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Inherited Metabolic Diseases

A Clinical Approach

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To our patients and their families

Preface

The field of inherited metabolic diseases has changed from a limited group of rare, untreatable, often fatal disorders to an important cause of acutely life-threatening and/or chronic debilitating but increasingly treatable illness. Unchanged is the orphan nature of these disorders with mostly relatively non-specific initial clinical manifestations.

The patient does not come to the physician with the diagnosis; the patient comes with a history, symptoms, and signs. This book starts with those and proceeds logically through algorithms from questions to answers. Special emphasis is placed on acutely presenting disorders and emergency situations. The rationale of the approaches presented in this book is based on extensive, collective clinical experience. It is imbedded in the environment of Springer Pediatric Metabolic Medicine and complements the disease-based approaches to *Inborn Metabolic Diseases* in the books edited by Jean-Marie Saudubray and colleagues as well as Nenad Blau and colleagues.

Important concepts with special emphasis to metabolic medicine which are rarely described in detail in medical textbooks such as structured communication, guidelines, patient associations, transition, concepts of dietary therapy, and adult and maternal care are delineated by experts. A system- and symptom-based approach to inherited metabolic diseases should help colleagues from different specialties to diagnose their patients and to come to an optimal program of therapy. For metabolic and genetic specialists, this book is designed as a quick reference for what may be (even for the specialist) infrequently encountered presentations.

Heidelberg, Germany — Georg F. Hoffmann
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General Introduction

Inborn errors of metabolism are often felt to be inaccessible as a huge group of numerous genetic defects that are understood only by the specialist. Many textbooks leave the uninitiated with complicated biochemical pathways, strange disease names, and the impossible task of reading through large lists of single enzyme and protein defects. This is unfortunate as metabolic disorders can be understood and remembered in clinically distinct disease groups that require similar investigations and therapeutic strategies. The aim of this book is to make inborn errors of metabolism accessible to the general clinician and to help with the differential diagnosis in individual patients.

Although the individual defects are rare, they represent an important differential diagnosis in many patients with a wide range of clinical symptoms and signs. We felt that there is a need for a textbook on metabolic disorders that is not disease-orientated but patient-orientated, that does not focus on individual disorders for which there are excellent textbooks but is designed to help the general clinician who is concerned with the care of a patient with a set of problems or specific symptoms and signs.

This book is composed of four distinct but interdependent parts:

1. The introduction (Part I) provides an overview of the major groups of metabolic disorders. Rather than discussing individual defects, it highlights the similarities of the diseases in each group to give simple advice on which investigations are indicated if a particular disease group is suspected.
2. The first major part (Part II) concentrates on clinical aspects and the differential diagnosis with regard to specific metabolic symptoms and signs. The clinician is guided to understand which clinical problems can be caused by which metabolic disorders and to utilize the most efficient diagnostic strategies. Core aspects of metabolic medicine such as structured communication, guidelines, transition, pregnancy, maternal care, and how to respond to various medical emergencies are covered. Therapeutic concepts are delineated and practical advice provided on the different treatment approaches required for individual diseases.
3. The second major part (Part III) is structured according to major organ systems and clinical symptomatology in metabolic disease. It outlines the correct approach in the context of specific symptoms and signs.

4. The diagnostic section (Part IV) provides an overview and detailed instructions on diagnostic procedures, their indication, requirements, interpretation, and problems. This is intended as a reference for clinicians planning investigations or tests in a particular patient.

We hope that this book will help to spread our experience that the universe of metabolic disorders is not a huge number of separate single enzyme and protein defects, but that there is a structure which makes this important group of disorders comprehensible to clinicians, for the benefit of the patients.

Contents

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Part I

Introduction to Inborn Errors of Metabolism

Disorders of Intermediary Metabolism

1

Johannes Zschocke

Key Facts

- The classical inborn errors of intermediary metabolism are defects in enzymes of the metabolism of amino acids, carbohydrates, and fatty acids or in mitochondrial energy metabolism (Fig. 1.1).
- Disorders of intermediary metabolism are often dynamic; they fluctuate with changes in the metabolic state of the patient and frequently allow successful therapeutic intervention.
- Most disorders of intermediary metabolism are readily diagnosed through basic metabolic investigations which include blood gases, glucose, lactate, ammonia, plasma amino acids, urinary organic acids, and an acylcarnitine profile.

There is no clear consensus which diseases should be regarded as inherited metabolic diseases. We like to use this term for inherited (occasionally de novo genetic) disorders of the biosynthesis or breakdown of substances within specific pathways that are usually recognized by specific biochemical tests and are sometimes treatable by metabolic intervention. This definition does not include disorders primarily affecting:

- Structural proteins and their modification
- Membrane channels or other membrane proteins as long as they do not have a primary function in a metabolic pathway
- Intra- or intercellular signal transduction as well as transcription factors
- Endocrine function or hormone biosynthesis

Inherited metabolic diseases are often multisystem disorders that show a dynamic or progressive clinical course and may be associated with a risk of acute metabolic decompensation. They are typically caused by partial or complete loss of function of a single enzyme, cofactor, or auxiliary protein and are mostly inherited as autosomal recessive or X-chromosomal traits. A dominant inheritance pattern is found for only few disorders that mostly involve biosynthetic metabolic pathways. There are different pathomechanisms depending on the metabolic function of the affected biochemical reaction, including:

- Accumulation of toxic substrates and their metabolites
- Accumulation of non-metabolized substrates
- Deficiency of a reaction product
- Overproduction of a reaction product
- Insufficient provision of cellular energy

The pathogenetic relevance of an inborn error of metabolism is not always easy to ascertain as clinical symptoms observed in a patient may be coincidental or the reason for performing the analysis in the first place. With regard to pathophysiology, clinical presentation, and diagnostic strategies, disorders of intermediary metabolism,

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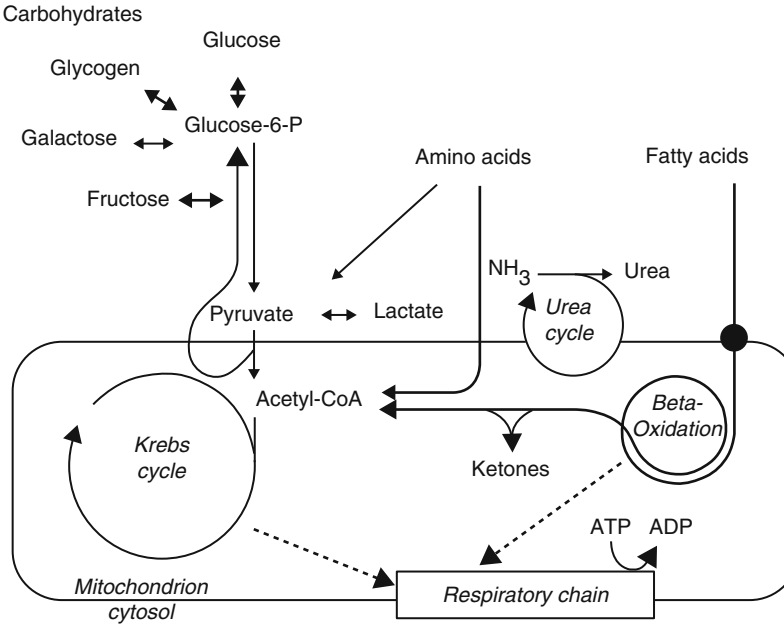

Fig. 1.1 Main pathways of intermediary metabolism

the focus of this chapter, may be distinguished from diseases that involve biosynthesis and breakdown of complex molecules which are summarized in Chap. 2.

1.1 Aminoacidopathies

Typical aminoacidopathies result from abnormalities in the breakdown of amino acids in the cytosol. In addition, several disorders involving mitochondrial enzymes such as branched-chain ketoacid dehydrogenase (maple syrup urine disease) or ornithine aminotransferase (gyrate atrophy of the chorioidea) are classified as aminoacidopathies as they do not involve CoA-activated metabolites. This distinguishes aminoacidopathies from the organic acidurias which are considered a separate group of disorders that affect processing of CoA-activated metabolites in the mitochondria and which have effects on other mitochondrial functions. Clinical symptoms of the aminoacidopathies are often caused by the accumulation of toxic intermediates that cause specific organ damage. Several defects of amino acid metabolism such as histidinemia are benign because the metabolites that accumulate are not toxic. Aminoacidopathies are diagnosed through the analysis of plasma (rarely urinary) concentrations of amino acids and sometimes of urinary organic acids. A majority is treatable through dietary restriction of the protein and of the amino acid involved in the defective pathway and by the avoidance or prompt treatment of catabolic states that lead to the breakdown of large amounts of protein. Another therapeutic strategy that has been successful in hepatorenal tyrosinemia is the inhibition of a biochemical step before the actual genetic deficiency, thereby changing a harmful disease into a more benign amino acid accumulation without the accumulation of the more damaging substances downstream.

1.2 Organic Acidurias

The classical organic acidurias are deficiencies of enzymes in the mitochondrial metabolism of CoA-activated carboxylic acids, most of which are derived from amino acid breakdown. In this way, they are distinguished from disorders of fatty acid oxidation which involve acyl-CoA esters produced in high concentrations during fasting and present different diagnostic and

therapeutic challenges. The term organic acidurias is preferred to the alternative term organic acidemias as they are most often detected by analysis of the urine. Biochemically, some of the reactions impaired in the organic acidurias are parallel to the dehydrogenase, hydratase, or ketothiolase reactions of the mitochondrial β-oxidation cycle. Clinical features are caused not only by the accumulation of toxic intermediates but also by a disturbance of mitochondrial energy metabolism and carnitine homeostasis; they may include encephalopathy and episodic metabolic acidosis. Organic acidurias are diagnosed through the analysis of organic acids in the urine or acylcarnitines in the blood. Treatment is similar to that of the aminoacidopathies and involves the dietary restriction of the relevant amino acid(s) and the avoidance of protein catabolism. However, as the defective enzymes are distant (more downstream) from the respective amino acids, restriction may not lead to a stoichiometric reduction of pathological metabolites. Unexpected fluctuations occur and complete return to normal intermediary metabolism is usually impossible. Supplementation with carnitine and sometimes other substances such as glycine (e.g., to form isovalerylglycine in isovaleric aciduria) are very useful adjuncts to the treatment.

Disorders of biotin metabolism are often included among the organic acidurias. Biotin is a cofactor of the mitochondrial carboxylases, and a deficiency of biotinidase or holocarboxylase synthetase leads to multiple carboxylase deficiencies. It is also usually diagnosed through urinary organic acid analysis. Biotinidase enzyme analysis of dried blood spots has been included into programs of neonatal screening as it is well treated with biotin supplementation.

1.3 Disorders of Ammonia Detoxification

The breakdown of protein produces large amounts of nitrogen in the form of ammonia that is highly neurotoxic but is normally converted to urea and excreted in the urine. Defects in enzymes of the urea cycle and other disorders of ammonia detoxification present clinically with encephalopathy and other symptoms of hyperammonemia. Metabolic investigations should include analysis of the amino acids in plasma and urine as well as urinary orotic acid. Treatment requires the reduction of protein intake in conjunction with the supplementation of essential amino acids, the avoidance of catabolic states, and the administration of benzoate and/or phenylacetate/phenylbutyrate which remove nitrogen in the form of alternative conjugates of amino acids such as glycine and glutamine.

1.4 Disorders of Amino Acid Transport

Deficiencies in the intestinal and/or renal transport of amino acids may be nonsymptomatic or cause symptoms because of deficient absorption of essential amino acids (e.g., tryptophan in Hartnup disease) or because of increased urinary concentration of unsoluble amino acids which causes nephrolithiasis (e.g., cysteine in cystinuria). These disorders are diagnosed by the quantitative analysis of amino acids in plasma and urine. Treatment depends on the clinical picture. Deficiency of essential amino acids is treated by supplementation with large amounts of these compounds or, in the case of tryptophan deficiency, supply of the cofactor nicotinic acid that is normally synthesized from tryptophan. Renal calculi in cystinuria can be prevented by treatment with a chelating agent such as penicillamine, which forms mixed disulfides with cysteine, and calculi once formed can be resorbed if they have not incorporated too much calcium.

1.5 Disorders of Peptide Metabolism

- The tripeptide *glutathione* and the *gammaglutamyl cycle* have multiple functions in cellular metabolism, ranging from amino acid transport across membranes to detoxification of peroxides. Deficiencies may cause neurological and hematological as well as metabolic

problems. Investigations should include the determination of organic acids in the urine and glutathione in various body fluids. Treatment is largely symptomatic; certain drugs should be avoided.

- Defective breakdown of *dipeptides* of histidine such as homocarnosine or carnosine may be found in patients with intellectual disability although the causative relationship is uncertain. Ulcers of the skin, particularly of the legs, are seen in prolidase deficiency. Investigations should include amino acid and peptide analysis of the urine.

1.6 Disorders of Carbohydrate Metabolism and Transport

The disorders in this group display a relatively wide range of clinical features and may cause clinical symptoms because of toxicity, deficiency of energy, hypoglycemia, or storage.

- *Disorders of galactose and fructose metabolism*: Defects in the cytosolic metabolism of galactose and fructose for glycolysis cause disease through accumulation of pathogenic metabolites. Children with galactosemia and fructosemia typically develop evidence of severe damage to the liver and/or kidney after dietary intake of lactose (milk, milk products) or fructose (fruit, sucrose), respectively. Treatment requires the elimination of the intake of galactose or fructose.
- *Disorders of gluconeogenesis and glycogen storage*: Typical metabolic features are hypoglycemia after relatively short periods of fasting and lactic acidemia. There may be variable organ dysfunction, most frequently hepatopathy. Glycogen storage leads to hepatic enlargement, which may be massive. In some disorders such as glycogenosis type III, there are elevations of the transaminases and creatine phosphate kinase and there may be clinical myopathy. Treatment includes frequent meals, cornstarch supplementation, or continuous overnight tube feeding to avoid hypoglycemia.
- *Disorders of carbohydrate transport*: There are a number of different glucose and other carbohydrate carriers, and clinical symptoms differ greatly depending on the tissue localization of the individual defect. Symptoms are frequently gastrointestinal or renal but also include the central nervous system (deficient glucose transport across the blood-brain barrier).

1.7 Disorders of Fatty Acid Oxidation and Ketogenesis

Mitochondrial fatty acid oxidation is required for the provision of energy during fasting, either through complete oxidation or through production of ketones in the liver that then serve as an alternative energy source for the brain. Disorders in this pathway typically present as hypoketotic hypoglycemia precipitated by fasting, leading to coma or epileptic seizures. In addition, some disorders cause severe liver disease and/or cardiomyopathy probably as a result of the accumulation of toxic metabolites. The diagnosis is best reached in the acute situation through the analysis of free fatty acids and the ketone bodies 3-hydroxybutyrate and acetoacetate, as well as the acylcarnitine profile and urinary organic acids. The diagnosis may be missed if samples are obtained in the normal interval between episodes or after the patient has been treated with intravenous glucose. Treatment consists of avoidance of fasting. Carnitine supplementation is mostly unnecessary and must be carefully balanced in some defects, particularly those that cause cardiomyopathy or hepatopathy.

1.8 Mitochondrial Disorders

Disorders of energy metabolism (usually summarized as mitochondrial disorders although enzymes deficient in organic acidurias or fatty acid oxidation disorders are also located in the mitochondrion) comprise a rapidly increasing number of molecularly defined diseases that cause primary or secondary impairment of the

production of adenosine triphosphate (ATP). Affected proteins include subunits of the respiratory chain complexes; components of the final pathways of substrate breakdown such as the pyruvate dehydrogenase complex and the Krebs cycle; at least 24 chaperones for the production of respiratory chain complexes; components of mitochondrial deoxyribonucleic acid (DNA), ribonucleic acid (RNA), and protein synthesis; numerous proteins involved in cofactor biosynthesis; as well as various other components of mitochondrial homeostasis.

Mitochondrial disorders typically manifest with symptoms and signs of an energy deficiency and a highly variable pattern of organ dysfunctions. In many cases, there are lactic acidemias and progressive neurodegenerative disease. Periods of metabolic stress such as intercurrent infections may trigger a deterioration of the patient's condition. The diagnostic work-up may be difficult and should include frequent measurements of blood lactate levels, cerebrospinal fluid (CSF) lactate, plasma amino acids and alanine, and often a search for mutations in mitochondrial DNA. Repeated, careful examinations of organ functions as well as imaging are essential. Recently, exome sequencing has emerged as the primary diagnostic approach in many patients. Treatment options are limited but usually include various vitamins and cofactors such as riboflavin, coenzyme Q, or thiamine. Heterozygous mutations in the genes of some Krebs cycle enzymes (e.g., fumarase) cause inherited cancer predisposition syndromes.

1.9 Disorders of Cobalamin and Folate Metabolism

Genetically determined or nutritional deficiencies of vitamin cofactors may affect various metabolic pathways and cause a wide range of clinical symptoms. They can frequently be satisfactorily treated by supplementation of the deficient substance. Of particular importance in intermediary metabolism are cobalamin (vitamin B_{12}) and folate which are essential for cytosolic methyl group transfer. The cellular methylation reactions require methyl group transfer from serine to *S*-adenosylmethionine involving the folate cycle, the cobalamin (vitamin B_{12}), and the methionine–homocysteine cycle. A disturbance in this pathway may be caused by methylcobalamin deficiency, a disturbance of the folate cycle, or by deficient remethylation of homocysteine to methionine. Most disorders of cobalamin metabolism as well as nutritional deficiency of vitamin B_{12} cause methylmalonic aciduria. Clinically, disorders of cytosolic methyl group transfer cause an encephaloneuropathy, often with additional hematological problems such as megaloblastic anemia and thromboembolic complications of hyperhomocysteinemia. The diagnosis involves the analysis of urinary organic acids, plasma amino acids (homocysteine), and levels of folate and cobalamin. Treatment includes supplementation of cobalamin and folate, in some situations with addition of betaine and methionine.

1.10 Disorders of the Transport or Utilization of Copper, Manganese, Iron, and Zinc

- *Disorders of copper metabolism*: Wilson disease causes a chronic hepatopathy and symptoms of central nervous dysfunction, while patients with Menkes disease suffer from neurological problems in conjunction with abnormalities of the hair, connective tissue, and bones. Diagnosis involves the analysis of copper and coeruloplasmin in serum, urine, and liver tissue. Treatment in Wilson disease is aimed at reducing copper load, while copper should be parenterally substituted in Menkes disease. A well-characterized variant of Menkes disease that is associated with milder mutations in the *ATP7A* gene is the occipital horn syndrome, also known as X-linked cutis laxa (OMIM: 304150). Another gene required for normal copper homeostasis has recently been identified based on the presence of abnormalities of copper metabolism found in an autosomal recessive disorder called MEDNIK (mental retardation, enter-

opathy, deafness, neuropathy, ichthyosis, and keratoderma) syndrome (OMIM: 609313). The phenotype combines clinical features of both Wilson and Menkes diseases.

- *Disorders of manganese metabolism*: Several patients have been reported with typical features of Wilson disease, including a liver dysfunction and a movement disorder that result from mutations in a manganese transporter and consequently hypermanganesemia.
- *Disorders of iron metabolism*: Patients affected with such disorders may present with iron-deficient anemia, e.g., due to insufficient intestinal absorption of iron, or with iron overload and liver dysfunction as in hemochromatosis. Secondary iron overload may be observed in some hemolytic anemias. Treatment is directed at substitution or removal of iron.
- *Disorders of zinc metabolism*: Acrodermatitis enteropathica is characterized by chronic skin problems, alopecia, and central nervous symptoms. It is diagnosed through reduced levels of zinc and alkaline phosphatase and is treated with supplementation of zinc.

Disorders of the Biosynthesis and Breakdown of Complex Molecules

2

Johannes Zschocke

Key Facts

- Disorders of the biosynthesis and breakdown of complex molecules typically show slowly progressive clinical symptoms often in several organ systems and are less likely to cause acute metabolic crises.
- Disorders in this group are not usually recognized by basic metabolic analyses but require specific investigations for their diagnosis.
- Disorders of purine and pyrimidine metabolism cause a range of nephrological, neurological, musculoskeletal, hematological, and other symptoms.
- Clinical features of lysosomal storage disorders include progressive neurological deterioration, dysmorphic features, and organomegaly.
- Peroxisomal disorders cause a wide range of neurological symptoms as well as hepatointestinal dysfunction, skeletal abnormalities, dysmorphic features, and others.
- Other relevant disease groups include disorders of the metabolism of isoprenoids and sterols, disorders of posttranslation protein modification, disorders of bile acid and bilirubin metabolism, and inherited cholestasis and porphyrias disorders of lipoprotein metabolism.

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2.1 Disorders of Purine and Pyrimidine Metabolism

Deficiencies in enzymes required for the biosynthesis or breakdown of purines and pyrimidines cause neuromuscular abnormalities, nephrolithiasis, gouty arthritis, or anemia and immune dysfunction. They may be recognized through increased or reduced urinary urea in relation to creatinine and urine microscopy or specifically through the analysis of urinary purines and pyrimidines. Some metabolites of pyrimidine breakdown are only recognized by urinary organic acid analysis. Nephrolithiasis may be treated or prevented by allopurinol; a high fluid intake is helpful. Some disorders of pyrimidine metabolism, notably orotic aciduria and 5′ nucleotidase overactivity disease (nucleotide depletion syndrome), are treatable with uridine or triacetyluridine. There is no effective treatment for most of the primarily neurological manifestations of disorders of purine metabolism.

2.2 Lysosomal Storage Disorders

Lysosomes contain a number of hydrolases required for the intracellular breakdown of large lipid and mucopolysaccharide molecules. If one of these enzymes is deficient, its substrate accumulates and causes enlargement and/or functional impairment of the organ system. Clinical features include progressive neurological deterioration, dysmorphic features, and organomegaly. There is usually no metabolic decompensation although

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acute symptoms (e.g., severe pain) are a major feature in some conditions. Investigations include careful roentgenographic examination of the skeleton for dysostosis multiplex, analysis of leukocytes and other cells for vacuoles, and assessment of parenchymatous organs. The urine may be investigated for abnormal glycosaminoglycans and oligosaccharides. In the past, specific enzyme studies were usually required to make the exact diagnosis; this has changed with the advent of massively parallel (next generation) sequencing techniques that allow the simultaneous analysis of all lysosomal genes. For many disorders, there is no specific therapy yet, but enzyme replacement therapy or bone marrow transplantation has been shown beneficial in several disorders:

- *Mucopolysaccharidoses* (*MPS*) typically cause progressive dysmorphic features, hepatomegaly, and psychomotor regression. Some forms are characterized by intellectual disability without major morphological abnormalities or severe skeletal symptoms with normal cognition. The MPS are usually recognized through the analysis of urine for glycosaminoglycans.
- *Oligosaccharidoses* may resemble the MPS, but many show more severe neurological symptoms and are more frequently symptomatic at birth (nonimmune fetal hydrops). The diagnosis is made through the demonstration of abnormal oligosaccharide patterns in the urine followed by molecular or enzyme analyses.
- *Sphingolipidoses* and *lipid storage disorders* usually present with progressive neurological deterioration. Hepatomegaly may be present, and skeletal deformities and dysmorphic features are rare. Other presentation patterns are found particularly in Fabry disease (pain and paresthesias, angiokeratomas, cardiomyopathy, renal failure) and non-neuronopathic Gaucher disease (hematoma, anemia, massive splenomegaly, abdominal/bone pain). The neuronal ceroid lipofuscinoses cause epilepsy, loss of vision, and progressive neurological deterioration and can be recognized by electron microscopy. *Mucolipidoses* combine clinical features of the mucopolysaccharidoses and sphingolipidoses and may reflect the deficiency of several lysosomal enzymes as a consequence of defective enzyme processing.
- *Lysosomal transport defects*: Cystinosis causes nephropathy and dysfunction of other organs including the thyroid gland and the eyes; it is diagnosed on the basis of increased cystine content of leukocytes. Sialic acid storage disease causes progressive encephaloneuropathy; it is recognized through elevated free sialic acid in the urine. Both of these disorders result from defective transport out of lysosomes. Cystinosis is treated with oral cysteamine, cysteamine eyedrops, and renal transplantation.

2.3 Peroxisomal Disorders

The biochemical roles of peroxisomes are diverse. Peroxisomal defects usually cause severe, progressive multisystem disorders.

- Defects of *peroxisome biogenesis* or the *activation and β-oxidation of long-chain fatty acids* cause progressive neurological disease, structural abnormalities as in Zellweger syndrome, and abnormalities in hepatic, intestinal, or adrenal function. They are usually recognized through the analysis of very long-chain fatty acids in blood or cultured fibroblasts. There is no effective treatment.
- *Refsum disease* is a defect in the metabolism of exogenous phytanic acid. It causes slowly progressive neurological, visual, and auditory abnormalities and often does not present until adulthood. It is diagnosed through the quantification of serum phytanic acid and is treatable by a diet restricted of phytanic acid.
- Defects of *ether-phospholipid biosynthesis* cause rhizomelic chondrodysplasia punctata characterized by proximal shortening of the limbs in addition to neurological and other manifestations. It is diagnosed through quantification of plasmalogens in erythrocytes. There is no effective treatment.
- Catalase deficiency is the only known defect of the *detoxification of oxygen radicals*. It

causes chronic ulcers in the oral mucosal membranes.
- Primary hyperoxaluria type I is the only known defect of *glyoxylate metabolism*; it causes nephrolithiasis and nephrocalcinosis. It is recognizable by organic acid analysis or high-performance liquid chromatography (HPLC) analysis for oxalate and glyoxylate. It has been treated by transplantation of the liver and kidney.

2.4 Disorders of the Metabolism of Isoprenoids and Sterols

Isoprenoids and sterols are essential in many cellular and developmental processes. Most defects of their synthesis are caused by enzyme deficiencies in the postsqualene portion of the pathway. Only mevalonic aciduria and hyperimmunoglobulinemia D syndrome, both due to mevalonate kinase deficiency, are found in the proximal part of the pathway.

- Mevalonate kinase deficiency is the only known defect of *isoprenoid biosynthesis*. It causes dysmorphic features, failure to thrive, developmental delay, and recurrent febrile crises. An attenuated variant causes hyper-IgD syndrome. Treatment is symptomatic.
- Defects of *sterol biosynthesis* cause various structural abnormalities including the dysmorphic features of the Smith–Lemli–Opitz syndrome and intellectual disability. Diagnosis involves plasma–sterol analysis. In Smith–Lemli–Opitz syndrome, specific treatment by cholesterol supplementation has been of limited success.

2.5 Disorders of Bile Acid and Bilirubin Metabolism, Inherited Cholestasis, and Porphyrias

- Genetic defects of *bile acid biosynthesis* cause symptoms either through bile acid deficiency or through deposition of precursors. The former causes progressive cholestasis and malabsorption, while the precursors can lead to progressive neurological dysfunction and xanthomas. The bile acid biosynthetic pathway is located partly in the peroxisomes and is affected by peroxisomal disorders. Diagnosis involves the analysis of urinary bile acids. Treatment with bile acids is effective in the bile acid deficiency states and to downregulate bile acid biosynthesis.
- Hem is metabolized to bilirubin and excreted together with bile acids in the urine. Genetic defects may involve specific enzymes or mechanisms of transport into the bile ducts. They cause indirect or direct hyperbilirubinemia. Specific treatment strategies have been developed for some disorders.
- Porphyrias are disorders of hem biosynthesis, frequently inherited as autosomal dominant traits. Neurotoxic metabolites accumulate in deficiencies affecting the first few steps of the pathway and typically cause intermittent acute symptoms such as abdominal pain triggered by various factors, in particular induction of hem-containing enzymes. Porphyrins accumulating in more distal enzyme deficiencies are associated with photosensitivity and dermatological symptoms. The diagnosis involves analysis of porphyrins and porphyrin precursors in urine, feces, or erythrocytes. Management entails the avoidance of precipitating factors.

2.6 Disorders of Posttranslation Protein Modification

Many proteins including enzymes, transport, and membrane proteins as well as hormones require glycosylation or other modifications in the Golgi apparatus or endoplasmatic reticulum to render them functional.

Congenital disorders of glycosylation (CDG) are caused by the deficiency of one of the more than 40 different enzymes involved in glycosylation. They lead to a wide range of structural abnormalities and disturbances of physiological functions. A disorder from the CDG group should be considered in all patients with unclear multisystem or neurological disorder. The diagnosis of *N*-glycosylation

disorders is usually made by isoelectric focusing of transferrin in serum. There is no effective treatment for most disorders of this group.

Glycosylphosphatidylinositol (*GPI*) *anchor-related diseases* are caused by impaired maturation and remodeling of GPI-anchored proteins in the ER and the Golgi. They are multisystem disorders with a range of clinical features including dysmorphism, skeletal abnormalities, severe neurological symptoms, epilepsy, and intellectual disability. Disorders in this pathway have mainly been solved by exome sequencing; a helpful marker for some of these disorders is alkaline phosphatase (ALP) which may be elevated or reduced in serum.

2.7 Disorders of Lipoprotein Metabolism

Many disorders of lipoprotein metabolism cause clinical symptoms through the deposition of lipid in tissues and premature atherosclerosis. Others cause gastrointestinal or peripheral neurological problems. They are recognized by quantification of cholesterol and triglycerides and through lipoprotein electrophoresis. Many disorders are open to dietary or pharmacological therapy.

- Elevated blood cholesterol levels in *hypercholesterolemias and mixed hyperlipidemias* cause lipid deposition in the form of xanthomas and xanthelasma. They lead to complications of premature atherosclerosis, especially myocardial infarction and cerebrovascular disease. Therapeutic options include diet, drugs, and lipid apheresis.
- *Hypertriglyceridemia* may be caused by genetic disorders that affect the utilization of chylomicrons and very low-density lipoproteins (VLDL). They may cause failure to thrive and abdominal symptoms and sometimes severe pancreatitis. These disorders require stringent restriction of dietary fat.
- Genetic disorders affecting *high-density lipoproteins* (*HDL) metabolism* cause a variety of clinical manifestations including premature atherosclerosis, neuropathy, nephropathy, and corneal clouding. Therapy is symptomatic.
- Genetic disorders in which there are *reduced LDL cholesterol and triglycerides* lead to symptoms of fat malabsorption. They are treated by restriction of fat and supplementation with fat-soluble vitamins.

Neurotransmitter Defects and Related Disorders

3

Georg F. Hoffmann

Key Facts

- Congenital neurotransmitter defects have become recognized as important causes of severe, progressive encephalopathies mostly of early onset.
- The clinical presentation of neurotransmitter defects can be quite distinctive. Patients suffering from nonketotic hyperglycinemia, defects of glutamate transport, or GABA-transaminase deficiency usually present with early-onset severe encephalopathy, dominated by seizures refractory to treatment. Defects in pyridoxine metabolism also present similarly; however, rational therapies have been developed with satisfactory or even excellent success.
- Defects in the biosynthesis of dopamine generally result in progressive extrapyramidal movement disorders, especially parkinsonism–dystonia and chorea. The spectrum of individual symptoms and course of disease, however, is wide, ranging from intermittent focal dystonia to severe, lethal infantile encephalopathies.
- The hallmark of succinic semialdehyde dehydrogenase (SSADH) deficiency is an increase of 4-hydroxybutyric acid in urine, blood, and CSF, leading to variable clinical symptoms that are the main findings of developmental delay, delayed speech, and hypotonia being nonspecific.
- Diagnosis of neurotransmitter defects usually requires investigations of the CSF.

3.1 Disorders of Glycine and Serine Metabolism

Nonketotic hyperglycinemia is one of the best known causes of early-onset epileptic encephalopathy. It is recognized via concomitant amino acid analysis of plasma and CSF. Glycine levels in both are elevated, and the CSF-to-plasma ratio is increased. Treatment with dextromethorphan, benzoate, or folate is of limited success. Disorders of serine biosynthesis cause neurological symptoms. They have been treated with serine and glycine supplementation.

3.2 Disorders of the Metabolism of Pterins and Biogenic Amines

Affected children suffer from progressive developmental retardation and epileptic encephalopathy. There may be specific symptoms of dopamine

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and/or serotonin deficiency, such as infantile parkinsonism, dopa-responsive dystonia, oculogyric crises, or disturbed temperature regulation. These diseases are sometimes recognized by hyperphenylalaninemias but many exclusively through the analysis of biogenic amines and pterins in CSF.

Disorders of tetrahydrobiopterin biosynthesis and recycling affect the hydroxylation of phenylalanine and have been called atypical or malignant phenylketonuria. The hydroxylations of tyrosine and tryptophan are also affected, leading to deficiency of both dopamine and serotonin. Investigations should include the analysis of biogenic amines, pterins, and amino acids in the CSF as well as amino acids in plasma and pterins in urine. The disorders are treated with L-dopa along with carbidopa and 5-hydroxytryptophan and/or tetrahydrobiopterin and tetrahydrobiopterin substitution.

Disorders of the biosynthesis of biogenic amines present similarly with progressive extrapyramidal symptoms and encephalopathy. The deficiency of biogenic amines is treated with of L-dopa along with carbidopa and 5-hydroxytryptophan and/or dopamine agonists.

Defects in monoamine transport leading to neurotransmitter disorders have been recently identified. Dopamine transporter deficiency syndrome and vesicular monoamine transporter 2 deficiency are both autosomal recessive disorders resulting in progressive movement disorders ranging from early-onset chorea/dyskinesia, orolingual dyskinesia, dystonia, oculogyric crises, and dystonic storms to parkinsonism-dystonia in childhood or even later presentations.

