophy, glaucoma, and Brushfield spots. Facial dysmorphia is in general less pronounced when compared to the neonatal-infantile form.

Adolescent-Adult Presentation Patients with this presentation are even more difficult to diagnose clinically compared to the childhood form. Sensory neural hearing loss and ocular features are important clues whereas additional signs and symptoms may be absent or occur later in life. Patients at the mildest end of the ZSD spectrum have mental retardation plus visual and hearing impairment and nonspecific symptoms including teeth and nail abnormalities as in Heimler syndrome. Cranial facial dysmorphia is usually absent or very subtle. Adrenal insufficiency is common although asymptomatic in more than 50% of the patients [64].

Metabolic Derangement

The impaired formation of peroxisomes in ZSD is associated with the loss of basically all peroxisomal functions. This is reflected in abnormalities in all peroxisomal biomarkers including elevated plasma levels of VLCFA, phytanic acid, pristanic acid, di-and trihydroxycholestanoic acid and pipecolic acid and reduced plasmalogen levels in erythrocytes.

Genetics

The ZSDs are genetically heterogeneous. So far mutations in 12 PEX genes have been identified in ZSD patients (Fig. 42.2) [65, 66].

Diagnostic Tests

Analysis of C26:0-lysoPC is the best initial laboratory test for ZSD [42], preferably in combination with the analysis of the other peroxisomal biomarkers which includes phytanic acid, pristanic acid, di-and trihydroxycholestanoic acid and pipecolic acid and plasmalogen levels in erythrocytes. Analysis of these metabolites in a single assay is now feasible. This type of peroxisomal biomarker analysis is extremely rewarding especially

since patients with a milder form of ZSD may only show minimal abnormalities restricted to a single biomarker to no abnormal biomarker at all [45]. Moreover, peroxisomal biomarkers may well be abnormal early in the life of ZSD patients but tend to normalize later [68]. This diagnostic problem requires the identification of novel, improved peroxisomal biomarkers. The generation of new tandem mass spectrometry-based platforms which allow the analysis of large groups of metabolites including the complete lipidome, has already proven its power in this respect [69–72].

Treatment and Prognosis

There is no curative therapy presently available for ZSD patients. Symptomatic therapy should include evaluation of feeding, hearing, vision, liver function, as well as neurological and developmental functions. Most patients benefit from the use of hearing aids, cochlear implants, or other assistive devices. Monitoring for adrenal insufficiency should be done routinely, and hormone replacement instituted when needed. Appropriate educational programs are recommended for the milder affected patients. It is important to ensure that adequate calories are provided which often implies feeding by means of a gastrostomy tube. A diet low in phytanic acid is advocated by avoiding cow's milk and other dairy products. Since many children have some degree of malabsorption, elemental formulas may be better tolerated. Coagulation and other synthetic liver functions should be monitored regularly, and supplementation with vitamin K and other fat-soluble vitamins is recommended. Liver dysfunction may lead to varices that respond to sclerosing therapies. Bile acid supplementation involving cholic acid (100 mg/day) has been advocated but [67]. Furthermore, an extensive double-blind, randomized trial revealed no beneficial effects of docosahexaenoic acid (DHA) disproving earlier claims to the contrary [73].

42.3 Disorders of Peroxisomal Fatty Acid α-Oxidation: Adult Refsum Disease

Although most FA including branched-chain FA can be broken down by β-oxidation, FA with a methyl-group at the 3-position first need to undergo α-oxidation to remove the terminal carboxyl group and generate a 2-methyl branched chain FA which can be β-oxidized. The enzymology of the 3-methyl branched-brain FA α-oxidation system involves five consecutive enzymes (Fig. 42.1) [13, 74]. So far only phytanoyl-CoA 2-hydroxylase deficiency which causes Adult Refsum Disease (ARD has been identified.

Clinical Presentation

Patients with ARD are asymptomatic at birth, show no obvious defects in growth and development, and usually present in late childhood with progressive loss of night vision, a decline in visual capacity and anosmia. After 10–15 years or more, patients may develop additional abnormalities including deafness, ataxia, demyelinating polyneuropathy, ichthyosis, fatigue, and cardiac conduction disturbances [75]. Short metacarpals and/or metatarsals are found in around 30% of patients. The full constellation of typical features as originally defined by Refsum in the 1940s which includes retinitis pigmentosa, cerebellar ataxia, and chronic polyneuropathy, is rarely seen in single patients with ARD. The polyneuropathy is of a mixed motor and sensory type which is asymmetrical, chronic, and progressive in untreated ARD. Over the course of years muscular weakness can become widespread with disability involving not only the limbs but also the trunk. Almost without exception patients have peripheral sensory disturbances, most often impairment of deep sensation, particularly perception of vibration and position/motion in the distal legs. Some patients develop cardiomyopathy which can be lethal in the absence of cardiac transplantation. Of note PHARC syndrome due to a/b-hydrolase 12 deficiency (ABHD12) may mimic severe forms of ARD (► Chap. 35).

Metabolic Derangement

Phytanoyl-CoA 2-hydroxylase deficiency causes the accumulation of 3-methyl branched-chain FA which includes phytanic acid as most typical representative.

Genetics

ARD is an autosomal recessive disorder caused by biallelic, mostly private mutations in *PHYH* [76]. In a few patients the molecular basis is different and involves *PEX7* [5, 77].

Diagnostic Tests

Phytanic acid is solely derived from exogenous sources and accumulates in a diet- and age-dependent way. Although phytanic levels may vary considerably in ARD patients reaching levels beyond 1000 micromol/L (normal:<10 micromol/L), plasma phytanic acid analysis is a very robust biomarker for ARD with no false negatives reported so far.

Treatment and Prognosis

Dietary restriction of phytanic acid is the mainstay of therapy. The main source of phytanic acid are dairy products, meats and certain fishes which must be eliminated from the diet rigorously in order to try and keep phytanic acid as low as possible, although levels never normalize completely. Green leaves and vegetables do not need to be restricted since the phytol present in the chlorophyll mol-

ecule cannot be released in humans. Dietary restriction of phytanic acid is extremely important and rewarding since several features may stabilize or even improve including the peripheral neuropathy, ataxia, ichthyosis, and cardiac abnormalities. However, the retinitis pigmentosa, deafness, and anosmia appear to be more refractory.

42.4 Disorders of Fatty Acid Chain Elongation and Fatty Acid/Alcohol/Aldehyde Homeostasis

Before the different fatty acids can be incorporated into the various classes of lipid species, fatty acids may have to undergo a number of modifications. This includes chain-elongation of LCFA either synthesized endogenously or taken up from dietary sources, to produce ULCFA required for the synthesis of certain ceramides and sphingolipids. However, FA have to undergo many additional modifications to generate a pool of acyl-CoA esters which can be used for lipid synthesis. These modifications include: (1.) activation to the corresponding acyl-CoA esters by one of the many ACS-enzymes including FALC4 and FATP4; (2.) synthesis of 2-hydroxyacyl-CoAs as mediated by the enzyme FA 2-hydroxylase (FA2H) for the incorporation into ceramides, sulphatides, and sphingolipids; (3.) desaturation to generate the large variety of mono-and polyunsaturated fatty acids as catalysed by stearoyl-CoA desaturases (SCD) and fatty acid desaturases (FADS); (4.) synthesis of ω-hydroxylated fatty acyl-CoAs by the enzyme CYP4F22 for the synthesis of w-O-acylceramides; (5.) acyl-CoA reduction of long chain acyl-CoAs to the corresponding long-chain fatty alcohols by the two enzymes FAR1 and FAR2 for the formation of ether lipids as part of the so-called Fatty Alcohol Cycle which also includes the enzyme fatty aldehyde dehydrogenase (FALDH/ALDH3A2); and (7.) chain elongation by the fatty acid chain elongation complex in the ER (◘ Fig. 42.1).

42.4.1 FACL4 Deficiency

FACL4 deficiency was first described in a family with Alport syndrome, elliptocytosis, and mental retardation as caused by a continuous deletion also encompassing *COL4A5* [78]. Subsequently, bi-allelic, pathogenic mutations in *FACL4* were found in five patients from two families with non-specific mental retardation [79] and low acyl-CoA synthetase activity with arachidonic acid as substrate in lymphocytes. A third family with four affected males was later described carrying a novel mutation in *FACL4* [80]. Since then, FACL4 deficiency has only been described in patients carrying different continuous deletions at Xq22.3-q23.

42.4.2 FATP4/ACSVL4/SLC27A4 Deficiency

FATP4 deficiency was first reported in 2009 in patients from different families affected by ichthyosis prematurity syndrome (IPS), an autosomal recessive disease characterized by premature birth and neonatal asphyxia followed by a life-long, non-scaly ichthyosis with atopic manifestations [81]. Symptoms may become milder during childhood mainly manifesting as dry and pruritic skin. Respiratory and/or food allergies are common. Only few additional patients have been described in literature [82].

42.4.3 Fatty Acid 2-Hydroxylase (FA2H) Deficiency

Recently, an in-depth clinical and retrospective neurophysiological and imaging study was reported in a cohort of 19 patients with bi-allelic mutations in FA2H to determine the phenotypic spectrum, define the disease course, and identify clinical and imaging biomarkers [83] (► Sect. 40.1.5).

42.4.4 CYP4F22 Deficiency

CYP4F22 deficiency was first identified as an autosomal recessive congenital ichthyosis (ARCI) gene in a series of 21 patients from different Mediterranean countries with erythroderma, severe scaling which was more pronounced on flexural areas, and palmoplantar hyperlinearity [84]. This was at a time when the function of CYP4F22 in the formation of ω-O-acylceramides, essential lipids for skin permeability barrier formation, was not yet known [85]. The phenotypic spectrum associated with CYP4F22 deficiency was enlarged by the finding of mutations in *CYP4F22* in two patients born as collodion babies who improved over time with little to no skin abnormalities remaining, which is a rare, uncommon autosomal recessive congenital ichthyosis subtype known as "self-healing collodion babies". In the meantime many CYP4F22-deficient patients showing a wide spectrum of clinical signs and symptoms have been identified.

42.4.5 Sjögren Larsson Syndrome (SLS)

- **Clinical Presentation**

The classical tetrad of abnormalities in SLS includes pruritic ichthyosis, spasticity, ophthalmological abnor-

malities and mental retardation. However, this full-blown phenotype is not observed in all patients and may only manifest later in childhood beyond three years of life. The skin in newborn infants with SLS may first exhibit an erythrodermic appearance, gradually evolving into a generalized ichthyosiform hyperkeratosis during infancy. The ichthyosis follows a generalized distribution pattern with sparing of the face and is different from that observed in other cornification disorders because of the striking pruritic character. Furthermore, there is bilateral spastic tetraparesis in SLS which affects the legs more than the arms. None of the adolescent patients are able to walk without support [86]. The neurological features often stabilize in adolescence. Motor performance in everyday functioning in most patients reaches the developmental age of 12 years at best. Mild to moderate dysarthria is observed in virtually all patients with a delay in language development. MRI studies invariably show normal gross anatomy of the brain with periventricular white matter abnormalities ranging from (very) mild to severely increased signal intensities on T2-weighted images. Myelination is generally slightly delayed and most patients show demyelination predominantly in the frontal white matter in childhood. Only at the age of >10 years slight cerebral atrophy may be seen. Beyond 1–2 years of age MRS shows highly characteristic, almost pathognomonic, abnormal resonances at 1.3 and 9.8–0.9 ppm in the cerebral white matter but not in the grey matter.

Metabolic Derangement

The enzyme deficient in SLS is the ER-bound fatty aldehyde dehydrogenase (FALDH) encoded by *ALDH3A2*. FALDH belongs to a large family of aldehyde dehydrogenases and accepts a wide range of fatty aldehydes [87]. FALDH plays a key role in the degradation of long-chain alcohols in line with the accumulation of long-chain fatty alcohols in SLS patients [88]. FALDH also plays a key role in LTB4 metabolism by catalysing the oxidation of the ω-aldehyde of LTB4 which is an obligatory step in the degradation of LTB4 [89]. Furthermore, FALDH is involved in sphingosine catabolism by catalysing the oxidation of trans-2-hexadecanal produced in the sphingosine-1-phosphate lyase reaction into hexadecenoic acid. Very recent work has pointed to yet another function of FALDH in the breakdown of sphingolipids especially C24–2-hydroxy-containing sphingolipids. After activation of the C24-hydroxy FA to the corresponding CoA-ester, the C24–2-hydroxy fatty acyl-CoA is cleaved into formyl-CoA and the C23-aldehyde by the ER-enzyme HACL2 after which the C23-aldehyde is dehydrogenated into the C23-acid by FALDH and further oxidized. In case of a deficiency of FALDH, the C23-aldehyde accumulates and is converted into the C23-alcohol [90].

Genetics

SLS is an autosomal recessively inherited lipid disorder caused by mutations in *ALDH3A2* and a range of missense, nonsense, splice-site and deletions have been identified [91].

Diagnostic Tests

The metabolic abnormalities in SLS include elevated levels of a range of long-chain fatty alcohols notably octadecanol, which should be measured as first line test in candidate patients [88]. This should be followed up by enzymatic studies in fibroblasts or preferably lymphocytes using one of a variety of different assays described in literature including the assay using pyrenedecanal as substrate [92].

Treatment and Prognosis

Treatment of SLS patients is focused on the spasticity and prevention of contracture development. One of the key problems in SLS patients is the striking pruritus which may originate from the impairment in LTB4 homeostasis. This finding inspired Willemsen and co-workers to try Zileuton, a drug known to inhibit leukotriene formation by blocking its biosynthesis. In an initial open label study 5 SLS patients exhibited reduced pruritus and improved behaviour [93]. A subsequent randomized, double-blind, placebo-controlled crossover study involving 10 SLS patients, however, only showed a significant improvement in one [90]. For a recent review see [94].

42.4.6 Fatty Acid Chain-Elongation Disorders

As described above fatty acids either derived from dietary sources or synthesized de novo by the FAS complex, can be converted into a large variety of longer-chain FA either saturated, or mono-or polyunsaturated. This is achieved through the consecutive action of the chain-elongation system in conjunction with different desaturases which can introduce double bonds at specific positions. Humans only express Δ9, Δ6, and Δ5 desaturase activities and the enzymes involved belong to two distinct families referred to as stearoyl-CoA desaturases (SCD) and fatty acid desaturases (FADS). The different isoforms of SCD catalyze the introduction of double bonds at Δ9 positions [95] whereas the different FADS enzymes mediate desaturation at positions Δ6 and Δ5 [96].

42.4.7 Acetyl-CoA Carboxylase 1 Deficiency

Acetyl-CoA carboxylases are biotin-containing enzymes which catalyze the carboxylation of acetyl-CoA to malonyl-CoA which is the building block for the two different chain elongation systems in the cytosol (FASN) and

endoplasmic reticulum, respectively. Humans express two different acetyl-CoA carboxylases encoded by *ACACA* and *ACACB* (▶ Chap. 27) which exert different roles in human metabolism. The cytosolic enzyme ACC1 encoded by *ACACA* provides the malonyl-CoA units for FA chain elongation whereas the mitochondrial outer membrane protein ACC2 encoded by *ACACB* generates the malonyl-CoA to control fatty acid oxidation flux via the malonyl-CoA sensitive enzyme carnitine palmitoyltransferase 1 (CPT1). So far only a single case of ACC1 deficiency has been reported in 1981 [97]. In this patient ACC activity was markedly deficient both in liver and fibroblasts (2% and 10%, respectively). No molecular analyses were done however which leaves some doubt about the diagnosis in this patient.

42.4.8 ELOVL4 Deficiency

ELOVL4 deficiency is associated with different clinical phenotypes and is inherited in both an autosomal as well as autosomal dominant form. In juvenile-onset, autosomal dominant Stargardt macular dystrophy (STGD3) a series of mutations have been identified in the last exon of ELOVL4. The onset of loss of vision ranges from 3–50 years with a mean age of 14 years. Over decades the macular lesion enlarges and visual acuity decreases to 20/300 to 20/800. All the mutations identified cause a frame-shift that results in the introduction of a premature stop codon. Current evidence holds that haploinsufficiency is not the key mechanism of pathogenesis as believed originally but rather the dominant negative effect exerted by the mutant ELOVL4 protein [98]. Interestingly, bi-allelic mutations in *ELOVL4* have been identified in patients with a completely different phenotype characterized by ichthyosis, seizures, mental retardation, and spasticity, in many respects, reminiscent of Sjögren Larsson Syndrome, although the neurologic phenotype is more severe [99]. The clinical phenotype associated with ELOVL4 deficiency has been further expanded with autosomal dominant spinocerebellar ataxia and erythrokeratodermia in a French-Canadian family [100] and a neuro-ichthyotic disorder caused by bi-allelic mutations in *ELOVL4* in three affected patients from a consanguineous Pakistani family [101]. See [102] for recent review.

42.4.9 ELOVL5 Deficiency

ELOVL5 deficiency was first described in an Italian family with a pure form of spinocerebellar ataxia (SCA), which is a genetically heterogeneous group of autosomal dominant neurodegenerative disorders involving the cerebellum [103]. Following the identification of a single missense mutation in *ELOVL5* in this family, two additional unrelated families carrying the same p.Gly230Val variant were identified in the same cohort of 456 SCA patients. Haplotype analysis revealed that at least two of the three families shared a common ancestor. A more extensive analysis of the three families was published later [104]. Since ELOVL5 is involved in the synthesis of polyunsaturated fatty acids of the ω3 and ω6 series, which includes arachidonic acid (C20:4) and docosahexaenoic acid (C22:6), the levels of these fatty acids were measured in serum from affected patients which revealed reduced C20:4 and C22:6 levels amounting to 30–40% of control [103]. Based on these findings a double-blind, randomized placebo-controlled trial was performed in 10 ELOVL5 deficient patients supplemented with 600 mg of DHA per day for 16 weeks, followed by an open-label study with overall 40 weeks of DHA treatment. After 16 weeks of treatment, a mild but significant improvement of cerebellar functions was noted which persisted over 40 weeks [105] and 104 weeks [106] of treatment. Remarkably, no differences in serum DHA levels were found before and after 40 weeks of DHA treatment.

42.4.10 ELOVL1 Deficiency

ELOVL1 deficiency has only been described in two apparently unrelated patients showing a similar phenotype characterized by pronounced ichthyotic keratoderma, spasticity, dysmorphic features and early onset neurological disease with mild hypomyelination [107]. Both carried the same heterozygous mutation in *ELOVL1* which leads to an amino acid substitution at position 165. Enzymatic studies revealed that the p.Ser165Phe variant was catalytically inactive and subsequent lipid analyses showed reduced levels of the C26-ceramide and sphingomyelin species in fibroblasts and increased C20- and C22-sphingomyelins [108]. An ELOV 1 homozygous mutation have been recently described in 2 siblings presenting with a severe hypomyelinating spastic dyskinesia with facial dysmorphic features and ichthyosis [109].

42.4.11 Trans-2,3-Enoyl-CoA Reductase Deficiency

So far only a single report has appeared on trans-2,3-enoyl-CoA reductase (TER) deficiency which describes a consanguineous family with five young adults with non-syndromic mental retardation [110]. Three patients had an intention tremor and slow rapid finger movements. There were no resting tremors, signs of ataxia, or other abnormal movements. Exome sequencing revealed a single variant resulting in a mutant protein and an altered sphingolipid profile in the mutant cells characterized by reduced C24-ceramide and sphingomyelin levels [111].

42.4.12 3-Hydroxyacyl-CoA Dehydratase Deficiency

3-Hydroxyacyl-CoA dehydratase (HACD1) deficiency has also been described in a single family, with six affected individuals all showing a severe myopathic phenotype at birth that gradually improved over time [112]. Cognition was within normal limits. Homozygosity mapping followed by exome sequencing revealed a nonsense mutation in *HACD1*, which is mainly expressed in skeletal, muscle and heart tissues, whereas *HACD2* and *HACD3* show ubiquitous expression. The nonsense mutation (Y258ter) is presumed to lead to a full loss of enzyme activity. No lipid analyses have been performed.

42.4.13 MFSD2A Brain DHA Transporter Deficiency

Recently two consanguineous families each with two affected members with homozygous variants in *MFSD2A* were identified. Patients exhibited a lethal microcephaly syndrome linked to inadequate uptake of lysophosphatidylcholine (LPC) lipids and presented with microcephaly, developmental delay and intellectual disability, hypotonia, hyperreflexia, spastic quadriparesis, seizures and death within the first few years. Brain imaging showed gross hydrocephalus, effacement of the cortical surface, and cerebellar and brainstem hypoplasia/atrophy. Exome sequencing on both families identified two rare protein-altering homozygous variants in the MFSD2A gene [113]. LPCs are synthesized by the liver and circulate via albumin at levels equivalent to unesterified FA in plasma, and serve as the chemical carrier for DHA uptake via MFSD2A. Lipid analysis in the probands pointed to elevated serum LPC levels, likely as a result of failed cellular uptake due to the lack of MFSD2A activity. Because MFSD2A can transport both DHA as well as other long chain fatty acids in their lysoPC form, it remains unknown to which degree defects in transport of specific LPCs contribute to the human phenotype [113].

42.5 Disorders of Eicosanoid Metabolism

The eicosanoids constitute a large variety of biologically active molecules derived from arachidonic acid after its liberation from cellular membrane phospholipids by cytosolic phospholipases through three main pathways including the cyclooxygenase (COX), lipoxygenase (LOX) and cytochrome P450 monooxygenase pathways [114]. The COX pathway generates the different prostaglandins (PG) including PGE2, PGD2, PGF2α, PGI2, and the thromboxane TXA2 whereas the LOX-pathway generates the different hydroxyl-eicosatetraenoic acids (HETE) including 5-, 8-, 12-, and 15-HETE plus the leukotrienes LTA4 (instable), LTB4, LTC4, LTD4, and LTE4. Finally the CYP450 pathway produces a series of epoxy-eicosatrienoic acids (EET). So far, only a few defects in eicosanoid metabolism have been described (◘ Fig. 42.3).

42.5.1 Cytosolic Phospholipase A2∝ Deficiency

Cytosolic phospholipase A2∝ (cPLA2∝) deficiency was first described in a patient with multiple small intestinal ulcerations in the context of a severe bleeding disorder [115]. Urinary analysis revealed a systemic deficiency in eicosanoid production as concluded from the finding of marked reductions in the urinary levels of PGE2, PGD2, PGI2, and TxA2 and metabolites derived thereof. Ex vivo studies showed that the COX-1 mediated production of TxB2 and 12-lipoxygenase catalysed production of 12-HETE from platelets was virtually fully deficient. Furthermore, there was a marked platelet aggregation defect in response to both ADP and collagen which could be fully corrected upon addition of exogenous arachidonic acid. Taken together, these findings suggested a defect in one of the early steps of eicosanoid biosynthesis most likely at the level of cPLA2α. This conclusion was supported by the subsequent finding of bi-allelic mutations in *PLA2G4A* encoding cPLA2α and its marked enzyme deficiency as measured in platelet homogenates [115].

42.5.2 15-Hydroxyprostaglandin Dehydrogenase and Prostaglandin Transporter Deficiency Causing Primary Hypertrophic Osteoarthropathy (PHOAR)

Clinical Presentation

Prostaglandin E2 (PGE2) is degraded through two main steps which includes transport of PGE2 across the plasma membrane mediated by SLCO2A1, SLCO3A1, and SLCO4A1 after which PGE2 is degraded by the enzyme 15-hydroxyprostaglandin dehydrogenase (15-PGDH) to produce PGEM which is then excreted into the urine. Mutations in *HPGD* encoding the enzyme 15-PGDH, were first identified in patients affected by hypertrophic osteoarthropathy (PHO), a disorder primarily affecting the skin and bones which occurs either in a familial, primary form (PHO), or, more commonly, secondary to other pathologies in association with chronic hypoxia in bronchopulmonary or cardiovascu-

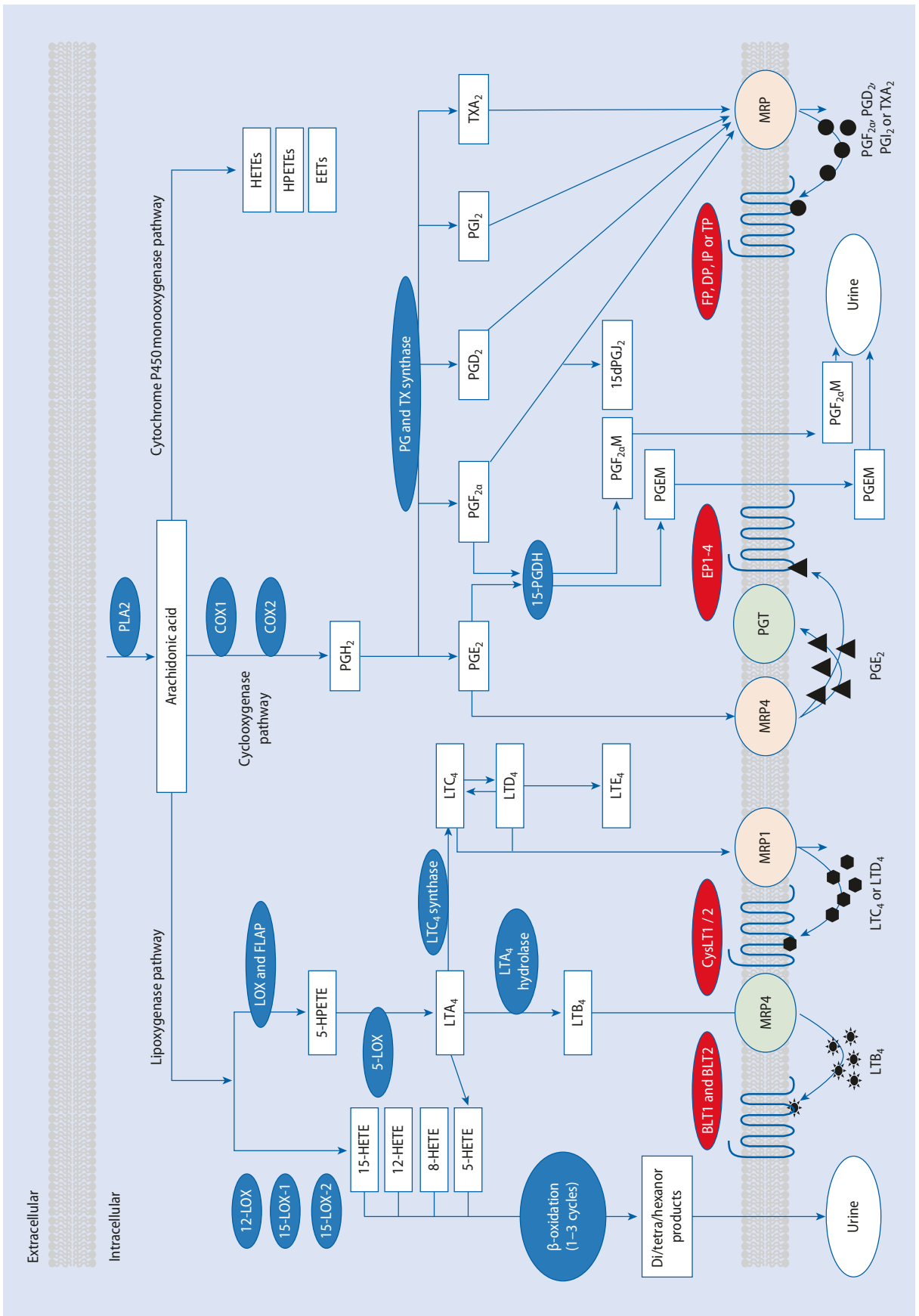

Fig. 42.3 Eicosanoid Metabolism

lar disease, and systemic inflammatory or neoplastic processes. In 2012 the identification of mutations in SLCO2A1 in PHO patients negative for mutations in *HPGD* was reported [116–118]. The two genetic forms of PHO are named PHOAR1 and PHOAR2, respectively. Key features of PHO include digital clubbing, periostosis with bone and joint enlargement, and skin changes such as pachydermia, abnormal furrowing, seborrhoea, and hyperhidrosis. Distinct developmental abnormalities have been reported in some PHOAR patients such as wide cranial sutures, Wormian bodies, and patent ductus arteriosus. In adults, when all major clinical symptoms are present, PHO is relatively easy to diagnose. However, the identification of patients with incomplete phenotypes may render correct diagnosis of patients much more difficult particularly in children.