3.3 Disorders of Gamma-Aminobutyrate Metabolism

Disorders of GABA metabolism include deficiencies of 4-aminobutyrate aminotransferase (GABA-transaminase and succinate semialdehyde dehydrogenase). These disorders cause central nervous dysfunction, often including seizures and encephalopathy. They are diagnosed through CSF analysis of amino acids and gamma-aminobutyrate (GABA). Urinary organic acid analysis may reveal 4-hydroxybutyric acid indicative of succinate semialdehyde dehydrogenase deficiency.

3.4 Disorders of Vitamin B_6 Metabolism

Pyridoxal phosphate (PLP, vitamin B_6) is a cofactor of all the transamination reactions and some decarboxylation and deamination reactions of amino acids and as such is also required for the biosynthesis of several neurotransmitters including dopamine and GABA. Intracellular deficiency may be caused by primary or secondary disorders in the biosynthetic pathway and leads to (neonatal) epileptic encephalopathy. In the well-known entity of vitamin B_6-dependent seizures, PLP is inactivated by delta 1-piperideine-6-carboxylate, which accumulates because of an enzyme deficiency in a different pathway. Disorders of vitamin B_6 metabolism are generally treatable with pyridoxine or PLP.

3.5 Related Neurometabolic Disorders

- *Defects in the biosynthesis or transport of creatine* – the central compound in cytosolic energy metabolism – manifest as neurometabolic disorders with progressive central nervous dysfunction. They are diagnosed through the analysis of creatine and guanidinoacetate in body fluids or nuclear magnetic resonance (NMR) spectroscopy of the brain, treatment centers on creatine supplementation.
- *Disorders of folate pathway and transport* often cause early-onset (epileptic) encephalopathies. In two disorders (dihydrofolate reductase and cerebral folate transport deficiencies), serum folate and plasma homocysteine concentrations are in the normal range. However, there is a marked depletion of 5-methyl-tetrahydrofolate (THF) in the CSF, and determination of folate in CSF should be always included when "neurotransmitters" are investigated.

- *Sulphite oxidase deficiency* is a cause of severe infantile seizures and encephalopathy. It is recognized through a sulphite stix test of the urine. Amino acid analysis of plasma and urine may be diagnostic but is less reliable. When it is caused by molybdenum cofactor deficiency, there is also xanthine oxidase deficiency, which may be detected by purine analysis of the urine. There is no specific treatment.
- Various *cerebral organic acidurias* including Canavan disease; L-2-, D-2-, and D-/L-2-hydroxyglutaric acidurias; 2-ketoglutaric aciduria; fumaric aciduria; and malonic aciduria present with central nervous dysfunction, which is usually progressive. General metabolic abnormalities are absent, but the specific metabolites are found on organic acid analysis of the urine. The molecular basis of these conditions has now been established. There is no specific treatment.

Part II

Approach to the Patient

When to Suspect Metabolic Disease

4

William L. Nyhan

Key Facts

- Careful clinical and family histories, repeated clinical examinations, and a sequential workup by routine laboratory and organ evaluation remain the best and most often the only way to diagnosis.
- While it is imperative to include disorders for which effective treatments are available, in cases of slowly progressive and by experience often incurable disorders, diagnostic procedures should be performed stepwise.
- Unexpected findings in the "routine" laboratory in patients with unusual and unexplained symptoms may be indicative of an inborn error of metabolism.
- Every child who is suspected of suffering from an inborn error of metabolism requires a careful evaluation of organ functions aided by routine laboratory and imaging investigations. The involvement of multiple organ systems is an especially strong indication of an inherited metabolic disease.
- As it can be very difficult to recognize a constellation which was not personally experienced, a second opinion should be sought in case of unexplained symptoms or disease courses.

4.1 History

4.1.1 Family History

A careful family history may reveal important clues that point toward the diagnosis of an inborn error of metabolism. Most metabolic disorders are inherited as autosomal recessive traits, which may be suspected if the parents are consanguineous or the family has a confined ethnic or geographic background. Carriers for particular disorders, and as a consequence affected children, may be more frequent in remote villages, close-knit communities (such as the Amish in Pennsylvania), certain ethnic groups (such as Ashkenazi Jews), or countries that have seen little immigration over many centuries (such as Finland).

Quite often specialist investigations are started only after a second affected child is born into a family. Older siblings may be found to suffer from a similar disorder as the index patient or may have died from an acute unexplained disease classified as "sepsis with unidentified pathogen," "encephalopathy," or "sudden infant death syndrome (SIDS)." The latter is a frequent feature in disorders of intermediary metabolism that may have acute lethal presentations, such as disorders of ammonia detoxification, organic acidurias, or fatty acid oxidation disorders.

In assessing medical records of previously affected but undiagnosed family members, it should be taken into account that the written clinical descriptions of complex conditions can be inconsistent and even misleading. Depending on the presumptive diagnosis at that time, important clinical clues may be missing. Parents are sometimes more reliable sources of information. On the other hand, the clinical expression of the same inborn error of

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metabolism may be variable even within families. Some more common Mendelian disorders are caused by a wide range of different mutations with different degrees of disease severity. Disease manifestations are especially variable in females with X-linked traits because of differences in the lyonization of the X chromosome in carrier females, e.g., ornithine transcarbamylase deficiency. Similarly, dominant disorders with variable penetrance may cause variable clinical problems in different members and generations even of one family, such as Segawa syndrome due to guanosine triphosphate (GTP) cyclohydrolase deficiency.

As a result of the successful treatment of disorders of intermediary metabolism in which toxic small molecules accumulate, an increasing number of relatively healthy affected women are reaching the reproductive age. If they become pregnant, there is a risk for their fetuses to be harmed by pathological amounts of toxic metabolites from the mother, although the children are themselves not affected but heterozygous. Especially important is maternal phenylketonuria (PKU), which is likely to become a major health problem. Some women at risk may not even know that they are affected with PKU, if they come from countries where newborn screening did not exist or if they have discontinued dietary treatment and medical follow-up in late childhood. The latter will however remember that they had followed a special diet, which should be specifically asked for. Several mothers have been found to suffer from mild PKU only after maternal PKU was diagnosed in one of her children. Other maternal conditions may cause "metabolic" disease in the neonate or infant postnatally, e.g., methylmalonic aciduria and hyperhomocysteinemia in fully breastfed children of mothers ingesting a vegan diet, which causes nutritional vitamin B_{12} deficiency.

4.1.2 Prenatal Development and Complications of Pregnancy

Toxic small molecules that accumulate in many disorders of intermediary metabolism do not harm the fetus because they are removed via the placenta and metabolized by the mother. Children affected with such disorders usually have a completely normal intrauterine development and are born with normal birth measurements at term. In contrast, disorders that interfere with cellular energy metabolism, e.g., mitochondrial disorders, may impair fetal organ development and prenatal growth, causing structural (in particular cerebral) abnormalities, dysmorphic features, and dystrophy. Structural abnormalities and dysmorphic features may be even more pronounced in disorders of the biosynthesis of complex molecules that are necessary for developmental pathways and networks. Notable examples are the defects of sterol biosynthesis that interfere with cholesterol-dependent signaling pathways of development and cause, for example, the Smith–Lemli–Opitz syndrome. Disorders affecting the breakdown of complex molecules such as lysosomal storage disorders cause specific dysmorphic characteristics as in the Hurler disease and, when severe, may already present at birth. An unusual prenatal disease manifestation is found in mothers carrying a fetus affected with defects of fatty acid β-oxidation, notably long-chain hydroxyacyl-CoA dehydrogenase (LCHAD) deficiency or carnitine palmitoyltransferase II deficiency. These mothers have an increased risk of developing acute fatty liver of pregnancy, preeclampsia, or hemolysis, elevated liver enzymes, and low platelets (the HELLP syndrome). Systematic studies in mothers showed that fetal LCHAD deficiency is present in a significant number of women with acute fatty liver of pregnancy but only in a very small proportion of the far more common HELLP syndrome. The neonates of such mothers should be screened for fatty acid oxidation disorders by acylcarnitine analysis.

4.1.3 Age of Presentation and Precipitating Factors

The "typical" ages of manifestation of different groups of metabolic disorders in the first year of life are depicted in Fig. 4.1. Disorders of intermediary metabolism that cause symptoms through the accumulation of toxic molecules ("intoxication") are usually asymptomatic in the first hours

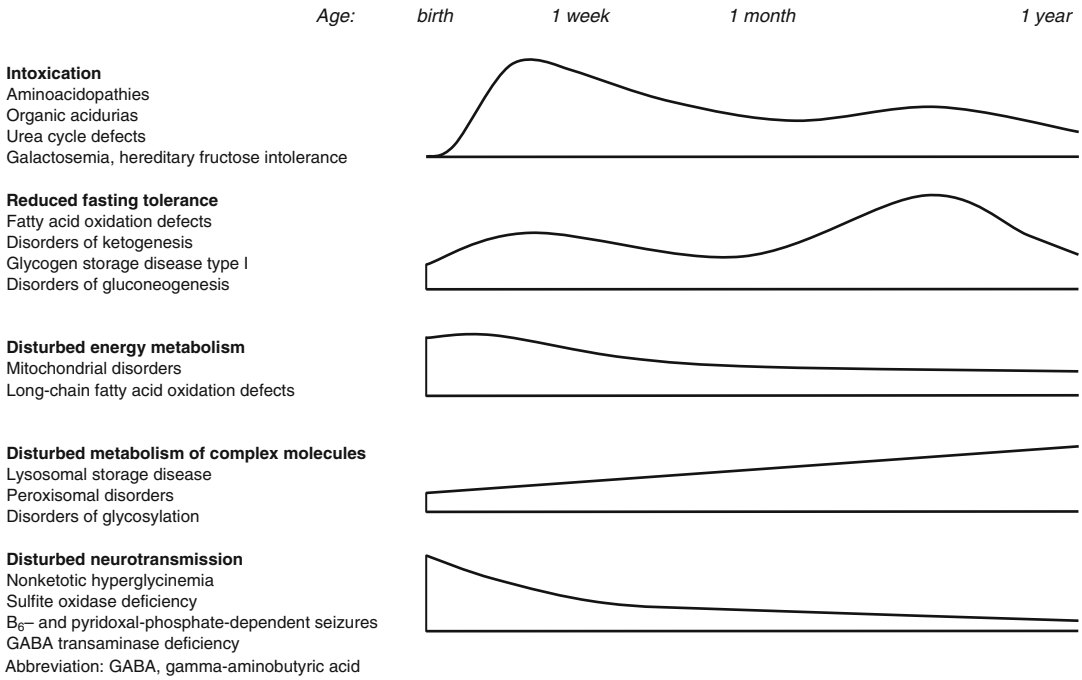

Fig. 4.1 Typical ages of manifestation of metabolic disorders in the first year of life

of life. They present after exposure to the respective substrate derived from catabolism or diet. Postnatal protein breakdown requires amino acid catabolism and nitrogen detoxification. Patients with acute aminoacidopathies (e.g., maple syrup urine disease (MSUD)), classical organic acidurias, or urea cycle defects most frequently develop progressive symptoms between days 2 and 5 of life. Subsequent risk periods include the second half of the first year of life (in particular, age 6–8 months) when solid meals with higher protein content are introduced and the children start to fast overnight and late puberty when hormonal changes and a reduced growth rate change the metabolic state. Important precipitating factors throughout life are catabolic states caused by infections, fever, vaccinations, high-dose steroid therapy, surgery, and accidents, as well as prolonged fasting.

Of the disorders of carbohydrate metabolism, galactosemia usually presents after the introduction of milk (which contains the galactose–glucose disaccharide lactose) in the first week of life, while children with hereditary fructose intolerance develop symptoms after the introduction of fruits, vegetables, and particularly table sugar (the fructose–glucose disaccharide sucrose) to the diet, often between 4 and 8 months of age.

Disorders with a *reduced fasting tolerance* include genetic defects of fatty acid oxidation and ketogenesis, as well as deficiencies in the production and release of glucose and its hormonal regulation. They typically present during periods of reduced food intake and/or increased energy requirement such as prolonged fasting or metabolic stress, and the age of presentation thus overlaps with the "intoxication" disorders. However, the disorders with reduced fasting tolerance are less frequently or less severely symptomatic in the postnatal period and more frequently present in association with infections in the second half of infancy.

Disorders of *energy metabolism* are frequently symptomatic at birth but may essentially present at any time of life, depending on the severity of the genetic defect and the organs involved. Acute decompensation in mitochondrial disorders may specifically be triggered by major alterations in carbohydrate intake or the ingestion of large amounts of rapidly absorbed carbohydrates, while long-chain fatty acids that interfere with energy metabolism in some β-oxidation defects

cause clinical features of a mitochondrial disorder during fasting periods. Another characteristic feature of mitochondrial disorders is a marked and frequently irreversible deterioration of the clinical condition with intercurrent illnesses.

Disorders in the *metabolism of complex molecules* rarely show acute metabolic crises but present with variable and often progressive organ dysfunction throughout childhood. There are usually no precipitating factors. The clinical presentation of *neurotransmitter defects and related disorders* depends on the ontogenetic expression of neurotransmitter systems and receptors. Affected children are often symptomatic immediately after birth, and there may even be symptoms of intrauterine epilepsy as evidence of prenatal disease manifestations. There are usually no precipitating factors.

4.2 Physical Examination

Every child who is suspected of suffering from an inborn error of metabolism requires a thorough physical examination and a careful evaluation of organ functions aided by routine laboratory and imaging investigations. In addition, hearing and vision should be examined at specialist appointments. Depending on the presenting symptoms and the clinical course, a reevaluation, especially a detailed physical examination, should be repeated every 6 months. The detection of additional manifestations is of great importance even if the patient does not complain of them, particularly if the final diagnosis is still unknown.

The involvement of multiple organ systems is one of the strongest arguments in favor of an inherited metabolic disease. This is especially true for defects of organelle metabolism such as mitochondrial or peroxisomal disorders or the quickly enlarging group of glycosylation defects or CDG syndromes. Structural abnormalities such as dysmorphic features or malformations may be caused by disorders in the metabolism of complex molecules as well as disorders affecting mitochondrial energy metabolism but are not usually observed in other disorders of intermediary metabolism. Generalized organomegaly is often indicative of a (lysosomal) storage disorder, while isolated hepatomegaly is observed in a great variety of enzyme defects. Urine color and body odor can provide diagnostic clues, as discussed later. A list of differential diagnoses of characteristic symptoms and signs is given in the appendix.

4.2.1 Unusual Odor

Unaccustomed odors can serve as alerting signals for several metabolic diseases (Table 4.1). The most commonly encountered is the sweet smell of acetone found in the acute ketoacidosis of diabetes mellitus and the organic acidemias. Other characteristic odors are that of *MSUD*, the acrid smell of *isovaleric acidemia* and *glutaric aciduria type II* and the odor of phenylacetic acid in *PKU*. The phenylacetic acid odor is much more prominent in patients with urea cycle defects, treated with sodium phenylacetate or phenylbutyrate. Very prominent unpleasant odors are found in *trimethylaminuria* and *dimethylglycinuria*. Odors can be very useful in suggesting a diagnosis or an appropriate test. It is also important not to discard a potential diagnosis because of the absence of the odor. Some people are sim-

Table 4.1 Diagnostic utility of unusual odors

Odor	Substance	Disorder
Animallike	Phenylacetate	PKU
Maple syrup	Sotolone	Maple syrup urine disease
Acrid, short-chain acid	Isovaleric acid	Isovaleric aciduria, glutaric aciduria type II
Cabbage, rancid butter	2-hydroxybutyric acid, 2-keto-4-methiolbutyric acid	Tyrosinemia type I, methionine malabsorption
Rotten fish	Trimethylamine, dimethylglycine	Trimethylaminuria, dimethylglycinuria

ply unable to detect some odors. Many physicians have never really been able to smell the ketotic patient. In other conditions, the acute metabolic crisis leads to a cessation of oral intake and vigorous parenteral fluid therapy, so that by the time the patient reaches the referral hospital, the odor has long since disappeared.

Remember

Diagnosis by smell is underutilized, but many are not good at it. Too, a characteristic odor maybe absent in a severely ill infant partaking nothing by mouth and receiving parenteral fluids.

The odor of maple syrup led to the recognition and original description of *MSUD*, before it was known that this was a disorder in the metabolism of the branched-chain amino acids. A keen sense of smell can still be useful in the detection of this disease, but the seriousness of the presentation of metabolic imbalance and a readiness to carry out an analysis of amino acids in plasma are such that most patients diagnosed today do not trigger the smell test. This is also true of acute exacerbation in established patients. Testing for urinary ketones with dinitrophenylhydrazine (DNPH) and organic acid analysis of the urine are also useful in the diagnosis of this disease. The odor of the patient with isovaleric acidemia has been described as like that of sweaty feet, but it does not smell anything like a locker room. The smell is penetrating, pervasive, and readily recognized. It is the odor of a short-chain volatile acid, and the same smell may be appreciated in patients with *multiple acyl-CoA dehydrogenase deficiency* (*glutaric aciduria type II*) during times of acute illness.

Now that screening of newborns for *PKU* is universal in developed countries, patients with this disease are not likely to be diagnosed because of the characteristic odor, but some of us have made the diagnosis in this way in patients born prior to the development of screening. The odor has variously been described as musty, barny, animallike, or wolflike. It is actually the odor of phenylacetic acid. Now that patients with defects in the urea cycle are treated with phenylacetic acid or its precursor phenylbutyric acid, specialists in inherited metabolic disease are quite accustomed to this odor.

Patients with *hepatorenal tyrosinemia* and other nonmetabolic patients with hepatic cirrhosis may have a very unpleasant odor that results from the accumulation of methionine.

The classic unpleasant odor is that of patients with *trimethylaminuria*. Trimethylamine is the odor of fish that is not fresh. The compound is a major end product of nitrogen metabolism of teleost fishes, which convert it to the oxide and employ the resulting compound to balance their osmotic pressure with surrounding seawater. In man, trimethylamine is formed from dietary trimethylamine oxide in fish and from choline absorbed from the intestine and transported to the liver, where the trimethylamine oxide is formed and ultimately excreted in the urine. Patients with trimethylaminuria have an inborn error in the metabolism of the oxide and defective activity of hepatic trimethlyamine *N*-oxide synthetase. The metabolic abnormality does not *appear to* produce a disease as we usually know one; its consequences are nevertheless terrible. An odor so unpleasant leads to social ostracism, poor performance in school, depression, and loss of employment. Suicide is a possibility. Diagnosis is important because a diet low in fish, liver, and egg yolks is usually sufficient to eliminate the odor. The diagnosis is made by identifying the compound by gas chromatography, gas chromatography–mass spectroscopy (GC–MS) or nuclear magnetic resonance (NMR) spectroscopy. Its excretion is increased by loading with choline, and this may be necessary for the diagnosis in patients who have found dietary ways of minimizing their odor. Following a morning specimen of urine, a 5 g oral administration of choline bitartrate in 3 doses over 24 h led to a 44-fold increase in trimethylamine excretion to 1.098 μmol/mg creatinine. Normal individuals excreted 0.0042–0.405 μmol/mg creatinine. The activity of the enzyme has been measured in the biopsied liver. It is a flavin-containing monooxidase, designated FMO_3. Several mutations in the gene have been identified.

Patients have been described in whom the odor of trimethylamine is mild or intermittent. Mutations have been identified in the FMO_3 gene on chromosome 1q23–25. For instance, the P153L mutation has been identified in patients with severe trimethylaminuria and no enzyme activity in vitro. The patients with the mild phenotype have had an allele with two common polymorphisms, E158K in which a 472G→A mutation coded for a lysine instead of a glutamate and E3086 in which a 923A→G mutation coded for glycine instead of glutamate. Patients have generally been heterozygous for this allele and a disease-producing mutation, but one patient has been homozygous for the variant allele. The variant allele is common in Caucasian populations; allele frequency was found to be 20 % in Germans.

Dimethylglycinuria is a newly recognized inborn error of metabolism that causes a fishy odor. The defective enzyme is the dimethylglycine dehydrogenase, which catalyzes the conversion of this compound to sarcosine. A missense mutation in the gene has been identified in an affected patient. Trimethylamine was absent from the patient's urine. He also complained of muscle fatigue and had elevated levels of creatine kinase in the serum. Dimethylglycine is most readily detected by $_1$H-NMR spectroscopy. Its presence was confirmed by ^{13}C-NMR spectroscopy and by GC–MS of nonextracted urine, but the compound could not be detected by GC–MS after the usual ethylacetate extraction.

4.2.2 Color of the Urine or Diaper

Physicians since at least the time of Hippocrates have recognized that the color of the urine may be the clue that leads to the diagnosis. It was Garrod's recognition of the significance of the dark urine of patients and families with alkaptonuria that led to the conceptualization of the inborn errors of metabolism.

Alkaptonuria is recognized surprisingly infrequently in this way, and many patients reach adulthood and clinical arthritis before the diagnosis is made. This is the result of many factors, among them is that the black pigment forms with time and oxygen and that flushing does away with both. In a patient in whom one seeks to make this visual diagnosis, it is best to alkalinize and shake the urine and look with excellent light for the fine black precipitate. In times past when infants wore cloth diapers, which were laundered with strong alkaline soap, the conditions were perfect, and the diagnosis could be made by the appearance of black pigment in the diaper. Now they wear plastic disposable diapers, many of which turn pink on contact with alkaptonuric urine. So, we can still make the diagnosis early by examining the diaper.

Alkaptonuric urine also gives a positive test for reducing substance and is glucose-negative, and this may be an alerting signal for the diagnosis. Homogentisic acid also reduces the silver in photographic emulsion, and alkaptonuric urine has been used to develop a photograph, an interesting qualitative test for the diagnosis. The diagnosis is confirmed by quantitative analysis of homogentisic acid in the urine.

Remember

The diagnostic black pigment of alkaptonuria is often missed. A pink color may be seen in plastic diaper or a positive test for reducing substance may be alerting (See Table 4.2).

4.2.2.1 Examination of the Urine for the Significance of Color

Urine has a normal amber color that is the color of the pigment urochrome. Pale, dilute, or watery urine results from a plentiful fluid intake or diuresis as in diabetes mellitus or diabetes insipidus or in the recovery phase of a tubular necrosis. Very dark urine or concentrated urine results from dehydration. Pale urine with a high specific gravity suggests diabetes mellitus. Dark urine with a low specific gravity suggests the presence of urobilin or bilirubin and is best checked by analysis of the blood for bilirubin. Very bright yellow urine may be seen in infants who ingest large amounts of carotene, but the skin of such infants is usually carotenemic. Urine may, of course, be red because of hematuria, but this is readily recognized by microscopic analysis, and such a specimen is not the subject of differential

Table 4.2 Syndromes of abnormally colored urine or diapers

	Conditions	Confirmation
Dark brown or black urine or diapers		
Alkaptonuria (plastic pampers become red)	Standing, alkaline	Homogentisic aciduria and clinitest positive
Melanuria		Disseminated melanotic sarcoma
Red urine or diapers		
Hematuria		Microscopic
Hemoglobinuria		Guaiac and benzidine
Beets (anthocyanins)		History
Congenital erythropoietic porphyria		Blood, urine, stool, uroporphyrin, and uroporphyrin-III cosynthase (CEP) activity
Red dyes (Monday morning disorder, rhodamine B)		History
Red diaper syndrome (Serratia marcescens)	24–36 h of oxidation after passage	Culture Neomycin Rx
Phenolphthalein		History
		pH sensitive
Green–blue–purple urine		
Blue-diaper syndrome (indigotin)		Tryptophan malabsorption
		Indicanuria
		Indole-acetic aciduria
Biliverdin (obstructive jaundice)		Serum bilirubin
Methylene blue (ingestion, Rx)		History
Purple urine bag syndrome		Urine culture and catheter removal
Orange sand		
Urate overproduction (urates may stain diaper red in neonatal period)		Chemical assay for uric acid, blood, and urine
		Hypoxanthine-guanine phosphoribosyl transferase (HPRT)

diagnosis by color. Free hemoglobin in the urine appears brown or black as methemoglobin is formed. The most famous example of this is the blackwater fever of malaria.

4.2.2.2 Dark Brown or Black Urines

In addition to alkaptonuria, *hemoglobinuria* and *myoglobinuria* both produce brown or dark urine and both are detected by the dipstix for hemoglobin or by the benzidine test. Hemoglobin in the urine is often accompanied by hematuria. Hemoglobinuria in the absence of red cells in the urine is accompanied by evidence of hemolysis, such as anemia, reticulocytosis, or hyperbilirubinemia, while myoglobinuria is often accompanied by muscle pains or cramps and elevation of creatine phosphokinase and uric acid. An attack of myoglobinuria should signal a work-up for a disorder of fatty acid oxidation (Chap. 28). It is also seen in enzyme defects localized to the muscle, such as myophosphorylase deficiency (McArdle disease) and myodenylate deaminase deficiency. *Melanuria* is seen in disseminated melanotic sarcoma.

4.2.2.3 Red Urine

Porphyrias are the major metabolic cause of red urine. Congenital erythropoietic porphyria is an autosomal recessive disease caused by mutations in the gene for uroporphyrinogen synthase. Uroporphyria and coproporphyria are found in the urine. It manifests a variable phenotype from nonimmune hydrops fetalis to a mild adult-onset form with only photosensitive cutaneous lesions.

The disease is often first recognized because of a pink, red, or brown stain in the diapers. These patients also develop erythrodontia in which a red fluorescence of the teeth is visible with ultraviolet illumination.

Red urine may also be seen following the ingestion of large quantities of colored foods. The anthrocyaninuria of beet ingestion is quite common. Blackberries have also been associated with red urine. Red dyes, such as rhodamine B, used to color foods and cold drinks have led to red urine of so many children after a weekend party that the condition was termed the Monday morning disorder of children. Phenolphthalein in laxatives may also cause red urine. In the neonatal period, distinct red spots in the diaper were seen where crystals of ammonium urate dried out. In previous days when cloth diapers were used and accumulated for a while before laundering, a red diaper syndrome was recognized in which the color developed after 24 h of incubation and came from the growth of the chromobacterium, *Seratia marcescens*, which does not produce pigment in the infant's intestine, but only after aerobic growth at 25–30 °C.

Urine described as vin rose is seen in patients with toxicity from iron injections treated with deferoxamine. Red stools may also be seen after the ingestion of red crayons, in some patients receiving cefdinir and many but not all patients receiving oral iron.

4.2.2.4 Green or Purple or Blue Urine

Blue pigment in urine-containing urochrome usually leads to a green color. Blue color was seen in the blue diaper syndrome. This disorder of the intestinal absorption of tryptophan was described in two siblings who also had hypercalcemia and nephrocalcinosis. When tryptophan is not efficiently absorbed, intestinal bacteria convert it to indole metabolites that are absorbed and excreted in the urine. The blue color comes from the oxidative conjugation of two molecules of indican to indigotin, or indigo blue, a water-insoluble dye. The excretion of indole products is increased by an oral tryptophan load. The condition must be very rare because further patients have not been reported since the initial report in 1964. Indoles including indican are also found in the urine of patients with Hartnup disease, in which there is defective renal tubular reabsorption, as well as intestinal absorption of a number of amino acids including tryptophan, but blue diapers or urine have not been observed.

Biliverdin, the oxidation product of bilirubin, is excreted in the urine, and so green urine may be seen in jaundiced patients, particularly those with chronic obstructive jaundice.

Benign pigments such as methylene blue, found in some tablets, are excreted in urine and if a sufficient quantity is taken, will color the urine. Indigo-carmine is another blue dye that may find its way into food stuffs.

A purple urine bag syndrome has been encountered, predominantly in elderly catheterized patients. It has variously been related to bacteriuria and constipation. It disappeared with removal of the catheter and urine bag.

4.3 Routine Laboratory Investigations

Unexpected findings in the "routine" laboratory require critical evaluation. Particularly in patients with unusual and unexplained symptoms, they may be indicative of an inborn error of metabolism and can help to direct specific diagnostic investigations. Table 4.3 gives a noncomprehensive collection of such sometimes unexpectedly obtained laboratory abnormalities that may be suggestive of certain metabolic disorders.

4.4 When Not to Suspect a Metabolic Disease

Inborn errors of metabolism may be considered in the differential diagnosis of a great variety of clinical problems, and at times it can be difficult to decide that specialist metabolic investigations are not warranted. Whether or not certain specialist investigations are indicated quite obviously also depends on secondary factors such as the local or national availability, the costs of the test, the likelihood of litigation, and the personal experience of the clinician. It is imperative to

Table 4.3 Routine laboratory investigations

Finding	Indicative of
Anemia (macrocytic)	Disturbances in cobalamin or folic acid metabolism or transport
Reticulocytosis	Defects of glycolysis and disorders of the γ-glutamyl cycle
Vacuolization of lymphocytes	Lysosomal storage disorders
↑ Alkaline phosphatase	Hypoparathyroidism, defects of bile acid synthesis
↓ Alkaline phosphatase	Hypophosphatasia
↓ Cholesterol	A-, hypobetalipoproteinemia, sterol synthesis defects, and peroxisomal disorders
↑ Triglycerides	Glycogen storage disorders and lipoprotein disorders, e.g., lipoprotein lipase deficiency
↑ CK	Mitochondrial disorders; fatty acid oxidation defects; glycogen storage disease types II, III, and IV; glycolysis defects; muscle-AMP-deaminase deficiency; and dystrophinopathies
↑ α-Fetoprotein	Ataxia telangiectasia, hepatorenal tyrosinemia, neonatal hemochromatosis, viral hepatitis, and ataxia telangiectasia
↓ Glucose in CSF	Mitochondrial disorders and glucose transport protein deficiency
↑ Uric acid	Glycogen storage disorders (including Fanconi–Bickel disease), fructose intolerance, disorders of purine metabolism, fatty acid oxidation defects, and mitochondrial disorders
↓ Uric acid	Disorders of purine metabolism and molybdenum cofactor deficiency
↓ Creatinine	Creatine synthesis defects
↑ Iron, transferrin	Hemochromatosis and peroxisomal disorders
↓ Copper (in plasma)	Wilson disease, Menkes disease, aceruloplasminemia, and MEDNIK syndrome
↑ Copper (in plasma)	Peroxisomal disorders
↑ Copper (in urine and in the liver)	Wilson disease and peroxisomal disorders
↓ Ceruloplasmin	Wilson disease, Menkes disease, and aceruloplasminemia
Hypothyroidism and hypoparathyroidism	Mitochondrial disorders and CDG syndromes

Mental retardation, enteropathy, deafness, neuropathy, ichthyosis, and keratoderma (MEDNIK) syndrome

AMP adenosine monophosphate, *CDG* congenital disorders of glycosylation, *CK* creatine kinase

exclude disorders for which effective treatments are available, while in cases of slowly progressive and by experience often incurable disorders, diagnostic procedures should be performed stepwise depending on the results of the first investigations and the appearance and development of signs and symptoms with time. The diagnosis of some metabolic disorders involves procedures that are stressful, such as sedation or lumbar puncture, or potentially dangerous for the child (e.g., fasting or loading studies) and that are often also stressful for the parents. Psychosocial factors should be taken into consideration when the diagnostic work-up is planned. The families need to be guided and supported. In the worst case, a specific diagnosis with a doomed prognosis that shatters the expectations of the parents can even damage the parent–child relationship. On the other hand, in almost all families, a specific diagnosis no matter how negative will be one of the most important supports for coping and of course is critical for timely genetic diagnosis in young families and appropriate counseling.

Specialist metabolic investigations are not usually indicated in children with moderate developmental delay, isolated delay in speech

development in early childhood, moderate failure to thrive, frequent infections, occasional seizures, e.g., during fever, or defined epileptic syndromes. An inborn error of metabolism is also unlikely in the healthy sibling of an infant who died of SIDS, provided that this child had been previously asymptomatic. Key factors in the evaluation of symptoms are their isolated appearance vs. the presence of additional pathology, however subtle, i.e., the lack or presence of additional neurological and/or systemic abnormalities, and a static vs. a progressive clinical course. Multisystem or progressive disorders are much more likely to be caused by inborn errors of metabolism. Expanded programs of newborn screening have simplified these issues.

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Patient Care and Treatment

5

William L. Nyhan and Georg F. Hoffmann

5.1 General Aspects

Care and treatment of patients with an inherited metabolic disease require both a detailed knowledge of the natural history of the diseases and a comprehensive understanding of the molecular basis and the pathophysiological consequences of gene defects. Continuous sympathetic company and guidance of patients and their families are essential for optimal outcome. Inherited metabolic diseases are chronic conditions that involve various different organ systems and often show progressive pathology. In addition, several genetic aspects such as passing on a disease to one's children, implications of consanguinity, the possibility of carrier detection, and prenatal or preimplantation diagnosis can create a severe psychosocial burden for individuals and families as a whole. This implies the need for an equally diverse multidisciplinary approach to care and treatment.

Primary correction of the genetic defect, i.e., gene or molecular therapy, is on the edge of becoming established for some inherited metabolic disease. Treatment is usually aimed at circumventing or neutralizing the genetic block, e.g., through the reduction of dietary phenylalanine in phenylketonuria. In addition, symptomatic treatment of the disease, such as medication for seizures or a portable electric wheel chair, is essential for outcome and improved quality of life of the patient and the family. The aim is to help the affected individual to achieve optimal development during childhood and maximal independence, social integration, and self-esteem as an adolescent and adult. This goal can only be achieved by a multidisciplinary approach. Care and treatment of the patient and the family should involve different medical specialties as well as associated professions such as dieticians, nurses, psychologists, physiotherapists, social workers, speech therapists, and teachers. Families may gain valuable emotional support and much practical advice by meeting other affected families. Ideally, a specialist in inherited metabolic diseases coordinates all aspects of care and treatment of the patient in close collaboration with the local family doctor or pediatrician.

The objective of this book is not to provide a detailed coverage of the diverse issues of the long-term care and treatment of inherited metabolic diseases. Treatment is discussed in detail

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where it is practically relevant for physicians particularly the emergency situation in which there is an acute presentation of a metabolic disorder. There is also a section on anesthesia, a subject seldom considered, but of major importance for patients with a variety of inherited metabolic diseases.