Metabolic Derangement

15-PGDH and the plasma membrane prostaglandin transporter SLCO2A1 are both involved in the degradation of PGE2 by catalysing the oxidation of PGE2 into 13,14-dihydro-15–keto-PGE2 (PGEM) and providing PGE2 to the enzyme 15-PGDH, respectively.

Genetics

Many, often private mutations in *HGPD* and *SLCO2A1* have been described. Although the clinical signs and symptoms associated with the two genetic forms of PHOAR are in general very comparable, the sex ratio as well as age of onset may differ markedly. Indeed, whereas mutations in *HPGD* have been described in both males and females at about equal frequency, mutations in the autosomal recessive gene *SLCO2A1* have been reported in males only, a conclusion supported by a recent study in a large cohort of patients [119].

Diagnostic Tests

Diagnosis of PHO can be done via urinary analysis of prostaglandins. In both genetic forms PGE2 levels are usually elevated in urine whereas the PGE2 metabolite PGEM, which is the product of the 15-PGDH reaction, is usually elevated in SLCO2A1 patients but very low to undetectable in 15-PGDH deficient patients. Although measurement of PGE2 and PGEM in urine is extremely helpful for the diagnosis, urinary levels of PGE2 and PGEM may also be completely normal particularly in older patients since levels tend to decrease with age and may even normalize. For this reason molecular analysis is often advocated as the primary diagnostic method.

Treatment and Prognosis

Treatment options have been focused on the reduction of PGE2 levels by inhibiting its formation which may include the use of etoricoxib, a selective COX-2 inhibitor [120].

42.5.3 Leukotriene C4 Synthase (LTC4) Deficiency

Only a single patient with LTC4 deficiency has been described [121]. The patient had hypotonia, psychomotor retardation, failure to thrive, and microcephaly. The disease was rapidly progressive and the infant died at six months of age. In plasma and cerebrospinal fluid elevated levels of LTB4 were found whereas the levels of LTC4, LTD4, and LTE4 were below the detection limit. Furthermore, formation of LCT4 in monocytes of the patient turned out to be fully deficient. Unfortunately, no follow-up studies have been performed due to the lack of material from the patient which implies that the true genetic defect has not been identified despite the fact that human LCT4 synthase has been identified at the protein and molecular level [122].

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Congenital Disorders of Glycosylation, Dolichol and Glycosylphosphatidylinositol Metabolism

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Contents

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Synthesis of N-Glycans

This complex synthesis proceeds in three stages, schematically represented in ◘ Fig. 43.1.

1. Formation in the cytosol of nucleotide-linked sugars, mainly guanosine diphosphate-mannose (GDP-Man), and also uridine diphosphate glucose (UDP-Glc) and UDP-*N*-acetylglucosamine (UDP-GlcNAc), followed by attachment of GlcNAc and Man units to dolichol phosphate, and flipping (indicated by circular arrows) of the nascent oligosaccharide structure into the endoplasmic reticulum (ER).
2. Stepwise assembly in the ER, by further addition of Man and Glu, of the 14-unit oligosaccharide precursor, dolichol pyrophosphate-*N*-acetylglucosamine$_2$-mannose$_9$-glucose$_3$.
3. Transfer of this precursor onto the nascent protein, followed by final processing of the glycan in the Golgi apparatus by trimming and attachment of various sugar units.

Introduction

Numerous proteins are glycosylated with monosaccharides and/or oligosaccharide structures, also termed glycans, attached to the polypeptide chain. Most extracellular proteins, such as serum proteins (transferrin, clotting factors …..), most membrane proteins and several intracellular proteins (such as lysosomal enzymes) are glycoproteins. The glycans are defined by their linkage to the protein: N-glycans are linked to the amide group of asparagine, and O-glycans are linked to the hydroxyl group of serine or threonine. Synthesis of *N*-glycans, schematically represented in ◘ Fig. 43.1, proceeds in three stages: formation of nucleotide-linked sugars, assembly, and processing. Synthesis of *O*-glycans involves assembly but no processing and occurs mainly in the Golgi apparatus. It forms a diversity of structures, such as O-xylosylglycans, O-N-acetylgalactosaminylglycans, O-mannosylglycans, O-fucosylglycans and O-glucosylglycans. Besides protein glycosylation, lipid glycosylation also exists, e.g. glycosylation of ceramide, which is essential for the biosynthesis of gangliosides. Finally, glycosylphosphatidylinositol anchors are glycolipids that tether more than 150 proteins to the outer leaflet of plasma membranes and contain a glycan core with one glucosamine and three mannoses.

Congenital disorders of glycosylation (CDG), first reported in 1980 [1], are due to defects in the synthesis of glycans and in the attachment of glycans to proteins and lipids. It is a rapidly growing disease family, as the number of known CDG has increased by about 50% (from 91 to 137) since the previous edition of this book. Thirty one disorders of N-glycosylation have been identified (◘ Table 43.1).

Thirty four disorders of O-glycosylation have been identified (◘ Table 43.2).

A third group are the defects in glycosphingolipid and glycosylphosphatidylinositol anchor glycosylation, with 25 disorders (◘ Table 43.3).

The fourth group comprises defects in multiple glycosylation pathways and in dolicholphosphate synthesis (47 disorders; ◘ Table 43.4).

In addition there are also congenital disorders of deglycosylation: the lysosomal disorders due to an enzymatic defect, and NGLY1 deficiency, due to defective N-glycanase, a cytoplasmic enzyme.

In 2008, a novel nomenclature for CDG was introduced that uses the official symbol of the defective gene (not in italics), followed by '-CDG' [2] (list of approved gene names at ► www.genenames.org). Descriptive names such as hereditary multiple exostoses and familial tumoral calcinosis may continue to be used alongside the novel designations. Only the novel nomenclature is used in this text.

Patients with CDG show a very broad spectrum of clinical manifestations. A number of them have at least one typical/characteristic sign/symptom (see ◘ Table 43.5).

CDG should be considered in any unexplained clinical condition, particularly in multi-organ disease with neurological involvement but also when non-specific developmental disability is the only presenting sign. Isoelectrofocusing (IEF) of serum transferrin is still the screening method of choice, but it is important to realize that it is able to detect only a limited number of CDG, namely *N*-glycosylation disorders associated with sialic acid deficiency [3]. The (partial) deficiency of sialic acid in these forms of CDG causes one of two main types of cathodal shift. A type 1 pattern indicates an assembly disorder, and PMM2-CDG or MPI-CDG (depending on the clinical presentation) should be considered first. If these are excluded, the next step could be dolichol-linked glycan analysis, or direct mutation analysis of a panel of genes known to be involved in CDG, or whole exome/genome sequencing (WES, WGS). A type 2 pattern indicates a disorder of processing. Protein-linked glycan analysis should next be performed in an attempt to identify the defective step, or CDG gene panel analysis or WES/WGS. In addition, IEF of serum apolipoprotein C-III (which is only O-glycosylated) can detect some *O*-glycosylation disorders [4].

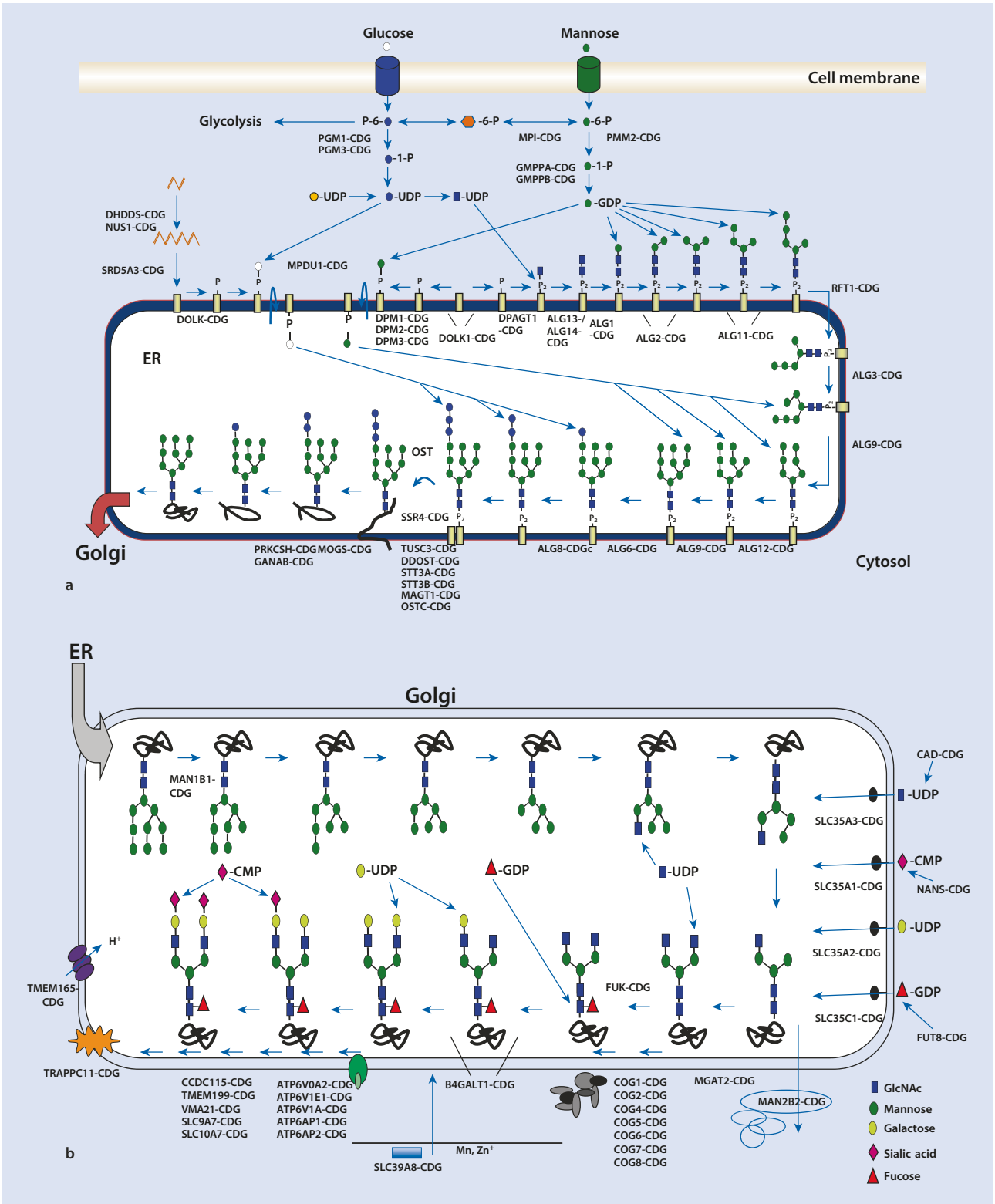

Fig. 43.1 *N*-glycans: **a** schematic representation of the assembly of *N*-glycans in the cytosol and the endoplasmic reticulum (ER). GDP guanosine diphosphate, UDP uridine diphosphate, OST oligosaccharyltransferase. **b** Schematic representation of the remodeling of N-glycans in the Golgi. CMP cytidine monophosphate

Table 43.1 Protein N-glycosylation disorders

Name	Main clinically affected organs and systems	Defective protein
MPI-CDG	Intestine, liver	Mannosephosphate isomerase
PMM2-CDG	Nervous system, fat tissue, and nearly all other organs	Phosphomannomutase 2
GMPPA-CDG	Autonomic nerve fibers of distal oesophagus (achalasia) and lacrimal glands (alacrimia), neurons (brain, hearing system, visual system)	Guanosine diphosphate mannose pyrophosphorylase A
GMPPB-CDG	Brain, skeletal muscles, eyes, heart	Guanosine diphosphate mannose pyrophosphorylase B
ALG13-CDG	Brain, eyes, liver	UDP-GlcNAc:Dol-P-GlcNAc-P transferase
ALG14-CDG	Neuromuscular junction (congenital myasthenic syndrome)	UDP-GlcNAc:Dol-PP-GlcNAc transferase
ALG6-CDG	Brain, and variable involvement of eyes, gastrointestinal system, liver, heart and skeleton	Glucosyltransferase 1
ALG3-CDG	Brain, skeleton	Mannosyltransferase 6
ALG12-CDG	Brain, skeleton, heart, genitalia and immune system	Mannosyltransferase 8
ALG8-CDG	Brain, and variable involvement of eyes, skin, liver and intestine	Glucosyltransferase 2
ALG2-CDG	Brain, eyes, skeletal muscles, neuromuscular junction (congenital myasthenic syndrome)	Mannosyltransferase 2
DPAGT1-CDG	Brain, neuromuscular junction (congenital myasthenic syndrome)	UDP-GlcNAc: Dol-P-GlcNAc-P transferase
ALG1-CDG	Brain, and variable involvement of eyes, heart, liver, beta cells, kidneys, gonads	Mannosyltransferase 1
ALG9-CDG	Brain, liver, kidneys, and variable involvement of adipose tissue, heart, skeleton, intestine	Mannosyltransferase 7/9
ALG11-CDG	Brain, hearing system	Mannosyltransferase 4/5
RFT1-CDG	Brain, hearing system	Flippase of $Man_5GlcNAc_2$-PP-Dol
MGAT2-CDG	Brain, skeleton, intestine, immune system	*N*-Acetylglucosaminyltransferase 2
TUSC3-CDG	Brain (non-syndromic autosomal recessive mental disability)	Oligosaccharyltransferase subunit TUSC3
MAGT1-CDG	Brain, immune system	Oligosaccharyltransferase subunit MAGT1 (magnesium transporter 1)
DDOST-CDG	Brain, eyes, liver	Oligosaccharyltransferase subunit DDOST
STT3A-CDG	Brain, gastrointestinal tract	Oligosaccharyltransferase subunit STT3A
STT3B-CDG	Brain, optic nerve, gastrointestinal tract	Oligosaccharyltransferase subunit STT3B
SSR3-CDG	Brain, lungs, gastrointestinal system	Brain, lungs, gastrointestinal system
SSR4-CDG	Brain, respiratory system, skeleton	Signal sequence receptor 4 of TRAP complex
MOGS-CDG	Brain, skeleton, immune system	Mannosyl-oligosaccharide glycosidase (glucosidase 1)

(continued)

Table 43.1 (continued)

Name	Main clinically affected organs and systems	Defective protein
MAN1B1-CDG	Brain, cranial skeleton, fat tissue	Golgi α1-2 mannosidase 1
MAN2B2-CDG	Brain, immune system	Alpha-1,6-mannosidase
GANAB-CDG	Kidneys, liver	Glucosidase II, alpha subunit
JAGN1-CDG	Neutrophils	Jagunal homolog 1
PRKCSH-CDG	Liver, kidneys	Glucosidase II, beta subunit
FUT8-CDG	Brain, skeleton	Fucosyltransferase 8

Table 43.2 Protein *O*-glycosylation disorders

Name	Main clinically affected organs and systems	Defective protein
Defects in O-xylosylglycan synthesis		
XYLT1-CDG	Brain, skeleton (short stature, advanced bone age), articulations (joint laxity), fat	Xylosyltransferase 1
XYLT2-CDG	Brain, eyes, heart, hearing system, bones	Xylosyltransferase 2
B4GALT7-CDG	Brain, skeleton (short stature, bowing of extremities), articulations (hyperlaxity, dislocations), skin (premature aging phenotype)	B-1,4-galactosyltransferase 7
B3GALT6-CDG	Skeleton (spondyloepimetaphyseal dysplasia with bone fragility, severe kyphoscoliosis), joints, skin (fragility, delayed wound healing)	B-1,3-galactosyltransferase 6
B3GAT3-CDG	Brain, aorta, heart, skeleton, joints, skin, teeth	B-1,3-glucuronyltransferase 3
EXT1-CDG (multiple cartilaginous exostoses)	Cartilage (osteochondromas of the ends of long bones)	Exostosin 1
EXT2-CDG (multiple cartilaginous exostoses)	Cartilage (osteochondromas of the ends of long bones)	Exostosin 2
EXTL3-CDG	Brain, skeleton, immune system	Exostosin-like glycosyltransferase 3
CSGALNACT1-CDG	Brain, skeleton	Chondroitin sulfate N-acetylgalactosaminyltransferase 1
CHSY1-CDG (Tentamy preaxial brachydactyly syndrome)	Brain, teeth, skeleton (particularly brachydactyly), hearing system	Chondroitin β-1,4-N-acetylgalactosaminyltransferase 1 (chondroitin synthase 1)
CANT1-CDG	Skeleton	Calcium-activated nucleotidase 1
DSE-CDG	Brain, skeleton, skin, heart	Dermatan sulfate epimerase
Defect in O-N-acetylglucosaminylglycan synthesis		
EOGT-CDG	Skin (aplasia cutis congenita), skeleton (terminal transverse limb defect)	EGF domain-specific O-GlcNAc transferase

Table 43.2 (continued)

Name	Main clinically affected organs and systems	Defective protein
OGT-CDG	Brain, skeleton, eyes	O-linked N-acetylglucosamine transferase
Defect in O-N-acetylgalactosaminalglycan synthesis		
C1GALT1C1-CDG	Erythrocytes, leukocytes, platelets	Core 1 beta-3-galactosyltransferase-specific chaperone
GALNT2-CDG	Brain, skeleton	UDP-N-acetyl-α-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase 2
GALNT3-CDG (familial hyperphosphatemic tumoral calcinosis)	Subcutaneous tissue (painful calcified masses)	UDP-N-acetyl-α-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase 3
Defect in O-xylosyl/N-acetylgalactosaminylglycan synthesis		
SLC35D1-CDG (Schnecken-becken dysplasia)	Skeleton (generalized; radiographic snail-like configuration of iliac bones) (stillborn or lethal in the neonatal period)	Solute carrier family 35 (UDP-glucuronic acid/ UDP-N-acetylgalactosamine dual transporter) member D1
Defects in O-mannosylglycan synthesis		
POMT1-CDG (cerebro-ocular dysplasia-muscular dystrophy syndrome)	Brain, eyes, skeletal muscles, heart	Protein O-mannosyltransferase 1
POMT2-CDG (cerebro-ocular dysplasia-muscular dystrophy syndrome)	Brain, eyes, skeletal muscles	Protein O-mannosyltransferase 2
POMGNT1-CDG (muscle-eye-brain disease)	Brain, eyes, skeletal muscles	Protein O-mannose β-1,2-N-acetylglucosaminyltransferase 1
POMGNT2-CDG	Brain, eyes, skeletal muscles	Protein O-mannose β-1,2-N-acetylglucosaminyltransferase 2
POMK-CDG	Brain, eyes, skeletal muscles	Protein-O-mannose kinase
B3GALNT2-CDG	Brain, eyes, skeletal muscles	Beta-1,3-N-acetylgalactosaminyltransferase 2
B4GAT1-CDG	Brain, eyes, skeletal muscles, urogenital system	Beta-1,4-glucuronyltransferase 1
FKTN-CDG	Brain, eyes, skeletal muscles, heart	Fukutin
FKRP-CDG	Brain, eyes, skeletal muscles, heart, skeleton	Fukutin-related protein
LARGE1-CDG	Brain, eyes, skeletal muscles	Acetylglucosaminyltransferase-like protein
CRPPA-CDG	Brain, eyes, skeletal muscles	Isoprenoid synthase domain-containing protein
RXYLT1-CDG	Brain, eyes, skeletal muscles, gonads	Ribitol xylosyltransferase 1
Defects in O-fucosylglycan synthesis		
POFUT1-CDG	Skin (progressive reticular hyper- and hypopigmentation)	Protein O-fucosyltransferase 1
LFNG-CDG (spondylocostal dysostosis type 3)	Axial skeleton, associated muscles	O-fucose-specific β-1,3-N-acetylglucosaminyltransferase
B3GLCT-CDG	Eyes (anterior eye chamber abnormalities), skeleton (short stature, cleft palate), and variable involvement of other organs	O-fucose-specific β-1,3-glucosyltransferase
Defect in O-glucosylglycan synthesis		
POGLUT1-CDG	Skin (progressive reticular hyper- and hypopigmentation)	Protein O-glucosyltransferase 1

Table 43.3 Defects of lipid glycosylation and of glycosylphosphatidylinositol anchor synthesis

Name	Main clinically affected organs and systems	Defective protein
Defects in lipid glycosylation		
ST3GAL5-CDG (Amish infantile epilepsy; salt and pepper syndrome)	Brain, hearing system, skin	Lactosylceramide α-2,3-sialyltransferase (GM3 synthase)
B4GALNT1-CDG (spastic paraplegia 26, autosomal recessive)	Brain, peripheral nerves (spastic paraplegia), gonads	B-1,4-N-acetylgalactosaminyltransferase 1 (GM2 synthase)
A4GALT-CDG	Erythrocytes	Alpha-1,4-galactosyltransferase
Defects in glycosylphosphatidylinositol anchor synthesis		
GPAA1-CDG	Brain, skeleton	Glycosylphosphatidylinositol anchor attachment protein 1
PIGA-CDG	Brain, heart, liver, kidneys, skin	UDP-GlcNAc:phosphatidylinositol N-acetylglucosaminyltransferase subunit
PIGB-CDG	Brain, eyes, skeleton, hearing system	Phosphatidylinositol glycan anchor biosynthesis class B protein
PIGC-CDG	Brain	Phosphatidylinositol glycan anchor biosynthesis class C protein
PIGG-CDG	Brain	Phosphatidylinositol glycan anchor biosynthesis class G protein
PIGH-CDG	Brain	Phosphatidylinositol glycan anchor biosynthesis class H protein
PIGK-CDG	Brain, skeleton, teeth	Phosphatidylinositol glycan anchor biosynthesis class K protein
PIGL-CDG (CHIME syndrome)	Brain, eyes, hearing system, heart, skin	GlcNAc-phosphatidylinositol de-acetylase
PIGM-CDG	Brain, hepatic veins	Dol-P-Man:phosphatidylinositol mannosyltransferase 1
PIGN-CDG	Brain, skeleton (including palate, fingers), cardiovascular system, kidneys	Glycosylphosphatidylinositol ethanol-amine phosphate transferase 1
PIGO-CDG	Brain, lips, fingers, toes, anus/rectum, hearing system, cardiovascular system	Glycosylphosphatidylinositol ethanolami-nephosphate transferase 3
PIGP-CDG	Brain	Phosphatidylinositol glycan anchor biosynthesis class P protein
PIGQ-CDG	Brain	UDP-GlcNAc:phosphatidylinositol N-acetylglucosaminyltransferase subunit
PIGS-CDG	Brain, skeleton, hearing system	Phosphatidylinositol glycan anchor biosynthesis class S protein
PIGT-CDG	Brain, eyes, heart, kidneys, skeleton	PIGT transamidase subunit
PIGU-CDG	Brain, eyes, skeleton	Phosphatidylinositol glycan anchor biosynthesis class U protein
PIGV-CDG	Brain, fingers, toes, and less frequent involvement of lips, palate, anus/rectum, hearing system	Dol-P-Man:phosphatidylinositol mannosyltransferase 2
PIGW-CDG	Brain, skeleton	Phosphatidylinositol acylase
PIGY-CDG	Brain, eyes, skeleton, muscles	Phosphatidylinositol glycan anchor biosynthesis class Y protein
PGAP1-CDG	Brain	Phosphatidylinositol deacylase
PGAP2-CDG	Brain	Phosphatidylinositol glycerol acylase
PGAP3-CDG	Brain, skeleton	Phosphatidylinositol glycerol deacylase

Table 43.4 Defects in multiple and other glycosylation pathways including dolichol metabolism defects

Defects in dolichol synthesis		
DHDDS-CDG (retinitis pigmentosa 59)	Retina	Dehydrodolichyl diphosphate
NUS1-CDG	Brain, eyes, skeleton	Nogo-B receptor (subunit of cis-prenyltransferase)
SRD5A3-CDG	Brain, eyes, heart, skin, joints	Steroid 5 α-reductase 3
DOLK-CDG	Brain, heart, skin	Dolichol kinase
Defects in dolichol utilization/recycling		
DPM1-CDG	Brain, eyes, skeletal muscles	GDP-Man:Dol-P-mannosyltransferase 1 (Dol-P-Man synthase 1)
DPM2-CDG	Brain, skeletal muscles	GDP-Man:Dol-P-mannosyltransferase 2 (Dol-P-Man synthase 2)
DPM3-CDG	Skeletal and cardiac muscles	GDP-Man:Dol-P-mannosyltransferase 3 (Dol-P-Man synthase 3)
MPDU1-CDG	Brain, eyes, skin	Man-P-Dol utilization 1
Defects in monosaccharide synthesis and interconversion		
GFPT1-CDG (limb girdle congenital myasthenic syndrome)	Neuromuscular junction, skeletal muscles	Glutamine:fructose 6-phosphate aminotransferase 1
PGM1-CDG	Skeletal muscles, skeleton, heart, liver	Phosphoglucomutase 1
PGM3-CDG	Brain, immune system, skeleton	Phosphoglucomutase 3
GNE-CDG (hereditary inclusion body myopathy)	Skeletal muscles (with sparing of quadriceps muscles), rarely cardiac muscles	UDP-GlcNAc 2-epimerase/Man-NAc kinase
FCSK-CDG	Brain, eyes, gastrointestinal system	Fucose kinase
G6PC3-CDG	Blood cells, immune system, skeleton, skin, cardiovascular system	Glucose-6-phosphatase, catalytic, 3
NANS-CDG	Brain, skeleton, hair	N-acetylneuraminic acid phosphate synthase
Defects in glycosyltransferases		
B4GALT1-CDG	Skeleton, eyes	B-1,4-galactosyltransferase
ST3GAL3-CDG	Brain	B-galactoside α-2,3-sialyltransferase 3
Defect in nucleotide-sugar synthesis *(see ► Chap. 32)* [48]		
CAD-CDG	Brain, intestine, kidneys, erythrocytes	Carbamoylphosphate synthetase 2
Defects in nucleotide-sugar transporters		
SLC35A1-CDG	Brain, heart, kidneys, platelets	CMP-sialic acid transporter
SLC35A2-CDG	Brain, eyes, gastrointestinal system, skeleton	UDP-galactose transporter
SLC35A3-CDG	Brain, skeleton	UDP-GlcNAc transporter
SLC35C1-CDG	Brain, cranial skeleton, neutrophils	GDP-fucose transporter
Defects in vesicular trafficking		
COG1-CDG	Brain, skeleton	COG component 1
COG2-CDG	Brain, liver	COG component 2
COG4-CDG	Brain, face	COG component 4
COG5-CDG	Brain, hearing system, vision, liver, bladder	COG component 5

(continued)

Table 43.4 (continued)

COG6-CDG	Brain, gastrointestinal system including liver, immune system	COG component 6
COG7-CDG	Brain, skeleton, skin, gastrointestinal system including liver, heart	COG component 7
COG8-CDG	Brain, eyes, peripheral nervous system	COG component 8
GORAB-CDG	Skeleton, skin	Golgi, RAB6-interacting
GOSR2-CDG	Brain, peripheral nervous system	Golgi SNAP receptor complex member 2
SEC23B-CDG	Erythrocyte lineage (secondary involvement of heart, liver, beta cells)	COPII component SEC23B
TRAPPC11-CDG	Brain, eyes, skeletal muscles, oesophagus	Trafficking protein particle complex, subunit 11
TRIP11-CDG	Skeleton, teeth	Thyroid hormone receptor interactor 11
VPS13B-CDG	Brain, eyes, skeleton, neutrophils	Vacuolar protein sorting 13 homolog B
Defects in Golgi pH and ion homeostasis		
ATP6AP1-CDG	Brain, liver, immune system	Vacuolar ATPase subunit 1
ATP6AP2-CDG	Brain	Vacuolar ATPase subunit 2
ATP6V0A2-CDG (autosomal recessive cutis laxa type II; wrinkly skin syndrome)	Skin (cutis laxa becoming less obvious with age), brain (mental development mostly normal), eyes, neuromuscular system, skeleton	Vacuolar ATPase V0 subunit A2
ATP6V1A-CDG	Brain, eyes	Vacuolar proton pump, alpha subunit 1
ATP6V1E1-CDG	Brain, skeleton, heart, skin	Vacuolar ATPase V1 subunit E isoform 1
CCDC115-CDG	Brain, liver	Coiled-coil domain-containing protein 115
SLC9A7-CDG	Brain	Solute carrier family 9, member 7
SLC10A7-CDG	Skeleton, teeth	Solute carrier family 10, member 7
SLC39A8-CDG	Brain	Solute carrier family 39, member 8
TMEM165-CDG	Brain, skeleton (particularly cartilage), joints, heart, liver, kidneys	Transmembrane protein 165
TMEM199 -CDG	Liver	Transmembrane protein 199
VMA21-CDG	Skeletal muscles	Vacuolar ATPase assembly factor VMA 21

Since at least 1% of the human genome is involved in glycosylation, there is no doubt that the majority of CDG have still to be discovered. We predict that these will include diseases that are due to defects in organ-specific glycosylation. Also, there is no doubt that known diseases with unknown aetiology will continue to be identified as CDG.

Only the most frequently reported protein N-glycosylation disorders will be described in more detail in this chapter. The most frequently reported disorders from the other groups will be summarized. For recent general reviews on CDG see [5–15] and for specific organ involvement in CDG see [16–20].

Table 43.5 Characteristic/typical signs/symptoms (in a syndromatic context) of selected CDG (present in at least 50 % of the patients)

Genes	Signs/symptoms
Disorders of N-linked protein glycosylation	
ALG2	Myasthenia
ALG9	Polycystic kidney disease
ALG11	Sensorineural hearing loss
ALG14	Myasthenia
DPAGT1	Myasthenia
FUT8	Recurrent pneumonia
GANAB	Polycystic kidney disease
GMPPA	Alacrimia
MAGT1	Epstein-Barr virus infections
MGAT2	Radio-ulnar synostosis
MOGS	Typical tetrasaccharide in urine
PMM2	Fat pads
RFT1	Sensorineural hearing loss
Disorders of O-linked protein glycosylation	
B4GALT7	Premature aging
B3GLCT	Anterior eye change abnormalities
EOGT	Aplasia cutis congenita
EXT1/2	Multiple exostoses
GALNT3	Tumoral calcinosis
LFNG	Spondylocostal dysostosis
POFUT1	Progressive reticular hyper- and hypopigmentation
POGLUT1	Progressive reticular hyper- and hypopigmentation
SLC35D1	Lethal skeletal phenotype (perinatal)
XYLT1	Advanced carpal and tarsal bone age
Disorders of multiple and other glycosylation pathways	
ATP6V0A2	Cutis laxa
CAD	Anisocytosis/poikilocytosis
DHDDS	Retinitis pigmentosa
DOLK	Cardiomyopathy
GNE	Myopathy excepted quadriceps
G6PC3	Neutropenia
MPDU1	Hyperkeratosis
PGM1	Rhabdomyolysis
SEC23B	Hemolytic anemia
SLC10A7	Amelogenesis imperfecta

(continued)

Table 43.5 (continued)

Genes	Signs/symptoms
SLC35A1	Macrothrombocytopenia
SLC35A3	Arthrogryposis
SLC35C1	High neutrophilia
SLC39A8	Manganese deficiency
SRD5A3	Coloboma
TMEM165	Abnormal cartilage
VPS13B	Intermittent neutropenia

Congenital Disorders of Glycosylation: Main Phenotypes

Most CDG are complex multisystem disorders with prominent neurological manifestations.

Diverse degrees of cognitive impairment (from severe encephalopathies to mild intellectual disability) and associated neurological signs, particularly cerebellar/ponto-cerebellar involvement, are frequent. Some epileptic encephalopathies may improve with treatment: galactose in SLC35A8-CDG or vit. B_6 in PIGO-CDG. Dysmorphism (with or without a distinctive phenotype), thrombosis, retinopathy, cardiac and hepatic involvement are also major features.

- **Muscular diseases** may present as pure myopathic forms or complex diseases:
  - **Congenital myasthenic syndromes:** ALG2-CDG, ALG14-CDG, DPAGT1-CDG, GFPT1-CDG
  - **Hereditary inclusion body myopathy:** GNE-CDG
  - **Muscle-eye-brain syndromes:** B3GALNT2-CDG, CRPPA-CDG, FKRP-CDG, FKTN-CDG, LARGE-CDG, POMGNT1-CDG/POMGNT2-CDG, POMT1-CDG/POMT2-CDG, RXYLT-CDG
- **Liver involvement**:
  - **Gastro-hepatic phenotype** including protein-losing enteropathy and hypoglycemias (hyper/normoinsulinemic) without or with minor neurological involvement: MPI-CDG, PMM2-CDG
  - **Liver involvement with microcephaly, developmental/intellectual disability, recurrent infections:** COG6-CDG
  - **Liver involvement with growth deficiency, hypoglycemia, malformations (particularly cleft uvula, cleft palate) and cardiac involvement:** PGM1-CDG
  - **Chronic, non-progressive mild hepatic steatosis, elevated serum aminotransferases and alkaline phosphatase, hypercholesterolemia, and low serum ceruloplasmin:** TMEM199-CDG
  - **Polycystic liver disease with or without polycystic kidney disease**: GANAB-CDG, PRKCSH-CDG, PMM2-CDG due to promotor defect
- **Ocular involvement**:
  - **Muscle-eye-brain syndromes: see above**
  - **Peters-plus syndrome:** anterior eye chamber abnormalities including Peters' anomaly (corneal clouding and iridolenticulocorneal adhesions), growth retardation, brachydactyly, intellectual disability and other symptoms: B3GLCT-CDG
  - **Nystagmus, coloboma, optic atrophy, neurological symptoms and skin abnormalities:** SRD5A3-CDG
- **Bone and connective tissue involvement**:
  - **Progeroid variant of Ehlers-Danlos syndrome**:B4GALT7
  - **Hyperphosphatemic syndromes:** hyperphosphatemic familial tumoral calcinosis (HFTC) or hyperphosphatemic hyperostosis syndrome (HHS): GALNT3-CDG
  - **Hereditary multiple exostoses**: EXT1/EXT2-CDG
  - **Cutis laxa type II:** ATP6V0A2-CDG (type IIA), ATP6V1A (type IID), ATP6V1E1 (type IIC)
  - **Spondylocostal dysostosis type 3:** LFNG-CDG
  - **Schneckenbecken dysplasia:** SLC35D1-CDG
- **Haematological involvement**:
  - **Congenital dyserythropoietic anemia type II:** SEC23B-CDG

Specific clinical signs are listed in Table 43.5

43.1 Congenital Disorders of Protein N-Glycosylation

Table 43.1

43.1.1 Phosphomannomutase 2 Deficiency (PMM2-CDG)

Clinical Presentation

PMM2-CDG is by far the most frequent CDG, with at least 900 patients known worldwide. The symptomatology can often be recognised shortly after birth. The nervous system is affected in all patients, and most other organs are involved in a variable way. The neurological picture comprises alternating internal strabismus and other abnormal eye movements, axial hypotonia, psychomotor disability, ataxia and hyporeflexia. After infancy, symptoms include retinitis pigmentosa, often stroke-like episodes and sometimes epilepsy. As a rule there is no regression. During the first year(s) of life, there are variable feeding problems (anorexia, vomiting, diarrhoea) that can result in severe failure to thrive. Other features are a variable dysmorphism, which may include a cherubs face, large, hypoplastic/dysplastic ears, abnormal subcutaneous adipose tissue distribution (fat pads, orange peel skin), inverted nipples and mild to moderate hepatomegaly, skeletal abnormalities and hypogonadism. Some infants develop a pericardial effusion and/or cardiomyopathy. At the other end of the clinical spectrum are patients with a very mild phenotype (no dysmorphic features, slight intellectual disability). Patients often have an extraverted and happy appearance. Neurological investigations reveal (olivoponto)-cerebellar hypoplasia, variable cerebral hypoplasia, and peripheral neuropathy (both axonal and demyelinating). Liver pathology is characterised by fibrosis and steatosis, and electron microscopy shows myelin-like lysosomal inclusions in hepatocytes but not in Kupffer cells. A special presentation is hyperinsulinemic hypoglycemia associated with congenital polycystic kidney disease without the typical clinical and diagnostic features of PMM2-CDG. It is due to a promotor variant (c.-167G>T) either homozygous or *in trans* with *PMM2* coding variants [21].

Metabolic Derangement

Phosphomannomutase (PMM) catalyses the second committed step in the synthesis of guanosine diphosphate (GDP) mannose, namely the conversion of mannose-6-phosphate to mannose-1-phosphate, which occurs in the cytosol (Fig. 43.1a). PMM2-CDG is due to the deficiency of PMM2, the principal isozyme of PMM. Since GDP-mannose is the donor of the mannose units used in the ER to assemble the dolichol-pyrophosphate oligosaccharide precursor, the defect causes hypoglycosylation, and hence deficiency and/or dysfunction of numerous glycoproteins, including serum proteins (such as thyroxin-binding globulin, haptoglobin, clotting factor XI, antithrombin, cholinesterase etc.), lysosomal enzymes and membranous glycoproteins.

Genetics

PMM2 deficiency is inherited as an autosomal-recessive trait caused by mutations of *PMM2*. At least 139 pathogenic and likely pathogenic variants (mainly missense) have been identified (► www.lovd.nl/PMM2). The most frequent mutation (c.422G>A) causes an R141H substitution and is present in 75 % of patients of Caucasian origin. This mutation is not compatible with life in the homozygous state. Its frequency in the Belgian population is as high as 1 in 50. The incidence of PMM2 deficiency is not known; in Sweden it has been estimated at 1 in 40,000.

Prenatal testing should only be offered in families with a documented PMM2 deficiency and variants in *PMM2*. It cannot be performed by any assay that determines the glycosylation of proteins, since this has been found to be normal in the foetus.

Diagnostic Tests

The diagnosis of congenital disorders of *N*-glycosylation in general (and of PMM2 deficiency in particular) is usually made by IEF and immunofixation of serum transferrin. Normal serum transferrin is mainly composed of tetrasialotransferrin and small amounts of mono-, di-, tri-, penta- and hexasialotransferrins. The partial deficiency of sialic acid (a negatively charged and end-standing sugar) in CDG causes a cathodal shift. Two main types of cathodal shift can be recognized. Type 1 is characterized by an increase of both disialo- and asialotransferrin and a decrease of tetra-, penta- and hexasialotransferrins; in type 2 there is also an increase of the tri- and/or monosialotransferrin bands. In PMM2 deficiency, a type 1 pattern is found. A type 1 pattern is also seen in secondary glycosylation disorders such as chronic alcoholism, hereditary fructose intolerance and galactosaemia. A shift due to a transferrin protein variant has first to be excluded (by IEF after neuraminidase treatment, study of another glycoprotein and/or investigation of the parents). The carbohydrate-deficient transferrin (CDT) assay is also useful for the diagnosis of sialic acid-deficient CDG. It quantifies the total sialic acid-deficient serum transferrin. A drawback is a not negligible number of false-positive results. Capillary zone electrophoresis of total serum is an interesting, rapid screening test. An abnormal result should be further investigated by serum transferrin IEF.

In addition to the above-mentioned serum glycoprotein abnormalities, laboratory findings include elevation

of serum transaminase levels, hypoalbuminaemia, hypocholesterolaemia, and tubular proteinuria. To confirm the diagnosis, the activity of PMM should be measured in leukocytes or fibroblasts. Note that the PMM activity in fibroblasts can be normal. Therefore, in case of a typical PMM2-CDG presentation and a normal PMM activity in fibroblasts, mutation analysis of *PMM2* is indicated.

Treatment and Prognosis

No effective treatment is available. The promising finding that mannose is able to correct glycosylation in fibroblasts with PMM2 deficiency could not be substantiated in patients. Recently, the first clinical trial was performed in this CDG, namely with acetazolamide. It was well tolerated and effective regarding the motor cerebellar syndrome. There is a substantially increased mortality (~20 %) in the first years of life, due to severe infection or vital organ involvement (liver, cardiac or renal insufficiency). Most survivors attain adulthood. Among the typical problems of this age group are ovarian insufficiency, growth retardation, thrombotic events, osteopenia/osteoporosis and skeletal deformities. In some patients the neurological phenotype is so mild that the diagnosis is only made in adulthood [22].

43.1.2 Mannose-Phosphate-Isomerase Deficiency (MPI-CDG)

Clinical Presentation

At least 35 patients have been described. Most have presented with hepatic-intestinal disease without notable dysmorphism, and without or with only minor neurological involvement. Symptoms started between the ages of 1 and 11 months. One patient had recurrent vomiting and liver disease that disappeared after the introduction of solid food at the age of 3 months. Two untreated, healthy adults have been reported. The classical phenotype consists of various combinations of recurrent vomiting, abdominal pain, protein-losing enteropathy, recurrent thromboses, gastrointestinal bleeding, liver disease and symptoms of (hyperinsulinaemic or normoinsulinaemic) hypoglycaemia. In 1985, four infants from Quebec were reported with a similar syndrome (Saguenay-Lac Saint-Jean syndrome) and retrospectively shown to have the same disease.

Metabolic Derangement

Mannose-phosphate isomerase (MPI) catalyses the first committed step in the synthesis of GDP-mannose, namely the conversion of fructose-6-phosphate to mannose-6-phosphate (Fig. 43.1a). Hence the blood biochemical abnormalities are indistinguishable from those found in PMM2 deficiency. Since the substrate of MPI, fructose-6-phosphate, is efficiently metabolized in the glycolytic pathway, it does not accumulate intracellularly.

Genetics

Inheritance of MPI deficiency is autosomal recessive. Sixty four pathogenic and likely pathogenic variants have been identified, including 36 missense variants.

Diagnostic Tests

Serum transferrin IEF shows a type 1 pattern. The diagnosis is confirmed by finding a decreased activity of MPI in leukocytes or fibroblasts and/or (a) pathogenic variant(s) in *MPI*.

Treatment and Prognosis

MPI deficiency is still the only known CDG that can be effectively treated. It should be noted that for a few other CDG there is a 'partial' treatment (e.g. fucose for SLC35C1-CDG and galactose for PGM1-CDG). Mannose is the therapeutic agent because hexokinases phosphorylate mannose to mannose 6-phosphate, thus bypassing the defect. An oral dose of 1 g mannose/kg body weight per day (divided into four to six doses) is usually effective. The clinical symptoms usually disappear rapidly but it takes several months before the transferrin IEF pattern improves significantly. However, in many patients this treatment does not control the liver disease. One patient was unable to tolerate mannose. Treatment with heparin led to temporary improvement, necessitating liver transplantation [23].

43.1.3 Glucosyltransferase 1 Deficiency (ALG6-CDG)

Clinical Presentation

ALG6-CDG is the second most common *N*-glycosylation disorder, with at least 89 patients identified. Clinical features in common with PMM2-CDG are developmental disability, hypotonia, ataxia, seizures, strabismus, nystagmus, and failure to thrive. A substantial number of patients showed skeletal abnormalities (brachydactyly, arachnodactyly, short arms, scoliosis), behavioural problems and nonspecific facial dysmorphism. There was usually no retinitis pigmentosa or cerebellar hypoplasia. A few patients have had protein-losing enteropathy, a consistent feature in MPI-CDG and ALG8-CDG.

Metabolic Derangement

ALG6-CDG is a defect in the attachment in the ER of the first of three glucose molecules to the dolichol-linked mannose$_9$-*N*-acetylglucosamine$_2$ intermediate

($Man_9GlcNAc_2$-P-P-Dol) (■ Fig. 43.1a). It causes hypoglycosylation of serum glycoproteins, because non-glucosylated oligosaccharides are a suboptimal substrate for the oligosaccharyltransferase and are, therefore, transferred to proteins with a reduced efficiency. For an unknown reason, the blood glycoproteins are unusually low (particularly factor XI, and coagulation inhibitors such as antithrombin and protein C). That the clinical picture in these patients is milder than that of PMM2-CDG patients may be because a deficiency in glucosylation of the dolichol-linked oligosaccharides does not affect the biosynthesis of GDP-mannose and, hence, does not affect the biosynthesis of compounds such as GDP-fucose or of glycosylphosphatidylinositol-anchored glycoproteins.

Genetics

Inheritance of this glucosyltransferase deficiency is autosomal recessive caused by variants in *ALG6*. The p.A333V and p.I299del are common protein alterations (► www.lovd.nl/ALG6).

Diagnostic Tests

This disease illustrates that, even in cases of mild psychomotor disability without any specific dysmorphic features, IEF of serum sialotransferrins should be performed. When a type 1 pattern is found, PMM2 deficiency and MPI deficiency must be considered first. If these enzymes show normal activities, the next step is the analysis of the dolichol-linked oligosaccharides (DLO) in fibroblasts, or mutation analysis of a panel of CDG genes, or WES/WGS.

Treatment and Prognosis

No efficient treatment is available. The oldest known patient is 38 years [24].

43.1.4 Mannosyltransferase 1 Deficiency (ALG1-CDG)

Clinical Presentation

ALG1-CDG is an autosomal recessive disease with a broad clinical spectrum, reported in 57 patients (belonging to 47 families). There is a predominant neurological involvement. Constant features are intellectual disability (mostly severe) and hypotonia (sometimes only in the infantile stage). The majority of patients show dysmorphism (such as facial dysmorphism, inverted nipples, fat pads, contractures, arachnodactyly), microcephaly (mostly from neonatal age), intractable seizures, visual disturbances, tremor, ataxia, severe infections/episodes of unexplained fever and cerebral abnormalities (cerebral infarct, general atrophy and/or periventricular white matter abnormalities). A number of other symptoms have been reported in one or a few patients.

Metabolic Derangement

ALG1 attaches the first of 9 mannose to the $GlcNAc_2$-P-P-Dol at the outside of the ER membrane. Biochemical abnormalities comprise decreased levels of serum LDL cholesterol, blood coagulation factor XI and anticoagulation factors antithrombin, protein C and protein S, as well as variable hypoalbuminaemia, increased serum transaminases, decreased serum cholinesterase and immunoglobulins, and endocrinological abnormalities (such as decreased serum IGF1 and IGFBP3).

Genetics

ALG1-CDG is an autosomal recessive disease. Seventy three pathogenic and likely pathogenic variants of *ALG1* been reported. The most frequent is c.773C>T (p.Ser258Leu).

Diagnostic Tests

Serum transferrin IEF shows a type 1 pattern, and analysis of short dolichol-linked oligosaccharides (DLO) in fibroblasts an increase of $GlcNAc_2$-P-P-Dol. The diagnosis has to be confirmed by mutation analysis of *ALG1*.

Treatment and Prognosis

No efficient treatment is available. Survival of reported patients ranged from 2 days to more than 20 years [25].

43.1.5 UDP-GlcNAc:Dol-P-GlcNAc-P Transferase Deficiency (DPAGT1-CDG)

Clinical Presentation

DPAGT1-CDG has been reported in 52 patients (from 25 families). It presents as one of two different phenotypes: an encephalopathy in the context of a multisystem disorder, or a congenital myasthenic syndrome. The multisystem presentation is usually a severe disease. All patients showed moderate to severe psychomotor disability, and most patients had microcephaly, hypotonia, and epilepsy. Less frequent symptoms were feeding difficulties, apnoea, respiratory insufficiency, chronic anaemia, cataracts, hypotrophy, hypertonia of the extremities, hypo- and hyperreflexia, joint contractures, and abnormal brain magnetic resonance imaging. The congenital myasthenic syndrome presentation is less frequent than the multisystem type. The first symptoms were noted between birth and 17 years. They suffered from a predominantly proximal muscle weakness with absent or minimal craniobulbar symptoms. The syn-

drome was mostly slowly progressive. Muscle cramps, difficulty in swallowing and chewing, and scoliosis have been reported in a few patients, as well as delayed motor development and intellectual disability.

43.1.6 Metabolic Derangement

DPAGT1-CDG is a defect in the attachment of the second GlcNAc to GlcNAc-P-P-Dol at the outside of the ER membrane. Biochemical abnormalities in the multisystem presentation comprise increased serum transaminases and creatine kinase, hypoproteinemia and decreased antithrombin. In the congenital myasthenic syndrome presentation serum creatine kinase levels were normal.

43.1.7 Genetics

DPAGT1-CDG is an autosomal recessive disease. Forty eight pathogenic and likely pathogenic variants of *DPAGT1* have been reported.

43.1.8 Diagnostic Tests

Serum transferrin IEF showed a type 1 pattern in all patients with the multisystem presentation, but only in about half of the patients with the congenital myasthenia syndrome presentation. The next step is to analyse a panel of genes known to be involved in CDG and, if this is normal, to perform WES/WGS.

43.1.9 Treatment and Prognosis

All patients with the congenital myasthenic syndrome presentation responded favourably to acetylcholinesterase inhibitors such as pyridostigmine. Ages at report ranged from 6 to 58 years. There is no efficient treatment for the multisystem presentation. Twenty-three patients died between 6 weeks and 5 years. Two siblings, who were 34 and 32 years old when reported, had a milder presentation [26].

43.1.10 Golgi α1-2 Mannosidase 1 Deficiency (MAN1B1-CDG)

- **Clinical Presentation**

MAN1B1-CDG has been reported in 34 patients (belonging to 21 families). They all showed mild to severe intellectual/developmental disability. Most patients also presented abnormal speech development and hypotonia. In the majority of patients there was facial dysmorphism and truncal obesity. Behavioural problems have been reported in about half of the patients, particularly verbal and physical aggression, autism, inappropriate sexual behaviour and overeating.

- **Metabolic Derangement**

MAN1B1-CDG is due to a defect in a Golgi mannosidase. Biochemical abnormalities such as increased serum transaminases and abnormal coagulation tests were present in only a few patients.

- **Genetics**

MAN1B1-CDG is inherited as an autosomal recessive disease. Sixty four pathogenic and likely pathogenic variants of *MAN1B1* have been reported.

- **Diagnostic Tests**

Serum transferrin IEF shows a type 2 pattern. Mass spectrometry of serum transferrin shows an accumulation of specific hybrid type *N*-glycans. The diagnosis should be confirmed by mutation analysis.

- **Treatment and Prognosis**

No efficient treatment is known. The oldest patient was 35 years at the time of report [27].

43.2 Congenital Disorders of Protein O-Glycosylation

43.2.1 Progeroid Variant of Ehlers-Danlos Syndrome (B4GALT7-CDG)

B4GALT7 is involved in the biosynthesis of the glycan moiety (glycosaminoglycans) of proteoglycans. All glycosaminoglycans, with the exception of keratan sulfate and hyaluronan, are connected to a serine residue of a core protein through a tetrasaccharide core linkage region. This tetrasaccharide linkage region, an O-linked glycosylation, is formed by the serial addition of a xylose, two galactoses and a glucuronic acid. B4GALT7 catalyses the attachment of the first galactose. As a consequence, these patients have defective synthesis of heparan, dermatan and chondroitin sulfate. At least 52 patients have been reported. They showed a short stature, developmental anomalies of the forearm bones and elbows, and bowing of the extremities, in addition to the classic features of Ehlers-Danlos syndrome (joint laxity, skin hyperextensibility and poor wound healing). In one family the phenotype included perinatal lethal skeletal dysplasia as well as cleft palate and radial ray defects. A specific entity, Larsen of Reunion Island syndrome,

is caused by a homozygous founder mutation. These patients have in addition multiple dislocations but no progeroid appearance [28].

43.2.2 GALNT3 Deficiency (GALNT3-CDG)

GALNT3-CDG is due to a defective UDP-N-acetyl-α-D-galactosamine: polypeptide N-acetylgalactosaminyltransferase 3. This autosomal recessive defect causes one of two syndromes: hyperphosphatemic familial tumoral calcinosis (HFTC) or hyperphosphatemic hyperostosis syndrome (HHS). At least 66 patients have been reported, mostly from Middle Eastern or African ancestry. It is a disturbance in the hormonal regulation of serum phosphate levels by FGF23. In HFTC this leads to the development of recurrent, painful calcified subcutaneous masses. This can be complicated by secondary infections and incapacitating mutilations. In HHS it leads to episodes of diaphysitis and cortical hyperostosis visualized on X-ray examination. The regulation of the phosphate metabolism involves phosphatonins, in particular FGF23. GALNT3 is necessary for the O-glycosylation of FGF23 thereby preventing the inactivation of this phosphaturic protein. Both phenotypes have been reported to occur in the same family [29].

43.2.3 Hereditary Multiple Exostoses (EXT1/EXT2-CDG)

Hereditary multiple exostoses is an autosomal dominant disease with a prevalence of 1 in 50,000 and is characterised by the formation of cartilage-capped tumours, known as osteochondromas, on the ends of long bones. These are often present at birth, but usually not diagnosed before early childhood. Their growth slows at adolescence and stops in adulthood. A small percentage of these lesions are subject to malignant degeneration. Complications may arise from compression of peripheral nerves and blood vessels.

The basic defect resides in a Golgi-localised protein complex, termed exostosin-1/exostosin-2 (EXT1/EXT2), which adds *d*-glucuronic acid and *N*-acetylglucosamine units in the synthesis of heparan sulfate. It has been hypothesized that mutations in these glycosyltransferases impair the synthesis of a glycosaminoglycan that exerts a tumor-suppression function. This would explain the higher risk of affected individuals to develop chondrosarcomas and osteosarcomas.

Mutations in *EXT1* and in *EXT2* have been identified and are responsible for over 70 % of cases of hereditary multiple exostoses (specific data base: ► http://medgen.ua.ac.be/LOVDv.2.0/home.php) [30].

43.2.4 Cerebro-Ocular Dysplasia-Muscular Dystrophy Syndromes, Types A1, B1, C1/A2, B2, C2 (POMT1-CDG/POMT2-CDG)

These are 2 of some 25 neuronal migration disorders known in humans. They are characterized by brain and eye dysgenesis associated with congenital muscular dystrophy. Male patients often have testicular defects. Psychomotor development is usually absent or very poor. The brain lesions consist of 'cobblestone' lissencephaly, agenesis of the corpus callosum, cerebellar hypoplasia, hydrocephaly and sometimes encephalocoele. The disease usually runs a fatal course before the age of 1 year, and only symptomatic treatment is available.

The metabolic derangement is an aberrant glycosylation of α-dystroglycan, an external membrane protein expressed in muscle, brain and other tissues. Most glycans of this heavily glycosylated protein seem to be *O*-linked via mannose, and they control the interaction with extracellular matrix proteins. Disrupted glycosylation of α-dystroglycan (and probably other glycoproteins) results in loss of this interaction and hence in progressive muscle degeneration and abnormal neuronal migration (overmigration) in the brain. In about 20 % of the patients this disrupted glycosylation is due to a defective *O*-mannosyltransferase-1 or -2, which catalyses the first step in the synthesis of the *O*-mannosylglycan core. This autosomal recessive defect is caused by mutations in *POMT1* and in *POMT2* [31, 32].

43.2.5 Muscle-Eye-Brain Disease, Types A3, B3, C3, RP76/A8,C8 (POMGNT1-CDG/POMGNT2-CDG)

Muscle-eye-brain disease is a neuronal migration/congenital muscular dystrophy syndrome similar to cerebro-ocular dysplasia-muscular dystrophy syndrome, but less severe and with longer survival. Defective POMGnT1 can also cause non-syndromic retinitis pigmentosa. The defects are in protein *O*-mannosyl-β-1,2-*N*-acetylglucosaminyltransferase 1 and 2, catalysing the second step in the synthesis of the *O*-mannosylglycan core. The disease is autosomal recessive and due to mutations in *POMGnT1* and in *POMGnT2* [33].