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Inherited Metabolic Diseases in the Context of Rare/Orphan Diseases

6

Georg F. Hoffmann

Inherited metabolic diseases have changed from a limited group of rare, untreatable, often fatal disorders to an important cause of acutely life-threatening and increasingly treatable diseases. Unchanged is the orphan nature of these disorders. In 1999 the World Health Organization (WHO) declared rare or orphan diseases as a major future health challenge followed by similar initiatives of the European Union. The prevalence was defined as less than four people affected per 10,000 people in the USA and less than five people per 10,000 in the European Union. Fortunately this difference does not hamper initiatives to develop worldwide. It has now become well established that rare diseases, including inherited metabolic diseases, are life-threatening or chronically debilitating diseases which such low prevalences that special combined and coordinated efforts are needed to address them. The aim is to tackle the severe reduction in an affected individual's quality of life and socio-economic potential and ideally finally to prevent significant early mortality and morbidity. It is estimated that between 8,000 and 10,000 distinct rare diseases exist today, affecting between 6 and 8 % of the population in total, i.e., between 27 and 36 million people in the European Union. Most of the people represented by these statistics even suffer from very rare diseases affecting one in 100,000 people or less. In 2006 the March of Dimes Birth Defects Foundation issued the first comprehensive global report on an important subset of rare diseases, genetic birth defects, showing incidences in the range of 40–80 per 1,000 live births worldwide (Christianson 2006). Challenges of rare diseases are especially high in inbred communities, determined by geographical, ethnical, or religious boundaries, e.g., isolated territories, better Amish communities, or Arabic countries. In the latter many local tribes were originally isolated until the beginning of the twentieth century, and, up to now, it is common to marry a first- or second-degree cousin. Resulting founder effects are responsible for a much higher prevalence of inherited diseases.

As >80 % of rare diseases have a genetic origin, current progress has been greatly fueled by technological advances, first of all molecular techniques and resulting medical progress. Diagnostically, next-generation sequencing allows for the fast generation of thousands to millions of base pairs of DNA sequence of an individual patient, the delineation of the whole genomic sequence with a realistic time frame and costs. The fast emergence and the great success of these technologies, first in research, hail a new era in genetic diagnostics. However, huge challenges need to be tackled, at the technical level, in terms of data management, for the interpretation of the results but even more in quality control, ethical issues, and simply training of (all) physicians in the strengths and weaknesses of these techniques (see

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Chap. 39). Technological advances are also allowing the extension of newborn screening to detect more than 50 inherited metabolic diseases (see Chap. 36). It is imperative to assess each condition found by screening for its impact as a screening disease. Increasing knowledge about the natural course of diseases and their variants, especially non-diseases, new treatment options, and analytical developments improving sensitivity and/or specificity of screening tests are some of the reasons for the need to continuously reassess expansion of screening for additional disorders. The policy of dealing with these objectives is different between screening programs and countries and still on debate with very different to-date results. In the USA an evidence development process for newborn screening was implemented to systematically evaluate new conditions nominated for addition to the uniform screening panel (Perrin 2010).

Among the orphan diseases, more than 700 inborn errors of metabolism currently known are especially important because of their relatively high frequency (about one in 100 births worldwide). Moreover, rationale therapies have already or will become available for many of these diseases in the foreseeable future. Landmark progress has been achieved by the development of enzyme replacement therapy, initially in patients with visceral type of Gaucher disease. Worldwide experience with more than 10,000 patients has clearly demonstrated its safety and effectiveness. Additional options for new/future therapies in patients with many different inherited metabolic diseases are inhibition of substrate synthesis (currently investigated for glycosphingolipidoses), chaperon-mediated enzyme enhancement, liver repopulation, transplantation of stem cells of various specificity, and finally (long-lasting) gene therapy.

Novel diagnostic and therapeutic possibilities for rare diseases, like extended newborn screening or enzyme replacement therapies, are expensive and can quickly become a significant challenge for the public health system. Until now almost all countries still lack an organized network of metabolic centers, which are capable of competent, comprehensive, and reliable diagnostic and therapeutic services. For example, it must be assumed that up to 30 % of patients with inherited metabolic diseases, which are diagnosable today, remain un-/misdiagnosed in Germany. The answer hopefully lies in Centers for Rare Diseases as umbrella organizations, which are being founded and now develop in many countries. In the necessary implementation process, regional differences like availability of funds, local pathology (particularly in isolated communities and societies with high consanguinity rates), and religious and geographical features must all be taken into account. Accordingly, specialized metabolic centers and appropriate metabolic networks should be established and properly maintained. The challenges to generate, implement, and utilize the exploding knowledge are huge and can only succeed through international collaboration. Firstly, metabolic physicians and scientists need to combine their efforts concentrating on well-conducted international registries, studies, and development of knowledge-based guidelines. Today significant differences exist in the diagnostic procedure, treatment, and monitoring of diseases resulting in a wide variation in outcome. Even for the disease with the most and longest experience in successful therapy, phenylketonuria, current international guidelines recommend different cutoffs for the indication of treatment at birth ranging from 400 µmol/L in the United Kingdom and 360 µmol/L in the USA to 600 µmol/L in Germany and France. Even greater are the differences of treatment recommendation later in life, e.g., from levels below 360 µmol/L in the USA to 1,500 µmol/L in France in adulthood. Hopefully, physician scientists will resolve these issues and the scientific progress will be paralleled by social, political, and economical ones as necessary prerequisites for transforming the full benefit of sciences to the people.

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Structured Communication and Behavioural Medicine

7

Peter Burgard

Key Facts

- Standard language even of educated people is insufficient for understanding diagnoses and treatment of inherited metabolic diseases. Therefore, communication with parents and patients requires teaching skills like explanation by analogy and visualisation.
- Comprehensive repeated information and counselling is ultimately necessary, because treatment has to be executed lifelong by patients and families themselves mostly at home or in their everyday life environment. New questions and demands arise in the course of the disorder or with age, making communication a continuous aspect of treatment and care.
- Information and counselling of patients and families should be given in close cooperation of all specialists involved.
- Scientific evidence and clinical experience allow the prediction of information needs and questions of patients, facilitating the preparation and training of communication skills and material supporting information transfer.
- Untrained professionals are at risk to overestimate their communicative competence and should be monitored by interprofessional supervision.

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7.1 General Considerations

The importance of communication, giving information and education of medical professions and other workers in preventive medicine of chronic disease has been stressed for a long time (Wilson and Jungner 1968).

Treatment and care of inherited metabolic diseases is mostly done at home either by the family or by the patients themselves, making information and training essential parts of the therapeutic regimen. However, the nomenclature and vocabulary of inherited metabolic diseases is neither part of standard everyday language nor basic medical terminology (Rolland and Walsh 2006). Aetiology, physiology and outcome cover the whole spectrum from genotype to enzymatic, metabolic and behavioural phenotypes, which even in the case of monogenic traits are not simple (Scriver and Waters 1999).

Education of patients and families requires a sound understanding and teaching strategies for at least ten basic concepts:

1. Food and food components
2. Chromosomes, genes and mutations
3. Inheritance
4. Productions and function of enzymes
5. Normal metabolism
6. Disease-specific disorders of metabolism
7. Diagnostic investigations for screening and confirmation of diagnosis
8. Principles of treatment
9. Measures for monitoring treatment and outcome
10. Principles of prognosis as a statistical and individual concept

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These teaching strategies include teaching by analogy and use of media to visualise principles of treatment and monitoring. Chromosomes can be introduced as cookbooks, genes as recipes – e.g. for enzymes – and mutations as errors in these recipes, ranging from minor typing errors to complete loss of a recipe, explaining the spectrum of biochemical and metabolic phenotypes. The distribution of a substrate or product in the human body can be visualised as a bathtub and the residual enzyme activity by the position of the spillway. Of course, both strategies well approved in scientific publications and presentations should be adapted to the patient's previous knowledge.

Remember

The vocabulary of inborn errors of metabolism is not part of standard language. Teaching strategies like explanation by analogy and visualisation, well established in the academic context, are appropriate for educating patients.

7.1.1 Communication Between Disciplines Involved in Diagnosis and Treatment

Although the ideal metabolic specialist is able to cover all the ten concepts, in practice the growing body of knowledge has led to a division of labour involving specialists from different disciplines like paediatric and adult metabolic medicine (often concentrated on particular disorders), neurology, genetics, dietetics and nutritional science, biochemistry, nephrology, ophthalmology, psychology, physiotherapy, occupational therapy, speech therapy and social work. Families and patients distribute information, i.e. different professions receive different particles of information, but are also sensitive to even slight differences of expressions and wording. For example, the prognosis of a nearly normal outcome might be interpreted as "not completely normal" or as "not distinguishable from normal in everyday life but by special investigations". In order to get complete information and to avoid unnecessary ambiguity, all disciplines involved must establish a metabolic team, coordinated by a skilled case manager, offering the patient a medical home (Serving the family from birth to the medical home. Newborn screening: a blueprint for the future – a call for a national agenda on state newborn screening programs 2000; Hinton et al. 2011).

Remember

Also communication between professionals and professional disciplines should be organised.

7.1.2 Communication in the Field of Inherited Metabolic Diseases

For the purpose of the present chapter, communication is divided into two facets:

A. Information as teaching, education, instruction and training of patients. Information is a primarily unidirectional process of communication, *operated* by a professional, communicating preferred (evidence based, guideline oriented) or recommended options. History taking should be regarded as collecting information.
B. Counselling as patient-centred assistance in decision-making and problem solving *supported* by a professional, where the patient is supported to find out his/her preferred option.

Communication is called structured when it has a defined goal, a subject and an agenda, and the process of communication is subject to quality control, i.e. an examination of the result in the light of the goal. Structured communication and information requires development and preparation of material, training and exercise.

Remember

Giving information in a structured way needs time, but is an investment in the future, making further communication more efficient.

7.1.3 Necessary Information and Interesting Questions

Apart from the initial manifestation and emergency situations which are treated on the ward, treatment is done at home requiring necessary and sufficient information for effective management.

For most patients and families, getting a diagnosis of an inherited metabolic disease is a unique existential experience. Therefore it is understandable that patients strive for getting comprehensive information and even ask questions which, from a professional point of view, are unnecessary or ill defined. Often the overall information can overwhelm the patient at the first encounter, and more specific themes must be repeated often several times.

Information, necessary and sufficient to understand a diagnosis, can be deduced from protocols, whereas patients' and families' questions can be forecasted from clinical experience.

Based on scientific research (Boyse et al. 2014; Buchbinder and Timmermans 2012) and clinical experience, faced with the first result of a diagnostic process, patients and families have the following nine prototypical questions:

1. *Terminology*: What does the names/abbreviations (phenylketonuria, PKU; MCADD; MMA; etc.) mean?
2. *Diagnostics*: How did you find out?
3. *Aetiology*: What is the cause?
4. *Nosology*: What kind of a disease is it?
5. *Symptomatology*: What will happen?
6. *Outcome*: Is it severe, dangerous, perilous and life-shortening?
7. *History and prognosis*: What can I expect? When will it go away?
8. *Treatment/cure*: What can be done (how, how often, how long, by whom)?
9. *Repetition and transmission*: Will others be affected?

During the first meeting, information should be given at full length by an experienced specialist. Whenever possible, graphic material should support verbal instructions.

Remember

Giving a diagnosis of an inherited metabolic disease is bad news. Bad news cannot be converted to good news, but they can be delivered in a good mode.

Giving first information over the phone should be restricted to make an appointment with the metabolic specialist. The use of new communication techniques (e.g., Skype) is still in the experimental phase, but might be an alternative in selected situations (Langenau et al. 2014).

Graphic material and a pencil should be handed out together with the invitation to make personal notes. This procedure has two reasons. First, information given over two channels – auditory and visual – will improve storage in memory. Second, take home material will increase retrieval of information and facilitate explanation given to other family members or caregivers, who preferably should be invited to participate in person.

In times of the Internet (unfortunately mixing up excellent and deceptive content), the apprehension that discussion of all items will result in information flooding is obsolete, as withholding or piecemeal information could be interpreted as incomplete information and cause mistrust. Parents and patients will appreciate if all aspects they perceive as necessary or important will be mentioned. The evaluation of the professionals' attitude as striving for complete information will outweigh incomplete understanding and retention of the material.

Remember

Information needs of patients can be divided into two parts: those necessary and sufficient for effective treatment and those satisfying the existential dimensions of a disease.

Delivering necessary information will increase competence, and answering questions will create trust.

7.1.4 Use of Ambiguous and New Terms

Not only everyday speech but also medical terms can be ambiguous. Phrases like "outcome is

nearly normal" or prognosis is "quite good" should be avoided, as the speaker cannot control whether the listener focusses on the adjective (normal, good) or the adverb (nearly, quite) neither the interpretation of their combination (nearly normal as hardly any difference vs. some difference will be obvious). Instead, achievement of developmental milestones or developmental tasks (e.g. degree of independence in adulthood) should be used to describe expected outcomes.

In many languages "treatment" also has the connotation of "cure", what is not appropriate for preventive measures in inherited diseases. The predominant meaning of treatment applies to therapeutic medicine, where a healthy person falls ill, illness (a fact by experience) is treated and health is re-established. In many cases treatment of inherited metabolic diseases is preventive, i.e. a healthy organism is treated in order to maintain health, making illness a fact by knowledge that in the case of good compliance will never become a fact.

Remember

Language is ambiguous, and information transfer needs quality control.

7.1.5 Communication with Children

Communication with children requires specific accommodation of the adult speaker. Professionals should abstain from "motherese" (a sing-song variation in high tone) especially in older children, but sentences should be short, simple and use present tense. Redundancy and repetition is much more appropriate, necessary and accepted than in adults. In particular children have longer response times when answering questions, what can be misinterpreted as refusal (General Medical Council 2007). Children's communication skills should not be underestimated. By the end of 4 years of age, they are able to communicate in a competent way, and with the start of formal schooling, they have to manage a substantial part of the day without parental support (Cahill and Papageorgiou 2007). Children should be directly involved into communication by treating professionals. In general, parents are medical laypersons needing instruction and support themselves. Using parents as extensions of the professional team will result in the paradox of a professional explaining a layperson how to deliver a message in a professional way to a target subject sitting side by side with the latter. Contrary to other rules and procedures in the child's educational environment, diagnostic and therapeutic measures result from medical necessity and not from parental principles or authority. Therefore, starting with preschool age, instructions regarding treatment should also be given directly to the patient, and instructions given to parents should be given in the presence of the patient.

By the start of puberty, it is helpful to distinguish chronological age and developmental age. Patients can well answer the question whether they feel themselves as a child, adolescent or adult or an amalgam of different developmental stages. Evaluation of developmental age is also helpful in interaction and communication with handicapped patients.

Some parents think that it is necessary to have special parenting skills raising a child with an inherited metabolic disorder. It may be helpful to explain that in principle raising a child with an inherited metabolic disease introduces new content, but does not require special parenting strategies.

Remember

Determining the patient's self-attributed developmental age as a child, adolescent or adult can be instrumental in guiding communication.

7.1.6 Nonverbal Communication

Communication is not restricted to the oral and written transfer of information, but also includes action. Acting as a metabolic team including the patient as an active integrated participant and not only as the target of recommendations is the "walk to talk" of cooperation, i.e. essential for successful cooperation. A well-functioning metabolic team is a model for successful cooperation,

motivating patients to act as a responsible and reliable partner.

Facilitating contacts with other families and patient advocacy groups provides positive role models and create opportunities for observational learning and imitation. However, it should also be explained that the same diagnosis has genetic variability and a broad range of phenotypic expression.

7.1.7 Management of Emotions

The most prevalent emotions in the initial stage of getting the diagnosis of an inherited metabolic disease are anxiety for metabolic crises and grieving for the loss of functional integrity of the child. Anxiety can be reduced by issuing an emergency pass and providing a 24/7 emergency number. Mothers may react with guilt, assuming to have made a mistake during pregnancy. Therefore, the mode of inheritance should not only be used to explain the condition but also to elucidate carriership. Particularly in the case of autosomal recessive traits, parents are immediately released from feeling guilt by learning that they too have inherited the gene.

Communicating the prevalence of carriership also can contribute to relief of being a member of an exceptional minority. For autosomal recessive disorders, following the Hardy-Weinberg law, the denominator X of the prevalence of carriership (1:X) can be calculated by dividing the denominator of the prevalence of homozygous individuals by four and extracting the square root from the result. As an example, given the prevalence of 1:10.000 for a given disorder, the denominator $X = 10.000$ divided by four is equal to 2.500, and the square root of 2.500 is 50, i.e. one in 50 individuals is a carrier. Using the inversion one can explain the prevalence of a homozygous genotype. Supposing equal prevalence of carriership in males and females, one of the possible $50 * 50 = 2.500$ couples will consist of two carriers. Statistically autosomal recessive inheritance will result in one homozygous case out of four offspring. Delineating a matrix with 50 lines (women) and 50 columns (men), most parents of a child detected by newborn screening will immediately understand what has happened to them and that in each occupied bus, plane or cinema, at least one other carrier can be expected.

Grieving for loss of integrity needs time and parents and patients should be given time for mourning. In practice psychotherapy is necessary only in a few cases. Psychotherapists may feel incompetent to disentangle technical problems due to the disorder and treatment on the one hand and insufficient coping skills due to personality. In these cases close communication between the metabolic specialist and the psychotherapist is recommended.

Shame for needing a special treatment or diet has become a minor problem since most Western societies became more and more “dietetic”, with nearly everyone having food preferences of food aversions. However, peer pressure might be strong, and families and patients should be trained how to explain treatment measures to others, e.g. grandparents, friends, teachers, etc.

Evaluations of the burden of disorders by professionals and patients revealed different perspectives on inborn errors of metabolism in both groups. Disorders rated as potentially very burdensome by experts were not rated accordingly by parents and vice versa (Gramer et al. 2014).

7.1.8 Talking About Numbers

Diagnosis and treatment of inherited metabolic disorders use a variety of numbers like diagnostic and monitoring parameters, quantitation of drugs, nutrient intake and other interventions (e.g. feeding schedules in MCAD deficiency). Probabilities and percentages should be communicated as natural numbers, i.e. instead of saying that the probability of recurrence of an autosomal recessive trait is 0.25 or 25 %, the expression of one in four will be more easy to understand. The statistical independence of repeated events should be explained or even demonstrated by analogy (e.g. repeatedly flipping two coins). Measurement results given as micromoles per litre will be meaningless for most laypeople, but mg/dl can be more easily explained and referred to nutrient intakes expressed in mg.

7.1.9 Newborn Screening

Newborn screening is a multidisciplinary programme of paediatric preventive medicine (see Chap. 36), requiring structured fast communication between different health care providers (obstetrical units, laboratories for screening and confirmation of positive screening results, inpatient and outpatient clinics responsible for treatment of confirmed patients), including families and patients (Burgard et al. 2012). Particular concern has been addressed to false-positive screening results. Contrary to the often-stated assumption that false-positive screening outcomes result in parental stress or trauma, empirical research has demonstrated that a real false-positive screening result experience is not as bad as imagined by those who have not experienced it (Prosser et al. 2008), and the willingness to pay for the avoidance of a positive screening result was not significantly different for parents with vs. without a false-positive result (Dixon et al. 2012). However, mothers of children with false-positive results referred to a metabolic centre had significantly lower stress scores than mothers of children not referred to a metabolic centre and that information given in person was associated with significantly less stress than information given by telephone or letter (Waisbren et al. 2003).

Remember

Real false-positive screening results are less stressful than non-concerned observers, probably including professionals, imagine it. Families with true-positive as well as false-positive results deserve comprehensive information and response to their questions.

7.1.10 Genetic Counselling

Genetic counselling (Evans 2006) is intrinsically intertwined with inherited metabolic disorders. Starting from an index case, genetic information might be required by various relatives, including parents, their siblings and partners concerning family planning, as well as individuals of different degree of kinship with possibly mild or late-onset phenotypes. In general correlations between phenotype and genotype are less than perfect. Although correlations are important for scientific reasons, parents and patients are interested in individual prognosis (Barker and Field 2014), what is usually only possible on the basis of metabolic investigations and long-term observation of the subject's history.

Remember

Genotype-phenotype correlations allow statistical predictions, but parents and patients are interested in individual prognosis.

7.1.11 Overestimation of Competence

Speakers should be aware that there is a tendency to overestimate one's own communication competence (Zell and Krizan 2014), probably resulting from the cognitive demands of speaking. Overhearers, i.e. observers attending the process of information transmission, give more accurate estimates of the success of communication than speakers themselves, who have few resources for simultaneously monitoring themselves and the listener (Keysar and Henly 2002). The situation may be even worse when the message is formulated ad hoc. Training of communication skills, creating and rehearsing well-tried scripts and using prefabricated material substantially improve communication. Training should also contribute to overcome egocentrism, i.e. understanding that patients have a different perspective from the one of professionals and how to find out what matters from the patients' point of view (Gramer et al. 2014).

Remember

Monitoring one's own communication might be difficult. Working in a tandem with another colleague can improve the rate of successfully transmitted information.

7.1.12 Behavioural Medicine

Behavioural medicine is defined as "…the interdisciplinary field concerned with the integration of behavioural, psychosocial, and biomedical science knowledge relevant to the understanding of health and illness, and the application of this knowledge to prevention, diagnosis, treatment, and rehabilitation" (Steptoe 2010) (p.v). In principle, integration and application of knowledge is information processing and communication. Inherited metabolic disorders are chronic conditions requiring treatment, monitoring and therefore communication for life. New questions and demands arise in the course of the disorder, including pregnancies necessitating intensified measures to protect the development of the embryo and foetus and require preconceptional training. As has been stated previously, treatment is predominantly done by parents and patients themselves, however, under the guidance of a professional interdisciplinary metabolic team. From this perspective diagnosis, treatment and monitoring of inherited metabolic medicine is behavioural medicine. Last but not the least, the programme of evidence-based metabolic medicine is impossible without effective and efficient structured communication.

Remember

Diagnosis and treatment of inherited metabolic disorder are deeply rooted in behavioural medicine.

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Guidelines and Follow-Up

8

Stefan Kölker

8.1 Guidelines

A guideline is not intended to serve as a standard of care. Standards of care are determined on the basis of all clinical data available for an individual case and are subject to change as scientific knowledge and technology advance and patterns of care evolve. Adherence to guideline recommendations will not ensure the correct diagnosis and satisfactory outcome in every case, nor should they be construed as including all proper methods of diagnostic work-up and care or excluding other acceptable methods aimed at the same results. The ultimate judgment must be made by the appropriate healthcare professional(s) responsible for clinical decisions regarding a particular clinical procedure or treatment plan. The judgment should only be arrived at following discussions of the options with the patient and their family, covering the diagnostic and treatment choices available.

Guidelines are an important tool of evidence-based medicine. Their major aim is to provide recommendations for diagnosis, therapy, and follow-up based on the best evidence available for a specific disease. Clinical practice guidelines enhance clinician and patient decision-making by clearly describing and appraising the scientific evidence as well as the likely benefits and harms behind clinical recommendations. Accepted criteria for the validity of guidelines were first defined by the US Institute of Medicine in 1990. Since then attributes such as validity, reliability, applicability, flexibility, clarity, multidisciplinary process, and documentation have formed the basis of guideline methodology such as described by SIGN 2014 (www.sign.ac.uk) and NICE. Significant steps include the establishment of multidisciplinary guideline groups, identifying key questions, systematic search for and review of relevant literature, grading of literature and formulation of evidence-based recommendations leading to the guideline draft, external peer review, and finalization. As per the SIGN methodology, evidence is rated from 1^{++} (based on high-quality randomized controlled trials [RCTs]) to three (case series and case reports) and four (expert opinions). Guideline recommendations are graded from A (the highest level, based on high-quality evidence) to C and D (based on level 3 and 4 evidences). Such graded evidence helps make guidelines more objective and also makes practitioners aware of the strength of the evidence used to devise the guidelines.

The development of evidence-based practice guidelines for rare diseases such as inherited metabolic diseases is a challenge. Available evidence exists mostly in the form of cohort studies, case series, case reports, or expert opinion papers,

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and therefore, available evidence from literature is mostly graded as level C or D. It has been debated that following the above described approach of SIGN and NICE might be unhelpful since it results in the development of recommendations with relatively low grades. However, there are strong refutations to this position: (1) The level of published evidence and grading of a recommendation do not necessarily correlate with its clinical relevance. For instance, although low phenylalanine diet for phenylketonuria has never been tested in a randomized controlled trial, and therefore, the level of evidence for this intervention has to be formally evaluated as relatively low, no metabolic specialist would doubt that this therapeutic intervention in general is extremely relevant for affected individuals. (2) The effect size of therapeutic interventions in patients with inherited metabolic diseases is often huge. Therefore, it can be reliably identified by a cohort study with low risk of confounding bias. There is no doubt that, if affordable and achievable, randomized controlled trials for rare inherited metabolic diseases should be performed, but this usually requires the support of the pharmaceutical industry. However, there are also examples of carefully designed n of one trial. (3) Low grading helps to identify the gaps in current knowledge thereby setting the scene for further research. (4) Setting standards of practice is important to minimize unnecessary variance or – even worse – trial and error. (5) Identification of alternative approaches is important since these are often required when adverse events occur, or a drug is not available in a national health system. (6) Practices based upon expert opinion of single physicians or centers with a long-standing experience do not gain wide appraisal and approval without independent and critical evaluation.

Over the last years, an increasing number of guidelines for inherited metabolic diseases such as for amino acid disorders, urea cycle disorders, organic acidurias, mitochondriopathies, and lysosomal storage disorders have been developed (Baumgartner et al. 2014; Blau et al. 2010; Häberle et al. 2012; Heringer et al. 2010; Kölker et al. 2011). Importantly, for some diseases, the use of these guidelines when evaluated after some years was shown to improve the outcome. Furthermore, the process of systematic literature review has stimulated research such as the conductance of observational clinical studies, the development of patient registers, and meta-analyses, thereby improving the evidence base of future recommendations.

8.2 Follow-Up

Follow-up of patients with inherited metabolic diseases is usually performed by a team of experts in a metabolic center. Regular therapy monitoring aims to evaluate relevant parameters that allow an estimation of the disease course, the benefit and relevant side effects of a therapy (risks and benefits), therapeutic efficacy (outcome), and its adequate or inadequate performance (adherence/nonadherence, mistakes). In children, monitoring must also include an evaluation of development, growth, and malformations. Therapy monitoring shall include those parameters that influence the decision on therapeutical interventions and has a preventive aim. Adverse changes should be detected at an early stage when they are still reversible. Potentially hazardous and invasive monitoring techniques should be used very cautiously. In addition, therapy monitoring shall be performed in a way that outcome can be evaluated. In general, an optimal (clinical or biochemical) parameter for therapy monitoring shall have the following qualities: (1) no or low risk and inconvenience for the patient, (2) high validity, reliability, and objectivity, (3) high sensitivity and specificity, (4) high predictive value, (5) broad availability, and (6) low cost. For many inherited metabolic diseases, reliable and predictive follow-up parameters are still unknown.

Clinical monitoring includes general pediatric parameters (e.g., anthropometrics, developmental milestones), neurological assessment, and specific psychological tests. Expertise from general pediatricians, metabolic specialists, and metabolic dieticians should be included in the evaluation of patients in an integrative way. In addition, consultations from other specialties (e.g., child neurologists, psychologists, physiotherapists)

should be added if applicable. The frequency of outpatient visits is adapted to various parameters such as age, the severity of the disease course, and adherence to treatment. Whereas outpatient visits might be required on a monthly basis for newborns and young infants, the frequency of such visits is more relaxed in adolescents and adults.

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Patient and Family Associations

9

Steve Hannigan

Key Facts

- A patient or family association is normally a registered charity or association.
- The first criterion that a charity, patient or family association must meet in order to qualify for charitable status is that it exists solely and exclusively for charitable purposes. This means that the organisation is not designed to generate private profit and is structured in such a way that assets and earnings are used for the purpose of giving.
- For many charities involved in inherited metabolic diseases, the primary function will be to provide information and support to patients and their families.
- Secondary functions will include raising awareness, funding research, education and fundraising.
- Charities may undertake any or all of the above functions.
- Part of the function of providing information is working with inherited metabolic diseases specialists to ensure that the information provided to patients or families is factually correct and up to date.

Information provided to patients and families must have been verified by an inherited metabolic diseases specialist to ensure that the information provided to is factually correct, is up to date and is in a family friendly format and not full of hard to understand medical terms.

Membership – many organisations provide a membership service. This meets two functions: One is to enable updated information to members through the organisations' database and the other is to raise much needed funds.

Bereavement services are one of the hardest areas to provide a service to families. The loss of a child is devastating to the family and to provide support at this very traumatic time needs very well-trained staff who are able to empathise with the parents or guardians.

Email networks are now a very essential service for families.

Conferences – many organisations provide an annual conference which will cover many areas of specific disorders or groups of disorders in the same area such as fatty acid oxidation disorders or glycogen storage disorders.

Social Media – Web sites and social media forums are now managed on a regular basis by many charities and are a vital area for the exchange of information.

9.1 What Can a Patient or Family Affected by an Inherited Metabolic Disease Expect from a Support Organisation

Information – the information provided to patients and families must have been verified by an inherited metabolic disease specialist to ensure that the

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information provided is factually correct, is up to date, is in a family friendly format and not full of hard to understand medical terms. This makes it a lot easier for the information to be understood and not misinterpreted. Many parents leave the first consultation with a specialist without having taken in a lot of the breadth and scope of the diagnosis. The information from support organisations is invaluable in supporting families especially through the first few weeks.

Membership – many organisations provide a membership service. This meets two functions: one is to enable updated information to members through the organisations' database and the other is to raise much needed funds. As part of many membership organisations, a regular quarterly or monthly newsletter is sent out to members.

Young People's Services – these services are often provided and are aimed specifically at those patients under the age of 16. Information will be provided to them on their disorder once approval has been given by their parents/guardians. Many organisations provide information and links via facebook and twitter.

Bereavement Services – this is one of the hardest areas to provide a service to families. The loss of a child is devastating to the family, and to provide support at this very traumatic time needs very well-trained staff who are able to empathise with the parents or guardians. Fortunately, many voluntary organisations ensure that support staff undertake counselling training.

Email Networks – are now a very essential service for families who have received a diagnosis of the inherited metabolic disease for themselves, their partner or one of their children as it can enable a charity to quickly put people in contact with other families who have the same or a similar diagnosis. The hardest group of people to put in contact with others are those who most likely have inherited metabolic diseases but are currently classed as 'undiagnosed'.

Conferences – many organisations provide an annual conference which will cover many areas of specific disorders or groups of disorders in the same area such as fatty acid oxidation disorders or glycogen storage disorders. Some organisations provide conferences especially focused at families who have received a diagnosis through national newborn screening. In all cases, the presentations are given by inherited metabolic diseases professionals with special expertise in this field.

Satellite meetings are a bonus to patients and families who cannot attend nationally organised conferences and are organised in a local venue so that members can attend, update their information and give valuable feedback to the charity on their role and/or activities.

Research is a vital part of many inherited metabolic diseases charities, but it must be recognised that many of these organisations are small and can only raise funds to support smaller projects or part of a large project, e.g. research funds in the area of €20,000–€25,000 per year.

Support Grants – financial support grants are often available to families in smaller amounts up to €300 for the purchase of items of equipment or to assist with travel costs to/from hospital.

Magazines or Newsletters – regular magazine and/or newsletters produced by the organisation help to keep members up to date with activities in many areas from specialist updates to fundraising events and activities. Many charities provide an in-memory section in their magazine to remember those who have recently passed on.

Social Media – Web sites and social media forums are now managed on a regular basis by many charities and are a vital area for the exchange of information.

Advocacy – National and International Advocacy and Representation is part of the larger picture for charities, and advocacy is a process by which a charity aims to influence decisions within economic and social systems or institutions. Advocacy can include many activities that an organisation undertakes including media campaigns, public speaking, commissioning services and publishing research.

9.2 Further Information

Many voluntary organisations have a Web site and avenues to social media links:

Climb (Children Living with Inherited Metabolic Diseases) is also the National Information Centre for Metabolic Diseases in the United

Kingdom, and their Web site (www.climb.org.uk) is a mine of information. There is also a freephone helpline (in the UK) on 0800 652 3181. Social media outlets can be a main source of information to families through Facebook and Twitter. Further details can be obtained from Climb by email (contact@climb.org.uk). Climb currently provides information on over 700 inherited metabolic diseases and works with patients, families and professionals throughout the world.

Eurordis (European Organisation for Rare Disorders) is a European non-governmental patient-driven alliance of patient organisations based in Paris and provides a host of information for many of the rare disorders organisations throughout Europe. Their excellent Web site (www.eurordis.org) covers many areas from disease-specific information to conferences and patient involvement. At the time of going to press, Eurordis represented 710 rare disease patient organisations in 63 countries covering over 4,000 diseases.

VKS (Volwassenen, Kinderen en Stofwisselingsziekten) provides excellent information via Web site (www.stofwisselingsziekten.nl), regular newsletters and family support to patients and families in the Netherlands. VKS can be contacted by email (info@stofwisselingsziekten.nl).

MPS (the Society for Mucopolysaccharide Diseases) hosts a very good Web site (www.mpssociety.org.uk) that provides invaluable support to families affected by MPS disorders. The society provides support, funds research and raises awareness for mucopolysaccharide and related diseases. They can be contacted by email (mps@mpssociety.org.uk).