43.2.6 O-Fucose-Specific β-1,3-Glucosyltransferase Deficiency (B3GLCT-CDG)

Variants in the B3GLCT gene have been found to underly Peters-plus syndrome. The major criteria of this autosomal recessive disorder, reported in at least 50 patients, are anterior eye chamber anomalies (mainly Peters' anomaly which consists of corneal clouding and iridolenticulocorneal adhesions), growth retardation, and brachydactyly. Intellectual disability is frequent. Cleft lip and/or palate, cardiac malformation, and facial dysmorphism, including external ear anomalies, are present in about half of the patients. No *B3GLCT* variant(s) has been found in patients with an incomplete phenotype [34].

43.3 Defects in Lipid Glycosylation and in Glycosylphosphatidylinositol Anchor Biosynthesis

43.3.1 GM3 Synthase Deficiency (ST3GAL5-CDG)

See ► Chap. 40 on sphingolipid disorders [35].

43.3.2 GM2 Synthase Deficiency (B4GALNT1- CDG)

See ► Chap. 40 on sphingolipid disorders [36].

43.3.3 PIGA Deficiency (PIGA-CDG)

This is an X-linked disorder of the glycosylphosphatidylinositol anchor biosynthesis. PIGA is one of seven proteins required for the first step of this biosynthesis, the transfer of *N*-acetylglucosamine from UDP-GlcNAc to phosphatidylinositol. Other names for this disease are 'multiple congenital anomalies-hypotonia-seizures syndrome 2' and 'ferro-cerebro-cutaneous syndrome'. At least 76 patients have been reported with less severe and severe phenotypes. The latter present with poorly responsive epilepsy (vit. B-responsive in some patients), hypotonia, multiple brain abnormalities, facial dysmorphism, and, in a minority, variable involvement of skin, heart, liver and kidneys. There is a high frequency of sudden death, especially in childhood. Some patients show systemic, still unexplained iron overload. Most patients show hyperphosphatasaemia and decreases of other GPI-anchored proteins such as CD16 and CD24 on blood cells. Of note is that somatic *PIGA* mutations cause the well-known paroxysmal nocturnal hemoglobinuria, an acquired disorder of bone marrow failure [37].

43.4 Defects in Multiple Glycosylation Pathways and in Other Pathways Including Dolicholphosphate Biosynthesis

43.4.1 Hereditary Inclusion Body Myopathy (GNE-CDG)

Hereditary inclusion body myopathy is an autosomal recessive disease that is allelic to the Japanese disorder 'distal myopathy with rimmed vacuoles' or 'Nonaka myopathy'. It usually begins after age 20 with muscle weakness that progresses over the next 10–20 years, sparing the quadriceps until the most advanced stage of the disease. Muscle histology shows rimmed vacuoles on Gomori's trichrome stain, small fibres in groups, and tubulofilaments without evidence of inflammation. Mutations have been identified in *GNE*, which encodes the bifunctional enzyme uridine diphospho-*N*-acetylglucosamine epimerase/*N*-acetylmannosamine kinase. This enzyme catalyses the first two steps in the biosynthesis of sialic acid. At least 164 variants have been reported. Oral administration of sialic acid, mannosamine and *N*-acetylmannosamine markedly improved muscle and renal hyposialylation in a mouse model. Trials are underway in patients [38].

43.4.2 Congenital Myasthenic Syndrome-12 (GFPT1-CDG)

Glutamine-fructose-6-phosphate transaminase 1 (GFPT1) is the initial and rate-limiting enzyme in the biosynthesis of N-acetylglucosamine, an essential substrate for O- and N-linked glycosylation. GFPT1-CDG is associated with a congenital limb-girdle myasthenic syndrome and has been described in at least 46 patients. It can be associated with leukoencephalopathy suggestive of mitochondrial disease. Most patients show tubular aggregates of the sarcoplasmic reticulum in type 2 fibres. There is an inconstant, mild increase of serum creatine kinase. It is not known whether serum transferrin IEF is abnormal. Inheritance is autosomal recessive. At least 32 variants have been reported. Most patients respond partially to cholinergic agents [39].

43.4.3 Steroid 5-α-Reductase Deficiency (SRD5A3-CDG)

This autosomal recessive disorder of the dolichol-phosphate synthesis (Fig. 43.2) has been reported in at least 38 patients. The clinical picture comprises mainly ophthalmological (mainly visual impairment, nystagmus, coloboma, optic atrophy) and neurological symptoms (mainly intellectual disability, hypotonia, spasticity, cerebellar ataxia, vermis atrophy). A minority of the patients show ichthyosiform skin lesions, kyphosis, contractures of the large joints, cardiac abnormalities, blood coagulation abnormalities, increased serum transaminases. The serum transferrin IEF shows a type 1 pattern in the large majority of the patients. At least 15 variants have been described. The oldest reported patient was 45 years [40].

43.4.4 COG6 Deficiency (COG6-CDG)

The conserved oligomeric Golgi (COG) complex consists of eight subunits, divided in two lobes. Lobe A comprises subunits 1–4, and lobe B subunits 5–8. These lobes are bridged by subunits 1 and 8. The COG complex plays an important role in Golgi trafficking and positioning of glycosylation enzymes. Mutations in all COG subunits, except subunit 3, have been reported in CDG patients. At least 28 patients have been described with COG6-CDG. Predominant clinical features are liver involvement, microcephaly, developmental/intellectual disability, recurrent infections, early lethality, and ectodermal symptoms (hypohydrosis predisposing to hyperthermia, and hyperkeratosis). Most of these symptoms are present in other COG-CDG. The closest overlap is with COG7-CDG, which has additional features such as malignant hyperthermia, adducted thumbs and cardiac defects. The patients show a type 2 pattern on serum transferrin IEF, and, in the patients tested, an abnormal pattern on serum apolipoprotein C-III IEF. Inheritance is autosomal recessive. At least 50 variants have been described. The splice site mutation c.1167-24A>G seems to be associated with a mild phenotype (ectodermal signs, mild developmental disability, no liver disease, no glycosylation deficiency). On the other hand, the loss-of-function mutations c.511C>T and c.1238_1239insA are associated with a severe phenotype including lethality between 1 and 15 months. Since the COG complex is most probably not only involved in glycosylation but also in other cellular functions, defects in this protein complex might be more appropriately called 'CDG-plus' [41].

43.4.5 Autosomal Recessive Cutis Laxa Type 2 (ATP6V0A2-CDG)

Patients with this disorder, also called 'wrinkly skin syndrome,' already have generalized cutis laxa at birth, but this becomes less obvious later on and may disappear with age. Furthermore, they show congenital or postnatal microcephaly, increased joint laxity, ophthalmological abnormalities (strabismus, myopia, amblyopia, etc.) and, rarely, cardiac defects. Mental development is mostly normal. There is a combined defect in *N*- and *O*-glycosylation demonstrated by a type 2 serum transferrin IEF pattern and an abnormal serum apolipoprotein C-III IEF pattern. Skin biopsy shows an abnormal elastic fibre structure and a decrease of elastin.

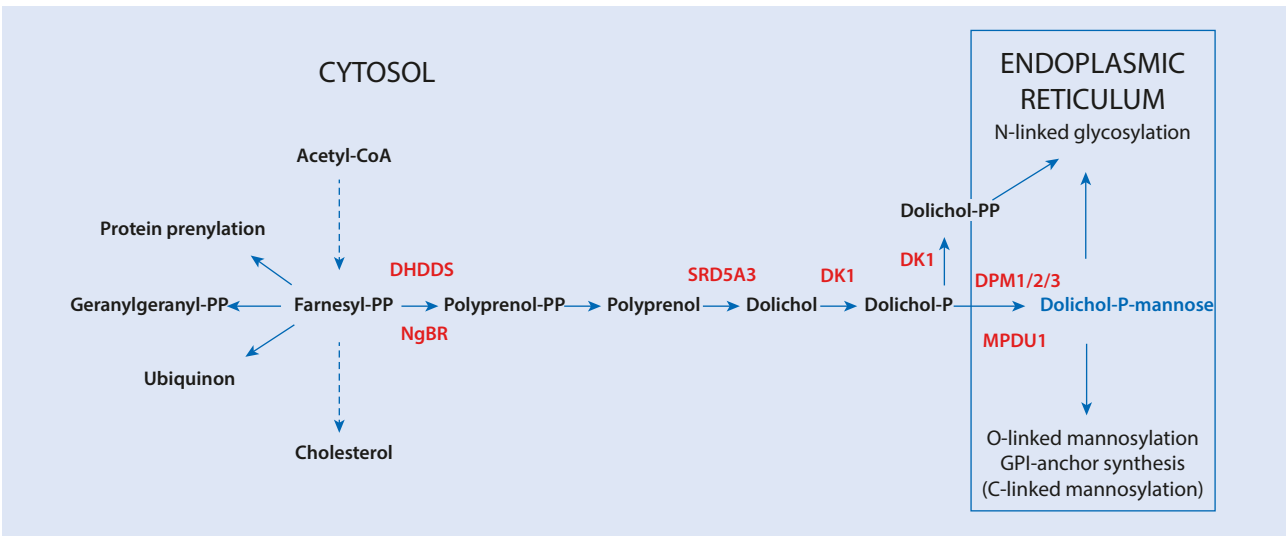

Fig. 43.2 Schematic representation of the dolichol synthesis and utilization/recycling

Two major (and closely related) functions of the V-ATPase V0 domain are (i) maintenance of the pH gradient along the secretory pathway by proton transport and (ii) regulation of protein transport through the facilitation of vesicle fusion. However, the exact mechanism by which mutations in the V-ATPase a2 subunit affect glycosylation remains to be elucidated. At least 35 variants have been reported. This seems to be another 'CDG-plus' [42].

43.4.6 Phosphoglucomutase 1 Deficiency (PGM1-CDG)

PGM1 is a key enzyme in glycogenesis and it is important for effective glycolysis during fasting. The disease has two major phenotypes: one is a myopathic glycogenosis (type XIV), and the other a multisystem presentation including growth deficiency, hypoglycemia, malformations (such as cleft uvula, cleft palate), and liver, cardiac and endocrine involvement. Contrary to most other CDG, PGM1 deficiency shows no or only minor neurological involvement. It is the only primary CDG that shows a defect in the assembly as well as in the processing of N-glycans (CDG-I/II). Serum transferrin IEF shows a type 2 pattern (in fact a combined type1/type 2 pattern), and mass spectrometry of serum transferrin a decreased galactosylation. Galactose supplementation improves glycosylation of patients' fibroblasts, and patients on oral galactose treatment show improved glycosylation and also clinical improvement. It has been shown by isotope studies that galactose therapy is effective through the replenishment of galactose-1-P and the nucleotide sugars UDP-glucose and UDP-galactose [43].

43.4.7 Golgi Homeostasis Disorders: TMEM199 and CCDC115 Deficiencies

Two new disorders with abnormal Golgi N- and O-glycosylation have recently been described. The affected proteins are involved in Golgi homeostasis. Both disorders show a type 2 pattern on serum transferrin IEF. The seven reported patients with TMEM199 deficiency showed only a chronic, non-progressive (over decades) liver disease with mild hepatic steatosis, elevated serum aminotransferases and alkaline phosphatase, hypercholesterolemia, and low serum ceruloplasmin [44]. In CCDC115 deficiency, the 11 reported individuals displayed a storage disease-like phenotype with hepatosplenomegaly, which regressed with age, highly elevated bone-derived alkaline phosphatase, elevated aminotransferases, and elevated cholesterol, in combination with abnormal copper metabolism and neurological symptoms. Two patients died of liver failure, and one was successfully treated by liver transplantation [45].

43.4.8 Manganese and Zinc Transporter Defect: SLC39A8 Deficiency

SLC39A8 (also known as ZIP8) is a divalent cationic membrane transporter important for the uptake of manganese (Mn) into cells. Compound heterozygous and homozygous mutations in *SLC39A8* have recently been described in patients with phenotypes ranging from cranial synostosis, hypsarrythmia, and disproportionate dwarfism to cerebellar atrophy, hypotonia, global developmental delay and recurrent infections. Serum Mn levels were reduced and transferrin IEF showed a type 2 pattern, due to dysfunction of the Mn-dependent enzyme ß-1,4-galactosyltransferase. Oral galactose supplements (up to 3.75 g/kg/d) corrected the glycosylation defect. Oral Mn therapy in two patients was associated with biochemical normalization and considerable neurological improvement. Close monitoring is important to avoid Mn toxicity [46]. See also ► Chap. 34.

43.5 Congenital Disorders of Deglycosylation (CDDG)

43.5.1 N-glycanase 1 (NGLY1) Deficiency

N-glycanase catalyzes deglycosylation of misfolded N-linked glycoproteins by cleaving the glycan chain before the proteins are degraded by the proteasome. It is a cytoplasmic component of the endoplasmic reticulum-associated degradation (ERAD) pathway. At least 22 patients have been reported [47]. All patients showed developmental disability, movement disorders and hypotonia. Common features comprised intrauterine growth restriction, alacrimia/hypolacrimia, chalazion, microcephaly, seizures, peripheral neuropathy, hyporeflexia, and liver involvement (increased serum transaminases and alpha-foetoprotein, cytoplasmic storage). Two patients died at 9 months and 5 years of age. Serum transferrin IEF is normal in this CDDG. Six of the 22 patients were homozygous for the c.1201A>T/p.R401X variant, that was associated with severe disease.

43.5.2 Lysosomal Storage Disorders

Besides the cytoplasmic protein deglycosylation disorder, NGLY1 deficiency, there are also lysosomal protein

deglycosylation disorders namely the lysosomal storage diseases due to enzymatic defects (sphingolipidoses such as GM1-gangliosidosis a.o., mucolipidoses such as MPS I a.o., oligosaccharidoses such as fucosidosis a.o.; see ► Chaps. 40 and 41).

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Disorders of Cellular Trafficking

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Contents

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Introduction

Cellular trafficking is essential to maintain critical biological functions. The machinery of proteins and the mechanisms that regulate membrane trafficking is immense and tend to be cell and tissue specific. Mutations in more than 300 genes are known to be associated with disorders of cellular trafficking and include those that affect: (i) membrane trafficking, that mediate the most important pathway to move cargo using membrane bound transport vesicles; (ii) membrane contact sites (MCS) or areas of close apposition between the membranes of organelles; (iii) Other mechanisms such as cytoskeleton mediated cargo/organelle transport, transcytosis, regulation of membrane phospholipids, and gap junctions.

Vesicle trafficking proteins coordinate vesicle formation, transport, tethering and fusion with the target membrane. Coat, Adaptors, Calveolins, GTPases, Rab, TBC, TRAPP, VPS and SNAREs are families of proteins that regulate these functions and are vulnerable to mutations.

Diseases of cellular trafficking affect all tissues and organs. The great majority of mutations cause a loss of function of transport machinery. Depending on the function and location of the affected protein, we have tentatively defined the following pathophysiological categories: vesicular trafficking disorders; organelle-related trafficking disorders (including MCS); cytoskeleton-related trafficking disorders; and autophagy disorders. Other membrane and vesicular trafficking such as glial and neuronal receptor trafficking disorders are brain specific.

The nervous system is especially vulnerable to these diseases since neurons are highly polarized and compartmentalized. In fact, neurons need sophisticated transport mechanisms to release cargos at the exact place at the exact moment. Early neurodevelopmental encephalopathies with congenital and post-natal microcephaly, often associated with brain malformations and epilepsy, are predominantly related to Golgi and cytoskeleton trafficking diseases. Motor disorders and dementias tend to have a late onset and are mostly related to dysfunctions in the endocytic pathway. They include spastic paraparesis, ataxia, Parkinson's disease, peripheral neuropathy (in particular Charcot-Marie-Tooth) and other neurodegenerative disorders such as amyotrophic lateral sclerosis.

Other tissues are also involved and often include well-defined syndromes with multisystem manifestations, such as familial hemophagocytic lymphohistiocytosis, Chediak-Higashi, Hermansky-Pudlak, ARC, Lowe, Cohen, CEDNIK and Vici syndromes. Certain biomarker analyses may be of help for the diagnosis and include copper and ceruloplasmin profiles (for Wilson and Menkes disease, MEDNIK syndrome, and SLC33A1 defect), serum transferrin isoforms (for CDG syndromes), and lysosomal-related testing in plasma and urine (for Mucolipidosis type II, Multiple sulphatase deficiency, and Yunis-Varon syndrome). Mutation analysis, via targeted Sanger or exome analysis, represents the most reliable method to diagnose disorders of intracellular trafficking. Mutation analysis via targeted Sanger or exome analysis is the only reliable method of diagnosis of an autophagy-related disorder. Thus far, there are very few treatments for this group of emerging disorders.

44.1 Cellular Mechanisms of Trafficking

Cellular trafficking is the exchange of signals and metabolites between cellular compartments. This exchange was first thought to occur by two broad mechanisms: diffusion or active transport through the cytoplasm and vesicular trafficking. A third mechanism that has been recently described is the exchange of signals and metabolites at regions where organelles form functional contacts [1]. Historically, and from De Duve to current times, different methods of study have been used to describe the ultrastructural cell anatomy. Today, cutting-edge structural methods, biochemical, cell-based and computational approaches, associated with the discovery of an increasing number of genes involved in these processes, pushes the understanding of trafficking and related diseases to unprecedent levels of detail and complexity [2].

44.1.1 Membrane Trafficking

Membrane trafficking encompasses the full range of processes that go into the movement of cargo using membrane bound transport vesicles (vesicular trafficking). This transport can take place within different organelles in the same cell, or across the cell membrane to and from the extracellular environment [3]. The underlying molecular machinery is estimated to comprise more than 2000 proteins [4]. Membrane trafficking can be divided into two pathways: exocytosis and endocytosis (◘ Fig. 44.1).

Exocytosis refers to the movement of cargo to the plasma membrane (PM) or out of the cell. Newly synthesized molecules (proteins, lipids or carbohydrates) move from the endoplasmic reticulum (ER) via the Golgi to the cell membrane or extracellular space. ER-derived cargo enters the Golgi in its cis cisterna (CGN) and moves through the medial and trans cisternae (TGN). In the trans Golgi, proteins destined for secretion are packed into secretory vesicles that subsequently fuse with the PM. In the Golgi, cargo is sorted not only to the PM but also to endosomes and lysosomes, or back to the ER [3, 5, 6] (◘ Fig. 44.1).

Endocytosis is the opposite movement: the cargo moves into the cell from the plasma membrane and can

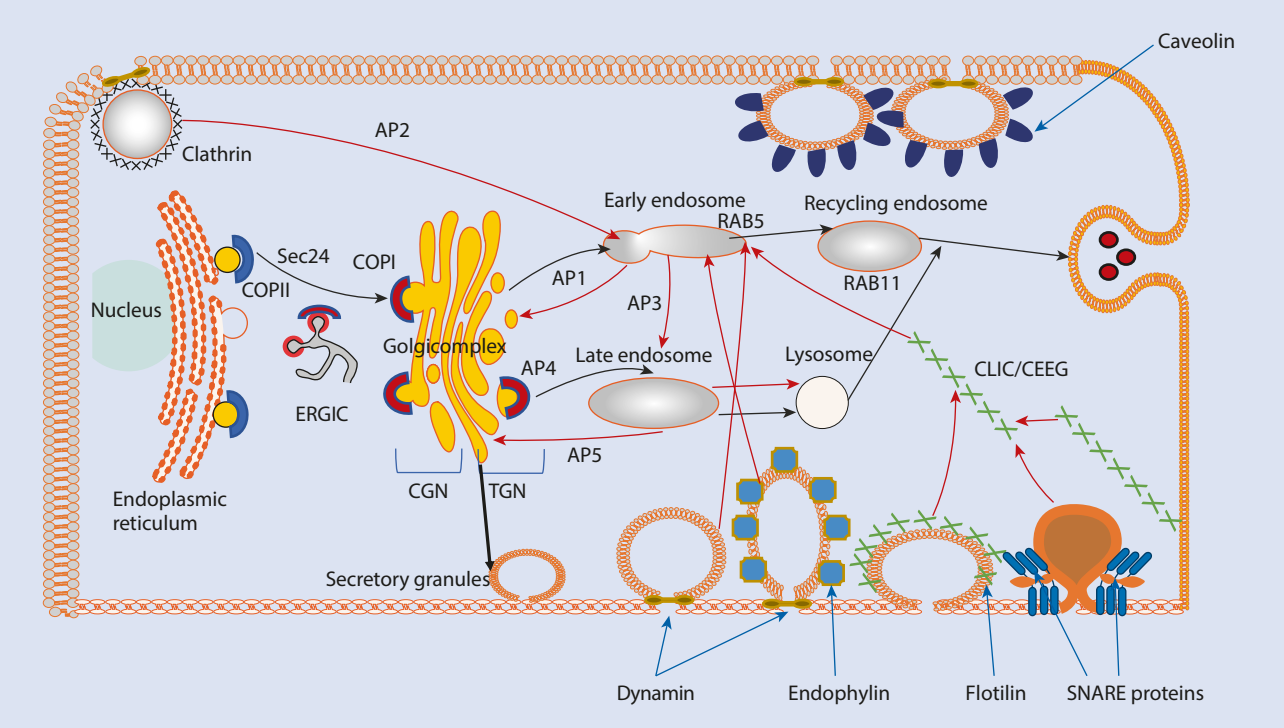

Fig. 44.1 Basic mechanisms of cell trafficking. Black arrows indicate the exocytic pathway. Red arrows indicate the endocytic pathway. AP1,2,3,4,5 subtypes of Adaptor Proteins; They mediate both the recruitment of clathrin to membranes and the recognition of sorting signals within the cytosolic tails of transmembrane cargo molecules. COPI Coat Protein I (vesicles are surrounded by COPI) COPII Coat Protein II (COPII vesicles bud off from the ER in the secretory or exocytic pathway towards the Golgi). CGN Cis Golgi Network; CLATHRIN Clathrin-coated vesicles mediate endocytosis from the plasma membrane to endosomal compartments and the Golgi; CLIC/CEEG The CLIC/GEEC (CG) pathway is a clathrin-independent endocytic pathway mediated by uncoated tubulovesicular primary carriers called clathrin-independent carriers (CLICs) which arise from the plasma membrane and later mature into early endocytic compartments called Glycosylphosphotidylinositol-anchored protein (GPI-AP) enriched compartments (GEECs). Dynamin the detachment of budded vesicles from the plasma membrane may be facilitated by the GTPase Dynamin, via membrane scission. ERGIC Endoplasmic Reticulum-Golgi Intermediate Compartment or tubule vesicular transporter. RAB5, RAB11 subtypes of RAB proteins (GTPase family). Sec24 Component of the coat protein complex II (COPII). It has two main functions, the physical deformation of the endoplasmic reticulum membrane into vesicles and the selection of cargo molecules for their transport to the Golgi complex. SNARE dock the transport vesicle at the correct membrane location. TGN Trans Golgi Network. Endophylin, Flotilin, Caveolin mediate endocytosis in an independent clathrin manner

be used for the uptake of nutrients or to direct cargo for recycling or degradation via autophagy. Cargo can be internalized at the PM via clathrin-coated vesicles, caveolar or raft-dependent routes [5]. These three internalization routes depend on the GTPase dynamin for fission of the forming PM vesicle. However, fluid-phase cargo can also enter the cell via a dynamin-independent process. In the endocytic pathway, proteins and membranes are internalized via a set of endosomes (early and late) to the lysosome, which is a major degradation site for internalized and cellular proteins [5, 6]. Cellular proteins can get to lysosomes either from the PM via the endocytic pathway or from the cytoplasm via the autophagy and the cytoplasm-to-vacuole targeting (CVT) pathways.

The main families of vesicle trafficking proteins include those that coordinate vesicle formation, GTPases of the Rab family, transport and tethering factors, and SNARE proteins that mediate vesicle fusion with the target membrane [7, 8] (see also Fig. 44.2).

- ***COAT and ADAPTOR proteins*** self-assemble on the membrane, helping to collect and concentrate the vesicle cargo. There are three well-characterised coat proteins: 1-**Clathrin-coated** vesicles mediate endocytosis from the PM to endosomal compartments and the Golgi. 2- **Coat protein I** (COPI) surrounds vesicles that mediate retrograde transport within the Golgi and towards the ER. 3- **COPII** vesicles bud off in the opposite direction, from the ER in the secretory or exocytic pathway towards the Golgi [3, 6, 9]. Central to the appropriate sorting of cargo, specific coat subunits (known as **cargo adaptors**) contain binding surfaces that recognize specific cargo proteins and are responsible for capture of specific cargo into the forming vesicles. Adaptor proteins are: (i) **Sec24**: cargo adaptor that contains multiple cargo binding sites to capture diverse set of proteins; (ii) **AP1, AP2, AP3, AP4 and AP5** that binds to different membranes (Fig. 44.1).

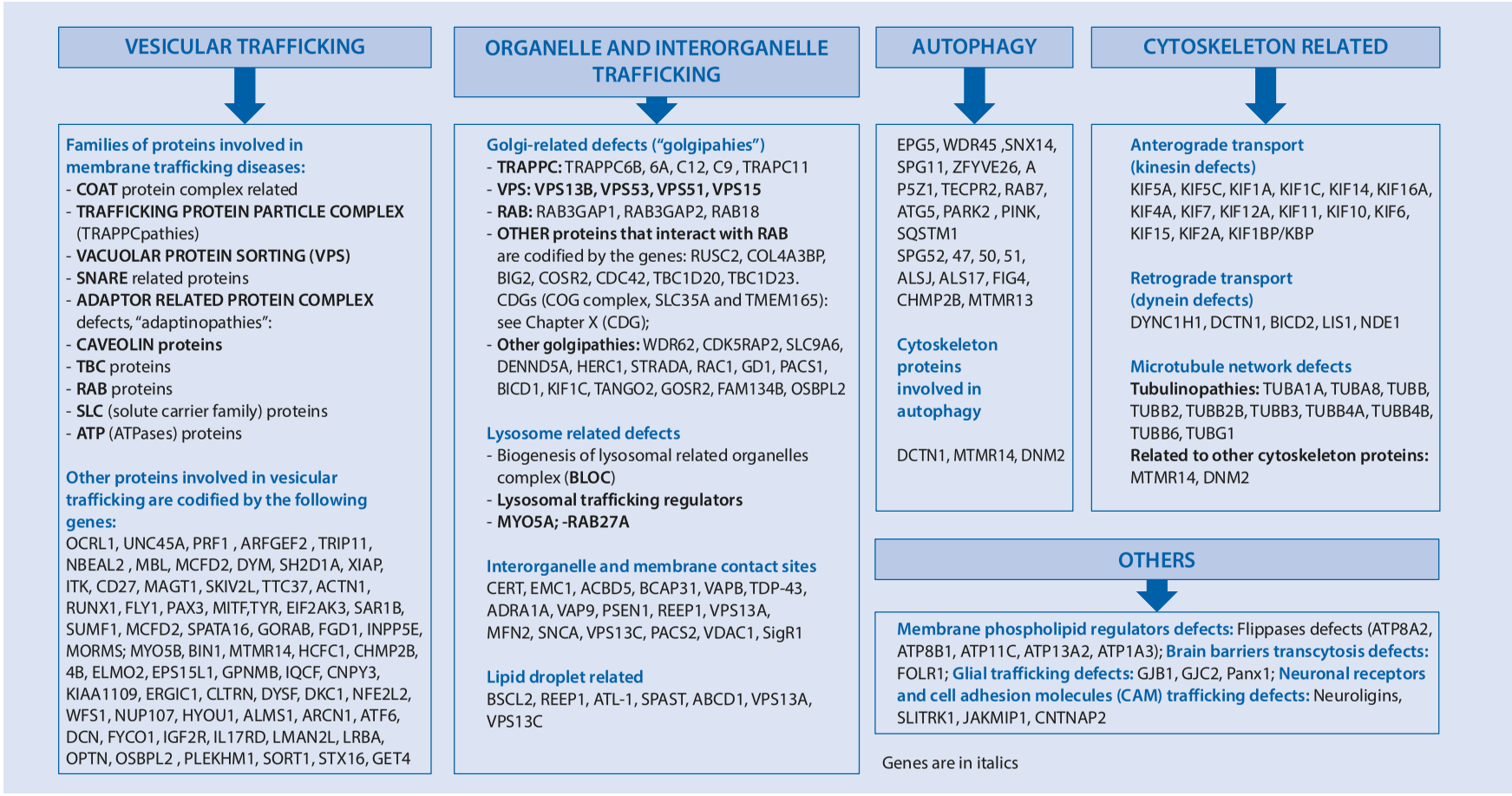

Fig. 44.2 Main families of proteins and genes involved in cell traffic diseases according to pathophysiological categories

- ***CALVEOLIN proteins***, a family of small proteins (18–24 kDa), are the principal components of caveolae membranes, forming caveolae by polymerization [10]. They are a structural component of transport vesicles derived from the trans-Golgi network. They function in signal transduction, endocytosis, transcytosis, cholesterol transport, and lipid homeostasis [11] by mediating clathrin non-dependent endocytosis (◘ Fig. 44.1).
- ***RAB proteins:*** the small soluble Ras-related proteins in the brain (Rab) comprise the largest family of small GTP binding proteins within the Ras superfamily with over 60 members. In membrane traffic they assist in a variety of steps: budding, movement docking, tethering and fusion of transport carriers operating between the endomembrane compartments of the cell [12]. They allow transport vesicles to recognise a specific target. Rab proteins can be expressed on both transport vesicles and target membranes, providing a further level of regulation. They are also important regulators of autophagy [13].
- ***TBC*** domain-containing proteins contain a conserved protein motif present in many eukaryotic proteins. They function as GTPase activating proteins (GAPs) for the small GTPase Rab, which can promote the hydrolysis of Rab-GTP to Rab-GDP [14].
- ***TRAFFICKING PROTEIN PARTICLE (TRAPP)*** participate in events upstream of vesicle fusion (secretory pathway), and in some aspects of vesicle transport to their correct intracellular target membrane. In humans, the TRAPP complex is formed by the core proteins TRAPPC1, 2, 3, 4, 5, 6, and 2L that self-assemble to form a stable core [15]. This TRAPP core interacts with a number of accessory proteins to form TRAPP II and III. Both of them participate in the secretory pathway and TRAPP III is also involved in autophagy [16].
- ***VACUOLAR PROTEIN SORTING (VPS)*** is a protein complex composed of VPS 26, 29, 35, 16 and 41 with an important role in trafficking transmembrane receptors toward the endosome compartment (from endosomes to the TGN) [17]. VPS16 and VPS41 are key components of the HOPS complex. The HOPS complex mediates autophagosome-lysosome and endosome-lysosome fusion through several different interactions with SNARE proteins, including catalysing the formation of the SNARE complex and protection of the trans-SNARE complex from disassembly once formed [18].
- ***SNARE proteins*** (soluble N-ethylmaleimide-sensitive factor attachment protein receptor) mediate membrane fusion by the formation of the SNARE complex assembly. SNAREs are divided in three families depending on the subcellular localization. Synaptosomal-associated proteins (SNAP) and syntaxins (STX) belong to the target SNARE (t-SNAREs) family and are located at target membranes. Synaptobrevin (SYB) (VAMP, from vesicle-associated membrane protein) are vesicle SNAREs (v.SNARE) enriched in vesicle membranes. The formation of the SNARE complex brings into close apposition the target and vesicle membranes during an exothermic process that overcomes the energy barrier required for membrane fusion [19] (see also ▶ Chap. 30, disorders of neurotransmission).
- ***CYTOSKELETON*** assist in bidirectional transport (anterograde and retrograde) between the compartments of the secretory pathway or the endocytic pathway [20]. Most vesicle traffic along microtubules using kinesin or dynein motors, although they can also use myosin II and myosin V motors to move along the actin network (▶ Sect. 44.2).

In this Chapter, we have schematically defined main groups of pathophysiogical categories (◘ Fig. 44.2) based on the function of the specific proteins known to be involved in human disease.

44.1.2 Membrane Contact Sites

Organelle communication at membrane contact sites (MCS) is gaining wide acceptance in multiple areas of cell biology [1]. Membrane contact sites are classically defined as areas of close apposition between the membranes of two organelles and can be homotypic (between identical organelles) and heterotypic (between two different organelles or two different membrane types) [21]. Well-studied heterotypic contacts involve the Endoplasmic Reticulum (ER) such as the ER-mitochondrial, ER-PM (plasma membrane), ER-Golgi, ER-peroxisomes and ER-lipid droplets (LDs). In the last years, organelle contacts that do not involve the ER have been discovered: LDs-peroxisomes, and membrane contact sites with mitochondria and other organelles (endosomes, lysosomes, PM, LDs, peroxisomes and mitochondrial inner and other membranes).

Recently, a consensus on the definition of MCS has been published [21]. The authors propose a set of unifying characteristics that they consider essential features:

- **Tethering:** a contact site is defined by the presence of tethering forces that arise from protein-protein or protein-lipid interactions and not by the distance between two organelles. The most common distance is from 10 to 80 nm distance although some contact sites have the capacity to be much larger (over 300 nm) [22].
- **Lack of fusion:** fusion is a characteristic of vesicular trafficking (such as SNARE proteins mediated fusion) but not of membrane contact sites.

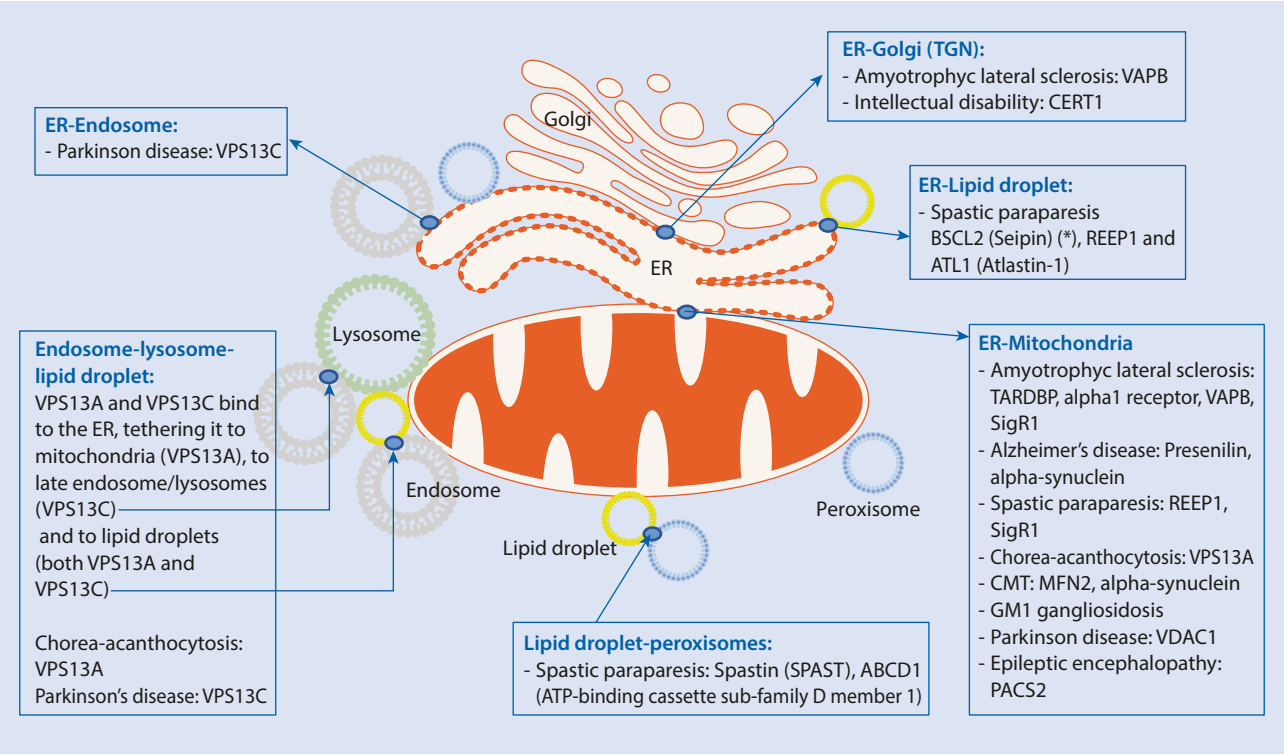

Fig. 44.3 Membrane contact sites and related diseases. Genes are written in italics followed by the correspondent protein. ABCD1 ATP-binding cassette sub-family D member 1, CERT1 ceramide transfer protein, MFN2 mitofusin 2, PACS2 phosphofurin acidic cluster sorting protein 2, REEP receptor expression-enhancing protein 1, SigR1 RNA polymerase sigma factor, TARDBP DNA-binding protein 43, VAPB vesicle-associated membrane protein-associated protein B/C, VDAC voltage-dependent anion-selective channel protein 1, VPS vacuolar protein sorting-associated protein. (*) Seipin related disorders include not only spastic paraparesis but a spectrum of clinical manifestations (lipodystrophy with neurodegeneration, epileptic encephalopathy) (► Chap. 35)

- **Specific function:** to date, three types of functions have been suggested: (i) the specific bidirectional transport of molecules such as ions, calcium, lipids, amino acids and metals; (ii) the transmission of signaling information important for remodeling activities such as organelle biogenesis, dynamics, positioning, fission and autophagy [23]; (iii): the positioning in *trans*, of enzymes so as to regulate their activity.
- **Defined proteome/lipidome:** contacts need a functional protein and/or membrane composition required for all the above functions and the maintenance of their architecture.

Metabolic pathways at MCS are tightly regulated. Mutations in proteins involved in these pathways may cause human diseases and are depicted in Fig. 44.3.

44.1.3 Other Types of Cellular Trafficking

Transport across the cytoskeleton is an important mechanism of molecular and organelle trafficking. This is ruled by microtubules, motor and adaptor proteins that associate and interact composing multi-protein complexes with specific functions.

Extracellular vesicles or neuronal exosomes is a type of vesicular membrane trafficking that mediates intercellular communication (i.e communication between neurons and other cells). They are released from multivesicular bodies to the extracellular space. There are no monogenic disorders of extracellular vesicles described so far. However, they have been highlighted for their diagnostic potential of neurodegenerative diseases such as Alzheimer disease, since they can be studied to asses -omic information in the CSF of patients [24].

Transcytosis is a phenomenon that is present in many different cells, from neurons to intestinal cells. Steps along this pathway include endocytosis (adsorptive or receptor-mediated internalization), intracellular vesicular trafficking, and exocytosis [25]. Several receptors capable of inducing receptor-mediated transcytosis are present in the BBB (blood brain barrier) (insulin, transferrin, lipoprotein receptors) and in the BCSFB (blood cerebrospinal fluid barrier) (folate receptor FOLR1) [26]. FOLR1 mutations cause an early-onset neurodegenerative disease (► Sect. 28.3.3, Fig. 44.2, Table 44.1).

Table 44.1 EARLY ONSET ENCEPHALOPATHIES: clinical signs may appear during the first year of life

MICROCEPHALY as prominent sign In general, these diseases associate prominent BRAIN STRUCTURE/ BRAIN IMAGE alterations and are GLOBAL developmental encephalopathies with multiple NRL symptoms, in particular EPILEPSY/ EPILEPTIC ENCEPHALOPATHY. They may have EXTRA-NRL signs (skeletal, multisystem, well-defined genetic syndromes)	GOLGIPATHIES	
	GENES INVOLVED	*COPB2, COPD, CDK5RAP2, ZNHIT3, AP4E1, WDR62, SLC9A6, AP1S2, DENNDA, RAC1, DYM*, RAB proteins (*RAB3GAP1, 2, RAB18*), TRAPPCpathies (*TRAPPC9,11,12,6A, 6B, 4), COL4A3BP, AP1S1 ARFGEF2, CDC42, VPS13B,VPS53, VPS51, VPS1, TBC1D23, TBC1D20*
	SPECIFIC CLINICAL FEATURES	SOME CAUSE WELL-DEFINED GENETIC SYNDROMES: *SLC9A6*: mimics Angelman Synd. TAU neuronal inclusions; *AP1S2*: Pettigrew Syndrome, EE and ID, Dandy-Walker, BBGG abnormality; DYM: Dyggve-Melchior-Clausen syndrome (ID, dwarfism); RAB proteins (*RAB3GAP1, 2, RAB18) and TBC1D20*: Warburg-Micro Syndrome, Marsof Syndrome, ID, progressive spasticity, axonal neuropathy, microphthalmia, cataracts; *VPS13B:* Cohen Syndrome, ID, obesity, neutropenia and retinopathy;*AP1S1:* MEDNIK Syndrome, high copper excretion; (▶ Chap. 39) *CDC42*: Takenouchi-Kosaki Syndrome, ID, EE, optic atrophy, macrothrombocytopenia, lymphedema
	CYTOSKELETON disorders	
	GENES INVOLVED	Kinesin (Anterograde Transport) deficiencies and NDE1 deficiency (Dynein Retrograde Transport): (NudE neurodevelopmental protein 1): *KIF5C, KIF10, KIF2A, KIF14, KIF16A, KIF7, KIF15*, Kinesin-binding protein *KIF1BP/KBP, KIF5A, NDE1* Tubulins (microtubule network): *TUBA1A, TUBA8, TUBB, TUBB2A, TUBB2B, TUBB3, TBCD, TUBG1*
	SPECIFIC CLINICAL FEATURES	OFTEN ASSOCIATE CORTICAL DYSPLASIA + HYPOMYELINATION +/–OTHER BRAIN MALFORMATIONS *KIF14*: Meckel syndrome; *KIF16A*: plus blindness; KIF7: Acrocallosal and Joubert syndrome; *KIF15*: with thrombocytopenia; *KIF1BP/KBP*: Golderg-Shprintzen syndrome; *KIF5A*: Neonatal seizures, SP. NDE1: Microhydranencephaly, Lissencephaly *TUBA1A*: Lissencephaly; *TUBB3*: complex cortical dysplasia with other brain malformations
	VESICULAR TRANSPORT	
	GENES + Other signs	*IER3IP1*: microcephaly, epilepsy and diabetes;
MACROCEPHALY Is rare in these group of disorders	GOLGIPATHIES	
	GENES + Other signs	*RAB39B* (is also a SV protein): with ID, EE and autism; *HERC1*: with ID, EE, hypotonia, ataxia *RAC1* that may produce MICROCEPHALY can also cause MACROCEPHALY
	VESICULAR TRANSPORT	
	GENES + Other signs	*TBCK* (RABGTPase binding): MACROCEPHALY or normocephaly, hypotonia, dysmorphy, brain atrophy
NEONATAL SEIZURES Are rare in these group of disorders, however epilepsy beyond the neonal period is common	VESICULAR TRANSPORT	
	GENES + Other clinical signs	*CNPNY3* (ER/cochaperone): NEONATAL-First months of life SEIZURES-EE. Progressive brain atrophy. Hippocampal malformation. (ER, Lysosome, Autophagosome, Dendrites /endo-lysosomal): NEONATAL SEIZURES, EE, post-natal MICROCEPHALY, SPASTICITY, ID, Parkinsonism. Brain atrophy, thin CC. *HCFC1* (ER, Nucleus): NEONATAL SEIZURES (some cases), EE, microcephaly, choreoathetosis, ID. Cortical malformations (some cases). High Homocisteine and MMA (CblC mimicking). *ATAD1*: AMPA receptor trafficking defect. NN Hypertonia +/–seizures
	CYTOSKELETON disorders	*KIF5A* (Kinesin, Anterograde Transport): INTRACTABLE NEONATAL MYOCLONUS

(continued)

Table 44.1 (continued)

	INTERORGANELLE-MCS	*PACS2*: ER-Mitochondria MCS defect. Seizures difficult to treat during the first year. Dysmorphism+cerebellar dysgenesis
COMPLEX encephalopathy with MULTISYSTEM INVOLVEMENT	VESICULAR TRANSPORT	
	GENES + Other signs	WITH ARTHROGRYPOSIS: *SLC35A3* (is a CDG), *ERGIC1, KIAA1109* (Alkuraya-Kucinskas syndrome (arthrogryposis, Dandy-Walker))
		WITH DIVERSE ORGAN INVOLVEMENT: *SUMF1* (Multiple sulfatase deficiency), *SNAP29* (CEDNIK syndrome; is a SV), *OCRL*: Lowe syndrome, Dent disease 2 and 1 (*CLCN5*); *TBCD*: Hypoparathyroidism-retardation-dysmorphism syndrome; *TBCE:* Encephalopathy, progressive, with amyotrophy and optic atrophy
		WITH RHABDOMYOLISIS (and Epilepsy and microcephaly): *TRAPPC2L* (ER-Golgi transport): may have also late-onset presentation
	GOLGIPATHIES	
	GENES + Other signs	WITH RHABDOMYOLISIS (and Epilepsy and microcephaly): *TRAPPC11 and TANGO2* (may present with metabolic crises, mild hyperammonaemia and hypoglycemia, long QT, but also other late NRL forms such as ID, spastic paraparesis and myastheniform symptoms)
		WITH DIVERSE ORGAN INVOLVEMENT: *VPS15* (ID, renal failure, retinitis pigmentosa, dysmorphy), *FLNA* (ID, EE, PV heterotopia, frontometaphyseal dysplasia, connective tissue abnormalites (Ehlers-Danlos like)); *ATP7* (TGN): MENKES Syndrome (mild variants: occipital Horn Syndrome, Peripheral Neuropathy); *AP3B1*: Hermansky-Pudlak syndrome 2 (albinism, infections)
	ORGANELLE AND INTERORGANELLE TRAFFICKING	
	GENES + Other signs	WITH ARTHROGRYPOSIS: *VPS33B and VIPAS39* (lysosome-interorganelle): ARC1 and ARC2
		WITH DIVERSE ORGAN INVOLVEMENT: Seipinopathies (► Sect. 35.2.2): include not only SP but EE, lipodystrophy and Celia's disease (neurodegeneration); *VPS33A*: MPS plus, Lysosome related
		WITH RETINAL DISTROPHY: Interorganelle trafficking and Membrane Contact Sites: -*EMC1:* cerebellar atrophy, visual impairment and psychomotor retardation; -*ACBD5*: retinal dystrophy with leukodystrophy
	AUTOPHAGY	
	GENES + Other signs	*EPG5*: VICI Syndrome: agenesis of the corpus callosum, cutaneous hypopigmentation, bilateral cataract, cleft lip and palate, and combined immunodeficiency. ID, seizures
BRAIN IMAGE FEATURES OF EARLY ONSET Encephalopathies		

BRAIN ATROPHY	THIN CC CC AGENESIS	CORTICAL MALFORMATIONS	POSTERIOR FOSSA	LEUKODYSTROPHY	BBGG
ATP6AP2, AP4E1, TBCD, VPS1, TBCK, TRAPPCpathies	THIN CC: Most Golgipathies with MICROCEPHALY Tubulinopathies with hypomyelination Others: *ATP6AP2* CC AGENESIS: *CDK5RAP2*	Most Golgipathies with MICROCEPHALY Tubulinopathies *NDE1* (Dynamin): Microhydranencephaly, Lissencephaly	Cerebellar Atrophy: *ZNHIT3* (PEHO-Like Syndrome), *AP4E1, SLC9A6* PCH: *VPS53, 51, TBC1D23, PCLO* (with optic atrophy) Joubert malformation: *AP4E1* Dandy-Walker: *AP1S2, KIAA1109* Other cerebellar dysplasia: *PACS2*	HYPOMYELINATION: Tubulinopathies *VPS11; FOLR1, SLC33A*: CCHLND. Low serum ceruloplasmin and copper *GJB1, GJA12*: Pelizaeus-Merzbacher-like DEMYELINATION: Pannexin (Panx1): Demyelination, *ACBD5*	*AP1S2, AP1S1*

OTHER NEUROPEDIATRIC DISEASES that may appear or are mostly detectable beyond the first year of life		
VESICULAR TRAFFICKING		*PAX3, MITF, TYR*: Waardenburg syndrome (different types); *NPC1, NPC2*: Niemann-Pick disease, type C1/C2; *EIF2AK3*: Wolcott-Rallison syndrome; *FGD1*: Aarskog-Scott syndrome; *INPP5E*: Mental retardation, truncal obesity, retinal dystrophy, and micropenis MORMS; *GET4* (VT): 1 case, EE, thin CC, brain atrophy (ER to Golgi RT, Syntaxin 5, TA protein); Psychiatric: *CLTN* (collectrin): Hartnup-like; *GD1l*: X-linked ID; *PACS1*: Schuurs-Hoeijmakers syndrome (SHMS): ID; *SCARB2*: epilepsy with or without renal failure; *CERT*: ID (also ER-TCN MCS dysfunction)
AUTOPHAGY		*WDR45*: Autistic traits, Rett-like phenotype, evolution towards NBIA
ASTROCYTIC TRAFFICKING		CAM: Neuroligins*: NLGN1, NLGN3, NLGN4*: autism spectrum disorders; NORMALLY LATE-ONSET: adolescence, adulthood: *SLITRK1*: obsessive compulsive disorder, *SLITRK4:* schizophrenia
POST-SYNAPTIC RECEPTOR TRAFFICKING		*JAKMIP1* (disorder of GABA receptor trafficking)—synaptopathy spectrum; *CNTNAP2*: autism
PREDOMINANT MOTOR DISORDERS		
ATAXIA **Most of them are SCA with onset in adolescence and adulthood** **May associate SP, epilepsy**	VESICULAR TRAFFICKING	*VPS13D* (SCA4), *RUBCN* (SCA15), *SIL1* (Marinesco-Sjoegren Syndrome: SCA with Congenital Cataract and ID; *AMP1* (Synaptic Vesicle disorder)): spastic ataxia, also causes a congenital myasthenic syndrome
	GOLGIPATHIES	*SCYL1* (SCA21), *GORS2* (progressive myoclonic epilepsy with ataxia); *ATG5*: SCA
	AUTOPHAGY	*SNX14, ATG5* (both SCA)
	CYTOSKELETON disorders	*SPTBN2* (SCA5 and 14), *KIF1C* (spastic ataxia)

(continued)

Table 44.1 (continued)

SPASTIC PARAPARESIS (SPG) Most of them complex and late-onset	AUTOPHAGY	*SPAST* and *ABCD1*: FA trafficking from LDs into peroxisomes (different types of SP: SPG4, 52, 47, 50, 51) *SPG11* (spatacsin): Type1 SP type 11; Other phenotypes: CMT2; ALS type5. Spastizin *(ZFYVE26)*: SP type 15 or Kjellin syndrome *TECPR2*: SP type 49; *AP5Z1*: SP type 48		
	CYTOSKELETON disorders	*REEP1* (SPG31), *KIF1C* (spastic ataxia 2, SPG 58); *KIF5A* (various phenotypes all AD: SPG10, CMT2, ALS); *KIF1A* (SPG30)		
	GOLGIPATHIES	*TANGO2, SLC33A1*(SPG42), *AP* related genes are also Golgipathies, *ATL1* (atlastin)		
	VESICULAR TRAFFICKING	*AP4B1, AP4E1*(Complex Spastic Paraparesis), *WASHC5* (SPG8), *NIPA1* (magnesium transporter): SPG6; MAST syndrome: *SPG21*; *SPART*: Troyer Syndrome, SPG20. Infantile onset: *AP4E1, AP4M1, AP4S1. APSZ1*: progressive SP; *TGFBR1*:onset in the first decade; *VPS37A*: early onset SP with pectus carinatum and hypertrichosis; *ARL6IP1, UBAP1*		
	ORGANELLE AND INTERORGANELLE	*BCSL2* (Seipin), *REEP1*, Atlastin-1 (*ATL-1*) (Organelle interplay Lipid Droplet, FA incorporation in LD): complex spastic paraparesis		
	GLIAL TRAFFICKING	Connexin47 (C47), *GJA12*: SP, Leukodystrophy		
PARKINSONISM and other MOVEMENT DISORDERS Most of them pediatric or juvenile parkinsonism and other early-onset movement disorders	AUTOPHAGY	*FIG4*: Yunis-Varon syndrome; type 2 (SDCO): Striatonigral degeneration, childhood-onset; *PRKN:* PARKIN deficiency (Parkinson Disease 2); *PINK1*: Parkinson Disease 6: *NADGP*: Neurodegeneration with ataxia, dystonia and gaze palsy		
	VESICULAR TRAFFICKING	*VPS13C*: Early-onset PARKINSONISM (may also cause Leigh-like features); *ATP6AP2*: Early-onset PARKINSONISM; *VPS13A*: chorea-achantocytosis; *VPS16, VPS4*: early-onset dystonia. These are also lysosome-related disorders; *GAK. LRRK2:* diversity of clinical phenotypes; *RME-8; SYNJ1*: pediatric-juvenile onset PD; *VPS16*: adolescence-onset dystonia; *VPS26A*: atypical PD, no L-Dopa response. *VPS35*: parkinsonism.		
	FLIPPASES	*ATP8A2*: cerebellar ataxia and atrophy, ID, chorea, severe hypotonia, optic atrophy; *ATP1A3*: Cerebellar ataxia, areflexia, pes cavus, optic atrophy, and sensorineural hearing loss (CAPOS), Alternating hemiplegia, Dystonia 12 (all AD); Rapid-onset parkinsonism Kufor-Rakeb Synd/ SP: *ATP13A2*		
	INTERORGANELLE-MCS	alpha-Synuclein, *VDAC1, SigR1* (Parkinson Disease); *BCAP31*(deafness, dystonia and cerebellar hypomyelination),		
	CYTOSKELETON disorders	*TUBB4A*: Torsion dystonia 4 (DYT4), Hypomyelinating leukodystrophy; *TUBB6*: congenital facial palsy with ptosis and velopharyngeal dysfunction; *ACTB*: dystonia, juvenile onset		
	GOLGIPATHY	*RAB39B*: Early-onset parkinsonism		
AMIOTROPHYC LATERAL SCLEROSIS (ALS)	AUTOPHAGY	*CHMP2B*: ALS type 17; *Spatacsin* (ALS type5 (SPG11 Type3)); *ALSJ* (ALS2); *FIG*: FIG4 deficiency: type 1 (BTOP): Polymicrogyria bilateral temporo-occipital; type 2 (ASL11): ASL type 11; type 3 *(CMT4J)*: CMT type 4		
	INTERORGANELLE/MCS: *VAP9, VAPB, SigR1*: ALS and SMA		CYTOSKELETON disorders: *KIF5A*	VESICULAR TRAFFICKING: *OPTN*

SPINAL MUSCLE ATROPHY (SMA)	CYTOSKELETON disorders: *BICAUDAL; DYNC1H1*		INTERORGANELLE: *VAPB*	VESICULAR TRAFFICKING: *ATP7*
PERIPHERAL NEUROPATHY Most of them are Charcot-Marie-Tooth (CMT) subtypes	AUTOPHAGY	*DCTN1* deficiency type 1: Neuropathy, distal hereditary motor, type VIIB; *FIG*, *SPG11*, *RAB7:* CMT2; *TMR13*		
	CYTOSKELETON disorders	*KIF1A*: Hereditary sensory neuropathy type IIC (AR), spastic paraparesis 30 (AR); *DCTN1* (Dynactin1/P150): Distal hereditary motor neuropathy Perry syndrome (AD); *KIF5A*: SPG10; CMT2 ALS; *DNM2*: CMT with neutropenia		
	GOLGIPATHIES	*FAM134B*: neuropathy hereditary sensory and autonomic type IIB; *RAB18, 39B, RAB7, RAB3GAP1*: CMT.		
	VESICULAR TRAFF: *ATP7*; *TGF* (HMSNO), *LITAF, MTMR2, 5, SH3TC2*		GLIAL TRAFFICKING: *Connexin32 (C32):* CMT	
DEMENTIA	INTERORGANELLE-MCS	Presenilin, amyloid precursor protein: Alzheimer disease; *TMEM106B*: FTD and a hypomyelination disorder		
	AUTOPHAGY	*TBK1* deficiency (TANK binding kinase 1): susceptibility to encephalopathy infection-induced; FTD; type 2 (FTD3): Frontotemporal dementia; *C9orf72*: frontotemporal dementia. Also involved in endosomal trafficking		
	VESICULAR TRAFFICKING	*CHMP2B, 4B*: Frontotemporal dementia, cataracts; *TREM2:* Nasu-Hakola disease (early-onset fronto-temporal dementia and recurrent bone fractures); *BIN1*: progressive dementia and amyloid deposition		
OTHER DISORDERS	Glial trafficking defects: Connexin32 (C32) (*GJB1*): Multiple Sclerosis; Connexin47 (C47) (*GJC12*): Leukodystrophy, Multiple Sclerosis; *Pannexin (PANX1*): Demyelination. Deafness related defects: *WFS1, UNCA5A, AP1S1, VPS33B, VPAS39, RFTL, MYH9,PAA3,MTF,TYR,SUMF1,MYC58,RAB23,MYO5B, BCAP31*			

AD autosomal dominant, *AR* autosomal recessive, *ARPHM* periventricular heterotopia with microcephaly, *ARC* arthrogryposis, cholestasis and renal dysfunction type 1, *AT* anterograde transport, *BBGG* basal ganglia, *CAM* cellular adhesion molecules, *CC* corpus callosum, *CCHLND* congenital cataracts, hearing loss, and neurodegeneration, *EE* epileptic encephalopathy (patients may exhibit different degrees of epilepsy severity), *Enc* encephalopathy, *DMC* Dyggve-Melchior-Clausen syndrome, *ID* intellectual disability, *HMSNO* hereditary motor and sensory neuropathy, *INH* inheritance, *MRXSCH* mental retardation X-linked syndrome Christianson, *MRD48* mental retardation autosomal dominant type 48, *MIC* microcephaly congenital or postnatal, *MACRO* macrocephaly, *MCS* membrane contact site, *MEDNIK* mental retardation, enteropathy, deafness, peripheral neuropathy, ichthyosis, and keratoderma, *mma* methylmalonic acid, *NRL* neurological, *PM* primary, congenital microcephaly, *POM* post-natal onset microcephaly, *PEHO-Like syndrome* progressive encephalopathy with edema, hypsarrhythmia, and optic atrophy infantile cerebellar optic atrophy-like (PEHO Like syndrome), *PCH* pontocerebellar hypoplasia, *PD* Parkinson disease, *PM* primary, congenital microcephaly, *POM* post-natal microcephaly, *MIC* PM or POM microcephaly, *SCA* spinocerebellar ataxia, *SP* spastic paraparesis, *RT* retrograde trafficking, *SP* spastic paraparesis, *Synd* syndrome, *TGN* trans Golgi network, *TKS* Takenouchi-Kosaki syndrome, *V* vesicle, *SV* synaptic vesicle, *XL* X-linked, *ER* endoplasmic reticulum, *VT* vesicular trafficking

Regulation of membrane phospholipids by phospholipid flippases (P4-ATPases contribute to membrane transport (see also ▶ Chap. 35)). Flippases translocate specific phospholipids from the exoplasmic to the cytoplasmic leaflet of membranes [27]. Several of these flippases are involved in neurological and hematological disorders (◘ Fig. 44.2, ◘ Table 44.2).