PND (the Paediatric Neurotransmitter Disease Association) has been established as a non-profit organisation that has been instrumental in helping to provide information and to link families and professionals involved in the diagnosis, care and research related to neurotransmitter disorders, www.pndassoc.org. The nonketotic hyperglycinaemia (NKH) International Family Network Web site (www.nkh-network.org/) provides an international resource for NKH patients with active chapters in North America and Germany. NKH International Family Network (www.nkh-network.org/) is a patient association that provides support for families throughout the world. Similar function with the focus on research projects is provided by the AADC Research Trust focusing on patients with aromatic l-amino acid decarboxylase deficiency (www.aadcresearch.org/).

10 Adolescence, Emerging Adulthood, and Problems of Transition

Ursula Plöckinger

10.1 Transition of Healthy Adolescents and Young Adults

Adolescence, defined by the United Nations as the age between 12 and 19 years (Bitzer 2013), is a critical developmental phase, characterized by increased sociocultural turbulence and vulnerability. Two of its hallmarks are the search for an identity separate from that of the family of origin and the redefinition of relationships with adults in parental and caring roles (Tsybina et al. 2012). Development of adolescence into adulthood poses many challenges like rapid physical growth, puberty and sexuality, development of personal identity, autonomy, independence and interpersonal relationships, planning for the future, and risk taking behavior (Flume 2009; Fredericks 2009). How a young adult manages these life experiences will influence the degree and direction of personality maturation (Bleidorn 2012). According to the social investment theory, age-graded life transitions in early adulthood have the potential to stimulate personality change, as they force individuals to invest in and commit to new social roles (Roberts et al. 2005). How they negotiate these demands and behavioral tasks will probably affect the timing, the direction, and the degree of personality trait change. Transitional tasks and role demands create a reward structure promoting self-regulated and consistent changes in behavior that, if extended, may cause changes in traits. That is, behavioral changes (besides changes in thoughts and feelings) take on a significant mediational role as they account for the path through which prolonged environmental effects will change neuroanatomical structures or gene expression and thus change personality traits (Roberts et al. 2005). To give an example, in healthy adolescents, graduation, taking place at about 18 years of age, is one transitional process where the adolescent assumes full responsibility for a task that has important consequences for the rest of the adult life. The investment in and commitment to these adult social roles in the work domain are related to increase in conscientiousness, i.e., specific personality traits. Similarly, the transition to the first serious partner relationship is associated with sustainable decreases in neuroticism and increases in conscientiousness (Bleidorn 2012).

Young or emerging adulthood, defined as the late teens into late twenties (Burt and Paysnick 2012), is characterized by distinctive features like identity exploration in areas of relationships and work; instability as evidenced by changes in residence, jobs, and relationships; the development of self-reliance needed for adulthood; a

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feeling of "in-between" wherein individuals express ambivalence in describing themselves as adults; and the perception of the available possibilities and variety of life choices (Betz et al. 2013). Thus, young adulthood represents a second period of significant change. Important aspects of brain development continue into and through the young adult years, including myelination of areas in the prefrontal cortex. In parallel to this brain development, increased skills in the area of executive functioning are observed. The term executive functioning comprises cognitive flexibility, inhibitory control, and working memory. Both, cognitive flexibility and inhibitory control, are of particular relevance for the developmental task of emerging adulthood, which include controlling one's behavior in service of rules or obligations of the society and making appropriate decisions for long-term stability and success (Burt and Paysnick 2012).

Young adulthood has been shown to be a period of declining healthy behavior, i.e., adequate exercise and sleep, eating breakfast, maintaining a healthy weight, and not smoking or binge drinking (Dummer et al. 2012; Frech 2012; Poobalan et al. 2012). Health-care utilization is exceptionally low in male young adults (Irwin 2010).

In conclusion, in healthy adolescents and young adults, transition is a formative period starting around puberty with the transition from childhood to adolescence and a second phase from adolescence into young adulthood and further on into mature adults. These developmental phases are characterized by formative environmental influences on behavior and personality traits that will shape the future personal and professional life. Thus, these transitional phases demonstrate a wide range of behavioral flexibility and instability, as well as risk-taking and unhealthy behavior until finally a mature adult may emerge.

10.2 Transition of Adolescents with Chronic Diseases

Over the last 40–50 years, advances in pediatric medicine from cardiology, oncology, kidney and liver diseases, as well as inherited metabolic diseases, among others, have resulted in a quickly growing number of successfully treated, socially capable, and integrated children with chronic conditions who would have previously died early and/or developed severe disabilities. These children now grow up to become adults in steadily increasing numbers. Today, nine of every ten children diagnosed with special health-care needs survive into adulthood. In the United States (US), the group of 12–17 years adolescents with special health-care needs (ASHCN) account for 40.8 % or 4.5 million adolescents which is the largest percentage of all children with special health-care needs. In Australia, nearly two-thirds of youth and emerging adults have a chronic condition of which approximately one-third is disabling (Betz 2013). No data are available for the European countries. These numbers will increase worldwide as today's generation of adolescents is the largest in history. Nearly, half of the global population is less than 25 years old (Bitzer 2013).

Adolescents with chronic diseases and special health-care needs do now confront additional challenges during transitional years. They do not have only to manage "normal transition" but also to confront additional difficulties related to their medical problems. Their chronic disease sets them apart from their peer groups, at a time when conformity with peers is important in many ways. Depending on the severity of health restrictions, participation in normal young adults' activities, romantic relationships, and professional opportunities may be hampered or impossible, further increasing the burden of these transitional years. Superimposed on these difficult times, ASHCN will have to adjust and manage the transition from pediatric into the adult health-care system. Taking into account, the young patients' experiences and expectations further emphasize the importance of health-care transition. As Flume (2009) observed, "transition offers the patient a positive sense of a future; while staying in child-centered care sends a subtle message that adulthood may be an unrealistic expectation." Furthermore, "it is highly unlikely that an adult with a health-care problem would ask for a pediatrician to deal with the problem. Why should we

expect any less for the adolescent or young adult with a chronic disease?" (Flume 2009)

10.2.1 Transition

In the context of this article, transition is defined as the "purposeful, planned movement of adolescents and young adults with chronic physical and medical conditions from child-centered to adult-oriented health-care systems" (Blum et al. 1993). Recently, an extended definition describes "a dynamic, lifelong process that seeks to meet their individual [referring to adolescents] needs as they move from childhood to adulthood. The goal is to maximize lifelong functioning and potential through the provision of high-quality developmentally appropriate health-care services that continue uninterrupted as the individual moves from adolescence to adulthood. It is patient-centered, and its cornerstones are flexibility, responsiveness, continuity, comprehensiveness, and coordination" (Pediatrics, Physicians et al. 2002). In addition, "special health-care needs" are defined as "the need of those who have or are at increased risk for a chronic physical, development, behavioral, or emotional condition and who also require health and related services of a type or amount beyond that required by children in general" (Betz et al. 2013). There is now an increasing awareness that proper transition is important. Yet evidenced-based data are rare, and most publications deal with the process of preparing transition with the ultimate goal of transfer from the pediatric into the adult health-care system.

10.2.2 Transfer

In this setting, transfer describes a one-time process, i.e., transferring the responsibility of care from a pediatric to an adult care setting. Transfer per se is thus part of, but clearly not the endpoint of the transitional process. The ongoing transition from young to mature adulthood falling into the responsibility of the adult health-care system is less evident but probably not is but as important (Fig. 10.1). However, data on this part of the transitional process are nonexistent (Suris et al. 2009).

10.2.3 Health-Care Settings

Awareness of the differences between pediatric and adult health-care is essential to understand why there is a need for transition at all. Pediatric health-care is primarily "family-centered." Pediatricians deal with the parents as their primary contacts. Parents have all the knowledge and take over responsibility for their child's disease far into adolescence. Subsequently, young adults may have major deficiencies in knowledge about their disease and health-care, as well as limited self-care skills (Anthony et al. 2009; Annunziato and Shemesh 2010). In addition, pediatricians as well as parents may be reluctant to depart from their responsibilities in the care of

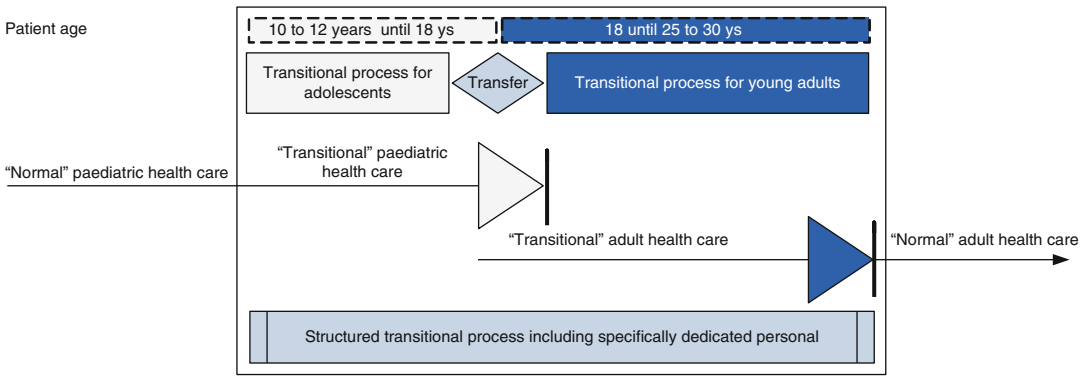

Fig. 10.1 Transition, trajectory, and stakeholders

these young adults, fearing for the quality of care in an adult health-care setting.

Adult health-care is primarily patient-centered. Ideally, the patient is a partner in all disease- and health-care-related issues. The health-care setting aims to "empower" the patient thus providing the basis for informed, rational, and responsible decisions by the patient. It is assumed that the patient has self-management skills as discussing one's illness, medical treatment, and schedule. Further basic expectations are that the patients attend appointments, adhere to medications and treatment recommendations, engage in a healthy lifestyle, and have a basic understanding of anatomy and her or his chronic disease, as well as health history (Fredericks 2009; Annunziato et al. 2011). The paradigm of "being cared for" changes to "taking care of oneself." Successfully, managing this change is already difficult for healthy adolescent, even more so for the adolescent or young adult with chronic disease and special health-care needs.

10.3 Results of Pediatric Transition Programs for Specific Diseases

Within the last 10 years, published data report the outcome of disease-specific pediatric transition programs providing information up to or beyond the transfer into adult health-care. The following examples should highlight some of the still unsolved problems:

1. *Renal allograft* loss significantly increases in adolescents (11–17 years) relative to younger recipients. In children, chronic kidney disease and glucocorticoid therapy may result in short stature. These short children have difficulties with behavior, cognition and life achievements, lower scores on physical and intellectual functions; part of it is due to the morbidity before transplantation or transplantation complications. Medical risk factors of transplantation include increased cardiovascular risk, infection, especially sexually transmitted disease due to immunosuppression, and malignancies. Overall, these complex medical conditions and their implications relay to the less favorable medical outcome during transition (LaRosa et al. 2011). In a review by Fredericks' nonadherence with immunosuppressive medication among adolescents, renal or liver transplant recipients ranged from 5–71 to 17–53 %, respectively, and was four times higher than among adult transplant recipients (Fredericks 2009). Poorer adherence correlated significantly with increasing age of the young adults, when parental supervision decreased. Most of the risk factors for nonadherence with therapy stem from socioeconomic and environmental variables.
2. Childhood *cardiac diseases* display different clinical manifestations and therapeutic necessities in childhood, adolescence, and young adults. Adult cardiologists are less familiar with some type of congenital heart diseases outside specific centers and thus are reluctant to take care of these patients (Rakhmanina et al. 2010). Up to one-half to three-quarters of the patients fail to continue regular follow-up when they have grown up, with far-reaching consequences. Being lost to follow-up is associated with significant morbidity (Moons et al. 2009).
3. Similar problems occur with the different etiologies in *liver disease* in the pediatric population compared to adult patients. Adults with pediatric liver diseases differ in treatment, complications, and extrahepatic conditions (Rook and Rosenthal 2009). Without a broad and comprehensive knowledge of the common childhood liver diseases and the different implications compared to adult etiologies, these patients will be at risk of insufficient care with probably higher morbidity.

Problems get even worse if we look on some of the rare inherited *metabolic diseases*. Previously, these have often led to severe disabilities and/or early death. An increasing number of early diagnosed and well-treated patients is now emerging mainly as a consequence of newborn screening, and soon the majority of metabolic patients will be adults (Table 10.1).

Table 10.1 Age-related epidemiology of inherited metabolic diseases in Germany

Number of patients in 2050 in different age groups	
Age group	Number of patients
0–10 years	~4.750
11–20 years	~4.700[a]
21–30 years	~5.500[a]
31–60 years	~12.200[a]
0–60 years	~26.700[a]
Adults only	
20–60 years	~17.250
0–60 years	~26.700

[a]Estimated on the basis of the predicted birth development (Federal Office for Statistics, Germany) with a total prevalence of inherited metabolic diseases of 1:1.200 (Birth cohort 1990–2050)

A questionnaire dealing with problems from maple syrup urine disease (MSUD) in eight patients and their parents highlights some of the problems encountered in transition of patients with metabolic diseases. MSUD is due to the deficiency of the branched-chain α-keto acid dehydrogenase complex. Subsequently, the metabolism of leucine, isoleucine, and valine is impaired, and these amino acids accumulate in plasma and body tissue resulting in neurotoxicity and negative effects on cognition and adaptive and psychosocial functioning. Therapy consists in lifelong dietary restriction of leucine, isoleucine, and valine, aiming at normal plasma concentrations of these amino acids and avoiding nutritional deficiencies. Similar dietary restrictions occur in many other disturbances of amino acid metabolism. Most of the young adults are still treated at pediatric departments, a fact many feel embarrassed about. Yet specific knowledge, as with many other inborn errors of metabolism, is not available within the adult health-care system. In addition, keeping up lifelong dietary restrictions, being different from their peer groups, and having difficulties explaining the disease are seen as major problems by these young adults. Developmental delays, recurrent hospitalizations, and school absences due to illness interfere with academic achievements and fatigue with professional success (Packman et al. 2012).

4. Available data indicate that *many patients are lost* during and after the transfer process. Despite a formal transition program in Canada, including 18-year-old patients with complex congenital heart defects, transition succeeded in only 47 % and failed in 27 %, with failure defined as no follow-up appointment since the age of 18 years. Only half of the patients had had a discussion of their future adult care while still under pediatric care. In Quebec, despite the presence of a structured transition program for patients with juvenile idiopathic arthritis, transition was only successful in 48 %. In Manitoba, 40 % of type 1 diabetes patients was lost to follow-up prior to implementing a transition program. In the USA, the Cystic Fibrosis Foundation (CFF) requires that CFF-designated centers create an adult program when the center treats more than 40 patients over the age of 18 years. Despite this goal, in 2010, only 18 % of centers had programs for the development of self-management tools, and only 10 % had written self-management goals to assess readiness for transition (Philpott 2011).
5. With some inborn errors of metabolism, due to the condition, time of diagnosis, treatment opportunities, and the extent of parental support, cognitive development may be delayed or impaired. Transition of *cognitive-disabled ASHCN* poses a special challenge on the adult health-care system. Low intelligence, deficits in concentration, memory, and executive function reduce the ability of decision-making, planning, organizing, and information processing. Self-organization of health-care, dietary regimen, or adherence with prescribed medications may be severely impaired. On the other hand, everyday management may be sufficiently dealt with, and thus no legal guardians dealing with health-care issues are ascribed. In contrast to children, where parents are legally responsible for supplying the appropriate health-care, these young adults are on their own, despite being severely handicapped with respect to successful management

of their health-care issues. Outside specialized-centers, the adult health-care system is ill-provided for the care of patients with impaired cognition. Neuropsychological evaluation for the determination of the patient's capacity for decision-making, communication, and information processing is not readily available. Even with professional psychological/psychiatric support dealing with inconsistencies, mood swings, and "irrational" behavior, treatment goals may be difficult to realize in the adult care setting (Herzer et al. 2010).

In conclusion, the combination of the physiological transitional process from adolescence to mature adulthood and the transition from the pediatric to the adult health-care system in adolescents with special health-care needs may well aggravate the medical course of many severe diseases and negatively influence the final outcome. The lack of adequate communication and professional psychological skills, specific medical knowledge, and adequate socioeconomic structures of the adult health-care system enhances these problems.

10.4 Transition and the Adult Health-Care System

Bringing together the concept of the emerging adult, with all its instabilities while adjusting to the new role of an adult person and the adult health-care system relying on the idea of dealing with mature adults, it is easy to see that both realities do not fit well. The following problems can be delineated:

1. The *adult health-care system* is more or less working on demand, i.e., the patient has to deal with many different partners of the health-care system that are related in one way or another with the management of his/her disease. Most patients have to coordinate and organize their health-care, sometimes supported by their family doctor or general practitioner. This responsibility already overwhelms even many experienced mature patients. Only rare specialized centers (i.e., comprehensive cancer centers, breast centers, etc.) caring for specific diseases offer an interdisciplinary approach with standard operating procedures and case managers taking care of all disease-related issues. The young adult may well feel lost outside these interdisciplinary centers.
2. Most adult patients are well *beyond 40 years of age*. Physicians working in the adult health-care setting often lack training to deal with young, emerging adults. Communication may be a problem from both sides: mood swings, problems with reliability, and adherence may interfere with the relationship between the physician and the young adult. In the adult health-care setting, the focus of the health-care providers is primarily on issues centered on diagnosis and therapy. The normal psychological, developmental, interpersonal, and socioeconomic problems of early adulthood are, if considered at all, experienced as unrelated to the primary health-care problem. Dealing with these issues is time-consuming, besides the professional experience of most health-care providers and therefore will often be "best avoided." In addition, financial incentives by the health-care system for investing time and knowledge in these "paramedical" problems are lacking. Thus, at the time, being in the adult health-care system is not well adjusted to deal with young adult patients resulting in missed chances or even severely impaired medical outcome.

10.5 Transition Within the National Health-Care Systems

Results of national questionnaires indicate that problems of transition are increasingly recognized worldwide. In parallel, the number of publications dealing with transition increased considerably within the last 10 years (Fig. 10.2). In Germany, only 8 of 36 university departments for endocrinology and diabetes offer a transition program for adolescents (Lausch and Reincke

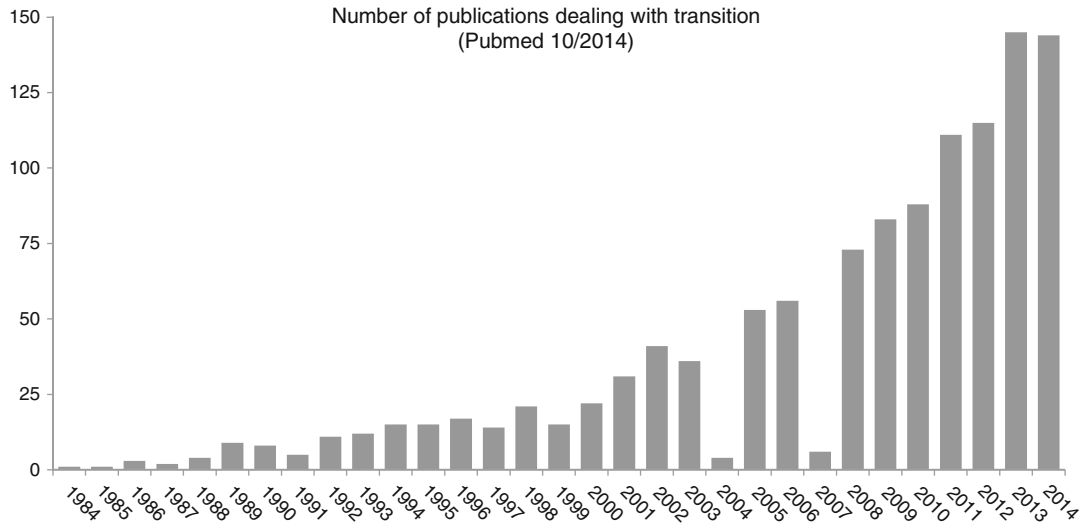

Fig. 10.2 Number of publications dealing with transition (Retrieved from PubMed 1.3.2015)

2004). In a similar investigation, 13 of 136 pediatric and adult endocrinologists reported having a common transition clinic with their respective partners for patients with childhood onset of growth hormone deficiency (Dorr et al. 2009). The situation is even worse for patients with inborn errors of metabolism – there are only two centers with a basic transition program, one in Leipzig (Mutze et al. 2011) and the other at the author's institution in Berlin.

Already in 2009, the expert group for the assessment of the development in health-care (Sachverständigenrat zur Begutachtung der Entwicklung im Gesundheitswesen) recommended appropriate financing of the transitional process, support of an interdisciplinary and multiprofessional approaches on transition, the development of specific quality measures, as well as an increased networking of participating institutions (Gesundheitswesen 2009). In 2011, the Federal Physicians Organization (Bundesärztekammer) acknowledged the existence of interdisciplinary centers caring for children with chronic diseases (Sozialpädiatrische Zentren, SPZ) and suggested similar institutions dealing with ASCHN (Bundesärztekammer 2011). A working group, initiated in 2012, and comprising representatives of the scientific societies for pediatrics, internal medicine, and neurology support a diagnosis-independent transition program working via a web-based transition manager (http://transitions-medizin.de /index.php/home/neuigkeiten#). This Berlin transition program is supported by the German Pediatric Society (DGKJ-Jahrestagung 2015). Tellingly, a grant from the Robert Bosch Foundation finances the project that thus unfortunately lacks sustainability. Yet, for a justiciable claim, any transition program will have to be embedded into the respective health-care legislature (Sozialgesetzbuch V). To increase awareness, the German Society for Transition, set up in 2011, has published a website ("Between – Fit für den Wechsel," www.between-kompass.de) informing adolescents and their parents on all issues of transition. In conclusion, in Germany, efforts are under way to improve the situation, yet no official, legally reliable setting is available so far.

In the Netherlands, physicians dealing with metabolic diseases initiated a network (www.investof.nl), primarily to exchange specific expertise and cooperate scientifically. Each of the participating centers has its own protocol for transition. Again, no integration of the transition process into the national health-care system has been achieved so far (Hollak and van Spronsen 2014).

The situation is even worse in Hong Kong. There are no data on transition and virtually no structured system or guidelines regarding when

and how to transfer adolescent chronic patients to adult services. Results of a questionnaire on transitional practice revealed that less than 10 % of subjects had ever received any transition information from health-care providers (Wong et al. 2010).

In the United States of America, a joint consensus paper by the American Academy of Pediatrics, the American Academy of Family Physicians, and the American College of Physicians-American Society of Internal Medicine was published in 2002 (American Academy of Pediatrics, Physicians et al. 2002). However, the Institute of Medicine report, Adolescent Health Services: Missing Opportunities documents significant shortcomings in creating a comprehensive health-care system for adolescents, describing the current system as consisting of "separate programs and services that are often highly fragmented, poorly coordinated and delivered in multiple public and private settings" (Park et al. 2011).

In the United Kingdom, the national health service (NHS) has set up recommendations and guidelines for transition of adolescents with special health-care needs "Getting it Right for Young People. Improving the Transition of Young People with Long Term Conditions from Children's to Adult Health Services" (Protheroe 2009). Additional recommendations are available via the Internet from the Royal College of Paediatrics and Child Health: Adolescent Health Program (AHP) (www.rcpch.ac.uk/AHP) and the Royal College of Nursing (Royal College of Nursing 2004). The initiatives of the NHS aim to ensure that young people do not miss out on health-care during the transfer between pediatric and adult services. Thus, while not everything may be perfect, the national health-care system of the UK has taken care of the problem in setting up and supporting a nationwide project on transition.

10.6 Problems in Transition

10.6.1 Pediatric Transition

Investigations in disease-specific problems of transition may demonstrate the underlying pattern and allow for a more generalized approach in delineating the problems with transition in ASHCN.

Despite increasing awareness, available guidelines for pediatric transition programs are in need of further improvement. As an adult physician, the author feels not justified to in-depth criticism. There are excellent disease-specific programs available, yet what is needed are the implementation of general programs with a structured background and the obligation of documentation and evaluation by the health-care system. Every child with special health-care needs will profit from a simple yet mandatory transition program. In contrast, at the time being, adequate care is available only for those children in transition to adolescence, cared for by ambitious caretakers who deliver individualized but mostly structural non-sustainable care.

10.6.2 Adult Health-Care System: Status and Suggestions

In the adult health-care setting, the situation is even worse. There is almost no existing structure to deal with transition from young to mature adulthood. The dire consequences are felt by the young adults not taken properly care of. The unsolved issues are related to the lack of medical knowledge and experience in dealing with specific pediatric diseases on the one hand and in addition to the lack of psychosocial support for this specific group of patients.

10.6.2.1 Medical Knowledge

In general, medical knowledge of diseases originating in the pediatric population, as well as experience in how to deal with these patients in adulthood, is still insufficient. This has been shown in cardiac, hepatic diseases, and especially so in inborn errors of metabolism where adult specialists are almost nonexistent. This list however is by far not complete, and many other conditions as childhood cancer survivors, pediatric HIV-positive adolescents, neuromuscular and neurologic diseases, and many more can be added. To bridge this knowledge gap, a close cooperation of pediatric and adult health-care

providers is necessary. Consequently, what is asked for is a transition process of patients *and* knowledge from the pediatric to the adult health-care system. In addition, medical education with the respective curricula for medical students will have to be set up, preparing future health-care providers with necessary information and knowledge. Medical guidelines for adult diseases should include, if appropriate, specific medical demands and standard operating procedures for the subgroup of medical entities manifesting during childhood and highlight the possible differences in treatment compared to similar conditions with first manifestation in adulthood. A similar approach has been suggested by the German National Action Program for Rare Diseases (NAMSE) to increase awareness and knowledge in the field of rare diseases (National Action League for People with rare Diseases NAMSE Coordinating Office 2014).

These basic actions could be the backbone of successful transitional care. Once these fundamental structures are set up, there should be growing trust of pediatricians into the successful transition of their patients, as well as growing confidence in adult health-care providers to deal appropriately with young adult patients. In the best of all worlds, the time gained by structural support could be invested into the time spent on the individual patient. Ideally, this would on the one hand help the pediatrician to let their patients go, as it would on the other hand help patients and the parents if they can be convinced that proper care is provided in the adult health-care setting.

10.6.2.2 Adult Health-Care System Expectations

If we accept the paradigm of a second phase of transition, i.e., the transition from young into mature adulthood, much of the responsibility of a successful transition will have to take place in the adult medical setting. Physicians dealing with adults outside the emergency departments mostly deal with patients older than 40 years, i.e., mature adults. Adult patients are considered as partners in health-care decision-making, in a best-case scenario empowered by their physicians to deal with their disease in a meaningful, rational, and responsible way, pondering long-term over short-term advantages, being able to successfully manage the intricacies of the respective insurance systems, appointments, and prescriptions.

These expectations interfere with the capabilities of most young adults with special health-care needs:

- Adolescents and young adults are in general not worried about transition. Executive functions, seated in the prefrontal cortex as planning and organization, the ability to imagine a future based on foreseeable issues are still developing. Overprotectiveness of parents have resulted in disengagement of managing one's own health-care, as with other things the "parents will take care of it" (Anthony et al. 2009).
- In addition, this attitude, together with the fact that most communication related to the disease has been between the pediatrician and the parents, may give way to wide gaps in knowledge about the disease in young adults, a misconception rarely acknowledged by the adult health care providers, establishing false premises for the communication between patient and health care providers.
- Sexuality and pregnancy are rarely discussed outside the gynecological departments. However, health-care providers for ASHCN will have to learn to take up these issues. Risk behavior is the highest in the age group of emerging adults. At this age, unprotected sexual intercourse has a probability of conception of 90 % within 1 year. In those 15–19 years old, 85 % of births are unplanned. Young adults between 15 and 24 years represent 25 % of the sexually active population, yet they account for 50 % of new cases of sexually transmitted infections each year, with half of these due to HIV (Bitzer 2013). With ASHCN, sexually transmitted infections may pose a high risk in those immunocompromised due to their disease or medications. An unplanned pregnancy without genetic counseling and/or proper metabolic control in those with inherited metabolic diseases may pose a risk on the developing child or induce severe metabolic derangements in the mother. Physiological

adaptations during pregnancy may aggravate the course of the disease, and interdisciplinary management of the pregnancy and parturition should be available. In this age group, sexuality and pregnancy should be discussed on a regular basis, and all health-care providers should be familiar with these issues.

In conclusion, expectations of the adult health-care providers will have to be adjusted to fit the reality of ASHCN. Every team member should be trained in dealing with young adults to provide age- and psychosocially adjusted health-care. Unfortunately, the adult health-care system is not yet prepared to deal with these problems, even worse, the situation is not even acknowledged. Thus, if no problem is realized, no solution needs to be found.

10.6.3 Possible Solutions

Physicians dealing with young adults with special health-care needs should speak out in advocacy for their patients. The interaction of the physiological transition process from young adulthood to mature adults with the transition from pediatric health-care into adult health-care is a psychological, developmental, and socioeconomic problem resulting in many cases in unfavorable medical outcome. Proper health-care settings providing committed personal to support these young adults or mentally challenged patients are necessary.

By no means will every young adult need the full armarium of psychosocial support. Thus, one of the first tasks to be taken upon is to analyze those factors indicating patients at risk. Low socioeconomic background, lack of primary and professional education, problems within the family, and language problems in those with migration background can be easily classified as risk factors.

Transition managers with a variety of professional backgrounds will be able to set up a structured support system that should allow for individualized support and educational help.

Risk classification and outcome scores and definition of the goals to be achieved, together with medical outcome analyses, should build the basis of such a system. At each step, the descriptions of the basal situation, the goals to be supported, and the means used should be based on broad categories that give room for individualization without complicating the system.

Documentation should be predefined as regular evaluations that will allow for further implementations and improvements.

Flexibility and a predefined increase in complexity should allow for a strategy of providing support as needed on a rational basis.

For such a system to be effective, national stakeholders will have to work together in planning and executing the project according to the national health-care systems and providing the necessary financial means.

In conclusion, the development of transition management in young adults with specific health-care needs should use a stepwise and nationwide approach. Initially, improved medical education of adult health-care providers and simple actions like standardized transfer would provide the elementary basics. This will by no means suffice all ASCHN. The evaluation of individual needs and preparedness for transition by pediatricians, later sociopsychological support by transition managers for those in needs, should substantially improve medical outcome. Defining their personal goals and working to minimize the impact of physical illness on those goals should yield broad dividends (Burt and Paysnick 2012). These programs can be easily set up for different diseases at specific institutions to evaluate their efficacy and the financial means needed for proper support, before set-up as a nationwide project.

Documentation and centralized evaluation are cornerstones for further developments and thus should be built in from the very beginning.

Proper transition for adolescents and young adults with special health-care needs is indispensable as these young adults will share our future and will give testimony on the way we care for those in needs.

The failure to deliver transitional care to young people, whom modern medicine allowed to survive and develop successfully until adulthood, will result in lost life opportunities and reduce the

chances to participate with a significant role in our society.

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Pregnancy and Maternal Care

11

Elaine Murphy

11.1 Introduction

Factors, including improved medical care, increased awareness of metabolic conditions and newborn screening with early treatment, have led to an increased number of patients with inherited metabolic disease (IMD) surviving to adulthood and wishing to have children of their own (Lee 2006). Although many women have successful pregnancies, with an excellent outcome, these patients present various challenges from the reproductive perspective. Apart from PKU, for which there is substantial evidence for management guidelines during pregnancy, information on pregnancy for most of the other inherited metabolic conditions comes either from isolated case reports or small case series, and no single centre is likely to have enough experience with any single condition to provide definitive guidelines for management. Although in general long-term outcome for these (mostly) autosomal recessive conditions following pregnancy is assumed to be good, there are in fact little data on the long-term follow-up of children born to mothers with inherited metabolic disease.

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Table 11.1, whilst not exhaustive, lists some of the specific issues that need to be taken into account when a woman with an inherited disorder of metabolism plans a pregnancy. If at all possible, the care of women with an inherited disorder of metabolism in pregnancy should be discussed/managed together with a physician and/or dietitian with expertise in this field. The Society for the Study of Inborn Errors of Metabolism – Adult Metabolic Physicians Group (SSIEM-AMG: http://www.ssiem.org/amp/contact.asp) can provide contact details for specialist centres worldwide. Similarly, the British Inherited Metabolic Disease Group (BIMDG: www.bimdg.org.uk) can provide details of centres within the UK.