Gap junctions-hemichannels constitute a type of membrane trafficking between astrocytes and oligodendrocytes. Connexins and Pannxenins are proteins, hemichannels, involved in this kind of transport. Mutations in genes encoding for some subtypes of these proteins can cause neurological disorders [28] (◘ Fig. 44.2, ◘ Table 44.1), as described in ▶ Sect. 44.2.5.

◘ **Table 44.2** Extraneurological manifestations of trafficking disorders

	Pathophysiological category	Genes
Immunohaematological		
Immune Dysfunction Chediak-Higashi syndrome *(LYST)* Griscelli syndrome *(MYO5A, RAB27A)* Takenouchi-Kosaki syndrome *(CDC42)* Vici syndrome *(EPG5)* Wiskott-Aldrich syndrome *(WAS)*	Vesicular Trafficking	*DKC1, FLI1, HYOU1, JAGN1, MAGT1, NBAS, PACS1, PRF1, RAB27A, STXBP2, TMEM173, TTC37, UNC13D, VIPAS39, VPS13B,*
	OIT	*COG1, COG4, COG6, MYO5A, SLC35A1, SLC35C1*
	VOIT	*AP3B1, AP3D1, BLOC156, COPA, LAMTOR2, LRBA, LYST, STX11, VPS33B, VPS45*
	Autophagy	*EPG5, TBK1*
	Cytoskeleton	*WAS*
	Others	*ITK, MBL2, NFE2L2, SH2D1A, SKIV2L, XIAP*
HLH Chediak-Higashi syndrome *(LYST)* Familial HLH *(PRF1, STX11, STXBP2, UNC13D)* Griscelli syndrome *(MYO5A, RAB27A)* Lymphoproliferative syndrome *(CD27, ITK, SH2DA1, XIAP)* STING-associated vasculopathy *(TMEM173)*	Vesicular trafficking	*AP3B1, CD27, ITK, LYST, NBAS, PRF1, RAB27A, STX11, STXBP2, TMEM173, UNC13D*
	OIT	*MYO5A*
	Others	*SH2D1A, XIAP*
Specific Erythrocyte Abnormalities Congenital dyserythropoietic anemia type II *(SEC23B)* SLC35C1-CDG, lack of erythrocyte H-antigen (*SLC35C1*) Rabenosyn-5 deficiency, macrocytosis, megaloblastoid erythropoiesis *(RBSN)*	Vesicular trafficking	*RBSN, SEC23B*
	OIT	*SLC35C1*
	Cytoskeleton	*TBCE*
Specific Platelet Abnormalities Hermansky-Pudlak syndrome *(AP3B1, DTNBP1, BLOC1S3, BLOC1S6)* Macrothrombocytopenia *(MYH9)* Platelet-type bleeding disorder *(ACTN1, FLI1)* Wiskott-Aldrich syndrome *(WAS)*	Vesicular trafficking	*DTNBP1, FLI1, NBEAL2,*
	VOIT	*BLOC1S3, BLOC1S6, LRBA, TMEM165*
	Vesicular trafficking and cytoskeleton	*CDC42*
	Autophagy	*AP3B1*
	Cytoskeleton	*ACTN1, MYH9, WAS*
	Others	*RUNX1*
Specific White Blood Cell Abnormalities Charcot-Marie-Tooth disease 2M*:* Neutropenia *(DNM2)* Chediak-Higashi syndrome *(LYST)* NBAS deficiency *(NBAS): Pelger-Huet anomaly* Severe congenital neutropenia *(JAGN1, VPS45, WAS)*	Vesicular trafficking	*JAGN1, NBAS, VPS13B*
	OIT	*SLC35C1*
	VOIT	*LYST, VPS45*
	Cytoskeleton	*WAS*
	Autophagy and cytoskeleton	*DNM2*

Table 44.2 (continued)

	Pathophysiological category	Genes
Skin & hair		
Hypopigmentation Griscelli syndrome *(MYO5A, RAB27A, MLPH)* Hermansky-Pudlak syndrome *(AP3B1, AP3D1, BLOC1S3, BLOC1S6, DTNBP1)* Menkes disease *(ATP7A)* Vici syndrome *(EPG5)* Waardenburg syndrome *(MITF, PAX3)*	Vescicular trafficking	*DTNBP1, GPNMB, RAB27A, TYR*
	VOIT	*AP3B1, AP3D1, ATP7A, BLOC1S3, BLOC1S6, LYST,*
	Autophagy	*EPG5*
	Cytoskeleton	*MLPH*
	OIT and cytoskeleton	*MYO5A*
	Others	*MITF, PAX3*
Hyperpigmentation Alstrom syndrome *(ALMS1)* Craniolenticulosutural dysplasia *(SEC23A)*	Vescicular trafficking	*GPNMB, SEC23A*
	Cytoskeleton	*ALMS1, KIF1B,*
Ichthyosis ARC syndrome *(VIPAS39, VPS33B)* CEDNIK syndrome *(SNAP29)* MEDNIK syndrome *(AP1S1)* Multiple sulfatase deficiency *(SUMF1)*	Vesicular trafficking	*SNAP29, SUMF1, VIPAS39*
	VOIT	*AP1S1, VPS33B*
Nodular/papular lesions Lowe syndrome *(OCRL)* Lymphoproliferative syndrome X-linked *(XIAP)*	OIT	*AP1S3, OCRL*
	Others	*XIAP*
Eruptions Dyskeratosis congenita X-linked *(DKC1)* MEDNIK syndrome *(AP1S1)* Palmoplantar punctate keratoderma *(AAGAB)* Wiskott-Aldrich syndrome *(WAS)*	Vescicular trafficking	*AAGAB, DKC1, FLI1, GPNMB, PRF1, RAC1, TMEM173*
	OIT	*AP1S1, ATP2C1, COG6*
	Cytoskeleton	*WAS*
Laxity Geroderma osteodysplasticum *(GORAB)* Menkes disease *(ATP7A)* Wrinkly skin syndrome *(ATP6V0A2)*	Vesicular trafficking	*ATP6V0A2, NBAS*
	OIT	*COG7, GORAB, SLC39A13*
	VOIT	*ATP7A*
Increased skin thickness – lipodystrophy CDG syndromes *(COG4, COG6, COG7, SLC35C1, TMEM165)* Lipoid proteinosis *(ECM1)* MPS-plus syndrome *(VPS33A)* Mucolipidosis II *(GNPTAB)*	Vesicular trafficking	*KIAA1109*
	OIT	*SLC35C1*
	VOIT	*GNPTAB, TMEM165*
	Cytoskeleton	*ECM1, KIF11*
	VOIT and autophagy	*VPS33A*
Vascular skin abnormalities Craniolenticulosutural dysplasia *(SEC23A)*	Vesicular trafficking	*SEC23A*
Hair abnormalities Menkes disease *(ATP7A)* Trichohepatoenteric syndrome *(SKIV2L, TTC37)* Warburg micro syndrome *(RAB3GAP1, RAB18, TBC1D20)* Yunis-Varon syndrome *(FIG4, VAC14)*	Vesicular trafficking	*DTNBP1, RAB18, RAB3BAP1, SEC23A, TTC37, TYR*
	OIT	*COG4, COG7, CTNS*
	VOIT	*AP1S1, ATP7A, BLOC1S3, CAV1, TBC1D20*
	OIT and cytoskeleton	*MYO5A*
	Autophagy	*EPG5, FIG4, VAC14*
	Cytoskeleton	*ALMS1, KIFBP, MLPH*
	Others	*MITF, PAX3, SKIV2L*

(continued)

Table 44.2 (continued)

	Pathophysiological category	Genes
Musculoskeletal		
Muscular abnormalities Acute recurrent myoglobinuria *(LPIN1)* Centronuclear myopathy *(MTMR14)* Danon disease *(LAMP2)* Marinesco-Sjogren syndrome *(SIL1)* *MECRCN (TANGO2)* Vici syndrome *(EPG5)*	Vesicular trafficking	*DYSF, LPIN1, PIK3R4, RFT1, SIL1, SLC33A1, STRADA, TMEM173, VIPAS39*
	OIT	*ACBD5, COG8, CTNS, SLC35A1, SLC35C1*
	VOIT	*ARCN1, CAV3, ERGIC1, LAMP2, LYST, TANGO2, TMEM165, TRAPPC11, TRAPPC2L, VPS33B*
	Autophagy	*EPG5, MTMR14, SQSTM10, TBCD, VPS13D*
	Cytoskeleton	*ACTB, BIN1, KIF5C, TUBB3*
	Autophagy and cytoskeleton	*DNM2*
Skeletal dysplastic changes Dyggve-Melchior-Clausen disease *(DYM)* Multiple sulfatase deficiency *(SUMF1)* Spondyloepiphyseal dysplasia tarda *(TRAPPC2)* Yunis-Varon syndrome *(FIG4, VAC14)*	Vesicular trafficking	*ATPV06A2, CDC42, EIF2AK3, HYOU1, PIK3R4, RAB18, RAB3GAP1, RAB3GAP2, RFT1, SEC23A, SUMF1, TRAPPC2, VPS13B*
	OIT	*COG1, COG6, COG7, DYM, PACS1, RAB33B, SLC35A2, VPS33A*
	VOIT	*AP3D1, ARCN1, ATP7A, GNPTAB, TBC1D20, TMEM165, TRIP11*
	Autophagy	*FIG4, VAC14*
	Cytoskeleton	*ACTB, DYNC2H1, KIFBP*
Other skeletal signs Paget disease *(SQSTM1)* Primary intraosseous vascular malformation *(ELMO2)* STING-associated vasculopathy *(TMEM173)*	Vesicular trafficking	*ELMO2, TMEM173*
	VOIT	*COPA, LRBA*
	VT and AUTOPHAGY	*SQSTM1*
Structural bone abnormalities Hypoparathyroidism-retardation-dysmorphism syndrome *(TBCE)* Osteopetrosis type 1, 3 *(PLEKHM1, TCIRG1)* Waardenburg syndrome *(PAX3)*	Vesicular trafficking	*AP2S1, HERC1, NBAS, UNC45A*
	OIT	*COG1, CTNS, GORAB*
	VOIT	*IL17RD, OCRL, STX16*
	Autophagy	*TBCE*
	VOIT and autophagy	*PLEKHM1*
	OIT and autophagy	*SQSTM1, TCIRG1*
	Cytoskeleton	*ALMS1*
	Others	*PAX3*
Arthrogryposis ARC syndrome *(VPS33B, VIPAS39)* Neurogenic multiplex arthrogryposis *(ERGIC1)* Progressive encephalopathy brain atrophy and thin corpus callosum *(TBCD)*	Vesicular trafficking	*KIAA1109, VIPAS39*
	OIT	*SLC35A3*
	VOIT	*ERGIC1, VPS33B*
	Cytoskeleton	*BICD2, KIF5C, TBCD*
Joint laxity SLC35A1-CDG *(SLC35A1)* Cutis laxa type IIA *(ATP6V0A2)* SSASKS syndrome *(SLC10A7)* Warburg micro syndrome *(RAB3GAP1)*	Vesicular trafficking	*ATP6V0A2, RAB3GAP1*
	OIT	*SLC35A1*
	VOIT	*ATP7A, SLC10A7*

Table 44.2 (continued)

	Pathophysiological category	Genes
Digestive		
Chronic liver disease ARC syndrome *(VPS33B, VIPAS39)* CDG type II *(COG2, COG4, COG5, COG6, COG7, COG8, RFT1, TMEM165, TMEM199)* MEDNIK syndrome *(AP1S1)* Trichohepatoenteric syndrome *(SKIV2L, TTC37)*	Vesicular trafficking	*DCK1, NGLY1, RFT1, TMEM199, TTC37, UNC45A, VIPAS39*
	OIT	*COG2, COG4, COG5, COG6, COG7, COG8*
	VOIT	*AP1S1, CAV1, IGF2R, NPC1, NPC2, PEX13, TMEM165, TRAPPC11, VPS33A, VPS33B*
	Cytoskeleton	*ALMS1, DYNC2H1*
	Others	*ITK, SKIV2L*
Acute liver failure and hepatitis-like attaks Familial HLH *(PRF1, UNC13D, STX11, STXBP2)* RALF (recurrent acute liver failure) *(NBAS, SCYL1)* Wilson disease *(ATP7B)*	Vesicular trafficking	*ATP7B, BCAP31, HYOU1, NBAS, PRF1, UNC13D*
	OIT	*COG4, STX11*
	VOIT	*SCYL1,*
	Others	*SH2D1A, STXBP2, XIAP*
Hepato(spleno)megaly Mucolipidosis II *(GNPTAB)* Multiple sulfatase deficiency *(SUMF1)* Niemann-pick disease type C *(NPC1, NPC2)* Trichohepatoenteric syndrome *(SKIV2L, TTC37)*	Vescicular trafficking	*EIF2AK3, PRF1, RFT1, SUMF1*
	OIT	*COG4*
	VOIT	*AP3B1, AP3D1, CAV1, GNPTAB, LYST, NPC1, NPC2, TRAPPC11, VPS33A, VPS45*
	VOIT and autophagy	*PLEKHM1, TCIRG1*
	VT and cytoskeleton	*CD27*
	Others	*ITK, SKIV2L*
Gastrointestinal signs Chylomicron retention disease *(SAR1B)* Goldberg-Shprintzen megacolon syndrome *(KIFBP)* MEDNIK syndrome *(AP1S1)* Microvillus inclusion disease *(MYO5B)*	Vesicular trafficking	*HYOI1, TTC37, UNC45A,*
	VOIT	*AP1S1, ARCN1, LRBA, SAR1B, TMEM165*
	OIT	*COG4, COG6, COG8, SLC35A2*
	Autophagy	*FIG4, VAC14*
	Cytoskeleton	*KIFBP, MYO5B, WAS*
	Others	*SKIV2L, SLC2A10, XIAP*
Cardiological		
Congenital heart disease Carpenter syndrome *(RAB23)* Saladino Noonan syndrome *(DYNC2H1)* Schuurs-Hoeijmakers syndrome *(PACS1)*	Vesicular trafficking	*RAB23, STRADA, VPS13B*
	OIT	*COG1, PACS1, SLC35A1*
	VOIT	*ARCN1*
	Cytoskeleton	*DYNC2H1, FLNA*
Cardiomyopathy Danon disease *(LAMP2)* Familial hypertrophic cardiomyopathy *(CAV3)* Martsolf syndrome *(RAB3GAP2)* Vici syndrome *(EPG5)* Wolfram syndrome *(WFS1)*	Vesicular trafficking	*RAB3GAP2, WFS1*
	VOIT	*CAV3, GNPTAB, TANGO2, LAMP2*
	Cytoskeleton	*MYO5B*
	Others	*NFE2L2*
Arrhythmia Long QT syndrome type 9 & 11 *(CAV3, AKAP9)* *MECRCN (TANGO2)*	VOIT	*CAV3, TANGO2*
	OIT	*AKAP9*

(continued)

Table 44.2 (continued)

	Pathophysiological category	Genes
Other cardiac abnormalities MATINS *(macrothrombocytopenia and granulocyte inclusions with or without nephritis or sensorineural hearing loss) (MYH9)*	Cytoskeleton	*MYH9*
Ocular		
Cataract Lowe syndrome *(OCRL)* Multiple sulfatase deficiency *(SUMF1)* Vici syndrome *(EPG5)* Warburg micro syndrome *(*RAB3GAP1, *RAB18, TBC1D20)* Yunis-Varon syndrome *(FIG4, VAC14)*	Vesicular trafficking	*DKC1, KIAA1109, RAB18, RAB23, RAB3GAP1, RAB3GAP2, RNF13, SEC23A, SIL1, SLC33A1, SUMF1, WFS1*
	VOIT	*AP1S1, ARCN1, FYCO1, GNPTAB, INPP5E, ORCL, PEX13, TBC1D20, TRAPPC11*
	Autophagy	*EPG5, FIG4, VAC14*
	VOIT and autophagy	*CHMP4B*
	Cytoskeleton	*ACTB, ALMS1, KIF11, KIF1B, TUBG1*
Retinopathy Alkuraya-Kucinskas syndrome *(KIAA1109)* Choroideremia *(CHM)* Cohen syndrome *(VPS13B)* Leber amaurosis with deafness *(TUBB4B)* Wolfram syndrome *(WFS1)*	Vesicular trafficking	*ATXN2, CHM, KIAA1109, PIK3R4, SUMF1, TYR, VPS13B, WFS1*
	OIT	*SLC35A2*
	VOIT	*AP3B2, BLOC1S3*
	Autophagy	*AP5Z1, FIG4, VAC14*
	VOIT and autophagy	*SPG11*
	Cytoskeleton	*ALMS1, KIF11, TUBB, TUBB4B*
	Cytoskeleton and autophagy	*ZFYVE26*
	VOIT and cytoskeleton	*INPP5E*
Corneal abnormalities Carpenter syndrome *(RAB23)* Congenital stromal corneal dystrophy *(DCN)* Cystinosis nephropathic *(CTNS)* Multiple sulfatase deficiency *(SUMF1)*	Vesicular trafficking	*DCN, RAB23, SUMF1, TRAPPC2*
	OIT	*CTNS*
	VOIT	*GNPTAB, TBC1D20*
Hypopigmentation Chediak-Higashi syndrome *(LYST)* Hermansky-Pudlak syndrome *(AP3B1, AP3D1, DTNBP1)* Waardenburg syndrome *(MITF)*	Vesicular trafficking	*DTNBP1*
	VOIT	*AP3B1, AP3D1, LYST*
	Others	*MITF, PAX3*
Coloboma Baraitser-Winter syndrome *(ACTB)* Pontocerebellar hypoplasia type 11 *( )* Schuurs-Hoeijmakers syndrome *(PACS1)*	VOIT	*TBC1D23*
	OIT	*PACS1*
	Cytoskeleton	*ACTB*
Other ocular abnormalities Achromatopsia-7 *(ATF6)* Glaucoma open angle *(OPTN)* Goldberg-Shprintzen megacolon syndrome *(KIFBP)*	Vesicular trafficking	*NGLY1, RAB18, RAB3GAP1, SFB2WFS1*
	OIT	*ATF6, COG5, SLC39A13*
	VOIT	*LAMP2, OCRL, OPTN, TMEM165*
	Cytoskeleton	*KIF14, KIF1B, KIFBP, TUBG1*

Table 44.2 (continued)

	Pathophysiological category	Genes
Renal		
Tubulopathy ARC syndrome *(VIPAS39, VPS33B)* SLC35A1-*CDG (SLC35A1)* Dent disease type 2 *(OCRL)* Lowe syndrome *(OCRL)*	Vesicular trafficking	*VIPAS39, VPS33B*
	OIT	*SLC35A1*
	VOIT	*OCRL*
	Autophagy	*EPG5*
Chronic kidney disease CDG type II *(COG1, COG6, COG7, SLC35A1, SLC35A2)* Cystinosis nephropathic *(CTNS)* Lowe syndrome *(OCRL)* Wolcott-Rallison syndrome *(EIF2AK3)*	Vesicular trafficking	*EIF2AK3, LPIN1, STRADA*
	OIT	*CTNS, SCARB2, SLC35A1, SLC35A2*
	VOIT	*OCRL, VPS33A*
	Cytoskeleton	*KIF14, WAS*
	Others	*NUP107*
Renal dysplasia/malformation Alkuraya-Kucinskas syndrome *(KIAA1109)* Saladino-Noonan syndrome *(DYNC2H1)* Trichohepatoenteric syndrome *(TTC37)* Zellweger syndrome *(PEX13)*	Vesicular trafficking	*KIAA1109, RAB23, TTC37, WFS1*
	OIT	*PACS1*
	VOIT	*PEX13, VPS33A*
	Cytoskeleton	*DYNC2H1*
Endocrine, reproductive, neoplasia		
Endocrinological abnormalities Cohen syndrome *(VPS13B)* (short stature,truncal obesity) Kenny-Caffey syndrome *(TBCE)* (hypoparathyroidism) Osteopetrosis type 1 & 3 *(TCIRG1, PLEKHM1)* Pseudohypoparathyroidism IB *(STX16)* Wolfram syndrome *(WFS1)* (diabetes)	Vesicular trafficking	*STRADA, VPS13B, WFS1*
	VOIT	*LRBA, PLEKHM1, STX16, TANGO2*
	VOIT and AUTOPHAGY	*TCIRG1*
	Cytoskeleton	*TBCE*
Genital/reproductive abnormalities Carpenter syndrome *(RAB23)* Globozoospermia 6 *(SPATA16)* Marinesco-Sjogren syndrome *(SIL1)* Martsolf syndrome *(RAB3GAP2)* Warburg micro syndrome *(RAB3GAP1 RAB18, TBC1D20)*	Vesicular trafficking	*PANX1, RAB18, RAB23, RAB3GAP1, RAB3GAP2, RAC1, SIL1, WFS1*
	VOIT	*TBC1D20*
	Autophagy	*PRKN, VAC14*
	Cytoskeleton	*TBCE*
	Others	*SPATA16*
Neoplasia Adenocarcinoma of lung *(PRKN)* Hepatocellular carcinoma somatic *(IGF2R)* Lymphoproliferative syndrome 1 & 2 *(ITK, CD27)* Pheochromocytoma *(KIF1B)*	Vesicular trafficking	*CD27, DKC1, ITK, MAGT1*
	OIT	*COG1, COG4, COG6, SLC35A1, SLC35C1*
	VOIT	*IGF2R*
	Autophagy	*PRKN*
	Cytoskeleton	*KIF1B*
	Others	*RUNX1, SH2D1A, XIAP*

OIT organelle/interorganelle trafficking, *VOIT* vesicular and organelle/interorganelle trafficking, *HLH* hemophagocytic lymphohistiocytosis, *CDG* congenital disorder of glycosylation

44.2 Cellular Trafficking in the Nervous System: Polarization and Compartmentalization

Neurons are the largest known cells, with complex and highly polarized morphologies. As such, neuronal signaling is highly compartmentalized, requiring sophisticated transfer mechanisms to convey and integrate information within and between sub-neuronal compartments [29].

Organelles, proteins and RNAs are actively transported to synaptic terminals for the remodelling of pre-existing neuronal connections and formation of new ones [30]. Mechanisms of intraneuronal communication are tightly regulated and disclose a high susceptibility to genetic mutations that produce both early-onset neurodevelopmental and late-onset neurodegenerative diseases [29].

The specialized domains of neurons are located in the cytosol, axons, axon growth cones, axon initial segment, nodes of Ranvier, dendrites (proximal and distal), and synapses (pre-synaptic and postsynaptic specializations). Each domain contains specific sets of proteins and plays distinct functions that rely on regulated trafficking from biosynthetic and endosomal compartments. These mechanisms may vary depending on different cell types and brain regions. Abnormalities in trafficking mechanisms in glial cells have also been described to cause neurological disorders.

44.2.1 Trafficking Defects in the Neuronal Soma (ER-Golgi-PM-Endosome-Lysosome-Autophagosome)

▪ Exocytic Pathway Defects

As already mentioned, the exocytic pathway moves cargo from the ER through the Golgi to the PM. There are two major steps in the exocytic pathway mediated by vesicles: ER-to-Cis Golgi and Trans Golgi-to-PM.

The great length of neuronal projections and the need for precise spatiotemporal control over membrane and secreted protein localization make neurons particularly vulnerable to defects in each of the ER-Golgi compartment export and trafficking. Here we describe only some of the most representative diseases according to the main affected proteins and subcellular compartments (◘ Figs. 44.2 and 44.3 ◘ Table 44.1).

1-ER-to-Cis Golgi defects include mutations in genes affecting the COPII machinery**.** They are linked to diverse neurological diseases (◘ Figs. 44.2 and 44.3, ◘ Table 44.1):

I. Most are early-onset neurodevelopmental disorders with congenital or post-natal microcephaly (+/− cortical and other brain malformations), intellectual disability, and white matter disorders. They are related to diverse type of proteins such as COP, SEC, TRAPPC, TBC, VPS and RAB (RAB GTPases, RAB-effectors, RAB-regulating proteins) [7, 8]. *TANGO2* defects belong also to this localization but may associate rhabdomyolysis episodes and other clinical manifestations mimicking fatty acid oxidation disorders [31–33] (▶ Sect. 1.4.6).
II. Late-onset presentations can appear in late childhood, adolescence and adulthood related with motor dysfunctions such spastic paraparesis (i.e. *TECPR2, TANGO2, SLC33A1* defects) [34], and Charcot-Marie-Tooth (CMT) disease (i.e *TFG, CNPNY3* defects) [35]. *TREM2* is a trafficking defect between the ER and ERGIC, linked to a syndrome characterized by early-onset fronto-temporal dementia and recurrent bone fractures (i.e. Nasu-Hakola disease, also known as polycystic lipomembranous osteodyplasia with sclerosing leukoencephalopathy) [36].

2-Trans Golgi-to-Plasma Membrane defects involve complex severe encephalopathies such as diverse adaptinopathies: MEDNIK syndrome (AP1S1 adaptor protein defect) (full description in ▶ Sect. 34.1.4), *AP1S2* (Pettigrew Syndrome) [37], *ARFGEF2* (microcephaly, periventricular heterotopia), and *ATP7* mutations (Menkes Syndrome), and complex spastic paraparesis (*AP4B1, AP4E1*) [38].