11.2 The Preconception Period

When a woman with an inherited disorder of metabolism reaches child-bearing age, she should be counselled with regard to the potential impact of pregnancy on her condition, as well as the impact of her condition on pregnancy and the outcome for her children. As with any woman planning a pregnancy, pre-pregnancy advice includes starting folic acid supplementation, stopping smoking, limiting caffeine and alcohol intake and optimising weight, diet and general physical health (Seshadri et al. 2012). Many women with an inherited disorder of amino acid or energy metabolism are treated with a modified

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Table 11.1 Issues to be considered when a woman with an inherited disorder of metabolism plans a pregnancy

Pre-pregnancy		During pregnancy and labour		Postpartum	
Issue	Examples	Issue	Examples	Issue	Comment
Fertility	Galactosaemia	Metabolic control	Nausea and vomiting ('morning sickness') – any disorder of energy metabolism, e.g. FAOD, GSD, UCD, MSUD, organic acidaemias, disorders of ketone body metabolism	Metabolic control	Puerperal stress; involution of the uterus; lactation – risk period for decompensation of disorders of protein or energy metabolism
Risk of miscarriage	Conditions with poor metabolic control, e.g. PKU, homocystinuria	Effect on foetus	Maternal PKU syndrome	Contraception	Discuss if required
Medications	Possible teratogenicity, e.g. statins, ACE inhibitors, some anticonvulsants		Growth retardation (secondary to any condition in which there is protein and/or calorie restriction or recurrent hypoglycaemia)	Long-term outcome of the child	Consider follow-up for children born to mothers with rare conditions where long-term outcome remains uncertain
Genetic counselling	Advice re reproductive options and options for antenatal and postnatal diagnosis	Effect on mother	Worsening of underlying condition, e.g. dyslipidaemia in LPL deficiency, haemorrhage of adenomatas in GSDI or secondary comorbidities (see Table 11.5)		
Metabolic control	Time for optimisation, e.g. PKU, urea cycle disorders, homocystinuria	Other maternal issues	Maternal learning difficulties, support network		
Nutritional issues	Optimisation of weight, ensure adequate vitamin and mineral supplementation in those on restricted diets	During labour	Ensure energy requirements are met in any disorder of protein and energy metabolism, especially UCD, MSUD, organic acidaemias		
			Consider impact of skeletal muscle involvement, e.g. GSD III, mitochondrial disorders, acid maltase deficiency		
			Consider impact of cardiac involvement, e.g. GSD III, mucopolysaccharidoses		

FAOD fatty acid oxidation disorders, *GSD* glycogen storage disorders, *UCD* urea cycle disorders, *MSUD* maple syrup urine disease, *GA1* glutaricaciduria type I, *PKU* phenylketonuria, *HCU* homocystinuria, *LPL* lipoprotein lipase

diet, which, depending on the specific condition, may be low in protein, high in carbohydrate, low in fat or high in fat. In this context, the nutritional requirements of pregnancy will therefore need to be carefully managed. The goal is to optimise metabolic control and nutritional status if possible *prior* to pregnancy.

Medications may need to be altered if the patient is prescribed any potentially teratogenic drugs, e.g. angiotensin-converting enzyme (ACE) inhibitors, angiotensin receptor blockers or certain anticonvulsants. The impact on maternal health of these alterations needs to be discussed. A decision to stop other specific medications, e.g. sodium benzoate and sodium phenylbutyrate in women with symptomatic urea cycle disorders, enzyme replacement therapy in Gaucher disease, or biotin in biotinidase deficiency, is likely to have significant detrimental effects on maternal and consequently foetal health. Successful pregnancies have been described with the use of these and other specialist medications (including enzyme replacement therapies for the lysosomal storage disorders, Gaucher disease, Fabry disease and acid maltase deficiency (Mendez-Figueroa et al. 2010a; Lamb et al. 2013; Granovsky-Grisaru and Belmatoug 2011; Hendriksz et al. 2005; Kalkum et al. 2009; de Vries et al. 2011). Table 11.2 shows specific treatments used in a number of lysosomal storage disorders, with data on animal toxicity and use in human pregnancy. Many manufacturers will keep a registry of pregnancies occurring on their product, and it is worth contacting them directly for further advice if no specific information is available in the literature.

All patients with known cardiac disease should have a review with a cardiologist to define risk and plan surveillance during pregnancy. If there is severe cardiomyopathy, pregnancy may even be contraindicated (Thorne et al. 2006). Similarly, women with epilepsy, respiratory disease or other issues such as significant skeletal disease need specialist pre-pregnancy advice and may require additional support and monitoring.

Many disorders of metabolism are autosomal recessive in inheritance; in non-consanguineous families, there is a very low risk of having an affected child, but patients are often anxious regarding the risks to their children, and genetic counselling can be offered at this stage. Preimplantation genetic diagnosis is now widely available for a number of (usually) X-linked inherited disorders of metabolism, e.g. ornithine transcarbamylase deficiency, adrenoleukodystrophy Fabry disease. Antenatal diagnosis is also available and may have a role in detecting autosomal recessive conditions in communities where there is a founder effect or a high rate of consanguinity. Similarly, patients with inherited metabolic disease need to understand that, even with a rare autosomal recessive condition, the risks of having a child with the condition are considerably higher than that of the general population.

Maternally inherited mitochondrial disorders affect approximately 1 in 8,000 people (Chinnery 1993). These heterogeneous conditions can cause variable phenotypes including cardiac and hepatic failure, defects in energy metabolism, blindness, deafness, loss of motor skills and premature death. Their management is largely supportive, and specific treatment is limited to coenzyme Q_{10} supplementation in individuals with defects of CoQ_{10} biosynthesis. In 2013/2014 the Human Fertilisation and Embryology Authority provided advice to the UK Government regarding the use of enucleated donated oocytes with normal (wild-type) mitochondria to be used as recipients of nuclear DNA from intending mothers to overcome transmission of mitochondrial disorders (http://www.hfea.gov.uk/docs/Mitochondria_replacement_consultation_-_advice_for_Government.pdf and http://www.hfea.gov.uk/docs/mitochondria_scientific_review_update_-_call_for_evidence_2014.pdf). In February 2015, the UK Parliament approved regulations to allow mitochondrial donation treatment. These regulations came into force in October 2015.

11.3 Fertility and Miscarriage

In general, fertility is not a major issue in the majority of inherited disorders of metabolism. A well-recognised exception however is classic

Table 11.2 Specific medications used in the management of lysosomal storage disorders

Medication (brand name)	Condition for which treatment indicated	Toxicity in animal pregnancy studies	Used safely in human pregnancy (literature reports)
Miglustat (Zavesca)	Gaucher disease; Niemann-Pick disease type C	Yes (dystocia, preimplantation loss)	No literature reports
Agalsidase beta (Fabrazyme)	Fabry disease	No	Yes
Agalsidase alfa (Replagal)	Fabry disease	No	Yes
Laronidase (Aldurazyme)	Mucopolysaccharidosis I (Hurler-Scheie disease)	No	No literature reports
Idursulfase (Elaprase)	Mucopolysaccharidosis II (Hunter disease)	No	No literature reports (personal unpublished observation – safely used from third trimester)
Galsulfase (Naglazyme)	Mucopolysaccharidosis VI (Maroteaux-Lamy disease)	No	No literature reports
Imiglucerase (Cerezyme)	Gaucher disease	Not known	Yes
Velaglucerase alfa (VPRIV)	Gaucher disease	No	Yes
Alglucosidase alfa (Myozyme)	Acid maltase deficiency (Pompe disease)	No	Yes

galactosaemia (#230400). Prompt recognition of the condition with removal of lactose from the diet results in resolution of liver and kidney involvement, but patients remain at risk of long-term complications including movement disorders, speech delay, cognitive problems and, in the majority of women, premature ovarian insufficiency (POI). POI may manifest as delayed puberty, primary or secondary amenorrhoea or oligomenorrhoea. The precise pathophysiology is currently uncertain.

In a retrospective, cross-sectional survey of the long-term outcome of 270 individuals with classic galactosaemia, 81 % of girls and women had signs of premature ovarian insufficiency (Waggoner et al. 1990). Most women developed oligomenorrhoea and secondary amenorrhoea within a few years of menarche. Only 5 out of 17 women over the age of 22 years had normal menstruation. Hormonal treatment is therefore needed to promote pubertal development and/or to manage secondary amenorrhoea in many women.

Fertility is possible however for some women with classic galactosaemia, and a number of successful spontaneous pregnancies have been reported. In a small cohort of 22 patients, 9 tried to conceive and 4 were successful (Gubbels et al. 2008). Other pregnancies have occurred following follicule-stimulating hormone (FSH) therapy (Rubio-Gozalbo et al. 2010).

To date, there are no data on the use of techniques such as ovarian tissue cryopreservation, or mature oocyte cryopreservation, in women with galactosaemia (van Erven et al. 2013). The majority of women who have undergone these procedures worldwide have done so prior to chemotherapy or other gonadotoxic therapies and so have had normal healthy ovaries. The ovaries of many girls with galactosaemia are probably already damaged at a young age, and so the success rate of these techniques may well be lower. Embryo cryopreservation is a well-established infertility treatment, but relies on ovarian stimulation, which may be inadequate in many women with galactosaemia.

These options, including alternatives such as the use of donor oocytes or adoption, should be discussed with parents, girls and women with galactosaemia. It is important to be aware that

legislation regarding fertility may differ between countries, and the involvement of an institutional ethics committee, particularly if treatment of a child or the use of an experimental technique is proposed, is advisable.

Another condition for which reduced fertility has been suggested is glycogen storage disease type I, caused by either deficiency of the catalytic subunit of glucose-6-phosphatase (GSD Ia, #232200) or of the endoplasmic reticulum of glucose-6-phosphate translocase (GSD Ib, #232220). It has been noted that many women with GSD I have polycystic ovaries and irregular menstrual cycles (Lee et al. 1995; Sechi et al. 2013). However, recent data have suggested that this appears to have little impact on fertility (Sechi et al. 2013).

There is an increased risk of miscarriage, usually associated with maternal poor metabolic control, recognised in maternal PKU, and possibly other conditions including those associated with hypertyrosinaemia, hyperhomocysteinaemia and some disorders of energy metabolism. Regular discussion with women of reproductive age regarding the value of good metabolic control around the time of conception and the early pregnancy period is an important part of the adult IMD clinic (see Chap. 10). Prompt treatment of intercurrent illness, significant nausea and/or vomiting and avoidance of known triggers of metabolic decompensation are critical in supporting women with a known disorder of protein or energy metabolism through a pregnancy. Similarly, effective and appropriate means of contraception should be discussed with those women who do not wish to plan a pregnancy.

11.4 Teratogenicity

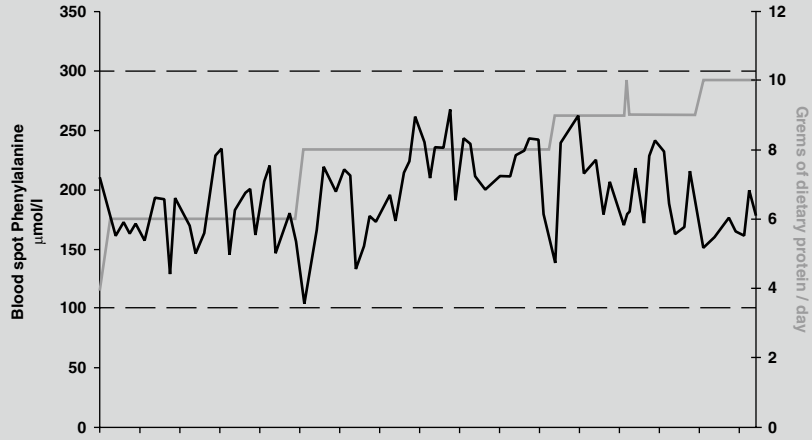
Patients diagnosed on newborn screening with PKU – treated appropriately with a low protein diet and phenylalanine-free amino acid, vitamin and mineral supplementation from an early age, with good control of phenylalanine levels – have normal intellectual development. There is no impact of well-treated PKU on fertility, and many women with treated PKU will wish to have a pregnancy.

However, in the early 1960s, it was recognised that high maternal phenylalanine levels are also teratogenic to a developing foetus in utero (Mabry et al. 1963). The maternal PKU (mPKU) syndrome includes developmental delay (92 %), microcephaly (73 %), cardiac defects (12 %), low birth weight (40 %) and dysmorphic features in the children born to mothers with untreated classic PKU (Lenke and Levy 1980). Unlike some harmful substances which affect only a single trimester, excess phenylalanine is associated with a significant increased risk of congenital heart disease in weeks 0–8; brain, foetal, and postnatal growth retardation, wide nasal bridge, and anteverted nares in weeks 8–12; and neurologic deficits throughout all 40 weeks of pregnancy in a dose-dependent manner (Rouse et al. 1997).

Fortunately, the mPKU syndrome is entirely preventable if women with PKU maintain strict metabolic control of their phenylalanine levels throughout pregnancy. The Maternal Phenylketonuria Collaborative Study (MPKUCS) established that if maternal PKU levels are maintained less than 360 μmol/L, there is no harmful effect on the foetus (Koch et al. 2003). At levels greater than 360 μmol/L, childhood developmental indices decreased by about three points for every 60 μmol/L rise in average maternal phenylalanine level. Target levels of phenylalanine vary slightly from country to country – in the UK the target range during pregnancy is 100–250 μmol/L; they are 120–360 μmol/L in Germany and the USA (Trefz 2014). As in childhood, lowering and maintenance of maternal phenylalanine levels are achieved by a combination of a diet low in natural protein, supplemented with a phenylalanine-free amino acid mixture, vitamins and minerals. Monitoring is by regular phenylalanine blood spot measurements. Depending on the maternal phenylalanine level, maternal dietary protein restriction may need to be very strict in the first trimester, and then, as the foetus grows and protein tolerance increases, protein intake can be increased in the second and third trimesters. Case Report 1 shows an example of phenylalanine levels and changes in prescribed dietary protein

Case Report 1: Pregnancy in a Woman with Well-Controlled Phenylketonuria

This 28-year-old woman with PKU had a planned pregnancy. Dietary control was optimised before conception and maintained throughout pregnancy. Target phenylalanine levels (upper and lower limits) are indicated by the dashed lines. Blood spot phenylalanine levels (three measurements per week during pregnancy) are indicated by the solid black line. Prescribed dietary protein intake (g/day) is indicated by the solid grey line. As the pregnancy progressed, protein tolerance increased and dietary protein intake was increased to allow appropriate foetal growth. A healthy baby girl was born at 37 + 5 weeks weighing 3,090 g.



Learning point

Poorly controlled maternal PKU is highly teratogenic to the developing foetus, but good maternal control of phenylalanine levels is associated with a normal foetal outcome.

intake throughout pregnancy in a woman with PKU.

From a young age, girls with PKU should be educated and advised to plan their pregnancies, obtain good metabolic control of their phenylalanine levels prior to conception if desired and use appropriate contraception to avoid any unplanned pregnancy. The risk of the mPKU syndrome is likely to be greater in patients lost to follow-up. Reports suggest that up to 10 % of patients with PKU in the reproductive age group can be lost to follow-up (Waisbren et al. 1998). All women with PKU should therefore be offered the opportunity to be seen at specialist adult metabolic unit, so that they can be kept under review throughout their child-bearing years.

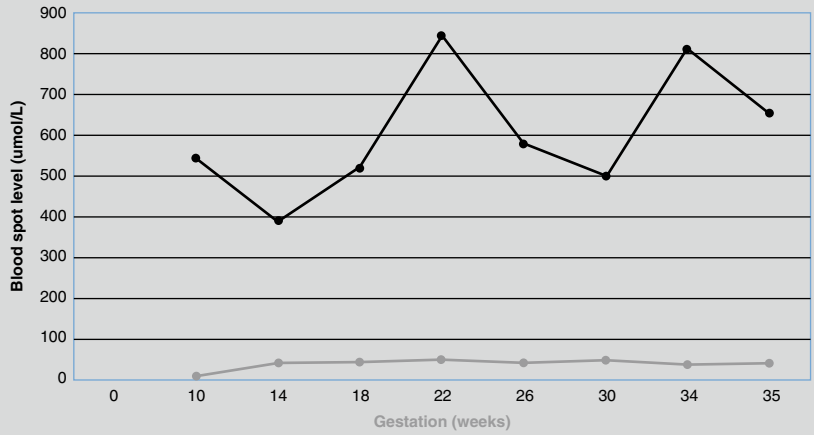
The potential teratogenicity of other abnormal/elevated metabolites in women with IMD is less clearly defined or unknown. For most conditions and most metabolites, there is simply not enough experience available to allow us to draw any firm conclusions about teratogenicity. For example, although the majority of women with classic homocystinuria have successful pregnancies, complications including spontaneous abortion and foetal abnormalities have been described (Langendonk et al. 2012; Levy et al. 2002; Calvert and Rand 1995). Interpreting the significance of these cases is complicated by the fact that many patients with marked hyperhomocysteinaemia may also have secondary B12 deficiency and/or folate deficiency. In the general population, talipes has been shown to be associated with higher maternal total homocysteine levels, possibly as a marker of low folate status (Vollset et al. 2000). In addition to the well-recognised reduction in neural tube defects, maternal folic acid

supplementation has also been shown to reduce the incidence of foetal congenital cardiac defects and limb abnormalities (Simpson et al. 2010). In theory, any foetal anomalies known to result from maternal folate deficiency could also be a risk with maternal B_{12} deficiency, as both vitamin deficiencies result in impairment of homocysteine remethylation and secondary hyperhomocysteinaemia. Advice to women with conditions causing hyperhomocysteinaemia therefore is to maximise metabolic control prior to pregnancy and ensure adequate B12 and folate supplementation.

Again, although successful pregnancies have been reported, there are some data to suggest that high maternal tyrosine levels, such as those that occur in the disorders of tyrosine metabolism, tyrosinaemia type I and type II, may have adverse effects on foetal neurodevelopment (Francis et al. 1992; Cerone et al. 2002; Vanclooster et al. 2012; Fois et al. 1986). With appropriate treatment, maternal tyrosine levels can be lowered, but there is little consensus as to the target range of tyrosine levels required in pregnancy to protect the foetus (Case Report 2 and Table 11.3).

Case Report 2: Pregnancy in a Woman with Tyrosinaemia Type II

This patient was diagnosed with tyrosinaemia type II in childhood and had struggled with dietary adherence during adolescence and adulthood. She had multiple symptomatic plantar and palmar keratotic lesions. She presented at 10 weeks gestation with tyrosine levels of 544 µmol/L (normal reference interval 35–95). She was advised to maintain tyrosine levels between 200 and 600 µmol/L throughout pregnancy, but dietary compliance was very variable and tyrosine levels fluctuated. A baby girl was born at 35 weeks, by caesarean section for oligohydramnios and intrauterine growth retardation (IUGR). She weighed 2.27 kg, with a head circumference of 31 cm. She also had tyrosinaemia type II (consanguineous parents). Two additional pregnancies, untreated (tyrosine levels unknown, but likely to have been greater than 1,000 µmol/L), ended in spontaneous miscarriage by 15 weeks. Blood spot tyrosine (solid black line) and phenylalanine (solid grey line) levels are shown.



Learning point

Elevated maternal tyrosine levels appear to be teratogenic to the developing foetus. Data are limited but suggest that maternal tyrosine >1,000 µmol/L may be associated with microcephaly, developmental delay and seizures.

Table 11.3 Summary of the pregnancies reported in women with tyrosinaemia type II

Mother	Maternal IQ	Child(ren) outcome	Maternal tyrosine in pregnancy (μmol/L)	Reference
1	Borderline	1. Microcephaly, neuromotor retardation	1655	Fois et al. (1986)
		2. Microcephaly, neuromotor retardation, convulsions	1655	
2	Normal	1. Normal	Mild hypertyrosinaemia	Fois et al. (1986)
		2. Normal	Mild hypertyrosinaemia	
		3. Normal	Mild hypertyrosinaemia	
3	Normal	1. Normal	Not known[b]	Chitayat et al. (1992)
		2. Normal		
4	Low normal	1. Normal, birth weight 3.18 kg, head circumference (HC) 34 cm	100–200	Francis et al. (1992)
5	Not reported	1. Microcephaly, maxillary hypoplasia, DQ of 72 (at 1 year 4 months)	Not known[c]	Cerone et al. (2002)
		2. Microcephaly, speech delay	1302	
6	Low average	1. Miscarriage (<15 weeks)	Not known[c]	Personal observation
		2. IUGR, birth weight 2.27 kg, HC 31 cm[a]	389–845	
		3. Miscarriage (<15 weeks)	Not known[c]	

[a]This child was born to consanguineous parents and also had tyrosinaemia type II (See also Case Report 2)
[b]Tyrosine levels were not measured during pregnancy, but at the time the woman was untreated. Her off-treatment tyrosine levels were reported as 801–1092 μmol/L
[c]Tyrosine levels were not measured, but as the women were untreated, and their untreated levels were known to be >1000 μmol/L, then this is likely to reflect levels during pregnancy

Although the number of reported pregnancies is small, there is no definitive evidence that the elevated organic acids found in disorders such as methylmalonic acidaemia (MMA), propionic acidaemia (PA) or glutaricaciduria type I (GA1) are teratogenic, and successful pregnancies have been reported (Langendonk et al. 2012; Deodato et al. 2002; Diss et al. 1995; Wasserstein et al. 1999; Van Calcar et al. 1992) (Case Report 3). Similarly, there is currently no evidence to suggest that the abnormal acylcarnitine levels found in women with disorders of fatty acid oxidation are teratogenic (Table 11.4).

11.5 Specific Issues

11.5.1 Dietary Restrictions

As mentioned above, many women with an inherited disorder of metabolism are treated with a modified diet. The foetus is dependent upon the maternal circulation for its nutritional needs. A constant and adequate supply of glucose is important, since it is the main energy substrate for foetal metabolism and is essential for normal growth and development (Morriss et al. 1974). Adaptations in maternal metabolism during a normal pregnancy that occur under the influence of placental hormones are designed to ensure an adequate supply of nutrients and energy to the unborn foetus (Butte 2000). The first half of pregnancy is an anabolic state and is associated with increased insulin sensitivity and fat deposition in adipose tissue. The nutritional demands of the foetoplacental unit increase in the second half of the pregnancy, and maternal metabolism responds by adopting an insulin-resistant state (Butte 2000; Scholl et al. 2001). This results in reduced glucose uptake by peripheral tissues and the increased use of free fatty acids as a metabolic fuel, thereby preserving maternal glucose for foetal metabolism. In addition, hepatic glucose production is increased

Table 11.4 Pregnancies and pregnancy-related complications in women with fatty acid oxidation disorders

Disorder	Successful pregnancies (live-born infant) reported	Specific pregnancy-related complications in *affected* women	Specific pregnancy-related complications in *heterozygote* women	References
2,4-dienoyl-CoA reductase (DECR) deficiency (#616034)	No pregnancies reported			
Carnitine deficiency, systemic primary (#212140)	Yes	Worsening of cardiac arrythmia, increased syncope. Postpartum deterioration		De Biase et al. (2012), El-Hattab et al. (2010), Boudin et al. (1976)
Carnitine palmitoyltransferase I (CPT1) deficiency (#255120)	Yes	HELLP syndrome	AFLP. Hyperemesis	Innes et al. (2000), Ylitalo et al. (2005)
Carnitine-acylcarnitine translocase (CACT) deficiency (#212138)	No pregnancies reported			
Carnitine palmitoyltransferase II (CPT2) deficiency (#255110)	Yes	Postpartum rhabdomyolysis		Slater et al. (2009), Lilker et al. (2006)
Very long-chain acyl-CoA dehydrogenase (VLCAD) deficiency (#201475)	Yes			Mendez-Figueroa et al. (2010b)
Long-chain 3-hydroxyacyl-CoA dehydrogenase (LCHAD) deficiency (#609016)	No pregnancies reported		Pre-eclampsia, AFLP, HELLP. Delivery of premature, growth restricted (IUGR) infants	Wilcken et al. (1993), Yang et al. (2002)
Mitochondrial trifunctional protein (MTP) deficiency (not including isolated LCHAD deficiency) (#609015)	No pregnancies reported		AFLP	Kobayashi et al. (2014)
Medium-chain acyl-CoA dehydrogenase (MCAD) deficiency (#201450)	Yes	AFLP	HELLP[a]	Nelson et al. (2000), Lang (2009)
Short-chain acyl-CoA dehydrogenase (SCAD) deficiency (#201470)	Yes		AFLP. HELLP	van Maldegem et al. (2006, 2010), Bok et al. (2003), Waisbren et al. (2008)

(continued)

Table 11.4 (continued)

Disorder	Successful pregnancies (live-born infant) reported	Specific pregnancy-related complications in *affected* women	Specific pregnancy-related complications in *heterozygote* women	References
3-hydroxyacyl-CoA dehydrogenase (HADH) deficiency (#231530) (also known as SCHAD deficiency)	No pregnancies reported			
Medium-chain 3-ketoacyl-CoA thiolase (MCKAT) deficiency (#602199)	No pregnancies reported			
Mitochondrial electron transfer defects				
Multiple acyl-CoA dehydrogenase (MADD) deficiency (also known as glutaricaciduria II) (#231680)	Yes	Recurrent neonatal death		Trakadis et al. (2012), Williams et al. (2008), Harpey et al. (1983)

[a]Successful uncomplicated pregnancies in women with MCAD deficiency are known, so the limited reports of HELLP syndrome may simply represent the background rate in the general population

Case Report 3: Pregnancy in a Woman with Propionic Acidaemia

A 31-year-old woman with propionic acidaemia wished to plan a pregnancy. Cardiology review revealed normal rhythm and a normal echocardiogram. She had developed seizures several years previously, but otherwise had not had an episode of metabolic decompensation for many years. Treatment included a self-selected low protein diet (0.55–0.6 g/kg/day) and lamotrigine 150 mg twice daily. Prior to conception she was also advised to start multivitamins (including folic acid 5 mg daily) and carnitine supplementation (1.5 g twice daily). Prior to supplementation, total and free carnitine levels were low (total carnitine 19 μmol/L (26–62) and free carnitine 9 μmol/L (22–50), respectively). Because of her restricted diet and some pregnancy-associated nausea with reduced dietary intake, MMA/PA coolers (methionine, threonine, and valine-free and low-isoleucine protein substitute) were commenced in the first trimester and adjusted throughout pregnancy to provide 1.2 g/kg total protein daily. Interval foetal growth was acceptable. The first trimester was complicated by two seizures which resolved with improved adherence to medication and an increase in lamotrigine to 200 mg twice daily. Total carnitine levels remained normal throughout pregnancy. She had a booked induction of labour (covered with 10% dextrose intravenously) at 40 weeks. A healthy baby girl (2,920 g; 25th centile) was delivered at term. There were no postpartum complications or metabolic decompensation.

Learning point

The frequency of seizures may increase in some women during pregnancy. In women who are planning a pregnancy ensure an anti-epileptic medication safe in pregnancy is used. An increase in dose of antiepileptic medication may be required, and higher doses of folic acid (i.e. 5 mg/day) are usually recommended.

to meet the growing demands of both the foetus and the mother (Kalhan et al. 1979). Additionally, in pregnant women, decreases in glucose, insulin and alanine and increases in ketone body production that occur in a fasting state are seen hours before they occur in a non-pregnant state (Butte 2000).

Women with defects of energy metabolism may not be able to match glucose production with the increasing demands of pregnancy, e.g. in conditions such as the hepatic glycogen storage disorders or disorders of ketone body metabolism (Langendonk et al. 2012; Ramachandran et al. 2012). The risk of maternal hypoglycaemia is usually greatest in the early morning, after the overnight fast. During acute maternal hypoglycaemia, the foetal metabolic rate is maintained by oxidation of intracellular (foetal) glucose (Hay 2006). During prolonged hypoglycaemia, however, the metabolic rate is maintained by oxidation of glucose derived from foetal glycogenolysis and gluconeogenesis. Thus protein breakdown and amino acid oxidation are increased, and net gain in terms of protein synthesis is diminished, resulting in intrauterine growth retardation. The foetal programming hypothesis suggests that prolonged intrauterine exposure to hypoglycaemia or poor nutrition that leads to restricted foetal growth may result in long-term/permanent changes in foetal metabolism, which may be responsible for an increased predilection to diseases such as type 2 diabetes, the metabolic syndrome and cardiovascular disease in later life (Petry and Hales 2000). Prolonged maternal ketosis may also result in a lower IQ in offspring (Rizzo et al. 1991). In the context of a woman with an inherited disorder of energy metabolism (e.g. a glycogen storage disorder) susceptible to hypoglycaemia, minimising such potential risks to the foetus is therefore advisable. Treatment options include the use of a slow-release starch, e.g. uncooked cornstarch (UCCS) taken orally before bed, with/without another dose during the night as needed to act as a source of glucose and maintain normoglycaemia. Regular meals are encouraged during the day. Maternal 24-h metabolic profiling can be useful in titrating the dose of UCCS and adjusting dietary requirements.

Protein requirements increase in the second and third trimester (Picciano 2003). Women on a therapeutic restricted protein diet (e.g. women with PKU, homocystinuria, tyrosinaemia, a urea cycle defect or an organic acidaemia) may be at risk of protein-energy malnutrition and micronutrient deficiency at this time resulting in intrauterine growth retardation and children born with low birth weight. In women with urea cycle disorders, who are often protein averse, or with an organic acidaemia where appetite is often poor, increasing oral intake appropriately can be a challenge and requires specialist dietetic input. In any woman on a restricted diet, vitamin and mineral supplementation should be considered. Regular monitoring of foetal growth is also advised.

11.5.2 Nausea and Vomiting in Pregnancy

Pregnancy, if complicated by nausea and vomiting, can lead to acute metabolic decompensation in women with inherited disorders of energy metabolism due to reduced calorie intake and difficulties taking essential supplements and medications (examples of such conditions include the urea cycle defects, the hepatic glycogen storage disorders, disorders of ketone body metabolism, the organic acidaemias and fatty acid oxidation defects). Although rare, if prompt treatment is not given, such decompensation can be sufficiently severe so as to lead to maternal and/or foetal death (Langendonk et al. 2012; Schimanski et al. 1996). In other conditions, e.g. PKU, reduced oral intake does not lead to acute maternal illness, but will result in higher levels of metabolites such as phenylalanine, thereby increasing the risk of foetal teratogenicity.

Nausea and anorexia must therefore always be taken seriously in such conditions, and effective antiemetic medication and dietary support should be given early on. The majority of women known to metabolic services will have an oral emergency regimen to start at home in such circumstances. If the oral emergency regimen is not tolerated, then women may require hospital admission for intravenous treatment. Oral and emergency guidelines for the management of many inherited disorders of metabolism are available on the British Inherited Metabolic Disease Group website www.BIMDG.org.uk.

11.5.3 Thrombotic Risk

Pregnancy is a prothrombotic period, and presentation of hypercoagulable conditions, e.g. homocystinuria during pregnancy, and the postpartum period with a thrombus is well described (Levy et al. 2002; Calvert and Rand 1995; Novy et al. 2010) (Case Report 4). Classic homocystinuria caused by cystathionine beta-synthase deficiency is rare (1 in 200,000 world; 1 in 65,000 in Ireland) – but in the untreated state (when total homocysteine is typically >100 μmol/L) approximately half of all patients will have a vascular event by the age of 30 years (Naughten et al. 1998; Yap et al. 2001). However, in those patients already known to have the disease and who are on treatment, the risk of a vascular event is substantially reduced (in one international study: from 112 expected to 17 actual events in 158 patients; relative risk 0.09, 95 % CI 0.036–0.228; $P < 0.0001$) (Yap et al. 2001).

The specific thrombotic risk in pregnancy for women with well-treated homocystinuria is not known, and there are no definitive guidelines for management in pregnancy. Practice varies worldwide, but guidelines for similar conditions with a high lifetime risk of thrombosis (e.g. homozygosity for factor V Leiden) suggest anticoagulation with low molecular weight heparin throughout pregnancy and for 7 days to 6 weeks postpartum: (http://www.rcog.org.uk/files/rcog-corp/GTG37aReducingRiskThrombosis.pdf).

11.5.4 Learning Disability

A variable degree of learning disability is associated with a number of inherited metabolic diseases, e.g. poorly treated PKU, or following childhood decompensation with hyperammonaemia (e.g. urea cycle defects) or hypoglycaemia

Case Report 4: Transverse Venous Sinus Thrombosis Presenting in a Pregnant Women Subsequently Diagnosed with Homocystinuria

A 33-year-old woman presented in 11th week of pregnancy with a severe headache secondary to a right transverse venous sinus thrombosis. On MR venogram, she had a filling defect in the right transverse venous sinus. She was found to have severe hyperhomocysteinaemia, with plasma total Hcy 214 μmol/L (reference interval 0–15 μmol/L), free Hcy 30 μmol/L (normally not detected) and Met 73 μmol/L and free Hcy was no longer detectable – consistent with pyridoxine(B6)-responsive homocystinuria. The remainder of her pregnancy was uneventful, and she delivered a normal, healthy child. Heparin was continued for 2 months postpartum. Postpartum warfarin was continued until the venous sinus became patent again 2 years later.

Learning points

1. *Pregnancy and the postpartum period is a risk for thrombosis in women with uncontrolled homocystinuria.*
2. *Consider anticoagulation in pregnant women with homocystinuria (there is no consensus currently as to when in pregnancy this should be started and how long it should be continued in the postpartum period).*

(fatty acid oxidation defects, glycogen storage disorders). Women with inherited metabolic disease who also have learning difficulties may need additional social and psychological support during pregnancy and following childbirth. These women may be difficult to manage, as they are not always engaged effectively with health services, and may not take medications or other treatment as prescribed, which can put them at increased risk of metabolic decompensation.

11.5.5 Secondary Comorbidities

The potential for impaired organ function is wide and varied and beyond the scope of this chapter. Table 11.5 lists some of the issues that may need to be considered with specific disease examples. Pregnancy may lead to worsening of an underlying condition, e.g. cardiomyopathy in GSD type III, dyslipidaemia, particularly hypertriglyceridaemia, lipoprotein lipase (LPL) deficiency, growth and even haemorrhage of hepatic adenoma in GSD type I. Resection of the adenoma should be considered if a female with GSD I has a lesion over 5 cm and wants to get pregnant. Alternatively, a pre-existing complication may influence factors such as timing and mode of delivery, e.g. skeletal dysplasia in certain lysosomal storage disorders.

11.5.6 Association of Foetal FAOD and Maternal Illness in Pregnancy

An association between foetal long-chain 3-hydroxyacyl-CoA dehydrogenase (LCHAD) deficiency and maternal acute fatty liver of pregnancy (AFLP) and the haemolysis, elevated liver enzymes and low platelets (HELLP) syndrome was first reported in 1993 (Wilcken et al. 1993). These complications have now been reported in a significant number (20–70 %) of the heterozygous mothers of affected foetuses with LCHAD or mitochondrial trifunctional protein (MTP) deficiencies (Ibdah et al. 1999; Yang et al. 2002) (Table 11.4). Carnitine palmitoyltransferase (CPT) type 1A, medium-chain acyl-CoA dehydrogenase (MCAD) and short-chain acyl-CoA dehydrogenase (SCAD) deficiencies have been associated with maternal liver disease in single case reports (Innes et al. 2000; Nelson et al. 2000; Matern et al. 2001). AFLP is overall a rare condition, with an incidence of 5 cases per 100,000 pregnancies in the UK, but carrying a foetus with a fatty acid oxidation disorder (FAOD) is estimated to increase the risk by 18-fold (Knight et al. 2008; Browning et al. 2006).

Table 11.5 Pre-existing organ involvement or issues that may impact on the management of pregnancy, labour and delivery

Issue	Clinical problem	Specific disease examples
Liver	Adenoma growth and/or haemorrhage	GSD I
	Impaired function	Hepatic GSDs, UCD, Wilson disease
Cardiac	Valvular disease	Mucopolysaccharidoses/mucolipidoses
	Impaired ventricular function	Fabry disease, GSD III, mucopolysaccharidoses/mucolipidoses
	Dysrhythmia	Fabry disease, carnitine transporter deficiency, propionic acidaemia
Respiratory	Sleep-disordered breathing	Acid maltase deficiency, mucopolysaccharidoses/mucolipidoses
	Thoracic deformities	Mucopolysaccharidoses/mucolipidoses
	Narrowed airways – difficult intubation	Mucopolysaccharidoses/mucolipidoses
Orthopaedic	Pelvic/hip/knee involvement	Mucopolysaccharidoses/mucolipidoses/X-linked hypophosphataemia
	Ligamentous laxity	Mucopolysaccharidoses/mucolipidoses
	Spinal involvement/fusion	Mucopolysaccharidoses/mucolipidoses
Endocrine	Thyroid dysfunction	Mitochondrial disorders
	Diabetes	Mitochondrial disorders
Renal	Proteinuria	GSD I
	Impaired renal function	GSD I, methylmalonic acidaemia, glutaricaciduria I
Muscle	Weakness	Acid maltase deficiency, mitochondrial disorders, some fatty acid oxidation disorders
	Risk of rhabdomyolysis	CPT II deficiency, VLCAD
Coagulation	Increased thrombotic risk	Any condition associated with hyperhomocysteinaemia, e.g. HCU
	Postpartum bleeding	GSD I
Intellectual	Ability to adhere to treatment recommendations and care for child	Any metabolic condition associated with severe childhood decompensation, e.g. with hyperammonaemia, hypoglycaemia, encephalopathy or progressive neurodegeneration
Medications	Potential impact if medication stopped	In particular for management of epilepsy, hypertension, dyslipidaemia

CPT carnitine palmitoyltransferase, *GSD* glycogen storage disorders, *HCU* homocystinuria, *VLCAD very* long-chain acyl-CoA dehydrogenase deficiency, *UCD* urea cycle disorders

The pathogenesis of these complications is incompletely understood but is thought to relate to the shift of maternal metabolism towards ketosis in the last trimester as foetal metabolic demands increase, when the foetus uses maternal ketone bodies for lipogenesis and energy production. In the context of an affected foetus and a heterozygote mother, the foetoplacental unit is compromised; maternal abnormal metabolites (acylcarnitines) accumulate and are postulated to cause pre-eclampsia, AFLP and HELLP syndromes (Shekhawat et al. 2005).

Table 11.4 summarises pregnancy data – both in affected women and in women who are heterozygous for a FAOD mutation. Catabolism, e.g. caused by nausea and vomiting, infection or fasting, should be avoided in pregnancy. The postpartum period can be a risk factor for decompensation with rhabdomyolysis in conditions such as CPT II deficiency (personal observation) and care should be taken to maintain metabolic homeostasis and minimise the duration and stress of delivery. Teratogenicity has not been reported for maternal FAOD.

No pregnancies have been reported to date in women who have autosomal recessive LCHAD or MTP deficiencies. A number of these women are now surviving into adulthood and may wish to plan pregnancies of their own. It is not known what their

risk of developing similar pregnancy-related complications is, but it is likely to be significantly increased and very close monitoring is advised.

11.6 Monitoring During Pregnancy

The frequency and type of monitoring depends on the particular condition and its severity. For example, at our unit, pregnant women with PKU send in blood spot cards by post three times a week for measurement of phenylalanine levels. This allows changes in phenylalanine levels, e.g. due to lapses in dietary adherence, intercurrent illness or increased protein tolerance as pregnancy progresses to be identified quickly, and hence changes made to dietary protein or supplement intake to maintain stability of metabolic control (Maillot et al. 2008). Women with hepatic GSD III have 24-h metabolic profiling towards the end of the first trimester and fax/email the results of fasting home blood glucose and ketone levels regularly for review thereafter. In general, in addition to their obstetric care, pregnant women with inherited metabolic disease are seen routinely approximately once every trimester by the metabolic team. Access to the outpatient clinic is open, and women can be seen more frequently if needed. Facilities available at our hospital include a dedicated metabolic kitchen for dietary education if required. Regular telephone contact with the metabolic dietitian is arranged, and women are encouraged to email/fax through results of home monitoring. Hospital admission for optimisation of metabolic control or additional nurse or social support at home is very rarely needed but can be offered to women if required.

11.7 Labour and Delivery

Labour and delivery are times of increased energy requirement, and women often have poor oral intake for the duration of labour. We advise that these babies should be delivered in a hospital setting, and our policy is to prescribe additional energy supplementation (usually intravenous 10% dextrose @ 2 mL/kg/h) once labour is established for women with known defects of energy metabolism.

Euglycaemia should be maintained throughout the period of delivery. Blood glucose should be monitored two to four hourly, as well as additional parameters such as lactate and/or ammonia as appropriate for the condition. The aim is to keep blood sugars >3.5 mmol/L at all times. In the absence of other complications, vaginal delivery is usually possible. A planned induction at term may be desirable. Hemodynamic stress can be reduced by appropriate analgesia and decreasing patients' anxiety.

Women with skeletal muscle involvement may find the second stage of labour difficult, necessitating caesarean section. Deterioration of underlying organ damage, e.g. cardiomyopathy in women with GSD type III, is also possible. Patients with GSD type I may also be at risk of bleeding due to platelet dysfunction.

For any woman in whom specific issues can be anticipated, liaison with the obstetrician and obstetric anaesthetist prior to delivery is advised.

11.8 Postpartum

In women in who it is required, the intravenous infusion of 10% dextrose should be continued until women are able to maintain normal oral intake postpartum. Rebound hypoglycaemia in the neonate should be monitored if the mother received intravenous dextrose during labour.

Following delivery, there is a well-recognised risk period for acute decompensation of some metabolic conditions, particularly of disorders of urea cycle metabolism such as ornithine transcarbamylase (OTC) deficiency (Mendez-Figueroa et al. 2010a; Arn et al. 1990) (Case Report 5). Classically this decompensation occurs between days 3 and 14 postpartum. OTC deficiency, the most common of the urea cycle defects, is an X-linked condition. Many females are asymptomatic, but others can have recurrent episodes of hyperammonaemia from childhood or present later in adulthood for the first time during a period of metabolic 'stress'. The reasons for this are not entirely clear, but postpartum are thought to relate

to the relative metabolic stress of the changes of the puerperium and an increased protein load for catabolism following involution of the uterus. Care must be taken not to confuse the behavioural changes of hyperammonaemia for symptoms of postpartum psychosis or depression. It should be remembered however that the majority of women who are heterozygote for an OTC mutation go through pregnancy and labour uneventfully without anyone being aware of the underlying problem. Elevations in postpartum metabolites have also been described in other disorders of protein metabolism such as maple syrup urine disease and methylmalonic acidaemia (Wasserstein et al. 1999; Grunewald et al. 1998). There are no specific guidelines for management of this period in women at risk, but treatments used to date have included reduction of dietary protein intake, increase in ammonia-chelating medications (if appropriate), the use of oral or intravenous emergency regimens and hyperalimentation.

Case Report 5: Pregnancy in a Woman with Symptomatic Ornithine Transcarbamylase (OTC) Deficiency

A 29-year-old woman with symptomatic OTC deficiency wished to plan a pregnancy. She had been diagnosed in early childhood following a hyperammonaemic crisis. Physical examination was normal and she had a verbal IQ of 108, with a performance IQ of 67. Prior to pregnancy she was managed on a restricted protein diet of 0.67 g/kg/day, sodium benzoate 4 g four times daily, sodium phenylbutyrate 2 g four times daily, arginine 500 mg four times daily and a multivitamin supplement. She had occasional issues with non-adherence to recommended treatment with intermittent hyperammonaemia (>100 μmol/L), but she remained clinically well with no recent hospitalisations. She received pre-pregnancy counselling and opted not to consider preimplantation genetic diagnosis with in vitro fertilisation (IVF). She became spontaneously pregnant within 1 year of unprotected intercourse. An essential amino acid supplement and ω3 supplement were started in the first trimester, and she continued with her other medications as above. She had no issues with anorexia, nausea or metabolic decompensation in early pregnancy, and chorionic villus sampling showed that she was carrying an unaffected male foetus. Ammonia levels remained normal. The course of pregnancy was uncomplicated with good foetal interval growth. Dietary protein intake was increased to 60 g daily in the third trimester. At 40 + 4 weeks gestation she underwent induction of labour. Additional glucose polymer and intravenous dextrose were given to maintain caloric intake above 2,000 kcal/24 h. Opiate analgesia and an epidural were used for pain relief. A healthy baby boy was born, weighing 3,050 g with Apgar scores of 10, 10, and 10. Postpartum protein was reintroduced at 25–30 g per day for the first week, with additional oral glucose polymer to maintain calories. Ammonia rose on day 4, peaking at 91 μmol/L on day 7. Oral sodium phenylbutyrate was increased to 4 g four times daily, from day 4 to 12. She remained asymptomatic and was discharged home on day 8. Her son (now 3 years) has normal development.

Learning points

1. *In symptomatic women with urea cycle defects, medications such as sodium benzoate and sodium phenylbutyrate may need to be continued during pregnancy and appear to be safe.*
2. *Dietary protein/essential amino acid intake should be monitored and may need to be increased in the second and third trimesters to promote foetal growth.*
3. *The postpartum period is a risk for hyperammonaemia and metabolic decompensation in women with urea cycle defects. Regular clinical assessment, monitoring of ammonia levels and adjustment of therapy as needed are advised.*

11.9 Breastfeeding

Similarly, breastfeeding places energy demands on a mother, and women with inherited metabolic disease wishing to breastfeed need to ensure adequate energy intake. The energy requirements for breastfeeding are at least as large as those of pregnancy. Breastfeeding has been reported to cause hypoglycaemia in mothers with GSD, but with specialist dietetic input and providing nutritional requirements that can be met, some women have successfully breastfed. However, in symptomatic patients with urea cycle disorders and organic acidaemias, the combination of the catabolic state of the puerperium with the nutritional demands of breastfeeding and a poor calorie intake may be a potential trigger for metabolic decompensation, and breastfeeding women should be closely monitored.

Another important issue is the use of medications during breastfeeding. Currently, there is very little safety information available regarding many of the specific medications used, and women and their physicians need to discuss this on an individual basis.

Conclusions

In general, with appropriate treatment, the outcome of pregnancy for the majority of women with inherited metabolic disease appears to be good, and there are very few contraindications to pregnancy. Pre-existing maternal disease is however one of the commonest causes of maternal mortality in developed countries (Cantwell et al. 2011). This is particularly relevant to women with pre-existing inherited metabolic conditions and highlights the importance of pre-pregnancy counselling and co-management of high-risk medical patients by obstetricians and specialist physicians with an understanding of the relationship between pregnancy and specific diseases.

It is important not just to carefully monitor the foetus during pregnancy but also to follow up the babies of mothers with inherited metabolic diseases longer term to identify any subtle neurocognitive or developmental issues that might be associated with exposure to abnormal/elevated metabolites in utero.

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Metabolic Emergencies

12

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Key Facts

- The classic presentation of inborn errors of metabolism is with a free period of apparent health after uneventful pregnancy and delivery that may last days or even years, but it is followed by overwhelming life-threatening disease.
- The episode usually follows catabolism introduced predominantly by acute infection, sometimes after surgery. Increased dietary intake of precursors of toxic metabolites (e.g., protein), uterine involution during the postpartum period, menstruation, and drugs that interfere with mitochondrial metabolism (e.g., valproate) should be considered as alternative mechanisms.
- Initial laboratory evaluation needs only the routine clinical laboratory to establish acidosis or alkalosis, hyperammonemia, ketosis, hypoglycemia, or lactic acidemia and to detect acute organ dysfunction (e.g., acute renal failure, acute liver failure, cardiomyopathy, rhabdomyolysis, or pancreatitis).

The most demanding cases in the field of genetic disease that pose problems for rapid diagnosis and rapid initiation of effective treatment are patients who present with episodes of acute life-threatening illness. This is the mode of presentation of a considerable number of inherited metabolic diseases (Table 12.1). It is particularly characteristic of the organic acidurias, the disorders of the urea cycle, maple syrup urine disease, nonketotic hyperglycinemia, and the disorders of fatty acid oxidation. The lactic acidemias may present in this way, but usually the presentation is more indolent. Disorders which present with potentially lethal metabolic emergencies usually do so first in the neonatal period or early infancy. In fact, we have felt that prior to the advent of programs of expanded newborn screening, a large number of such infants probably die(d) without the benefit of diagnosis. An increasing number of patients with late onset of symptoms are also reported. Their clinical presentation including episodic psychiatric disease, movement disorders, and liver dysfunction differs from those with a neonatal disease onset, and therefore, these patients can again be easily missed.

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Table 12.1 Metabolic diseases presenting as acute overwhelming disease

Disorder	Detect	Definitive diagnosis (in addition to mutation analysis)
Maple syrup urine disease	Urinary 2,4-DNP (branched-chain keto acids), plasma branched-chain amino acids, urinary organic acid pattern	Branched-chain keto acid decarboxylase assay
Organic acidurias, e.g., isovaleric aciduria	"Sweaty feet odor," acylcarnitine profile, urinary organic acid pattern	Isovaleric acid in blood, assay of isovaleryl-CoA dehydrogenase
Methylmalonic aciduria	Acylcarnitine profile, urinary organic acid pattern, methylmalonic acid in blood, plasma homocysteine, and amino acids	Methylmalonyl-CoA mutase assay, complementation assay
Propionic aciduria	Hyperglycinemia, acylcarnitine profile, urinary organic acid pattern	Assay of propionyl-CoA carboxylase
Multiple carboxylase deficiency	Acylcarnitine profile, urinary organic acid pattern	Biotinidase, holocarboxylase synthetase assay
D-2-, D-2-/L-2-, and L-2-hydroxyglutaric aciduria	Urinary organic acid pattern	Isomer differentiation of 2-hydroxyglutaric acids
Urea cycle defects	Blood NH_3, plasma and urinary amino acids, urinary orotic acid	Assays of ornithine transcarbamylase, carbamoyl phosphate synthetase, *N*-acetylglutamate synthetase, argininosuccinate synthetase, argininosuccinate lyase, arginase, mitochondrial ornithine transporter (HHH syndrome)
Disorders of fatty acid oxidation	Hypoketotic hypoglycemia, acylcarnitine profile, urinary organic acid pattern	Enzyme assay, mutation analysis
Lactic acidemias	Lactate, blood NH_3, plasma amino acids (alanine, glycine, proline), urinary organic acid pattern	Enzyme assay; mutation analysis/next generation sequencing of mitochondrial and/or nuclear DNA
Hyperglycinemia, nonketotic	Glycinemia, CSF glycine	Glycine cleavage enzyme in liver
Methylenetetrahydrofolate reductase deficiency	Homocysteinemia, hypomethioninemia	Enzyme assay
Sulfite oxidase deficiency	Sulfite test, sulfocysteine, amino acid pattern	Sulfite oxidase assay
Molybdenum cofactor deficiency	Sulfite test, sulfocysteine, purine analysis	Mutation analysis
Adenylosuccinate lyase deficiency	Succinyladenosine, SAICA riboside	Enzyme assay
Lysosomal acid phosphatase deficiency	Lysosomal acid phosphatase	Enzyme assay
Adrenogenital syndrome	17-ketosteroids	Pregnanetriol, testosterone
Fructose intolerance	Fructosuria	Hepatic fructose-1-P-aldolase assay
Galactosemia	Urinary reducing substance	Galactose-1-phosphate-uridyl transferase assay

CSF cerebrospinal fluid, *2,4-DNP* 2,4-dinitrophenol, *HHH* hyperammonemia, hyperornithinemia, and homocitrullinuria, *MCAD* medium-chain acyl-CoA dehydrogenase, *SAICA* succinylaminoimadazole carboxamide

Episodes of acute illness and metabolic decompensation are often precipitated by acute infection and its attendant catabolism. Catabolism may also be induced by surgery or injury. The duress of birth may be sufficiently catabolic to induce an early neonatal attack. The diseases in which the fundamental defect is in an enzyme involved in the catabolism of a component of food, such as protein, are often characterized by a period of buildup of body stores of toxic intermediates until the levels are high enough to produce metabolic imbalance. Such a patient may have cycles of acute illness precipitating admission to the hospital and cessation of feedings and administration of parenteral fluids and electrolytes with recovery and discharge only to repeat the cycle until the diagnosis is made and appropriate therapy initiated, or the patient dies undiagnosed in such an episode. In the disorders of fatty acid oxidation, episodes of metabolic emergency are brought on by fasting. This can be when the infant begins to sleep longer, or more commonly, when intercurrent infection leads to vomiting or failure to feed.

The *classic presentation* of the diseases that produce metabolic emergencies is, for the initial acute presentation in infancy, often in the neonatal period, followed by recurrent episodes of metabolic decompensation usually with infection. Nevertheless, some patients with these diseases, usually those with variant enzymes in which there is some residual activity, may present first in childhood or even in adulthood. The diseases of fatty acid oxidation typically require fasting for 16–24 h before metabolic imbalance ensues. Some people reach adulthood without ever fasting that long. Nevertheless, the first episode may be lethal, regardless of age.

The *initial clinical manifestations* of metabolic emergency are often vomiting and anorexia, or failure to take feedings. This may be followed by rapid deep breathing in the acidotic infant. Compensatory respiratory changes are also observed in hyperammonemic patients. A characteristically ketotic odor may be observed in propionic and methylmalonic aciduria, a distinct acrid odor which really does not smell like "sweaty feet" in isovaleric aciduria or multiple acyl-CoA dehydrogenase deficiency, and the name-giving maple syrup-like smell in maple syrup urine disease (often intense in cerumen once the odor has left elsewhere). There may be rapid progression through lethargy to coma, or there may be convulsions. Hypothermia may be the only manifestation besides failure to feed and lethargy. Further progression is to apnea and, in the absence of intubation and assisted ventilation, death.

The *initial laboratory evaluation* (Tables 12.2 and 12.3) involves tests that are readily available in most hospital laboratories, particularly clinical chemistry. Most important in early discrimination are ammonia, blood gases, glucose, ketones in urine, and electrolytes. Based on this, the anion gap can be calculated. In general, it is advisable to get everything on the list on admission of the

Table 12.2 Initial laboratory investigations

Blood
Electrolytes
Blood gases: pH, pCO_2, HCO_3, pO_2
→Calculate anion gap:
$[Na^+] - ([Cl^-] + [HCO_3-])$ (normal range: 7–16 mmol/L)
Ammonia
Glucose
Lactate, pyruvate
3-hydroxybutyrate
Uric acid
Creatine kinase
Creatinine and/or cystatin C
Alanine and aspartate aminotransferase (ALAT, ASAT)
Complete blood count
Urine
Ketones
Reducing substance
Dinitrophenylhydrazine
Sulfites (sulfite test at the bedside in fresh urine)
Benzidine, guaiac
Store at −20 °C (−4 °F)
Urine
Heparinized plasma
CSF
Store at 4 °C (39 °F)
Heparinized whole blood

Table 12.3 Initial differential diagnosis of metabolic emergencies

Group of disorders	Organic acidurias	Maple syrup urine disease (except E3 defect)	Urea cycle defects	Mitochondriopathies	Fatty acid oxidation defects	Ketolysis defects	Glycogen storage disease, gluconeogenesis def.	Galactosemia, tyrosinemia type I
Free interval	Days/weeks	Days/inconst	Hours/days/inconst	(+)	Days/months	Months	Months	Days/weeks
↑ *Uric acid, CK*	(+)	–	–	(+)	(+) to ++	–	(+) to ++	–
↑ *Liver enzymes*	(+)	–	(+) to ++	(+) to ++	(+) to ++	–	(+) to ++	+++
↑ NH_3	+ to +++	–	+++	– to +	(+) to ++	–	–	(+)
Ketonuria	++	++	–	(+) to ++	–	+++	+	(+)
Hypoglycemia	(+)	–	–	(+)	+++	–	+++	(+)
↑ *Lactate*	(+) to ++	(+)	(+)	+ to +++	(+) to +++	–	++	(+)
Metabolic acidosis	+ to +++	–	Alkalosis/acidosis	+ to +++	+ to +++	++	++	(+)

(+) = inconstant

patient suspected of having a metabolic emergency. Metabolic acidosis and hyperammonemia are indicative of a classic organic aciduria. Hyperammonemia and alkalosis are characteristics of urea cycle defects, but normal pH or even metabolic acidosis does not exclude this disease group. Hypoglycemia along with elevation of uric acid and creatine kinase (CK) is seen in disorders of fatty acid oxidation or glycogen storage disorders but is also found in severely decompensated patients with organic acidurias. If ketones are absent from the urine, this is almost certainly the diagnostic category. The presence of ketonuria does not rule it out; the blood level of 3-hydroxybutyrate is a better indicator of the adequacy of ketogenesis. Hypocalcemia may be a nonspecific harbinger of metabolic disease.

Elevated levels of lactate in the absence of cardiac disease, shock, or hypoxemia are significant, and seen in classic organic acidurias and even hyperammonemias, as well as in the lactic acidemias of mitochondrial disease (Chap. 14). A normal pH in the blood does not rule out lactic acidemia; the pH usually remains neutral until lactic acid concentrations reach 5 mM. The blood count is useful in indicating the presence or absence of infection. More importantly, neutropenia with or without thrombocytopenia or even with pancytopenia is characteristic of classic organic acidurias, while patients with mitochondrial disease often develop thrombocytosis.

The dinitrophenylhydrazine (DNPH) test is positive in any disorder, in which there are large amounts of ketones in the urine. It is particularly useful as a spot indicator of the presence of maple syrup urine disease. A positive urine-reducing substance may be the first indicator of galactosemia. Today in many countries, this disorder is discovered through routine newborn screening for the defective enzyme. Chemical testing for blood in the urine is also useful in detecting hemoglobinuria and myoglobinuria, the latter indicating a crisis in a disorder of fatty acid oxidation. The test for sulfite in fresh urine indicates the presence of sulfite oxidase deficiency, either isolated or as a result of molybdenum cofactor deficiency, which may present with intractable seizures shortly after birth.

It is advisable in a seriously ill infant to save some blood and urine (Table 12.2), while this initial testing is taking place. Then if the initial indicators point to an area of metabolic disease, these samples can be processed appropriately, obviating such problems as a later lack of availability of urine in an infant in whom dehydration and shock lead to a renal tubular necrosis. Further testing requires a biochemical genetics laboratory expert in these procedures. Programs of proficiency testing indicate that not all laboratories that undertake these specialized procedures do them properly.

In the presence of metabolic acidosis suggesting organic aciduria, the assay of choice is organic acid analysis of the urine. A positive DNPH in the absence of metabolic acidosis or with mild acidosis indicates amino acid analysis of the plasma for maple syrup urine disease. Hyperammonemia without metabolic acidosis is most suggestive of a urea cycle defect. This suspicion should be followed up by amino acid analysis of the plasma and analysis of the urine for orotic acid. The latter can be done by organic acid analysis or better by a specific quantitative assay for orotic acid. In an infant found to have an elevated plasma concentration of glycine, the availability of simultaneously obtained CSF permits a definitive diagnosis of nonketotic hyperglycinemia. Following initial testing indicating a disorder of fatty acid oxidation, definitive testing requires an acylcarnitine pattern of the blood, organic acid analysis of the urine, and a medium-chain acyl-CoA dehydrogenase (MCAD) assay of the DNA. In most of these patients, quantification of concentrations of carnitine in blood and urine is useful.

Precise molecular diagnosis is made by enzyme assay, usually of lymphocytes or cultured fibroblasts, or by determination of the mutation in the DNA. DNA analysis is particularly useful in those situations in which there is a common mutation, such as the p.Ala985Gly mutation in the *ACADM* gene for MCAD deficiency in Caucasians.

12.1 Neonates

The classic presentation of the metabolic disorders that lead to medical emergencies is with life-threatening illness in the newborn period. These infants are usually born healthy, and there is classically a hiatus, or period of apparent well-being, before the onset of symptoms. This free period can be as short as 12 h, or even less; it is usually at least 48 h; it can be as long as 6–8 months (Fig. 4.1). Nevertheless, within 24 h of the first symptom, the infant is usually admitted to the intensive care unit and artificial ventilation begun. Clinically, neonatal metabolic crises cannot be differentiated from neonatal sepsis. To avoid delayed diagnosis and start of metabolic emergency treatment and to reduce the risk of mortality and severe morbidity, the possibility of a neonatal metabolic crisis should be considered in the differential diagnosis of all newborns who present with sepsis-like symptoms.

Remember

- Acute life-threatening neonatal illness following a hiatus in which the infant appears well is a strong indicator of the presence of inherited metabolic disease.
- The most important key to the diagnosis of a metabolic disease in such an infant is a high index of suspicion.
- Never forget to carefully evaluate the family history. Previous patients of the family might have remained undiagnosed.

There are some alerting signals (Table 12.4). The picture of overwhelming illness in a neonate most often calls to mind a diagnosis of sepsis. Some alert physicians have initiated a metabolic workup in such an infant once they have found no proof for this assumption. However, since the sequential workup of sepsis and metabolic disorders is still quite time-consuming, a parallel workup of sepsis and metabolic disorders (at least including ammonia, lactate, and blood gases) should be considered. In general, awareness toward metabolic disorders still needs to be increased. Especially in a full-term newborn with an uncomplicated delivery, septicemia is not common, and a metabolic emergency should be considered and investigated in parallel. This approach may well provide an earlier diagnosis than is unfortunately the standard outcome in the diagnosis of most neonates with metabolic disease. However, it should be kept in mind that some patients with metabolic disease may actually present in the newborn period with septicemia. Prior to the advent of newborn screening for galactosemia, the earliest diagnoses of this disease were often made by physicians who recognized that these patients present with neonatal *Escherichia coli* sepsis. We have also encountered positive blood cultures and clinical sepsis in citrullinemia, propionic aciduria, and other disorders. Cerebral hemorrhage and pulmonary hemorrhage may complicate the initial episode of metabolic imbalance; and intestinal hemorrhage triggers the production of toxic metabolites. Coagulopathy may be the first sign of the presence of hepatorenal tyrosinemia (tyrosinemia type 1).

Many metabolic diseases and particularly the organic acidurias and the urea cycle disorders present first with vomiting. This has led frequently to a diagnosis of pyloric stenosis or

Table 12.4 Clinical and biochemical features suggesting the presence of metabolic disease in an infant

Clinical presentation
Overwhelming illness in the neonatal period
Vomiting (exclude pyloric stenosis and other causes), but patients with inherited metabolic disease have been diagnosed as having pyloric stenosis, even in the operating room
Encephalopathy
Deep coma
Seizures, especially myoclonic
Hiccups, chronic
Unusual odor
Extensive dermatosis (especially candidiasis)
Family history of siblings dying early
Biochemical findings
Acute metabolic acidosis (increased anion gap)
Hyperammonemia
Massive ketosis
Hypoglycemia

duodenal obstruction, and a number of pyloromyotomies or other explorations have been carried out. The organic acidurias should not be missed this way by the alert physician, because for all such patients, results of electrolyte analysis are available, and pyloric stenosis causes hypochloremic alkalosis. A patient who appears to have pyloric stenosis and a metabolic acidosis has an organic aciduria even if someone can feel an olive.

Neonates with hyperammonemic encephalopathy due to urea cycle disorders often present with normal to mildly increased blood pressure. This finding may help to distinguish neonates with neonatal sepsis from those with sepsis-like appearance due to a metabolic crisis.

Remember

An infant who is thought to have pyloric stenosis, but is acidotic, must be worked up for metabolic disease.

Electrolyte analysis also serves to indicate the presence of adrenal insufficiency or the adrenogenital syndrome with salt loss, especially in the male, which may present in this way. Some patients present with poor sucking or a complete inability to feed. An odd smell may be very helpful in the diagnosis of metabolic disease (Table 4.1 in Chap. 4), especially isovaleric acidemia and maple syrup urine disease.

The critically ill infant is often first seen in coma in an intensive care unit. An algorithmic approach to the infant in coma is shown in Fig. 12.1. Initial evaluation of NH_3, pH, and electrolytes permits early separation into those with elevated ammonia and no acidosis, most of whom have urea cycle defects (Table 12.3; Chap. 17). Similarly, those in whom the ammonia is elevated or normal, but there is metabolic acidosis and usually massive ketosis, commonly have organic aciduria. Patients with lactic acidemia and pyroglutaric aciduria may present with neonatal acidosis, but not usually with coma. The patient with maple syrup urine disease may be convulsant or opisthotonic as well as comatose, but there is usually little or no acidosis, and the DNPH test is positive.

Comatose patients without hyperammonemia or acidosis and a negative DNPH most commonly have nonketotic hyperglycinemia. The identical presentation can be seen in babies suffering from the treatable disorders of pyridoxine metabolism or from sulfite oxidase deficiency, molybdenum cofactor deficiency, adenylosuccinate lyase deficiency, methylenetetrahydrofolate reductase deficiency, or leukotriene C_4-synthase deficiency (see Chap. 27). A urinary sulfite test must therefore be done in every child presenting with catastrophic encephalopathy, and specific tests for homocysteine in blood as well as urinary purine analysis should be ordered. Leukotrienes are best analyzed in CSF, which must be stored at −70 °C or, as an intermediate measure, in dry ice or under liquid nitrogen. If intractable seizures dominate, the clinical picture pyridoxine-responsive (B_6–) as well as pyridoxal phosphate-responsive seizures should be considered. A therapeutic trial pyridoxine and pyridoxal phosphate is followed, if negative, by the administration of folinic acid with 5 mg/kg/day in three doses intravenously or orally (see Chap. 27).

Severe neonatal/infantile epileptic encephalopathy is one indication for specialized CSF analyses testing metabolic pathways of brain metabolism, especially amino acids and neurotransmitters (see Sects. 27.7 and 27.13). Defects in the metabolism of biogenic monoamines are diagnosed this way and so is GABA transaminase deficiency. An electroencephalographic finding of a burst suppression pattern is characteristic of nonketotic hyperglycinemia, but it is also found in other metabolic disorders such as disorders of pyridoxine metabolism, propionic aciduria, or molybdenum cofactor deficiency causing sulfite oxidase deficiency.

The occurrence of ketosis and metabolic acidosis in the neonatal period is an almost certain indicator of metabolic disease. Ketosis is rare in newborns. Even the neonatal diabetic is not ketotic, so testing of the urine at this time is often not performed. However, this is a mistake because infants with organic aciduria have massive ketosis. A reasonable position might be that any infant admitted to a neonatal intensive care unit with life-threatening nonsurgical illness should

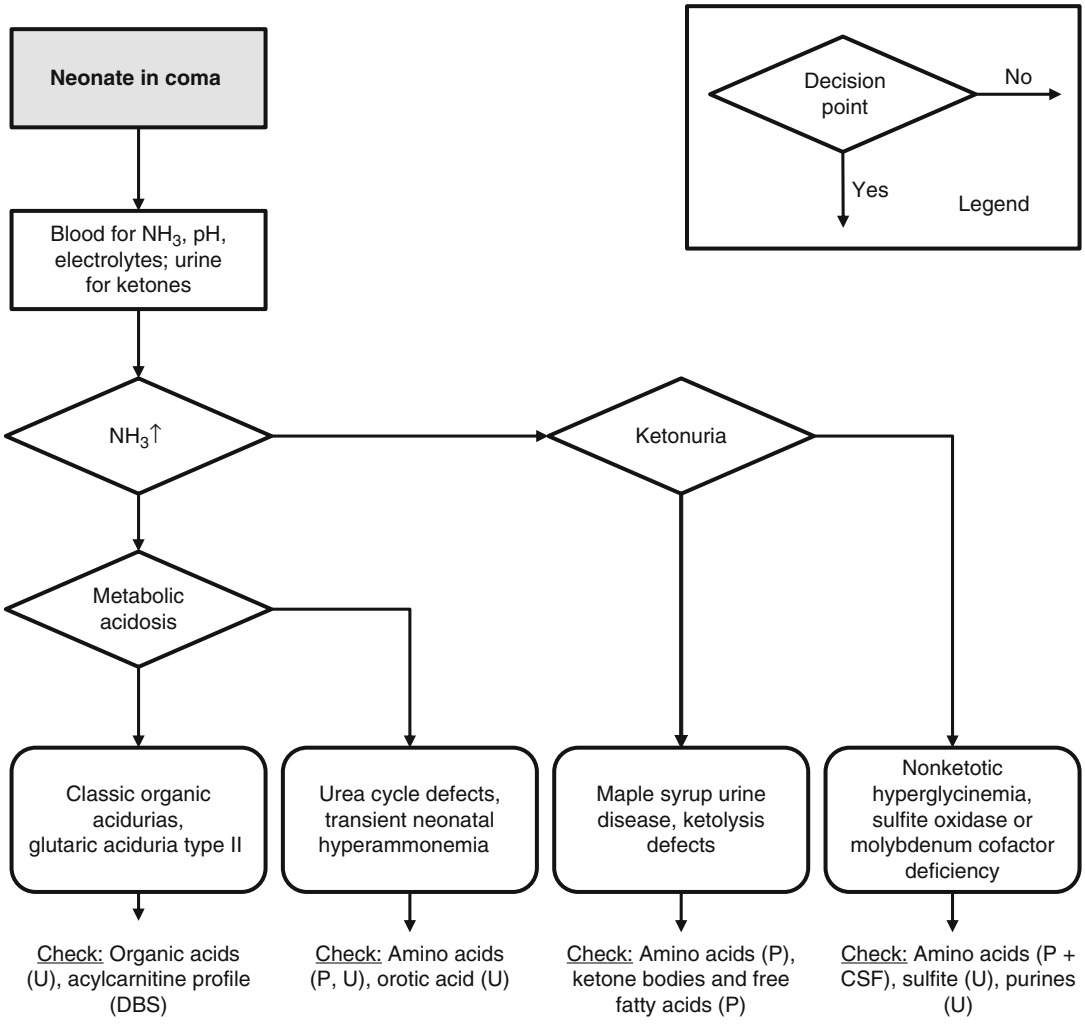

Fig. 12.1 The metabolic diagnosis of a newborn infant in coma

be tested for blood pH, NH_3, electrolytes, glucose, lactate, and ketonuria (Table 12.3). One could argue that valuable time would be saved by testing at the outset for amino acids in the blood, organic acids in the urine, and acylcarnitines in blood spots.