Several glycosylation defects (COG1, 7, 8) are related to trafficking alterations between the ER and the Golgi complex.

▪ Endocytic Pathway Defects

In the endocytic pathway, cargo can be internalized at the PM by a number of routes: membrane receptors are mainly internalized via clathrin-coated vesicles whereas other proteins are internalized by caveolar or raft dependent routes [5]. Early and late-endosomes to lysosomes belong to the clathrin-coated endocytic pathway (◘ Figs. 44.1 and 44.4).

▪▪ Golgi to Endosomes Trafficking Defects

Diseases in this subcellular compartment often present as complex early-onset encephalopathies that often associate multisystem involvement. This is the case of Rabenosyn-5 deficiency and *SLC9A6, DENND5A,* and *ARFGEF2* mutations [39, 40].

▪▪ Late Endosome to Lysosome and Trafficking Defects

Endosomes and lysosomes are acidic organelles that degrade plasma membrane components, extracellular and intracellular macromolecules and cellular fragments. The endolysosomal vesicle and autophagosomes communicate to each other. Therefore, some proteins involved

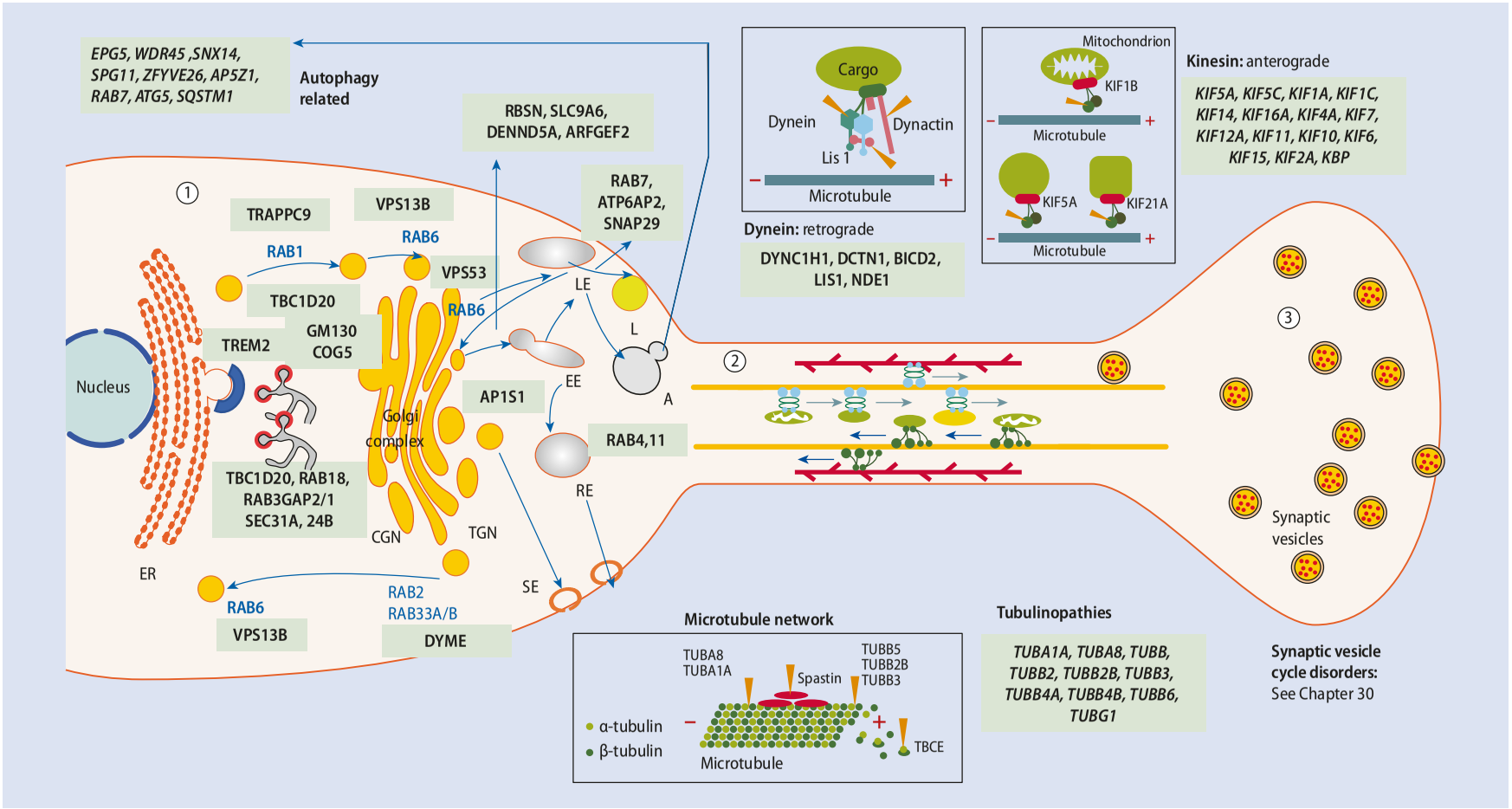

Fig. 44.4 Disorders of neuronal trafficking. There are three neuronal compartments with specialized traffic mechanisms: 1-Cytoplasm; 2-Axon; 3-Presynaptic terminal. Here we include only some of the most representative genes responsible of neurological diseases and their predominant cellular localization (in the green squares). Highlighted in blue are RAB proteins that behave in different cellular exocytic and endocytic processes. A autophagosome, CGN cis Golgi network, EE early endosome, L lysosome, LE early endosome, RE recycling, endosome, SE secretory endosome, TGN trans Golgi network

in this pathway can also have roles in autophagy. Clinical presentations include neonatal seizures (*ATP6AP2*) [41], complex multisystem syndromes such as CEDNIK syndrome (*SNAP29* mutations) see below (▶ Sect. 1.6.2) and ARC syndrome (*VPS33B* and *VIPAS39* defects) [42] and peripheral neuropathy (CMT) as in *RAB7* defects (late endosome to lysosome: pathway) [43].

Lysosome biogenesis defects (type 7 and 8 Hermansky-Pudlak syndrome; see later) and several phosphoinositide-phosphatase-producing myopathies (▶ Sect. 35.5), Lowe syndrome and CMT types 4B1 and 4B2, also belong to this category.

3-Autophagy Defects

The autophagy pathway allows to engulf areas of the cytoplasm, including membrane-bounded organelles and deliver the material for degradation in the lysosome to generate nutrients (◘ Fig. 44.3). Disorders present as early complex encephalopathies with multisystem involvement such as VICI syndrome (*EPG5* mutations) see below [44], neurodevelopmental disorders with brain iron accumulation (WDR45 defect; full description ▶ Sect. 34.2.2), spastic paraparesis (spastin, *ABCD1*, spastacsin, spastizin, *TECPR2* and *AP5Z1* mutations), amyotrophic lateral sclerosis (*CHMP2B, FIG4* mutations), peripheral neuropathy (CMT disease) due to *DCTN*1, *FIG4*, spactacsin and *RAB7* mutations, parkinsonism and other movement disorders (*FIG4, PRKN*, *PINK1*, *RAB39B* mutations), and dementia (*TBK1*, *FTD3*) [45].

44.2.2 Axonal and Other Cytoskeleton Related Trafficking Defects

The cytoskeleton is responsible for moving vesicles throughout the cell and is composed by the following main elements:

- **Microtubules** are crucial for long-range intracellular transport and are dynamic structures consisting of heterodimers of alpha-tubulin and beta-tubulin [46].
- **Motor proteins**, including the myosin, dynein, and kinesin families of proteins, are responsible for the anterograde and retrograde transport of cargo. In general, myosins are actin-dependent motors, whereas dyneins and kinesins are microtubule-dependent motors [47].

Neurons shuttle diverse substances along axon microtubules through a bidirectional, ATP-dependent process known as axonal transport. Anterograde transport, from the cell body to the axon tip, is driven by kinesins and deliver substances such as RNAs, proteins and organelles towards synapses. Retrograde transport travels in the opposite direction and is dependent on dynein. It is essential for autophagy-lysosomal degradation and neurotrophic factor signalling.

In addition to motor proteins and microtubules, **motor adaptor proteins** compose intricate protein kinase signalling pathways (◘ Fig. 44.4). Dynactin, BICD2, Hook, LIS1 and NDEL1 are dynein-related adaptor proteins. Most of the kinesin adaptors including HAP1, JIP1 and TRAK1 are also adaptors for dynein [46].

Mutations in genes encoding components of the axonal transport machinery have been implicated in the pathogenesis of neurological diseases (◘ Figs. 44.2 and 44.4, ◘ Table 44.1). Most of them are late-onset motor diseases (◘ Table 44.1) (motor neuropathies, CMT, spastic paraparesis, ALS, SMA, CFEOM: congenital fibrosis of extraocular movements) and early-onset encephalopathies associated with cortical migration abnormalities (◘ Table 44.1).

44.2.3 Synaptic Vesicle Cycle Disorders

Synaptic vesicle disorders are a group of neurological diseases of cellular traffic located at the presynaptic terminal with involvement in neurotransmission. They include both exocytic and endocytic defects and are described in ▶ Chap. 30 [48].

44.2.4 Dendrites and Post-synaptic Neuron Compartment Traffic Defects

Neuronal dendrites are highly branched and specialized compartments with distinct structures and secretory organelles (e.g., spines, Golgi outposts), and a unique cytoskeletal organization that includes microtubules of mixed polarity [49].

Post-synaptic diseases linked to mutations in neurotransmitter receptors have been described in ▶ Chap. 30.

Dendritic membrane traffic includes specific proteins such as septins and cytoskeleton-based mechanisms with specific functions of great complexity that are fundamental for processes such as learning and cognition. The details of these unique mechanisms, mostly based on the spatial control related to the highly arborized morphology of dendrites, are beyond the scope of this Chapter.

44.2.5 Glia Trafficking Disorders

Commonly, glial cells communicate with each other and with neurons via gliotransmitters but can also communicate using hemichannels. Oligodendrocytes form extensive functional interactions among them and with astrocytes through special structures called gap junc-

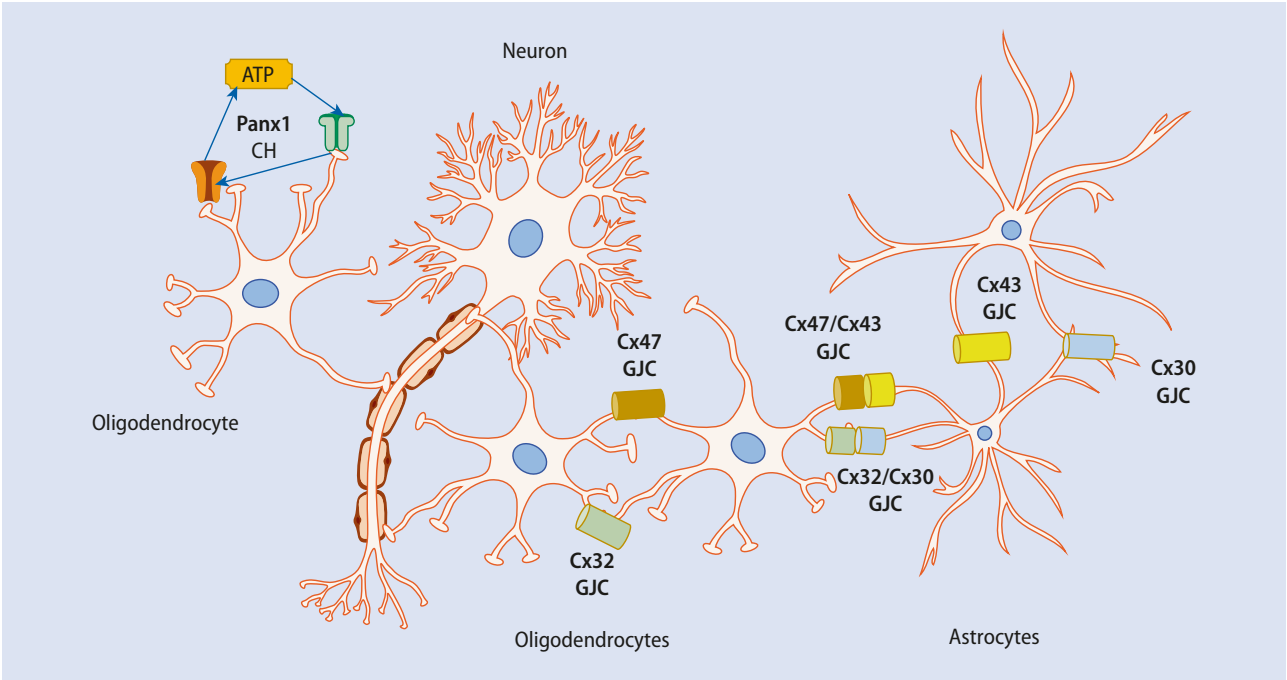

Fig. 44.5 Disorders of glial trafficking. Connexins (Cx) and Pannexins (Panx) in glial cells. GJC: Gap Junction. The opening of Panx1 CH (Panx1 channels) may lead to ATP release from the oligodendrocytes. Mutations in genes that codify for Cx32,Cx47 and Panx1 produce neurological diseases. Cx32 is codified by *GJB1* (Multiple Sclerosis). Cx47 is codified by *GJC2* (Leukodystrophy, Multiple Sclerosis). Panx1 is codified by *PANX1* (Demyelination)

tions. Gap junctions, composed of one or more of the 21-member connexin (Cx) family, function as intercellular channels and have the ability to pass small signalling molecules, metabolites, and electrical stimuli directly between contacting cells (51-Vejar, 2019). Connexins are integral membrane proteins that oligomerize into homomeric or heteromeric hexamers, also called connexons, in the ER or Golgi apparatus. Upon microtubule-dependent transport to the cell surface, connexons may function as hemichannels. Pannexons channels are similar than connexins but localized in the oligodendrocytes (Fig. 44.5).

Mutations in genes coding these trafficking proteins cause white matter disorders such as multiple sclerosis (Connexin32 (Cx32), *GJB1* gene, and Cx47, *GJA12* gene), hypomyelinating leukodystrophies (Cx32, Cx47, which causes Pelizaeus-Merzbacher-like disease), demyelinating leukodystrophies (Cx47 and Pannexin 1, *Panx1* gene), spastic paraparesis (Cx47) and peripheral neuropathy (Cx32, CMT disease) [28] (▶ Sect. 1.5).

44.3 Main Clinical Presentations of Cellular Trafficking Disorders

There are more than 300 different genes involved in human cellular trafficking diseases [50]. Due to the dimension and complexity of the topic, we will describe the general traits of clinical manifestations rather than reporting the exhaustive details of every genetic defect. See also Table 44.1. Cross reference is indicated each time a specific defect is described elsewhere in the book.

44.3.1 Neurological Manifestations

Most disorders of cellular trafficking exhibit neurological manifestations that may present as isolated nervous system diseases but also associated with symptoms involving other organs. They can appear at any age from the neonatal period to adulthood. Whereas early presentations tend to have a global involvement affecting multiple functions of the brain and sometimes the peripheral nervous system (▶ Chap. 1), late-onset diseases, starting beyond adolescence, are more likely to have predominant motor symptoms such as spastic paraparesis, motor neuron disorders and peripheral neuropathies (▶ Chap. 2). In general, they have a progressive character and there are almost no effective treatments.

Early-Onset Encephalopathies

These disorders are developmental encephalopathies that appear during the first year of life including the neonatal period. Frequent features include microcephaly, brain structural abnormalities, and the coexistence of multiple neurological signs, particularly epilepsy, sometimes refractory to antiepileptic treatment. They

may have multisystem involvement and some constitute well-defined genetic syndromes (see also ▶ Chap. 1).

Neonatal Manifestations

Neonatal seizures are rare in trafficking disorders. Five genes have been involved, most are vesicular transport defects and tend to associate congenital or post-natal microcephaly and brain malformations. Some examples include mutations in *CNPY3* (hipoccampal malformation) [51], *ATP6AP2* (post-natal microcephaly and brain atrophy), *HCFC1* (cortical malformation) and *PACS2* (ER-mitochondrial membrane contact site defect, that associates cerebellar dysgenesis) [52]. *KIF5A* mutations cause intractable neonatal myoclonus and is a cytoskeleton disorder [53].

Microcephaly

Both congenital and post-natal Microcephaly (POM), is a major manifestation of these diseases and the most frequent sign in developmental brain disorders [7]. Congenital microcephaly is related to disturbed mechanisms in cortical progenitor division and their subsequent survival and differentiation, whereas POM involves defective neuronal maturation, synaptic pruning, myelination and neurodegeneration [7, 54]. The Golgi apparatus and the cytoskeleton strongly regulates these processes. Around 30 genetic defects classified as "golgipathies" and 20 genes involved in cytoskeleton functions are responsible for disease (◘ Table 44.1). RAB, TRAPP and VPS proteins are amongst the most common involved in Golgi defects [55, 56], whereas kinesins and tubulins are the proteins involved in cytoskeleton disorders [57]. These diseases often associate cortical and other brain malformations and are global, severe, developmental encephalopathies with multiple neurological and extra-neurological sings. Well-defined genetic syndromes are depicted in ◘ Table 44.1.

Macrocephaly

It is rare in these disorders. Only four genes have been involved. In general, macrocephaly is associated to intellectual disability, autistic signs and epilepsy in different combinations. They include golgipathies such as mutations in *RAB39B* (that codifies for a synaptic vesicle protein, see ▶ Chap. 30), *HERC1* [58], *RAC1* (which may also produce microcephaly) [59], and the vesicular transport protein TBCK (which may also cause brain atrophy with normocephaly).

Complex Encephalopathies with Multisystem Involvement

They include around 30 defects. Arthrogryposis is a prominent sign in vesicular transport defects such as mutations in *SLC35A3* [60], *ERGIC1* [61] and *KIAA1109* (Alkuraya-Kucinskas syndrome) [62], and interorganelle trafficking defects such as mutations in *VPS33B and VIPAS39* that cause ARC1 and ARC2 syndromes respectively (ARC: Arthrogryposis, Renal involvement and Cholestasis) [63]. Rhabdomyolysis is often found in *TANGO2* mutations. Patients may have metabolic crises with rhabdomyolysis, mild hyperammonaemia, hypoglycaemia and long QT interval (abnormal cardiac rhythm) [31–33]. *TANGO2* is associated with a wide spectrum of neurological manifestations such as intellectual disability, spastic paraparesis, epilepsy and myasthenic symptoms, that are not always associated with metabolic crises [33]. Mutations in *TRAPPC11* may also cause myopathy in patients with intellectual disability, epilepsy and microcephaly [64].

Other important examples are Menkes disease (MD), caused by *ATP7A* mutations, and Vici syndrome (*EPG5*). MD is a disorder of copper metabolism but also a Golgipathy since ATP7A is a Golgi transport protein (▶ Sect. 34.1.3). Vici syndrome, caused by *EPG5* mutations [44], is a disorder of autophagy that includes early-onset severe neurological symptoms, agenesis of the corpus callosum, cutaneous hypopigmentation, bilateral cataract, cleft lip and palate, and combined immunodeficiency (see also below).

Other Disorders with Intellectual Disability and/or Neuropsychiatric Symptoms as Main Neurological Signs

That exhibit symptoms usually beyond the first year of life, include around 25 genes that belong to diverse pathophysiological categories (◘ Table 44.1). Here we highlight *WDR45* mutations, a disorder of autophagy that cause beta-propeller protein-associated neurodegeneration (BPAN). The most common form of presentation is intellectual disability and autism with hand stereotypies mimicking Rett syndrome with subsequent parkinsonism-dystonia in adolescence. NBIA (neuronal brain iron accumulation) with evident brain image characteristic abnormalities appears over time (▶ Sect. 34.2.3).

Several Disorders Have Biomarkers

- *SLC9A6* mutations (Golgipathy): mimics Angelman syndrome and has TAU neuronal inclusions [65].
- *CDC42* mutations (Golgipathy): Takenouchi-Kosaki Syndrome (ID, epileptic encephalopathy, optic atrophy, and lymphedema) associates also macrothrombocytopenia [66].
- *KIF15* mutations (cytoskeleton disorder): congenital microcephaly with thrombocytopenia.
- *HCFC1* mutations (vesicular transport defect): epileptic encephalopathy, microcephaly, choreoathetosis, cortical malformations and high homocysteine and MMA (CblX. See ▶ Sect. 28.2.1).

- *ER3IP1* mutations (vesicular transport defect): microcephaly, epilepsy and diabetes.
- *FOLR1* mutations (transcytosis defect): low folate levels in the CSF in patients with encephalopathy (▶ Sect. 28.3.2), myoclonic epilepsy and a progressive character;
- Menkes disease (*ATP7A* mutations), MEDNIK syndrome and CCHLND (congenital cataracts, hearing loss, and neurodegeneration) due to *SLC33A1* mutations, exhibit low serum ceruloplasmin and copper (▶ Sect. 34.1.4)
- Abnormalities on brain imaging are excellent biomarkers for several early-onset encephalopathies (◘ Table 44.1).

Motor Disorders

These tend to have a late-onset particularly if isolated as a clinical manifestation. Early-onset presentations are less common and appear in association with other signs such as intellectual disability, and epilepsy. Additionally, mutations in the same gene may cause different clinical phenotypes. Overall, they are neurodegenerative disorders (see also ▶ Chaps. 1 and 2).

Spastic Paraparesis

That tends to be complex (associated to other neurological and non-neurological signs). Around 30 genetic defects have been described. Autophagy and vesicular transport are the most common pathophysiological categories involved. The endocytic pathway is frequently affected. Genes associated with infantile onset SP include *AP4E1, AP4M1, AP4S1* [67]. Spastic paraparesis that develops during the first decade of life is caused by mutations in *TGFBR1 and VPS37A* (with pectus carinatum and hypertrichosis) [68].

Ataxia

It presents frequently as spinocerebellar ataxia (SCA) with onset in adolescence and adulthood. It may be associated with other symptoms such as spastic paraparesis, epilepsy and intellectual disability. There are about 11 genes involved [50].

Parkinsonism and Other Movement Disorders

There are about 20 genes responsible for parkinsonism. Endocytic defects [69] including autophagy are quite common. GAK, *SYNJ1*, VPS35 and RME8 mutations are some examples. The secretory pathway may also be affected and in this case other neurological signs are often associated. This is the case of RAB39B (early-onset parkinsonism, intellectual disability) [70]. ATP13A2 mutations cause a rapid-onset Parkinsonism (Kufor-Rakeb Syndrome). Hyperkinetic disorders include early-onset dystonia caused by VPS16 and VPS41 which have been recently reported [18] and chorea-acanthocytosis caused by mutations in VPS13A.

Motor Neuron Disorders

Amyotrophic lateral sclerosis (ALS) is a motor neuron disease that results in progressive degeneration of motor neurons. Dysregulation of endocytic transport that in turn affects lysosome function and autophagy is commonly involved as main mechanism of disease. An infantile-onset motor neuron disease, Spinal Muscle Atrophy (SMA) may be caused by mutations in BICD1, DYNC1H1, VAPB and ATP7 [71].

Peripheral Neuropathy

It presents in most cases as Charcot-Marie-Tooth (CMT) disease. Mutations in genes that involve the endocytic pathway (such as SH3TC2), cytoskeleton disorders (such as FIG4 mutations) and Golgipathies are common in these group of disorders [50]. DNM2 mutations causes CMT with neutropenia. FAM134B mutations produce hereditary sensory and autonomic neuropathy type IIB [72].

Dementia and Others

Alzheimer's disease (AD) and Frontotemporal dementia (FTD) are the most common forms of cognitive deterioration in cellular trafficking disorders. Mutations in Presenilin and BIN1 are associated with AD. CHMP2B and 4B mutations cause FTD with cataracts. TREM2 mutations [73] produce early-onset FTD and recurrent bone fractures (Nasu-Hakola disease).

44.3.2 Extra-Neurological Manifestations

The non-neurological manifestations, which may affect immuno-haematological, musculoskeletal, digestive, cardiological, renal, endocrine, reproductive, ocular, skeletal, renal, ocular, skin and hair systems are rarely isolated but more often part of a multisystem disease or a well-defined syndromic phenotype (◘ Table 44.2). Only a few characteristic syndromes are described below.

The most relevant manifestations of the immuno-haematological system are immune dysfunctions/deficiencies, familial hemophagocytic lymphohistiocytosis, and specific abnormalities of circulating blood cells. Vici syndrome, caused by autosomal recessive mutations in *EPG5,* was initially described as a new syndrome characterised by agenesis of the corpus callosum, skin and hair hypopigmentation, cataracts, cleft lip and palate, cardio/myopathy and immunodeficiency. It causes a complex immunological dysfunction, with selective involvement of memory B cells, affecting both innate and adaptive immunity [74].

Chediak-Higashi syndrome [75] is a rare autosomal recessive disorder caused by *LYST* (lysosomal trafficking regulator gene) mutations characterized by partial oculocutaneous albinism, severe immunodeficiency, mild bleeding, neurological dysfunction and lymphoproliferative disorder linked to defective natural killer lymphocytes and lethal in the absence of bone marrow transplantation. Differential diagnoses include oculocutaneous albinism, Hermansky Pudlak syndrome (see later) and Griscelli disease.

Skin and hair abnormalities are frequent and include a wide spectrum of clinical manifestations such as hypopigmentation/albinism, ichthyosis, eruptions, nodular and papular lesions, skin laxity, and lipodystrophy, and structural hair abnormalities with or without pigmentary changes.

Hermansky-Pudlak syndrome (HPS) is an AR multisytemic disorder characterized by an oculocutaneous albinism (abnormally light coloring of the skin, hair and eyes causing photophobia and nystagmus) and a bleeding tendency [76]. Other symptoms may include immune problems, pulmonary fibrosis, colitis and increased risk for skin cancer. There are ten types of HPS each caused by a different dysfunctional gene. **CEDNIK syndrome** (Cerebral Dysgenesis, Neuropathy, Ichthyosis, and palmoplantar Keratoderma) is an AR disorder linked to mutations of *SNAP29* which encodes a SNARE protein involved in vesicle fusion [77].

Musculoskeletal signs comprise myopathies with structural muscle abnormalities, recurrent myoglobinuria, skeletal dysplasia and structural bone abnormalities, arthrogryposis, and joint laxity**. Dyggve-Melchior-Clausen disease** caused by mutations of *DYM* is a rare, genetic spondylo-epi-metaphyseal dysplasia characterized by progressive short-trunked dwarfism, protruding sternum, microcephaly, intellectual disability and pathognomonic radiological findings (generalized platyspondyly, irregularly ossified femoral heads, a hypoplastic odontoid, and a lace-like appearance of iliac crests) [78].

Symptoms related to digestive system include acute and chronic liver disease and secretory diarrhea and inflammatory bowel disease. Recurrent acute liver failure (RALF) triggered by febrile infections is a common presenting sign in *NBAS* and *SCYL1* mutations. Mutations in *NBAS* (involved in retrograde transport) cause a complex disease with a wide clinical spectrum ranging from isolated RALF to a multisystemic phenotype including SOPH syndrome. Thermal susceptibility of the syntaxin 18 complex is the basis of fever dependency of RALF episodes [79]. *SCYL1* mutations underlie a syndrome characterized by RALF, peripheral neuropathy, cerebellar atrophy, and ataxia (◘ Table 44.1) [80].

Characteristic signs of cardiac involvement include congenital heart disease (such as Schuurs-Hoeijmakers syndrome due to *PACS1* mutations, cardiomyopathy (dilated and hypertrophic; i.e. Vici syndrome: *EPG5* mutations; Danon disease: *LAMP2*), and arrhythmia. *CAV3, AKAP9* and *TANGO2* mutations cause long QT syndrome.

The visual apparatus is frequently affected in disorders of cellular trafficking. Cataract, retinopathy, corneal abnormalities, pigmentary changes (see above **Hermansky-Pudlak and Chediak-Higashi syndrome**) and coloboma represent the major presenting clinical manifestations.