Remember

The urine of the ill newborn should always be tested for ketones. Massive ketonuria indicates an organic acidemia, while an absence of ketosis in a hypoglycemic infant leads to a diagnosis of a fatty acid oxidation defect.

12.2 Infancy

The infant with a metabolic emergency that presents after the neonatal period may have a period of some months of failure to thrive. Such an infant may feed poorly and vomit frequently, but the metabolic crisis is avoided until the advent of an intercurrent infection or a switch from human to cow's milk. Such an infant may then promptly develop the picture of life-threatening illness just like that of the newborn. The etiologies are often the same, organic acidurias, disorders of fatty acid oxidation, urea cycle disorders, hepatorenal

tyrosinemia type 1, glycogen storage disease, and fructose intolerance.

The disorders of fatty acid oxidation (Chap. 16) present classically at 7–12 months of age, consistent with the time infants begin to sleep longer or the timing of the first intercurrent infection that leads to prolonged fasting because of anorexia or vomiting. They can of course present first at any age in which these conditions are met. The infantile presentation of these diseases is with hypoketotic hypoglycemia. Clinically, there may be convulsions or coma. Cardiac arrhythmias are common. It is important to assess the level of glucose in the blood, and the adequacy of ketogenesis. A urine negative for ketones in the presence of hypoglycemia is very helpful, but if there are ketones in the urine, examination of blood concentrations of free fatty acids and 3-hydroxybutyrate is necessary to evaluate ketogenesis. X-rays of the chest, electrocardiography, and echocardiography should be carried out.

Muscle tone is affected in a variety of infants with metabolic diseases. These include muscular hypotonia in organic acidurias and disorders of fatty acid oxidation and muscular hypertonia (or alternating hypo- and hypertonia) in maple syrup urine disease, which are all classically complicated by metabolic emergencies. Thus, inclusion of a workup for metabolic disease in the hypotonic or floppy infant as well as in infants with rapidly increasing or fluctuating muscle tone may bring to light the more important need to treat an infant prior to the onset of that first episode of metabolic decompensation that so often leads to death or mental retardation.

Neutropenia, thrombocytopenia, and even pancytopenia are concomitants of a number of metabolic diseases, notably the organic acidurias and the cobalamin-related disorders. Recognition of this association in infancy may lead to an early diagnosis and forestall what is usually the most life-threatening crisis, the first.

The infant thought to have *Reye syndrome* is an excellent candidate for a diagnosis of an inborn error of metabolism. A single episode Reye syndrome was once relatively common, but it is not any longer, presumably related to the sparing use of salicylates in acute viral illness and the identification of overwhelming infectious, or metabolic, diseases resembling Reye syndrome in extended newborn screening. In fact, most infants considered to suffer from Reye syndrome – presenting with hypoglycemia, hyperammonemia, and elevated transaminases – today usually have an inherited metabolic disease such as MCAD (Chap. 16) or ornithine transcarbamylase (OTC) deficiency (Chap. 17). We have diagnosed the hyperornithinemia-hyperammonemia-homocitrullinuria syndrome (Chap. 17) in an infant thought to have Reye syndrome. The liver biopsy in this infant had the typical Reye picture of microvesicular steatosis, and so have infants with the other metabolic diseases. So a positive liver biopsy will not make the diagnosis. A metabolic workup is required.

Remember

An infant thought to have Reye syndrome has a metabolic disease until proven otherwise.

The differential diagnosis of primary *metabolic coma* in infancy overlaps with that of the neonatal period as shown in Fig. 12.1 and described earlier. In later infancy, patients with defective B_{12} metabolism, including cobalamin C disease, transcobalamin II deficiency, and the breast fed infant of a mother on a vegan diet, or a mother suffering from sometimes unrecognized pernicious anemia, may also present in this way. A patient in coma may also have hypoglycemia (Chap. 15). A very specific cause of hypoglycorrhachia is due to the defects of the glucose transporter 1 protein, the facilitative glucose transporter of the brain. This diagnosis, also termed De Vivo syndrome, can be anticipated from a pathologically decreased CSF glucose and CSF/blood glucose ratio <0.45, normal mean 0.7 ± 0.1, in the absence of pleocytosis or elevated CSF lactate. CSF lactate and alanine are in fact mostly decreased. Care must be taken to perform the lumbar puncture and determinations of blood sugar at least 4 h postprandially. Recurrent exaggerations of neurological and psychiatric symptoms often leading to metabolic coma are a major presenting feature of several late-onset

inborn errors of metabolism in older children and adults, e.g., urea cycle disorders. Clinical and laboratory presentations are especially variable and require a high index of suspicion on the part of the physician. These constellations and the most important diagnostic approaches are discussed in Chaps. 18 and 27.

12.3 Older Children and Adults

Any of the disorders, which present in early infancy, may develop repeated attacks of metabolic emergency at any age, despite generally successful therapy. Late-onset forms are also seen in many of the diseases that present classically in infancy. They are more common for the urea cycle defects, and determination of ammonia should be tested in every patient with unexplained fluctuating consciousness, behavioral abnormalities, psychiatric (postpartum) disease, or unexplained coma. The lateness of onset is usually a function of the fact that the variant enzyme resulting from the mutation has greater residual activity than that of the patient with the early neonatal presentation. Nevertheless, the dangerous nature of these diseases is clearly indicated by the fact that episodes of metabolic imbalance occurring in childhood, adulthood, or even old age may be quickly fatal. This is particularly true of urea cycle defects, and OTC deficiency is notoriously unpredictable. A series of readily manageable hyperammonemic episodes may be followed by one that leads promptly to cerebral edema, herniation, and death. We have also observed carbamoyl phosphate synthetase 1 deficiency leading to cerebral edema in a first episode in adults up to the age of 50 years. Branched-chain ketoaciduria has been reported in a small number of late-presentation patients. Again, as in the case of urea cycle defects, a late-onset patient is still in danger of dying from the first or a subsequent episode of metabolic imbalance.

The defects in fatty acid oxidation may present late, simply because the patient has never before fasted long enough to exhaust liver glycogen and call upon oxidation of fats. In this way, a MCAD deficiency can present first as a fatal episode of hypoketotic hypoglycemia in an adult. Other diseases of fatty acid oxidation may present later with acute rhabdomyolysis and cardiac arrhythmias. These patients usually have elevated levels of CK and uric acid at the time of the crisis. Others may present with acute cardiac failure, a consequence of year after year accumulated cardiomyopathy and the depletion of body stores of carnitine.

Mitochondrial diseases (Chaps. 14 and 42) may present at any age, but they more commonly present first in childhood or even adulthood. The first episode could be of coma with lactic acidosis and ketoacidosis. More commonly, particularly in the MELAS disease (Chap. 14), there is a stroke or stroke-like episode. Such episodes have also been seen in propionic aciduria, methylmalonic aciduria, OTC deficiency, CPS1 deficiency, and congenital disorders of glycosylation (CDG). Patients with mitochondrial disease often have abnormal neuroimaging studies. In addition to CT or MRI evidence of stroke, there are areas of increased signal in the basal ganglia and elsewhere. Many have the radiological appearance of Leigh syndrome. We have diagnosed NARP mutation in a patient who carried a radiological diagnosis of acute demyelinating encephalomyelitis. Basal ganglia lesions are also characteristic of propionic acidemia and methylmalonic acidemia.

13 Workup of the Patient with Metabolic Acidosis and Massive Ketosis

William L. Nyhan, Stefan Kölker, and Georg F. Hoffmann

Key Facts

- Massive ketosis in a neonate or young infant is a key to the diagnosis of a classic organic aciduria. Testing of the urine for ketones is a must in all ill infants.
- The most frequent organic acidurias are propionic aciduria, methylmalonic aciduria, multiple carboxylase deficiency, isovaleric aciduria, and 3-oxothiolase deficiency.
- Routine clinical chemistry reveals a low pH, a low bicarbonate, and an increased anion gap. The urine pH is low. Hyperchloremic acidosis and a normal anion gap mean intestinal losses or a renal tubular acidosis, the former with acidic urine and the latter with alkaline urine.
- Quantitative organic acid analysis by gas chromatography-mass spectrometry is essential in differential diagnosis.
- Acylcarnitine (MS/MS) profile may be a quicker route to diagnosis.

There are a number of metabolic diseases that present with acidosis (Table 13.1), most of them for the first time in the neonatal period. Metabolic acidosis may be caused by the accumulation of pathognomonic mono-, di-, or tricarboxylic acids, as in the case of lactic acidemia (Chap. 14) or pyroglutamic aciduria, or hawkinsinuria in infancy. However, in severe acidoses, the acidosis is often caused by a massive ketoacidosis, in which acetoacetic acid and 3-hydroxybutyric acid accumulate in the blood, and the urine tests strongly for ketones. These are classic metabolic emergencies. It is critical to make the diagnosis as soon as possible, and to get therapy started, even before the precise diagnosis is known (Chap. 19). It is important that testing of the urine for ketones be incorporated into the workup of a severely ill infant. Until the recognition of the organic acidurias, it was thought that ketonuria did not occur in the neonatal period, and testing for ketones early in life is often neglected. Its presence can signify an underlying metabolic diagnosis.

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Table 13.1 Metabolic diseases which may present with acute metabolic acidosis

Disorder	Detect or suspect	Definitive diagnosis (in addition to mutation analysis)
Propionic aciduria	Massive ketosis; methylcitrate, propionylglycine, and tiglylglycine in urine; hyperglycinemia	Propionyl-CoA carboxylase
Methylmalonic aciduria	Massive ketosis, methylmalonic acid in urine	Methylmalonyl-CoA mutase, complementation analysis
Isovaleric aciduria	Acrid smell ("sweaty feet" odor), massive ketosis, acylcarnitine profile, isovalerylglycine and 3-hydroxyisovaleric acid in urine	Assay of isovaleryl-CoA dehydrogenase
Multiple carboxylase deficiency	Lactic acidemia, hyperammonemia, acylcarnitine profile, hydroxyisovaleric acid, 3-methylcrotonylglycine, methylcitrate in urine	Biotinidase, holocarboxylase synthetase assays
Oxothiolase deficiency	Massive ketosis, tiglylglycinuria	3-oxothiolase
Methylcrotonyl-CoA carboxylase deficiency	3-methylcrotonylglycinuria	3-methylcrotonyl-CoA carboxylase
Maple syrup urine disease	Urinary 2,4-DNP, plasma branched-chain amino acids, urinary organic acid pattern	Branched-chain keto acid decarboxylase assay
Lactic acidemia	Growth retardation, ataxia, stroke, hyperalaninemia, lactic acid in blood, CSF, urine	Defective fructose-1,6-diphosphatase, pyruvate dehydrogenase, or decarboxylase, mitochondrial DNA, electron transport chain complexes
Pyroglutamic aciduria	Pyroglutamic acid in blood or urine	5-oxoprolien in urine, 5-oxoprolinase assay
Hawkinsinuria	Iodoplatinate	Cysteinyl-dihydrocyclohexyl acetic acid
Ketolysis defects	Ketosis	Cytosolic or mitochondrial acetoacetyl-CoA thiolase, succinyl-CoA:3-oxoacid CoA transferase
LCHAD deficiency	(Cardio)-myopathy, hypoglycemia, lactic acidemia, acylcarnitine profile	LCHAD assay

2,4-DNP 2,4-dinitrophenylhydrazones, *LCHAD* long-chain hydroxyacyl-CoA dehydrogenase

Remember

Testing for ketones in the urine is essential in any profoundly ill neonate. Massive ketosis is the hallmark feature of the organic acidoses.

Ketosis can be readily quantified by measuring the concentration of 3-hydroxybutyrate in the blood. Normally this is <1.0 mM in the nonfasting state. After a 24-h fast, levels of 2–3.5 mM are achieved in children. Infants and children with ketoacidosis may have levels over 7 mM (see Table 41.3).

Massive ketosis and metabolic acidosis are the hallmark features of the organic acidurias. Those most frequently encountered are propionic aciduria, methylmalonic aciduria, multiple carboxylase deficiency, isovaleric aciduria, and 3-oxothiolase deficiency (Table 13.1). 3-Hydroxyisobutyric aciduria causes episodic ketoacidosis and may otherwise mimic lactic acidemia. 3-Hydroxyisobutyryl-CoA deacylase deficiency, methacrylic aciduria, may also present with ketoacidosis and 3-hydroxyisobutyric aciduria.

The initial episode may begin with vomiting, anorexia, and lethargy, but progresses rapidly to life-threatening acidosis, dehydration, coma, and apnea. In the absence of intubation and assisted ventilation, the infant dies.

A clinical clue to the diagnosis is metabolic acidosis with vomiting. Some infants with organic aciduria have been thought to have pyloric stenosis, and some have been treated surgically; how-

ever, pyloric stenosis and its attendant vomiting lead to alkalosis. A patient who seems to have pyloric stenosis and has paradoxical acidosis has an organic aciduria.

These infants are often thought to have sepsis and some even have positive blood cultures. Septic infants can certainly be acidotic, but they are not ketotic, at least in the neonatal period. So an infant with real or apparent sepsis and massive ketonuria should be investigated and treated for an organic aciduria.

Patients with these disorders go on to have recurrent episodes of acidosis, always heralded by ketonuria. This is in response to the intake of the usual amounts of protein in patients prior to a specific diagnosis and the introduction of dietary therapies, and in response to infection in patients with an established diagnosis and receiving an appropriate therapeutic regimen.

The results from the clinical chemistry laboratory indicate severe acidosis. The arterial pH may be 6.9–7.2. The serum concentration of bicarbonate is low, and may be as low as 5 mEq/L or less. The anion gap is increased. The pH of the urine is <5.5. An acidosis with a high urinary pH signifies a renal tubular acidosis, but these disorders are usually chronic problems, not acute metabolic emergencies, and the anion gap is not increased. Hyperchloremia and a normal anion gap in an acidotic infant indicate renal tubular acidosis or intestinal losses of electrolyte. In the acute crisis of the organic acidurias, levels of lactic acid in the blood are also elevated, and this may contribute to the acidosis. There may also be hypoglycemia (Chap. 15), hypocalcemia, and hyperammonemia (Chap. 17), and each of these may be symptomatic. Hyperammonemia leads to respiratory alkalosis. An elevated ammonia in a patient with acidosis indicates that the diagnosis is an organic aciduria. Routine clinical hematology may also indicate the presence of an organic aciduria, especially in a very young infant. These disorders lead regularly to neutropenia, often to thrombocytopenia, and sometimes to anemia. Pancytopenia and acidosis are seen in infants with sepsis; they are also seen in infants with organic aciduria. If there are also large amounts of ketones in the urine, it is an organic aciduria. Chronic candidiasis may also indicate the presence of an organic aciduria. In a known patient with organic aciduria, abnormal hematological findings and moniliasis indicate a lack of metabolic control.

The definitive diagnosis of an organic aciduria is made by gas chromatography-mass spectrometry (GCMS). In this way, the presence of isovalerylglycine indicates the diagnosis is isovaleric acidemia; methylmalonate (methylmalonic aciduria); methylcitrate, 3-hydroxypropionate, and tiglylglycine (propionic aciduria); tiglylglycine and 2-methyl-3-hydroxybutyrate (3-oxothialase deficiency); and 3-hydroxyisovalerate, 3-methylcrotonylglycine, 3-hydroxypropionate, and methylcitrate (multiple carboxylase deficiency). Acylcarnitine (MS/MS) profile may be a quicker route to diagnosis.

Remember

If you suspect organic aciduria, order GCMS organic acid analysis of the urine and acylcarnitine profiling.

Among the organic acidurias, 3-oxothiolase deficiency is the most likely to be cryptic. For reasons that are not clear, the key metabolites may not be found in the urine at the time of the acute crisis, as they may be masked somehow by the massive quantities of acetoacetate and 3-hydroxybutyrate. On the other hand, when the patient is well, the tiglylglycine and 2-methyl-3-hydroxybutyrate may be missing from the urine. An isoleucine loading test will reveal the characteristic presence of these organic acid products of isoleucine, but may precipitate a metabolic crisis. Mutation analysis is increasingly replacing loading tests.

Quantification may also be important in diagnosis. For instance, the presence of 3-hydroxyisovalerate, 3-hydroxypropionate, and methylcitrate may suggest a diagnosis of multiple carboxylase deficiency, but these compounds are also found in propionic aciduria, because 3-hydroxyisovalerate increases in any patient with ketosis. The two are readily distinguished by quantification. In multiple carboxylase deficiency, the amounts of 3-hydroxyisovalerate are large and those of the other compounds small, while in propionic aciduria, the reverse is found. The distinction is important, because to send a patient home with biotin and no restriction of

protein intake, thinking that the diagnosis was multiple carboxylase deficiency, could be lethal in propionic aciduria.

Most of the organic acidurias may be detected by analysis of the blood or urine for acylcarnitines by tandem mass spectrometry. Today, an elevated plasma propionylcarnitine (C3) is often the first indication that the diagnosis is propionic acidemia or methylmalonic aciduria.

Once this biochemical diagnosis is made, definitive diagnosis may be undertaken at the level of the enzyme or the gene. Enzyme analysis can often be made by analysis of freshly isolated leukocytes, but for precision in diagnosis, especially at a distant laboratory, it is usually preferable to establish a fibroblast culture, send a confluent culture, and ensure enzymatic analysis of viable cells.

Most of the other disorders listed in Table 13.1, while causing acidosis, do not usually present as a metabolic emergency. Pyroglutamic aciduria may rarely present with severe acidosis without ketonuria, but more commonly the acidosis is mild or absent. Maple syrup urine disease (MSUD), on the other hand, presents as a metabolic emergency (Chap. 12), but acidosis is usually absent or minor. In MSUD, the DNPH test is very useful in detecting the presence of the large quantities of the keto acid derivatives of the branched-chain amino acids. GCMS analysis of the organic acids of the urine will identify these keto acids and also their hydroxy acids. The specific pattern is diagnostic of MSUD; as of course is the analysis of the amino acids of the plasma.

Ketoacidosis occurs, of course, in diabetes mellitus. This diagnosis is readily made clinically. A diabetic infant or child is not likely to be missed if routine clinical chemistry is employed. On the other hand, an occasional organic aciduria has been mistaken for diabetes when an isolated elevation of glucose occurred at the time of presentation in ketoacidosis. In these patients, there may be elevated ammonia or lactic acid in the blood, providing keys to the diagnosis. Hyperglycemia is also transient and responds exaggeratedly quickly to a small amount of insulin. Disorders of carbohydrate metabolism, especially glucose-6-phosphatase deficiency (glycogen storage disease (GSD) type 1), but also fructose-1,6-diphosphatase deficiency and glycogen synthase deficiency (GSD type 0), can have impressive levels of ketones in the blood. However, these disorders seldom present with an acute metabolic acidotic emergency. They present rather with hypoglycemia (Chap. 15). Ketosis is seldom considered in the lactic acidoses (Chap. 14). However, we have repeatedly observed crises of ketoacidosis in patients with episodic illness in electron transport abnormalities, such as the NARP mutation. These episodes respond to the administration of parenteral glucose and water (Chap. 19).

The disorders of ketolysis may present with a more or less pure ketoacidosis in which there is no hypoglycemia, hyperglycemia, organic aciduria, lactic acidemia, or hyperammonemia. It is thought that they have defective peripheral utilization of acetoacetate and 3-hydroxybutyrate. The prototype condition is cytosolic acetoacetyl-CoA thiolase deficiency. Actually, at least one patient with this disorder was reported to have moderate hypoglycemia; and others had elevated concentrations of lactate and pyruvate and a normal lactate to pyruvate ratio. Other ketolytic defects include the mitochondrial acetoacetyl-CoA thiolase deficiency and succinyl-CoA:3-oxoacid CoA transferase deficiency. This last enzyme catalyzes the conversion of acetoacetate to acetyl CoA. Patients with this disorder are ketotic in the fed condition. Recently, inherited deficiency of monocarboxylate transporter 1 has been described as another genetic cause of potentially lethal ketoacidosis resulting from deficient utilization of ketone bodies.

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Workup of the Patient with Lactic Acidemia: Mitochondrial Disease

14

William L. Nyhan, Stefan Kölker, and Georg F. Hoffmann

Key Facts

- Inborn errors characterized by lactic acidemia fall into two categories: abnormalities in gluconeogenesis and defects of oxidative phosphorylation. Distinction is important because management and prognosis are different.
- As a first step exclude factitious and secondary elevations of levels of lactic acid in order to focus on specifics of work-up.
- Ratios of lactate to pyruvate and 3-hydroxybutyrate to acetoacetate are useful in elucidating the area of metabolic defect.
- Postprandial rise or fall in lactate gives important information.
- A monitored fast has been required to distinguish oxidative defects from those of gluconeogenesis. Molecular methods have decreased the necessity for this.

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The lactic acidemias represent a family of disorders of pyruvate metabolism. Under these circumstances, large elevations of pyruvate concentration might be expected, but are seldom seen. Accumulating pyruvate does not lead to large elevations of pyruvate concentration; but rather to conversion to its two sinks, lactate and alanine (Fig. 14.1).

Genetically determined causes of lactic acidemia fall into two categories, defects in gluconeogenesis and defects in oxidation (Fig. 14.2). It is important in the work-up to distinguish clearly into which of the two categories each patient falls. The distinction is useful in determining optimal therapy, even in those patients in whom a molecular diagnosis remains elusive. Definitive diagnosis documents deficiencies in activity of a growing group of enzymes and mutations in DNA, first of mitochondrial and lately also of nuclear DNA (see also Chap. 42).

The first step in investigation of a patient with lactic acidemia is the documentation that the level of lactic acid in the blood or cerebrospinal fluid (CSF) is truly elevated. The most common situation in which the concentration of lactic acid in blood is elevated is factitious, the result of improper technique, the use of a tourniquet, or difficulty in drawing the blood. It is also true that levels are variable; even in patients with known mitochondrial disease, the concentration of lactic acid is not always increased. Lactic and pyruvic acids are located distant from many of the enzymatic steps that are defective, especially those of the electron transport chain.

For the evaluation of energy metabolism, lactate should be determined repeatedly throughout the day (especially before and after meals). It is

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useful to also determine levels of pyruvic acid and alanine in the blood, as well as lactic acid. Alanine is not falsely raised by problems of technique. It is important to be rigorous about methods of sampling and to obtain blood flowing freely without a tourniquet. The concentration of lactate and alanine in the CSF should also be determined, particularly in those who appear to have mitochondrial disease with neurological symptoms despite normal levels of lactate in the blood. Many have elevated concentrations of lactate in the CSF, while the plasma level is normal, slightly, or intermittently elevated. Lactate to creatinine ratios in urine are less sensitive. Raised urinary lactate does, however, support the significance of questionable increased lactate levels in blood. If urinary lactate is found more consistently elevated than blood lactate, predominant or even isolated disease of the kidney is to be considered. At first seemingly unrelated to the underlying mitochondrial disorders, patients often show a constant thrombocytosis and hypertrichosis.

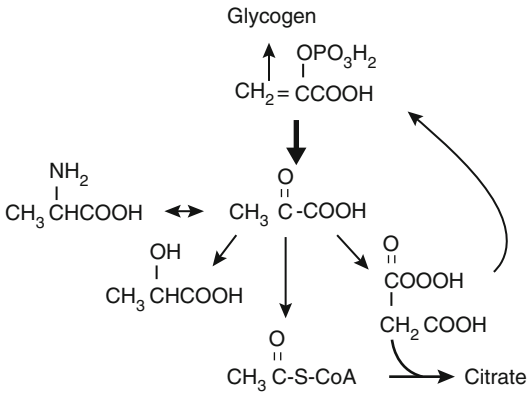

Fig. 14.1 Pathway of pyruvate metabolism with pyruvate in the center and lactate and alanine sinks to the *left*

Before embarking on a specific investigation for lactic acidemia, it is important to exclude conditions that cause secondary lactic acidemia. Patients with hypoxemia, hypoventilation, shock, or hypoperfusion are generally readily recognized as patients with sepsis or cardiac or pulmonary

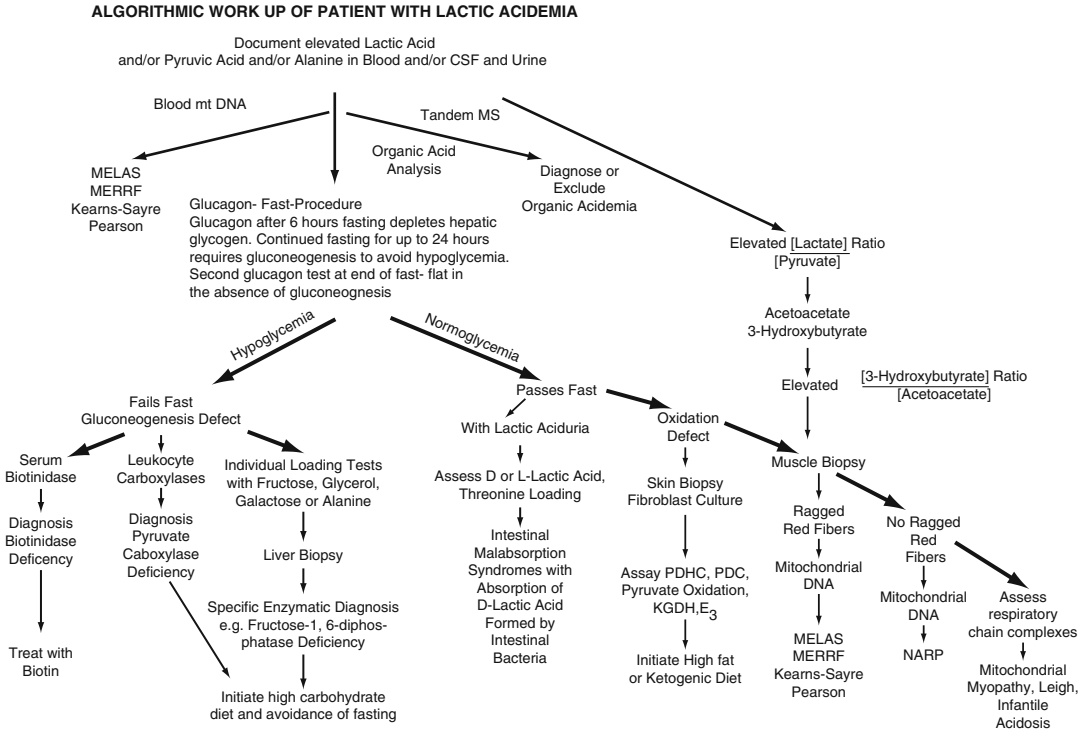

Fig. 14.2 An approach to the stepwise evaluation of a patient with lactic acidemia. E_3 estriol, *MELAS* mitochondrial encephalomyelopathy, lactic acidemia, and stroke-like episodes, *MERRF* myoclonus epilepsy with ragged red fibers, *MS* mass spectroscopy, *mt* mitochondrial, *NARP* neuropathy, ataxia, and retinitis pigmentosa, *PDHC* pyruvate dehydrogenase complex

disease. Anaerobic exercise also produces lactic acidemia, but this is seldom clinically relevant except in the convulsing patient. A variety of inherited metabolic diseases produces secondary lactic acidemia including propionic aciduria, methylmalonic aciduria, isovaleric aciduria, 3-hydroxy-3-methylglutaric aciduria, and pyroglutamic aciduria. Each of these conditions can be excluded by organic acid analysis. Oncologic patients may also present with elevated lactate which results from enhanced aerobic glycolysis (Warburg effect).

An uncommon cause of lactic acidosis is D-lactic acidemia, resulting from absorption of D-lactic acid produced by intestinal bacteria (see also Chap. 25). Most such patients have obvious malabsorption or short-gut syndromes, metabolic acidosis, and massive lactic aciduria, found by colorimetric test or by urinary organic acid analysis. Testing for lactate in routine clinical chemistry is now usually done in an enzymatic assay, which is specific for L-lactate, so this situation is often not even recognized. The discrepancy between urine and blood lactate levels, plus the history, is the key to diagnosis. A short course of treatment with oral neomycin or metronidazole will cause a dramatic fall in D-lactate production, and the lactic acidemia will disappear.

Remember

Before embarking on a workup for lactic acidemia, exclude factitious or secondary lactic acidemias, which are even more common, and even D-lactic acidemia.

14.1 Workup of a Patient with Lactic Acidemia (Congenital)

Once it has been decided that a patient has lactic acidemia, the redox status and the response to a carbohydrate load may be evaluated first. Lactate should be determined preprandially and postprandially together with pyruvate, 3-hydroxybutyrate, and acetoacetate, preferably from the same samples collected in tubes prefilled with perchloric acid. Plasma glucose must be determined as well. Elevated ratios of the cytosolic (lactate-pyruvate >20) as well as of the mitochondrial redox status (3-hydroxybutyrate-acetoacetate >3) point to a disturbance of oxidative phosphorylation. An elevated ratio of lactate-pyruvate without elevation of the 3-hydroxybutyrate-acetoacetate ratio is indicative of severe pyruvate carboxylase deficiency. In these patients, elevations of the amino acids citrulline and lysine may also be found, as well as hyperammonemia.

A postprandial rise of lactate (≥ twofold) occurs in pyruvate dehydrogenase deficiency and also in glycogenosis (GSD) types 0, III, and VI/IX. In primary defects of the respiratory chain, the redox state may become more abnormal; in addition, there may even be a rise of total ketone bodies (paradoxical ketonemia). A postprandial fall of lactate occurs in GSD type I and defects of gluconeogenesis.

The differentiation between problems in gluconeogenesis or in oxidative phosphorylation (Fig. 14.2) can be achieved by evaluating the response to a prolonged fast (Table 14.1). Fasting studies are not appropriate in the diagnosis of a child with a defect in fatty acid oxidation. An intravenous catheter is inserted to facilitate the drawing of samples. Prior to the initiation of fasting, blood is obtained for glucose, lactate, pyruvate, and alanine. In this method, 0.5 mg of glucagon is given intramuscularly at 6 h in order to deplete the liver of glycogen made from glucose, and the glucose response is determined at 15, 30, 45, 60, and 90 min. The response to glucagon should be a sizeable increase in glucose (>20 %) except in glycogenosis type I. As the fast is continued for 18–24 h, or until the development of hypoglycemia, the body is dependent on gluconeogenesis for the maintenance of normal levels of glucose in the blood. Hypoglycemia (blood glucose <45 mg/dL = 2.5 mM) at any time signals the conclusion of the fast. If the patient is asymptomatic, glucagon is given again. During this time if there is no rise in glucose, the defect is in gluconeogenesis. Glucose is given intravenously to restore normoglycemia without waiting for the usual interval of a glucagon test, and, in the presence of any symptoms, is given immediately without testing glucagon responsiveness. Concentrations of lactic and pyruvic acids, alanine, acylcarnitines, free fatty acids, and ketone bodies are determined at the end of the

Table 14.1 Twenty-four-hour fast for lactic acidemia

Protocol and specimens
Begin fast at time $T=0°$ at 4 p.m. The first 16 h are the least hazardous; so should happen overnight
T(ime) = 0° (4 p.m.): end of last meal with documented intake
$T=1°$ (5 p.m.): serum glucose, electrolytes, phosphate, uric acid (alanine and aspartate aminotransferases, creatine kinase). Lactate, pyruvate, 3-hydroxybutyrate, acetoacetate from perchloric acid tube. Plasma alanine. Plasma for acylcarnitines. Can be spotted on Guthrie card. Blood spot sample should be collected as backup
$T=6°$ (10 p.m.): blood glucose
$T=6°$ (10 p.m.): 1 mg glucagon is given i.m. or i.v. after flushing the line with 5 mL 5 % albumin
$T=6°15'$: blood glucose
$T=6°30'$: blood glucose
$T=6°45'$: blood glucose
$T=6°90'$: blood glucose
T = 9° (1 a.m.): blood glucose
If any glucose level from the 9° and after blood draws are
>85 mg/dL (4.7 mM), collect glucose levels q3 h
>65 but <85 mg/dL (3.6–4.7 mM), collect glucose levels q2 h
>50 but <65 mg/dL (2.8–3.6 mM), collect glucose levels q1 h
>40 but <50 mg/dL (2.2–2.8 mM), collect glucose levels q1/2 h
$T=15°$ (7 a.m.): serum glucose, electrolytes, phosphate, uric acid. Lactate, pyruvate, 3-hydroxybutyrate. Plasma alanine
$T=24°$ (4 p.m.) or at the time of development of hypoglycemia: serum glucose, electrolytes, phosphate, uric acid, creatine kinase (transaminases). Blood gases. Lactate, pyruvate, 3-hydroxybutyrate, acetoacetate collected in perchloric acid tube. Plasma alanine, free fatty acids. Plasma acylcarnitines. Collect urine for quantitative analysis of organic acids
If blood sugar is <40 mg/dL (2.2 mM), draw samples as above and in addition for insulin, growth hormone, and glucagon. Give 1 mg glucagon i.m. or i.v. and collect blood glucose at 15 min. If glucose rises, collect at 30 and 45 min followed by 3–5 mL 10 % glucose/kg b.w./h. In the presence of any symptoms or if glucose does not rise, give 2 mL of 20 % or 4 mL of 10 % glucose/kg b.w. intravenously as a bolus, followed by 3–5 mL 10 % glucose/kg b.w./h until normoglycemia is restored, and the patient is able to resume adequate oral intake

fast. In a hypoglycemic patient, concentrations of insulin, growth hormone, and glucagon are also obtained at the time the fast is terminated. It must be remembered that lactate will be raised in a struggling child, and as a result of a convulsion, as might occur with severe hypoglycemia. Errors in sample acquisition, technique, and sample handling all raise the lactate level measured by the laboratory.