Kidney disease may manifest as tubular dysfunction with or without renal failure, as chronic kidney disease, or with renal dysplastic changes. **Oculocerebrorenal syndrome of Lowe (OCRL)** is a rare X-linked multisystem disorder due to *OCRL* mutations, leading to phosphatidylinositol-4,5- bisphosphate accumulation. It is characterized by congenital cataracts, glaucoma, intellectual disabilities, seizures, postnatal growth retardation and renal tubular dysfunction of the Fanconi type (proximal tubular acidosis; phosphate wasting leading to renal rickets, osteomalacia and pathological fractures) with chronic renal failure [81] (▶ Sect. 35.5).

The endocrine and reproductive systems may also be involved. Finally, some disorders of cellular trafficking are associated with specific type of cancer or with increased risk of neoplasia (◘ Table 44.2).

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Appendices

Contents

Medications Used in the Treatment of Inborn Errors of Metabolism

Andrew A. M. Morris and Simon Jones

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The following table lists medication used in the treatment of IEM (Table 45.1). The list is not exhaustive. Drugs are listed alphabetically by the main name (e.g. L-arginine is listed under Arginine). Readers should be aware that many of these drugs are unlicensed and the evidence base for their use is limited; there have very seldom been controlled trials due to the rarity of the disorders. When using this table, readers are advised to consult the specific chapters indicated. While every effort has been made to ensure the accuracy of the information, before prescribing it is essential that the indications and dosage are checked against any local or national guidelines or formularies. The recommended doses related to body weight are generally those for paediatric patients, using these in adult patients may not be appropriate. For explanation of other abbreviations, see relevant chapters.

Table 45.1 Medication used in the treatment of IEM

Medication	Mode of action	Disorders	Recommended paediatric dose (unless otherwise stated)	Route	Remarks	Chapter(s)
Agalsidase alfa	ERT	Fabry disease	0.2 mg/kg alt weeks	IV		► 40
Agalsidase beta	ERT	Fabry disease	1.0 mg/kg alt weeks	IV		► 40
Aglucosidase alfa	ERT	Pompe disease	20 mg/kg alt weeks	IV	Higher doses are frequently used	► 5
Alirocumab	Increases LDL receptors by inhibiting PCSK9	Familial hypercholesterolaemia (heterozygotes and some homozygotes).	75 mg alt weeks (if necessary 150 mg) or 300 mg every 4 weeks	SC	If inadequate response to first line treatment and response to trial	► 36
Allopurinol	Xanthine-oxidase inhibitor	Disorders leading to hyperuricaemia (including PRPP synthetase superactivity, HGPRT deficiency and APRT deficiency)	Initial dosage 10–20 mg/kg per day in children and 2–10 mg/kg per day in adults	Oral	Adjust dose to minimum required to maintain normal plasma uric acid concentration	► 32
L–Arginine hydrochloride or free base	Replenishes arginine	Urea cycle disorders (except arginase deficiency)	UCDs <20 kg: 100–200 mg/kg/d (OCT & CPS deficiencies, HHH), 100–300 mg/kg/d (ASS & ASL deficiencies); UCDs >20 kg: 2.5–6 g/m^2/d, max 6 g/d	Oral	IV loading dose: 250 mg/kg (200–400 mg/kg in ASL deficiency) over 90–120 min; IV maintenance: 250 mg/kg/d (200–400 mg/kg in ASL deficiency)	► 19
		Citrin deficiency	Citrin deficiency: 5–15 g/d in adults.	Oral		
	Substrate of nitrous oxide	MELAS (unproven benefit)	MELAS: 300–500 mg/kg/d	Oral	Acutely in MELAS: 500 mg/kg IV over 90 mins, repeated after 2 hrs	► 10
Betaine	Remethylates homocysteine to methionine	Classic homocystinuria Remethylation defects	100–200 mg/kg/d in two to three divided doses, max 6–9 g/d	Oral		► 20, 28
Biotin	Co-factor for carboxylases Treatment of presumed transporter defect	Biotinidase deficiency; holocarboxylase synthetase deficiency; thiamine transporter 2 deficiency (biotin-responsive basal ganglia disease)	5–20 mg/d	Oral or IV	Holocarboxylase synthetase deficiency may require higher doses. Biotin-responsive basal ganglia disease should be treated with thiamine, with or without biotin	► 27, 29

(continued)

Table 45.1 (continued)

Medication	Mode of action	Disorders	Recommended paediatric dose (unless otherwise stated)	Route	Remarks	Chapter(s)
N-Carbamoylglutamate (Carglumic acid, Carbaglu)	Synthetic analogue of *N*-acetylglutamate; stimulates CPS	NAGS deficiency; CPS deficiency; hyperammonaemia associated with organic acidaemias	Acute hyperammonaemia: 100–250 mg/kg/d in four divided doses. Maintenance in NAGS deficiency 10 mg–100 mg/d	Oral or NG		▶ 18, 19
L-Carnitine	Replenishes body stores; removes toxic acyl-CoA intermediates from within the mitochondria	Primary and secondary carnitine deficiencies	100–200 mg/kg/d	Oral or IV	May be harmful in long chain fatty acid oxidation defects. Do not use racemic mixture.	▶ 12, 18, 22
Cerliponase alfa	ERT	Neuronal ceroid lipofuscinosis type 2 (CLN2) (tripeptidyl peptidase 1 deficiency)	300 mg alt weeks	ICV	Via implanted intracerebroventricular access device	▶ 40
Chenodeoxycholic acid	Inhibits cholesterol 7α-hydroxylase (rate-limiting enzyme in bile acid biosynthesis)	3β-Dehydrogenase deficiency	12–18 mg/kg/d for first 2 months then 9–12 mg/kg/d	Oral	Various combinations of chenodeoxycholic acid, cholic acid and ursodeoxycholic acid have been used in 3-ORD	▶ 38
		Δ^4–3-Oxosteroid 5β-reductase deficiency (3-ORD)	8 mg/kg/d			
		Cerebrotendinous xanthomatosis (CTX)	5 mg/kg/d (children) 750 mg/d (adults)			
		Oxysterol 7α-hydroxylase deficiency	25 mg/kg/d			
Cholesterol	Replenishes cholesterol	Smith-Lemli-Opitz syndrome	20–40 mg/kg/d in 3–4 divided doses	Oral	Doses of up to 300 mg/kg/d have been used	▶ 37
Cholestyramine	Bile acid sequestrant	Familial hypercholesterolaemia	Age ≥12 yrs: 12–24 g/d Age 6–11 yrs: 4 g up to 3 times daily Homozygous FH aged <6 yrs: 1–2 g up to 4 times daily	Oral	Possible vitamin A, D, and K deficiency with prolonged treatment. Other bile acid resins include colestipol & colesevelam	▶ 36
Cholic acid		Δ^4–3-Oxosteroid 5β-reductase deficiency (3-ORD); 3β-Dehydrogenase deficiency	6–8 mg/kg/d, initial doses may be higher	Oral	Maybe combined with chenodeoxycholic acid in 3-ORD	▶ 38
L-Citrulline	Replenishes citrulline and arginine	Used as an alternative to arginine in CPS and OCT deficiencies; LPI	CPS & OCT deficiency: 100–200 mg/kg/d, max 6 g/d in divided doses LPI: 50–100 mg/kg/d in 3–5 doses	Oral		▶ 19, 25

Copper histidine	Increases intracellular copper	Menkes disease	250 µg Cu twice daily to 1 year then 250 µg Cu/d in older children	SC	Only effective if started in first weeks of life. More than 3 years of treatment may not be necessary or desirable	► 34
Creatine monohydrate	Replenishes creatine	Guanidinoacetate methyltransferase deficiency	400–800 mg/kg/d in three to six divided doses	Oral	Given in combination with L-ornithine and low-arginine diet	► 9
		Arginine:glycine amidinotransferase deficiency	100–800 mg/kg/d in three to six divided doses	Oral		► 9
Cyclic pyranopterin monophosphate (cPMP)	Replenishes deficient product to allow production of molybdenum co-factor	Molybdenum co-factor deficiency type A	80–160 mg/kg/d	IV	Daily infusions	► 20
Cysteamine – given as cysteamine bitartate (Cystagon)	Depletes lysosomal cysteine	Cystinosis	1.3 g/m^2/d of free-base for children up to 12 yr, 2 g/d for older patients, given every 6 h. Max 1.95 g/m^2/d	Oral	Eye drops also required to prevent corneal deposits of cysteine	► 26
Dextromethorphan	NMDA channel antagonist	NKH	3–15 mg/kg/d in four divided doses	Oral	Doses up to 35 mg/d have been used	► 23
Diazoxide	Inhibits insulin secretion	Persistent hyperinsulinism	10–15 mg/kg/d (newborn); 10 mg/kg/d (infants), in three divided doses	Oral	Give lower doses (2–5 mg/kg/d) if risk of pulmonary hypertension	► 6
Dichloroacetate	Stimulates PDH activity by inhibiting PDH kinase	Primary lactic acidosis	25 mg/kg/d in two divided doses	Oral	May cause polyneuropathy with prolonged use	► 11
Disodium calcium edetate	Chelating agent	SLC30A10 deficiency	20 mg/kg/dose twice daily for 5 days/month	IV	Combined with oral iron supplementation	► 34
L-Dopa/carbidopa	Replacement of neurotransmitters	Disorders of L-dopa synthesis Diverse causes of L-dopa depletion (synaptic vesicle cycle disorders, POLG deficiency)	1–2 mg/kg increasing slowly to 10–12 mg/kg in four divided doses	Oral	Give as L-dopa /carbidopa (1:10 or 1:5) Monitor CSF HVA levels	► 1,16, 30
Eliglustat	Glucosylceramide synthase inhibitor	Gaucher Disease Type 1	84 mg daily (CYP2D6 poor metaboliser); 84 mg twice daily (CYP2D6 intermediate or extensive metaboliser)	Oral	Does not enter CNS; normally used as an alternative to ERT	► 40
Elosulfase alpha	ERT	MPS IVA (Morquio A)	2 mg/kg weekly	IV		► 41

(continued)

Table 45.1 (continued)

Medication	Mode of action	Disorders	Recommended paediatric dose (unless otherwise stated)	Route	Remarks	Chapter(s)
Empagliflozin	Lowers 1,5-anhydroglucitol by inhibiting SGLT2 (renal glucose transporter)	Neutropenia and neutrophil dysfunction in GSD Ib	Up to 10 mg (children) 10–25 mg (adults)	Oral		► 5
Entacapone	Prevents the peripheral breakdown of L-dopa	Disorders of BH_4 synthesis	15 mg/kg/d in two to three divided doses	Oral		► 16
Evolocumab	Increases LDL receptors by inhibiting PCSK9	Familial hypercholesterolaemia (heterozygotes and some homozygotes).	140 mg alt weeks or 420 mg monthly (420 mg alt weeks if necessary)	SC	If inadequate response to first line treatment and response to trial	► 36
Ezetimibe	Inhibits cholesterol absorption	Familial hypercholesterolaemia	10 mg/d	Oral		► 36
Fibrates	PPARα agonists: increase lipoprotein lipase & $apoA_1$, decrease $apoC_3$ & VLDL synthesis. PPARδ agonist (bezafibrate)	Hyperlipidaemias with hypertriglyceridaemia	Doses depend on age, response and which fibrate is used	Oral	Combination with statin increases the risk of rhabdomyolysis, esp. if renal impairment. Bezafibrate may reduce rhabdomyolysis in partial CPT II & VLCAD deficiencies	► 12, 36
Folinic acid	Provides accessible source of folate for CNS	DHPR deficiency; UMP synthase deficiency (hereditary orotic aciduria); methionine synthase deficiency and other disorders of cobalamin metabolism; hereditary folate malabsorption; cerebral folate transporter deficiency	5–15 mg/d	Oral, IV	Higher doses (up to 400 mg/d orally) have been used in hereditary folate malabsorption. Monthly IV doses of 20–25 mg/kg have been used in *FOLR1* deficiency. Use 5-Methyl tetrahydrofolate in MTHFR deficiency	► 16, 28
Galsulfase	ERT	MPS VI (Maroteaux Lamy)	1.0 mg/kg weekly	IV		► 41
Galactose	Restores missing compound	Phosphoglucomutase 1 deficiency; SLC39A8 deficiency	0.5–3 g/kg/d max 50g/d	Oral	Corrects abnormal glycosylation	► 43
G-CSF	Stimulates granulocyte production	Neutropenia in GSD Ib	0.5–5 μg/kg once daily or alt days	SC	Use the lowest effective dose to increase the neutrophil count to $>0.5 \times 10^9$/L	► 5

Glycerol phenylbutyrate	Converted to phenylacetate, which combines with glutamine to form phenylglutamine which has high renal clearance	Urea cycle disorders	5–12.4 g/m^2/d in 3 divided doses	Oral	1 ml contains 1.1 g glycerol phenylbutyrate	► 19
Glycine	Forms isovalerylglycine with high renal clearance	Isovaleric acidaemia	150 mg/kg/d in three divided doses	Oral	Up to 600 mg/kg/d during decompensation	► 18
	Replenishes glycine	3-phosphoglycerate dehydrogenase def; phosphoserine aminotransferase deficiency	200–300 mg/kg/d		Adjunct to treatment with serine	► 24
Glycocholic acid		Bile acid amidation defects 1 and 2	15 mg/kg/d	Oral		► 38
Haem arginate, (haematin, haemin)	Inhibits 5-aminolevulinic acid synthase	Acute porphyrias	3–4 mg/kg once daily for 4 days	IV		► 33
Hydroxocobalamin (vitamin B_{12})	Co-factor for methylmalonyl CoA mutase and methionine synthase	Disorders of cobalamin metabolism	IM 1–2 mg daily or 5 mg weekly (up to 10 mg daily sometimes given); oral 10 mg once or twice daily	IM or oral	IM dose may be reduced to once or twice weekly according to response	► 18, 28
D- or D,L-3-Hydroxybutyrate	Alternative fuel source, allowing reduced carbohydrate intake in GSD III, replaces deficient endogenous ketone body production in MADD	GSD III; MADD; other FAO disorders; Ketogenesis defects	300–2000 mg/kg/d in 3–6 divided doses	Oral	Possibly protects against cardiomyopathy in GSD III. May improve cardiomyopathy and leukodystrophy in MADD	► 5, 12,13
5-Hydroxytryptophan	Neurotransmitter replacement	Disorders of neurotransmitter synthesis	1–2 mg/kg, increasing gradually to 8–10 mg/kg in four divided doses	Oral	Monitor CSF 5HIAA levels	► 16, 30
Idursulfase	ERT	MPS II (Hunter)	0.5 mg/kg weekly	IV		► 41
Imiglucerase	ERT	Gaucher disease	30–60 U/kg alt weeks	IV	Lower doses are also used	► 40
Insulin	Promotes anabolism; inhibits catabolism	Acute decompensation in organic acidaemias, urea cycle disorders, MSUD, FAO disorders	0.05–0.2 IU/kg/h	IV	Always give with IV solutions containing glucose & with frequent blood glucose monitoring	► 4
Ketamine	*N*-Methyl-d-aspartate (NDMA) channel antagonist	NKH	15 mg/kg/d in neonates, 9 mg/kg/d in infants (range 1–30 mg/kg/d) in four divided doses	Oral or IV		► 23

(continued)

Table 45.1 (continued)

Medication	Mode of action	Disorders	Recommended paediatric dose (unless otherwise stated)	Route	Remarks	Chapter(s)
Laronidase	ERT	MPS1 (Hurler/Scheie or pre-HSCT in Hurler disease)	100 U/kg (0.58 mg/kg) weekly	IV		► 41
Libmeldy	Ex vivo stem cell Lentiviral gene therapy	Presymptomatic late infantile and early juvenile metachromatic leukodystrophy(MLD); early symptomatic early juvenile MLD	Gene corrected CD34 + ve autologous stem cells		Generally only available via approved centres	► 40
Lomitapide	Microsomal transfer protein inhibitor, reduces lipoprotein secretion	Homozygous familial hypercholesterol-aemia	Adults: initially 5 mg daily, increased at 4 week intervals, max 60 mg/d	Oral	Adjunct to other drugs ± lipoprotein apheresis. Often causes gastrointestinal & liver function disturbances.	► 36
L-Lysine-HCl	Increase plasma lysine; increase ornithine excretion (OAT deficiency)	Lysinuric protein intolerance; OAT deficiency	LPI: 20–30 mg/kg/d in three divided doses; OAT deficiency (adults): 10–15 g/d in 5 divided doses	Oral		► 25
Magnesium	Replenishes Mg	Primary hypomagnesaemia with secondary hypocalcaemia	0.5–1.5 ml/kg/d $MgSO_4$ 10% solution IV; oral maintenance 0.4–3.9 mmol/kg/d elemental Mg in three to five divided doses	IV or Oral		► 34, 43
Mannose	Improves glycosylation	Mannose phosphate isomerase deficiency (MPI-CDG)	1 g/kg/d in four to six divided doses	Oral		► 43
Mercaptopropionylg-lycine (Tiopronin)	Chelating agent	Cystinuria	10–20 mg/kg/d, up to max 1 g/d in three divided doses	Oral		► 25
5-Methyl tetrahydro-folate	Achieves measurable levels of CSF levels of 5-methyltetrahydrofolate	5,10-Methylenetetrahydrofolate reductase deficiency	15–60 mg/d	Oral	Available as calcium mefolinate	► 28
Metronidazole	Reduces propionate production by gut bacteria	Propionic and methylmalonic acidae-mia	10–20 mg/kg once daily	Oral	For a limited period (e.g. 10 days) each month	► 18
Migalastat	Pharmacological chaperone for mutant alpha-galactosidase A	Fabry disease with amenable mutations	123 mg alt daily (adult)	Oral		► 40
Miglustat	Inhibitor of glucosylce-ramide synthase, the first enzyme in glycosphingo-lipid synthesis	Gaucher disease; neurological manifestations of Niemann Pick C	Up to 300 mg/d (Gaucher) or 600 mg/d (NPC) in 3 divided doses	Oral	Only recommended for patients with mild to moderate Gaucher disease who are unsuitable for enzyme replacement therapy.	► 40

Nicotinamide	Replenishes deficiency state	Hartnup disease	50–300 mg/d	Oral		► 25
Nicotinic acid (niacin)	Inhibits free fatty acid release from adipose tissue, reducing synthesis of various lipids; increases HDL-cholesterol	Hyperlipidaemia	Adult dose: 100–200 mg 3 times daily, gradually increased over 2–4 weeks to 1–2 g three times daily	Oral	Seldom used due to flushing and other side effects	► 36
Nitisinone (NTBC)	Inhibits 4-hydroxyphenylpyruvate dioxygenase	Tyrosinaemia type I	1 mg/kg/d (2 mg/kg/d in liver failure) in one to two divided doses	Oral	Combine with low-TYR, low-PHE diet to maintain plasma TYR 200–400 µmol/l. Aim to maintain nitisinone levels >50 µmol/l in plasma or 20–40 µmol/l in whole blood	► 17
		Alkaptonuria	1–4 mg/d	Oral	Recommended in adults only, along with mild protein restriction. Avoid in pregnancy	► 17
Octreotide	Somatostatin analogue	Persistent hyperinsulinism	5–10 µg/d increasing up to 30–50 µg/d as required – given in 3–4 divided doses or by continuous pump (IV or SC)	IV or SC		► 9
L-Ornithine	Competitive inhibitor of AGAT – reduces guanidinoacetate production	Guanidinoacetate methyltransferase deficiency	400–800 mg/kg/d	Oral	Given in combination with creatine monohydrate	► 15
Penicillamine	Chelating agent	Wilson disease; cystinuria	Wilson disease: up to 20 mg/kg/d in divided doses (maintenance dose in adults 750–1500 mg/d); cystinuria: 30 mg/kg/d up to 3–4 g in 3–4 divided doses	Oral or IV	Give 1 hour before food. In Wilson disease give with pyridoxine 25 mg/d	► 25, 34
Pramipexole	Dopamine agonist	Adjunct to therapy in disorders of BH_4 synthesis	6–35 µg /kg/d in two divided doses	Oral	Allows reduction in L-dopa therapy	► 16
Pyridoxal-phosphate	Active co-factor	Pyridox(am)ine 5′-phosphate oxidase deficiency	30–60 mg/kg/d in 4–6 divided doses	Oral	Monitor liver transaminases and use lowest effective dose possible	► 29

(continued)

Table 45.1 (continued)

Medication	Mode of action	Disorders	Recommended paediatric dose (unless otherwise stated)	Route	Remarks	Chapter(s)
Pyridoxine	Co-factor	Pyridoxine-responsive forms of cystathionine β-synthase (CBS), γ-cystathionase & ornithine amino-transferase deficiencies; X-linked sideroblastic anaemia; primary hyperoxaluria type 1, GOT2 deficiency, AADC deficiency	50–500 mg/d. CBS deficiency: up to 10 mg/kg/d (max 500 mg/d).	Oral	Peripheral neuropathy can occur with high doses (>900 mg daily in adults)	► 20, 21, 33, 42
		Pyridoxine dependent epilepsy	Trial of 100 mg IV with EEG monitoring or 30 mg/kg/d for 3 days; maintenance 5–30 mg/kg/d (max 500 mg/d; infants 30 mg/kg/d, max 300 mg)	Oral	To prevent breakthrough seizures, dose may be doubled for the first 3 days of intercurrent infection	► 29
Riboflavin	Coenzyme	Mild variants of ETF/ETFQO deficiencies; ACAD9 deficiency; riboflavin transporter deficiencies RFVT1–3; FAD transporter defect & FAD synthase deficiency; dihydrolipoamide dehydrogenase deficiency; glutaric aciduria type I; L-2 hydroxyglutaric aciduria; trimethylaminuria	100–400 mg/d in two to three divided doses; up to 80 mg/kg/d has been used in RFVT2/3 deficiencies (Brown-Vialetto-van Laere syndrome)	Oral		► 11, 12, 14, 22
Sebelipase alfa	ERT	Lysosomal acid lipase deficiency (Wolman or CESD phenotypes)	1–5 mg/kg weekly for Infantile onset, 1mg/kg alternate weekly for later onset cases	IV		► 36
Selegiline (L-deprenyl)	Monoamine-oxidase-B inhibitor	As adjunct to therapy with 5HT & L-dopa in BH_4 defects	0.1–0.25 mg/kg/d in three to four divided doses	Oral		► 16
L-Serine	Replenishes serine	3-Phosphoglycerate dehydrogenase, phosphoserine aminotransferase, 3-phosphoserine phosphatase deficiencies GOT2 deficiency	up to 500–700 mg/d in six divided doses	Oral	190 mg/kg/d given to a pregnant mother whose fetus had 3-phosphoglycerate dehydrogenase deficiency	► 24

Sodium benzoate	Combines with glycine to form hippuric acid, which has high renal clearance – removes N_2 and reduces blood ammonia	Hyperammonaemia	250 mg/kg/d in divided doses (<20 kg) or 5.5 g/m^2/d (>20 kg); max 12 g/d	Oral	IV dose: 250 mg/kg over 90–120 min, followed by 250–500 mg/kg/d (<20 kg) or 5.5 g/m^2/d (>20 kg)	► 19, 20
	Reduces blood glycine levels	NKH	250–750 mg/kg/d in 3–6 divided doses	Oral		► 23
	Reduces glycine availability for guanidinoacetate synthesis	Guanidinoacetate methyltransferase deficiency	100 mg/kg/d in divided doses	Oral		► 15
Sodium phenylbutyrate	Converted to phenylacetate, which combines with glutamine to form phenylglutamine which has high renal clearance	Urea cycle disorders	Up to 250 mg/kg/d (<20 kg), 5 g/m^2/d (>20 kg); max 12 g/d	Oral	IV dose: 250 mg/kg over 90–120 min, followed by 250–500 mg/kg/d (<20 kg) or 5 g/m^2/d (>20 kg)	► 19
Sodium pyruvate	Restores hepatic cytosolic NADH/NAD$^+$ ratio	Adult Citrin deficiency	3–6 g/d in 3 divided doses	Oral		► 19
Statins	HMG-CoA reductase inhibitors	Hyperlipidaemias; simvastatin has been used in SLO syndrome and in lathosterolosis	Doses depend on specific statin, age & response	Oral	No benefit has been confirmed in SLO syndrome	► 36, 37
Taliglucerase alfa	ERT	Gaucher Disease type 1	60u/kg alt weeks	IV	Not licensed in EU	► 40
Taurine	To overcome lack of transporter	*SLC6A6* taurine transporter deficiency	100 mg/kg/d	Oral		► 20
Tetrahydrobiopterin (BH_4)	Replacement of BH_4	Disorders of BH_4 synthesis or recycling; BH_4 responsive forms of PAH deficiency	1–3 mg/kg/d in BH_4 defects; 5–20 mg/kg/d in PAH deficiency	Oral	May be contraindicated in DHPR deficiency	► 16
Tetrathiomolybdate	Chelating agent	Wilson disease	Bis-choline tetrathiomolybdate 15–60 mg/d for adults in divided doses	Oral	Not commercially available	► 34
Thiamine	Co-factor	Thiamine responsive variants of PDH deficiency, MSUD & complex 1 deficiency	50–1200 mg/d; 500–2000 mg/d in PDH deficiency	Oral		► 11, 18,
	To overcome lack of transporter	Thiamine transporter 2 deficiency (biotin-responsive basal ganglia disease); mitochondrial TPP transporter def	100–400 mg/d	Oral		► 29

(continued)

Table 45.1 (continued)

Medication	Mode of action	Disorders	Recommended paediatric dose (unless otherwise stated)	Route	Remarks	Chapter(s)
Triethylene tetramine (trientine)	Chelating agent	Wilson disease	Adult starting dose 0.9–2.5 g/d in 2–3 divided doses, usual maintenance dose 0.9–1.5 g/d. Child 20 mg/kg/d in 2–4 divided doses.	Oral	Give 1 hour before food. May reduce serum iron – if needed, iron supplements should be given separately	► 34
Triheptanoin	Anaplerotic substrate	Long-chain FAODs; PC deficiency	To provide 20–35% of daily calorie intake	Oral		► 11, 12
Ubiquinone (coenzyme Q10)	Replacement therapy	Inborn errors of CoQ_{10} synthesis	30 mg/kg/d, up to 3 g/d in adults	Oral	Has been used in other mitochondrial cytopathies, but unproven benefit	► 14
Uridine	Replenishes UMP	UMP Synthase deficiency (hereditary orotic aciduria); CAD deficiency	100–150 mg/kg/d in divided doses	Oral	In UMP Synthase deficiency adjust dose to maintain lowest possible output of orotic acid	► 32
Velaglucerase	ERT	Gaucher Disease Type 1	15–60 u/kg alt weeks	IV		► 40
Velmanase alfa	ERT	Alpha-Mannosidosis	1 mg/kg weekly	IV		► 40
Vestronidase alfa	ERT	MPSVII	4 mg/kg alt weeks	IV		► 41
Vitamin C	Co-factor; antioxidant	Hawkinsinuria; tyrosinaemia type III (4 hydroxyphenylpyruvate dioxygenase deficiency); Transient tyrosinaemia of the newborn; Glutathione synthase deficiency	100–1000 mg/d	Oral		► 17, 31
Vitamin E (alpha tocopherol)	Replenishes vitamin E stores; free radical scavenger	Abetalipoproteinaemia, Glutathione synthetase deficiency	100 mg/kg/d in abetalipoproteinaemia, otherwise 10 mg/kg/d	Oral		► 31, 36
Zinc	Increases Zn; impairs Cu absorption	Acrodermatitis enteropathica (AE); Wilson disease	Elemental zinc doses: AE: 1–2 mg/kg/d in infancy, thereafter 30–100 mg/d; Wilson disease: 50 mg/d (<5 yrs), 75 mg/d (children <57 kg), 150 mg/d (>57 kg & adults) in 3–4 divided doses	Oral	Sulphate or acetate salts can be used. Give 30 mins before food. Used for maintenance & presymptomatic treatment in Wilson disease.	► 34

Alt alternate, *max* maximum

Supplementary Information

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