Fed and fasted responses to glucagon can provide a discrimination between GSD type I and III. In suspected GSDs, it is useful to test the response to glucagon in the fed as well as in the fasted state. After a fast of 24-h or fasting to mild hypoglycemia, if a fast of 24-h is too long, administration of glucagon yields a flat response in GSD type I and III, while blood glucose rises normally in GSD type VI/IX. On the other hand, when glucagon is given 2, 3, or 6 h after a meal, the blood glucose rises in GSD type III, reflecting glucose molecules on the outer branches released by phosphorylase. In GSD type I, there is minimal production of glucose in response to glucagon, whereas lactate will increase markedly.

In the further workup of a patient with a defect in gluconeogenesis who fails the fasting test, it is convenient to assay biotinidase in serum or blood spot, and carboxylase activity in leukocytes or fibroblasts; in this way a definitive diagnosis of multiple carboxylase deficiency (due to holocarboxylase synthetase or biotinidase deficiency) or pyruvate carboxylase deficiency can be made. Patients with disorders of gluconeogenesis in whom these are not the diagnoses, such as those with fructose-1,6-diphosphatase deficiency, require liver biopsy for definitive enzyme assay or primary genetic testing, but the diagnosis will be suspected, and effective treatment can be instituted on the basis of fasting and loading data. Information as to the area of the defect may be obtained by loading tests, with fructose, alanine, or glycerol. Each compound is given by mouth as a 20 % solution 6–12 h postprandially in a dose of 1 g/kg.

Most patients who pass the fasting test have defects in pyruvate oxidation, reflecting mitochondrial dysfunction (see also Chap. 42). The elucidation of oxidation defects may be initiated by obtaining a skin biopsy for fibroblast culture. A diet high in fat and low in carbohydrate, with vitamin supplementation can be begun while waiting for sufficient quantities of cells for analysis. Fibroblasts may be assayed for defects in the

pyruvate dehydrogenase complex. Defects in the first enzyme of the complex, pyruvate decarboxylase or E1α, can also be tested for by mutational analysis. Measuring respiratory chain complex activities, and identifying the cause of the dysfunction, may require lengthy laboratory investigations. A more immediate answer may be obtained by the analysis of blood for abnormalities in mitochondrial (mt) DNA (Chaps. 39 and 42). Among the recognized mitochondrial diseases of electronic transport are the mtDNA depletion syndromes. mtDNA depletion syndrome is a genetically heterogeneous disease group. They are caused by defects in mtDNA maintenance (due to mutated TK2, SUCL2, SUCLG1, RRM2B, DGUOK, and TYMP) or mtDNA replication (due to mutated POLG and C10orf2). Clinically, mtDNA depletion syndromes are classified as myopathic, encephalopathic, hepatocerebral, and neurogastrointestinal, highlighting the broad clinical spectrum of affected individuals.

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Work-Up of the Patient with Hypoglycemia

15

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Key Facts

- Timely determination of blood concentrations of insulin, growth hormone, and cortisol at the time of hypoglycemia can elucidate endocrinologic causes of hypoglycemia. The endocrinologist may be the first consultant to see the patient and, if these determinations have not been made, often orders a control LED fast. It is important to educate these colleagues of the importance of obtaining metabolic testing (Fig. 15.1) under these circumstances.
- Liver disease is a common cause of hypoglycemia even in pediatric patients.
- The important distinction of ketotic vs. hypoketotic hypoglycemia is best made by determination of free fatty acids, acetoacetate, and 3-hydroxybutyrate at the time of hypoglycemia.
- Hypoglycemia after a short fast is the hallmark of a disorder of carbohydrate metabolism; after a long fast, it signifies a disorder of fatty acid oxidation.

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Hypoglycemia must be recognized promptly and treated effectively, if permanent damage to the brain is to be prevented. Treatment means bringing blood glucose to a normal concentration and maintaining it there. Rational treatment demands a specific diagnosis of the disease causing the hypoglycemia. Determination of the blood concentrations of insulin, growth hormone, and cortisol at the time of hypoglycemia leads to the definition of the classic forms of hypoglycemia. Liver disease must be excluded as a cause. The metabolic causes of hypoglycemia may be elucidated by the response to fasting and determination of the levels of free fatty acids, acetoacetate, and 3-hydroxybutyrate in the blood. This permits the distinction of ketotic hypoglycemia, which includes the disorders of carbohydrate metabolism and the transient disorder termed ketotic hypoglycemia, from hypoketotic hypoglycemia, which, in the absence of hyperinsulinemia, includes most of the disorders of fatty acid oxidation.

Acute hypoglycemia is a manifestation of a variety of different disorders. Its prompt recognition and reversal are critical because this absence of substrate for cerebral metabolism can lead to permanent damage of the brain just as surely as can lack of oxygen, since glucose is the primary energy substrate of the brain. The acute episode can also be fatal. Its management requires the provision of enough glucose to bring the blood concentration of glucose to normal and enough on a continuing basis to keep it there. Hypoglycemia is defined as a serum concentration under 50 mg/dL (2.8 mmol/L) or a concentration in whole blood under 45 mg/dL (2.5 mmol/L) at all ages. Low concentrations of glucose are so common in the neonatal period

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that neonatologists (used to) define hypoglycemia as a concentration under 30–35 mg/dL and in preterm infants <25 mg/dL. However, there is little evidence that the brain of the very young is any more tolerant of hypoglycemia, and we prefer to maintain concentrations of blood glucose >45 mg/dL in any age group.

The classic symptoms of hypoglycemia are sweating, pallor, irritability, and tremulousness, but there may be considerable variability even in the same individual, and convulsions or coma may be the initial manifestation, particularly in the neonate. There may be vomiting, but this may be the result of an intercurrent illness that induces the acute hypoglycemic episode. Headache, lethargy, altered behavior, or psychosis may be seen in older children and adults, while apnea, tachypnea, cyanosis, or hypothermia may occur in the newborn. Some individuals for whom very low levels of glucose are a chronic occurrence, for example, patients with GSD type I or 0 tolerate surprisingly low levels without symptomatology. Brains of these patients might be adapted to use alternative energy substrates such as lactate and ketone bodies. For the same reason, sudden drops in glucose levels are more apt to induce symptoms than those achieved slowly.

15.1 Algorithmic Approach to Diagnosis

Definitive diagnosis is the elucidation of the cause of the hypoglycemia. This is an essential feature of the design of therapy. It also permits prognostication of a transitory or a potentially recurrent nature.

The diagnostic work-up is ideally initiated when the patient is seen at the time of the acute attack of hypoglycemia at which time blood can be obtained for insulin, growth hormone, and cortisol to elucidate the common endocrine causes of hypoglycemia; tests of hepatic function to elucidate disease of the liver, a very common cause of hypoglycemia; as well as specialized tests, such as the blood concentrations of free fatty acids, acetoacetate, and 3-hydroxybutyrate, and plasma alanine, to elucidate whether hypoglycemia is ketotic or hypoketotic (Fig. 15.1). More often, we are called upon to evaluate the patient after treatment of the acute attack when normoglycemia has been restored. In this situation, a controlled monitored fast may be required to reproduce the hypoglycemic state and initiate the work-up.

The clinical history may guide the differential diagnosis. The age of the patient may help in certain types of hypoglycemia that are commonly encountered at different ages. Transient neonatal hypoglycemia is a condition of the first days of life and is seen particularly in preterm and small for gestational age (SGA) babies. Ketotic hypoglycemia is a common transient disease with onset usually between 1 and 2 years of age and disappearing by 6–8 years of age. Endocrine and metabolic causes may present first of any age, including the stressful first days of life, but there is a tendency to onset after 7 months of life when infants sleep longer and are more likely to acquire infectious illnesses that lead to anorexia or vomiting and hence fasting.

Patients with disorders of carbohydrate metabolism become hypoglycemic after short periods of fasting; 6–8 h leads to hypoglycemia in a patient with glycogenosis type I or glycogen synthase deficiency. On the other hand, even an infant may have to fast 16–24 h before stores of glycogen are exhausted, and fatty acid oxidation must be carried out to avoid hypoglycemia. Hypoglycemia resulting from the ingestion of a toxin, such as salicylate or ethanol, is usually evident from the history, but covert administration of insulin in the Munchausen-by-proxy-syndrome is more difficult to suspect. Hyperinsulinemia with a normal C-peptide gives this situation away.

Remember

Hypoglycemia after a short fast signifies a disorder of carbohydrate metabolism and, after a longer fast, a disorder of fatty acid oxidation.

The physical examination is useful in leading to the diagnosis of specific syndromes, in which hypoglycemia is common. Macrosomia in an infant with full rounded cheeks and a plethoric, edematous appearance is the alerting

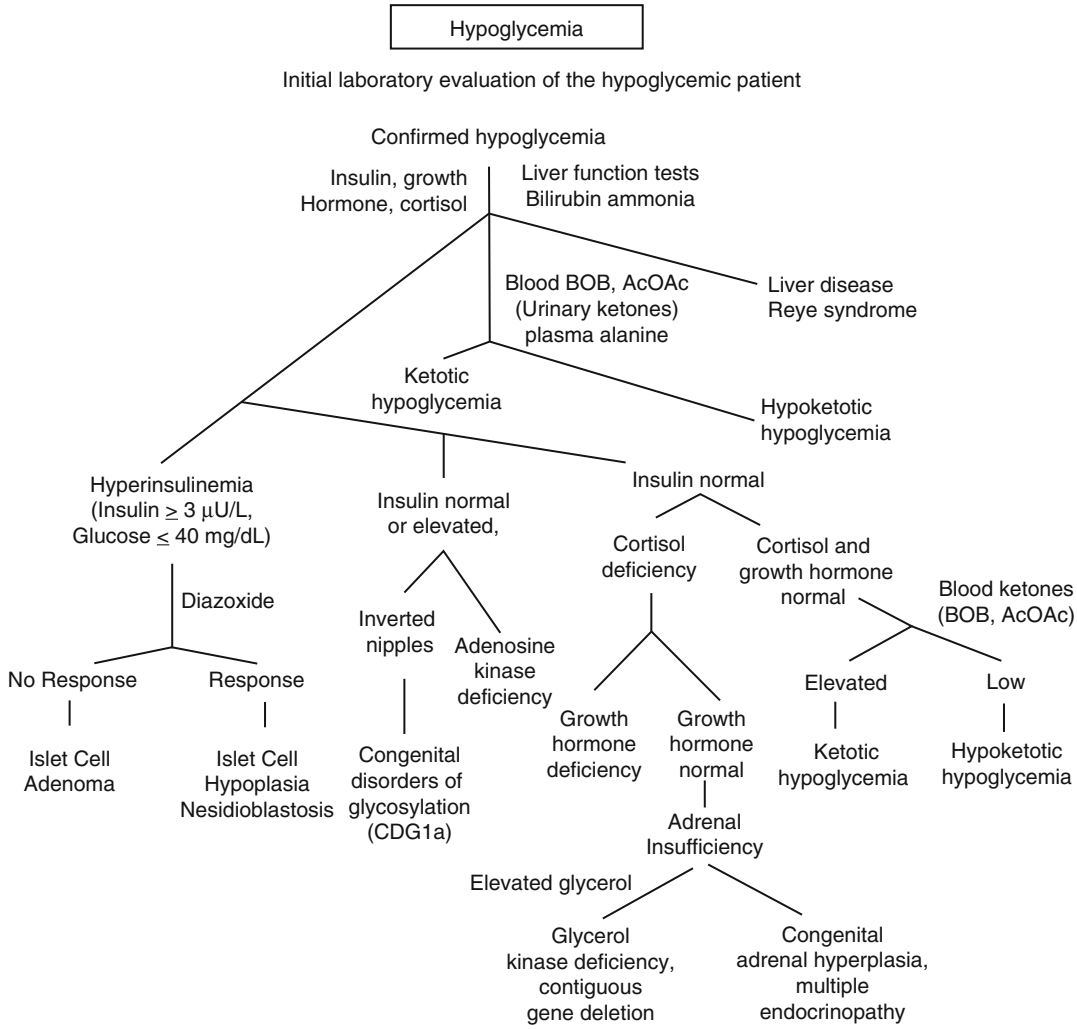

Fig. 15.1 An algorithmic approach to the definitive diagnosis of the cause of hypoglycemia

picture for the danger of hypoglycemia in the infant of the diabetic mother. These infants also have a behavioral phenotype of drowsiness, hypotonia, a long latency for response, and only brief periods of alertness. The most severe cases may also present with dysmorphism. Macrosomia and hypoglycemia are also seen in the *Beckwith–Wiedemann syndrome* along with macroglossia, omphalocele, and hepatomegaly. In both the syndromes, hypoglycemia is transitory, lasting 1–3 days; other syndromes associated with hyperinsulinism are congenital disorders of glycosylation (CDG), mostly in phosphomannose isomerase deficiency (MPI-CDG), adenosine kinase deficiency, where muscular hypotonia, liver dysfunction, and frontal bossing can point to the diagnosis, and Pearlman, Simpson–Golabi–Behmel, Sotos, and Usher syndromes. A micropenis may be the only external clue to the presence of panhypopituitarism. Some such infants have midline defects, such as clefts of the lip and palate. Hepatomegaly is characteristic of even very young infants with glycogen storage disease. In the older child, it can sometimes be differentiated from the hepatomegaly of acute liver disease by its lack of tenderness.

The initial laboratory evaluation of the hypoglycemic patient (Fig. 15.1) rests on tests readily available in the clinical laboratory. Hyperinsulinemia may be subtle but should be considered as relevant if plasma insulin is above 3 mU/L during hypoglycemia (40 mg/dL or less). Other diagnostic criteria are increased glucose demand (e.g., >8–10 mg/kg/min in newborns) and increase (at least 30% above the initial concentration) in serum glucose 10–30 min following administration of glucagon (100 μg/kg s.c. or i.m., max 1 mg). Many of the infants labeled as having idiopathic hypoglycemia, the infants of diabetic mothers, and those with leucine-sensitive hypoglycemia (hyperinsulinism–hyperammonemia syndrome, see below) have hyperinsulinism. *Persistent or recurrent hyperinsulinemic hypoglycemia* results from hyperplasia of the β-cells of the pancreatic islets, referred to as nesidioblastosis, or less commonly in older children an islet cell adenoma. In both the conditions, there is a relative absence of ketosis and a substantial risk of damage to the brain. Some patients without adenomas respond to diazoxide with a lessening of hypoglycemia. Most of either group with persistent hyperinsulinemia require surgical removal of most of the pancreas. In children, many with diffuse hyperinsulinism can be managed medically, while those with focal lesions require surgery as do adults with true adenomas. Distinction between focal and diffuse lesions of the pancreas has been difficult short of surgery; the use of [^{18}F] fluoro-L-dopa positron emission tomography (PET) appears to be useful in making that distinction. Hypoglycemia is also seen in patients with a variety of tumors that secrete insulin-like material.

An interesting cause of hyperinsulinemic hypoglycemia is known as the *hyperinsulinism–hyperammonemia syndrome* and is caused by mutations in the gene (*GLUD1*) for glutamate dehydrogenase (GDH). Mutations in this gene impair sensitivity to its allosteric inhibitor GTP, leading to gain-of-function enzyme activity and increased sensitivity to its activator leucine. Concentrations of ammonia in these patients, while clearly elevated, are not very high, and patients have not had symptoms attributable to hyperammonemia. Changes in protein intake do not change the hyperammonemia – some of these patients have epilepsy without hypoglycemia. Persistent hyperinsulinemic hypoglycemia of infants is also caused by mutation in the pancreatic β-cell sulfonylurea receptor gene (SUR1) and in the inward rectifier potassium channels gene (KIR6.2), as well as in the glucokinase gene (GK).

SUR1 and KIR6.2 are channelopathy genes. Channels are composed of four KIR6.2 subunits and four SUR1 subunits. Dominant mutations may lead to haploinsufficiency in which the channel may be unable to stay open enough to maintain depolarization of the membrane, or dominant negative mutation may lead to destruction of channel structure. Mutations in the same gene may lead to diabetes or hyperinsulinism. Some patients with the same mutations have hyperinsulinism in infancy and diabetes later.

Congenital hypoglycemia of infancy results from mutations in at least six different genes. These include *GLUD1*, *GCK*, *HADH*, and *SLC16A1*, but most are caused by mutations in one of two genes, *ABCC8* and *KCNJ11*, that code for major elements of the adenosine triphosphate (ATP)-regulated potassium channel of the pancreatic β-cells, which are major causes of hypoglycemia. In the Ashkenazi Jewish population, two founder mutations, c.3989-9 g>a and p.Phe1387del in the ABCC8 gene, account for the majority of mutations. In childhood, exercise-induced hyperinsulinism due to failed repression of SLC16A1 (MCT1 transporter) in pancreatic β-cells is another genetic cause of hyperinsulinemic hypoglycemia.

Congenital hypoglycemia of infancy is manifested as diffuse and focal; the latter is of therapeutic significance because it can be treated surgically by removal of a focal lesion. The diffuse form is autosomal recessive, and every β cell hypersecretes insulin. Patients with the focal form carry a recessive mutation on the paternal allele on chromosome 11, which is inherited. A somatic mutation occurs during the development of a β-cell precursor, leading to loss of the maternal short arm of the chromosome and an imbalance of imprinted genes. Clonal expansion provides an island of hypersecreting cells.

Patients with hypoglycemia and normal levels of insulin may have deficiency of cortisol with or

without growth hormone deficiency and may be diagnosed as having one of the classic endocrine disorders (Fig. 15.1).

Patients in whom endocrine evaluations are normal may have ketotic or hypoketotic hypoglycemia (Chap. 16). This distinction can sometimes be made on the basis of the presence or absence of ketonuria at the time of hypoglycemia. However, the presence of ketones in the urine may be misleading. Many patients with documented disorders of fatty acid oxidation have been missed because of the presence of positive tests for ketones in the urine. Quantitative assays of acetoacetate and 3-hydroxybutyrate in the blood along with the levels of free fatty acids clearly show that such a patient is hypoketotic at the time of hypoglycemia.

15.2 Ketotic Hypoglycemia

Ketotic hypoglycemia is the name given to a very common disorder of late infancy and childhood which seldom begins before 18 months and disappears by 4–9 years of age. Actually there are a number of disorders in which abundant amounts of ketones accompany hypoglycemia. Massive ketosis is the hallmark of the organic acidurias (Chap. 13) in which hypoglycemia sometimes accompanies the acute attack of ketoacidosis. The other molecularly defined conditions in which ketosis accompanies hypoglycemia are disorders of carbohydrate metabolism, notably GSD type I (see also Chap. 14).

The syndrome known as ketotic hypoglycemia presents classically with symptomatic hypoglycemia in the morning after a long fast, often precipitated by an intercurrent illness that causes the child to miss dinner. At the time of the hypoglycemia, tests of the urine for ketones are positive, and concentrations of acetoacetate and 3-hydroxybutyrate in the blood are elevated. Analysis of amino acids indicates the concentration of alanine to be low. This is often a hallmark of a problem with gluconeogenesis.

Physical examination is often unremarkable, but the patient may be short and have diminished subcutaneous fat, and there may be a history of low birth weight. Glucagon administered at the time of hypoglycemia is followed by little or no increase in glucose concentration.

In patients seen after recovery from hypoglycemia, the syndrome may be reproduced by fasting (see Sect. 41.2.1), but in some patients, this test may be negative. In these patients, a test of a ketogenic diet containing 67 % of the calories as fat initiated after an overnight fast may reproduce the syndrome, and again there is no response to glucagon. Although in more severe or familial cases a definitive inborn error of energy homeostasis is suspected this can rarely be ascertained. Three true inborn errors of metabolism that present with this constellation are the deficiency of GSD type 0 (see later) or of succinyl-CoA:3-oxoacid CoA-transferase (SCOT). In the latter, there is usually constant ketonuria even in the fed state. Recently, inherited deficiency of monocarboxylate transporter 1 has been described as another genetic cause of potentially lethal ketoacidosis resulting from deficient utilization of ketone bodies.

15.3 Disorders of Carbohydrate Metabolism

Patients with *GSD type III* or Cori disease, a consequence of deficiency of the debrancher enzyme, also have low blood concentrations of alanine consistent with their very active gluconeogenesis. They are distinguished on examination from those with ketotic hypoglycemia because they have quite large livers. They do not respond to glucagon after fasting, but they do respond to the fed glucagon test.

Patients with *GSD I* or von Gierke disease, by contrast, have very high concentrations of alanine. Hypoglycemia occurs early in life and is recurrent. Concentrations of lactate are high, as are those of acetoacetate and 3-hydroxybutyrate, and ketonuria is frequent. Hypercholesterolemia and hypertriglyceridemia lead ultimately to cutaneous xanthomata. Hyperuricemia may lead to gouty arthritis or renal disease. The liver is quite large, stature is short, and there may be a cherubic facial appearance. In these patients, the glucagon

test is flat under all conditions. The enzyme deficiency is in glucose-6-phosphatase.

Patients with disorders of gluconeogenesis, such as glycerol kinase deficiency, pyruvate carboxylase deficiency, pyruvate carboxykinase deficiency, or fructose 1,6-diphosphophosphatase deficiency, also tend to have high concentrations of alanine and lactate in the blood. Definitive diagnosis is by enzyme assay in leukocytes or fibroblasts in the case of fructose-1,6-diphosphatase deficiency of biopsied liver. They may be elucidated by the occurrence of hypoglycemia during a monitored fast (see Sect. 41.2.1) especially if glucagon is given after 6 h to deplete the liver of glycogen. The glycemic response to glucagon is normal at 6 h. Loading tests with fructose, glycerol, or alanine may clarify what enzyme to assay or gene to sequence. Patients with glycerol kinase deficiency may have adrenal insufficiency as part of a X-chromosomal contiguous gene deletion syndrome. Some may also have ornithine transcarbamylase deficiency and Duchenne muscular dystrophy.

15.3.1 Glycogen Synthase Deficiency

Deficiency of glycogen synthase (GSD type 0) is a rare, unique cause of hypoglycemia in which there is a distinctive pattern of biochemical abnormality. Patients have fasting hypoglycemia, usually without acidosis but with high concentrations of acetoacetate and 3-hydroxybutyrate along with ketonuria. Thus, this is a ketotic hypoglycemia. Concentrations of alanine and lactate are low at times of hypoglycemia, but feeding or a glucose tolerance test (see Sect. 41.2.3) leads to hyperglycemia and elevated concentrations of lactate in the blood. Glucagon during fasting has no effect on blood concentrations of glucose, lactate, or alanine, while the fed glucagon test yields a glycemic response (see Sect. 41.2.4). Molecular diagnosis by enzyme assay requires biopsied liver, but mutational analysis can define the defect in the gene.

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Approach to the Child Suspected of Having a Disorder of Fatty Acid Oxidation

16

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Key Facts

- Hypoketotic hypoglycemia signifies a disorder of fatty acid oxidation. An absence of ketones in urine at the time of hypoglycemia is an important clue, but the presence of ketonuria may be misleading. Blood levels of free fatty acids and 3-hydroxybutyrate may be required to differentiate.
- The acylcarnitine (MS/MS) profile is a very helpful clue to the diagnosis.
- Medium-chain acyl-CoA dehydrogenase (MCAD) deficiency resulting from the p.Ala985Gly mutation (ACADM gene) is so common that this test should be added to the initial work-up of a patient with hypoketotic hypoglycemia.

Initial presentation of disorders of fatty acid oxidation is usually with hypoketotic hypoglycemia. Hyperammonemia may suggest Reye syndrome but usually results from impaired synthesis of N-acetylglutamate due to the lack of acetyl-CoA. Sudden infant death syndrome (SIDS) is another acute presentation. Some patients present more chronically with myopathy or cardiomyopathy. Medium-chain acyl-CoA dehydrogenase (MCAD) deficiency is the only common disorder, and most patients have the same mutation. So a modern work-up may begin with assay of the DNA for the p.Ala985Gly mutation. Those not revealed in this way are now assayed by MS/MS for the acylcarnitine profile, and this may indicate the diagnosis and the appropriate enzymatic assay. Organic acid analysis should reveal the diagnosis in those with 3-hydroxy-3-methylglutaryl-CoA lyase deficiency. Those not elucidated by these measures are subjected to an algorithmic investigation, central to which is a prolonged monitored fast (Sect. 41.2.1) followed up by loading tests with specific lipids. Especially patients with 3-hydroxy-3-methylglutaryl-CoA synthase deficiency can be difficult to diagnose as in addition to hypoketotic hypoglycemia, there are no abnormal acylcarnitines and no specific elevations of organic acids.

Most patients with disorders of fatty acid oxidation present with fasting hypoglycemia which results from impaired ketogenesis (Chap. 15). The classic initial presentation is with hypoketotic hypoglycemia, usually at 6–12 months of age following a period of fasting of more than 12 h induced by vomiting or anorexia of an

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intercurrent respiratory or gastrointestinal infection. The hypoglycemia may be manifest as lethargy or a seizure. It may progress rapidly to coma. There may be hyperammonemia, and this may lead to diagnosis of Reye syndrome. Liver biopsy in such a patient may reveal microvesicular fat, and this may seem to confirm the diagnosis of Reye syndrome. Actually most patients we see these days, in whom a diagnosis of Reye syndrome has been made, have a disorder of fatty acid oxidation. A few have a urea cycle defect. Orotic aciduria, a hallmark of ornithine transcarbamylase deficiency, has been seen acutely in disordered fatty acid oxidation.

The other major presentation is myopathic (see also Chap. 28). This may be acute with muscle pains and rhabdomyolysis, with or without hypoglycemia. It may be chronic with weakness and hypotonia. A major common presentation is with cardiomyopathy. The first manifestations in some patients are those of congestive failure. Arrhythmias are also common, especially in the acute episode of metabolic imbalance.

A third presentation is with the SIDS. One scenario has been to make a diagnosis of a disorder of fatty acid oxidation, most commonly MCAD deficiency, in an infant and to obtain a history of a previous infant dying of SIDS. We have obtained blood samples saved from newborn screening programs representing the previous infant and made a diagnosis of MCAD deficiency by DNA analysis. This type of posthumous diagnosis has also been made by assay for octanoylcarnitine. Disorders of fatty acid oxidation have also been detected in studies of sudden infant death by assay of postmortem liver for deficiency of carnitine and the presence of key organic acids by gas chromatography-mass spectrometry (GC-MS).

In the patient presenting with hypoglycemia, it is really helpful in suggesting the diagnosis if the test for ketones in the urine is negative. In this situation, hypoglycemia is clearly hypoketotic. However, these patients may be treated with parenteral glucose in the emergency room, and the first urine analysis done hours or days after the hypoglycemia has resolved, and so this clue to the diagnosis is not available. More commonly the diagnosis may be missed because ketones are found in the urine at the time of acute illness, in particular in patients with deficient oxidation of short-chain fatty acids. These patients can readily be shown to be hypoketotic by analyzing the blood, but that does not exclude the possibility that the urine test for ketones may be positive. Documentation that hypoglycemia is hypoketotic is best done by quantification of the concentrations of free fatty acids, acetoacetate, and 3-hydroxybutyrate in the blood. This is most commonly done in the context of a controlled or monitored fast, because the stability of acetoacetate requires planning ahead for its analysis and is seldom considered at the time of the initial acute episode. Nevertheless, a good idea of the presence of hypoketosis can be obtained simply by the assay of 3-hydroxybutyrate and free fatty acids in the blood at the time of the acute hypoglycemic illness.

Remember

Do not be led away from a diagnosis of a disorder of fatty acid oxidation by the presence of ketones in the urine. Absence of ketones in a hypoglycemic patient is useful; their presence may be misleading.

Clues from the routine clinical chemistry laboratory that suggest the presence of a disorder of fatty acid oxidation are elevated levels of uric acid and creatine kinase (CK). Uric acid determinations are not regularly included in metabolic panels for pediatric patients, so it may have to be ordered separately and so may be the CK; levels over a 1000 U/L are commonly encountered on presentation. Alanine and aspartate aminotransferase levels may also be elevated. Analysis of organic acids in the urine may reveal dicarboxylic aciduria, and its pattern may provide direction as to the site of the enzymatic defect. During intervals between episodes of illness, these patients usually appear completely well. Furthermore abnormalities such as the dicarboxylic aciduria and elevations of uric acid and CK usually disappear completely. The patient is most often seen first in consultation after the initial hypoglycemia has been treated, and none of the abnormalities seen in the acute situation are present. Therefore, it has become important to develop a systematic algorithmic approach to the work-up (Fig. 16.1). In a

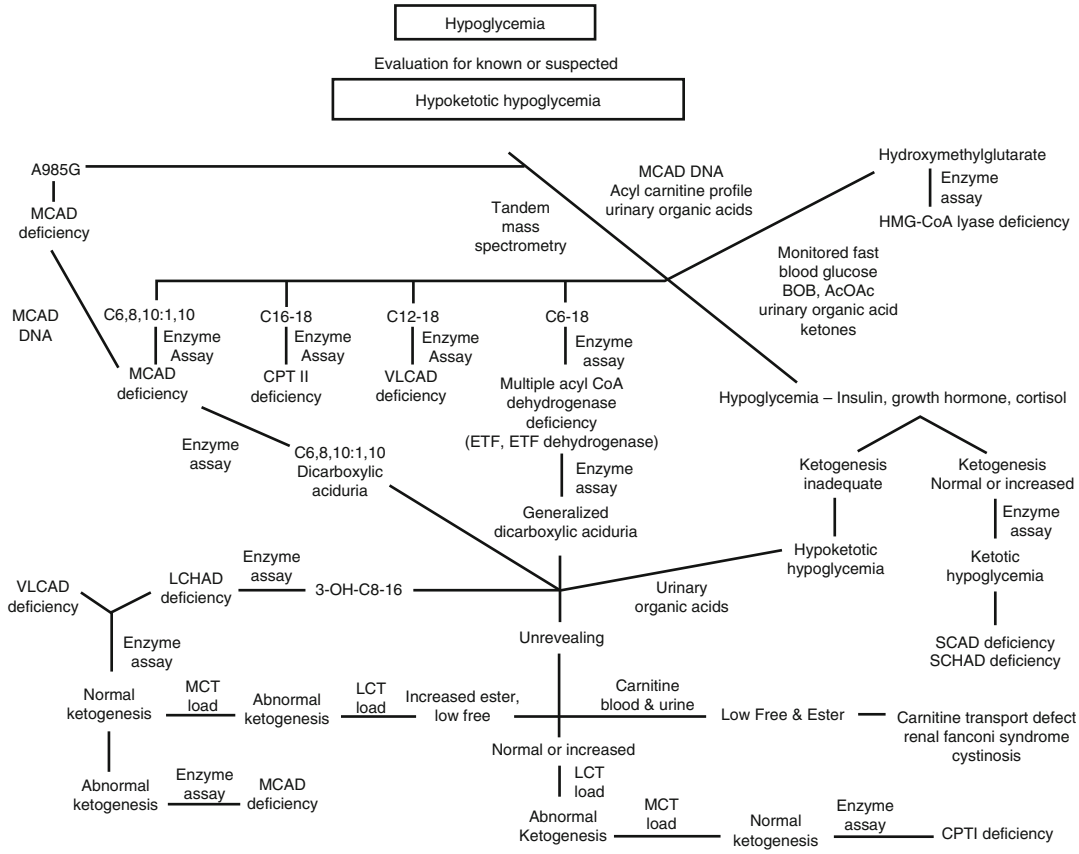

Fig. 16.1 An algorithmic approach to the work-up of a child with a possible disorder of fatty acid oxidation. *MCAD* medium-chain acyl-CoA dehydrogenase, *DNA* deoxyribonucleic acid, *CPT* carnitine palmitoyltransferase, *VLCAD* very long-chain acyl-CoA dehydrogenase, *ETF* electron transfer flavoprotein, *BOB* 3-hydroxybutyric acid, *AcOAc* acetoacetic acid, *HMG* 3-hydroxy-3-methylglutaric acid, *SCAD* short-chain acyl-CoA dehydrogenase, *SCHAD* short-chain hydroxyacyl-CoA dehydrogenase, *MCT* medium-chain triglycerides, *LCT* long-chain triglycerides, *LCHAD* long-chain hydroxyacyl-CoA dehydrogenase

patient suspected of having a disorder of fatty acid oxidation, the algorithm starts with an assay of the DNA from a blood sample for the common mutation in MCAD deficiency, assay of the blood for an acylcarnitine profile, and assay of the organic acids of the urine.

MCAD deficiency is the only common disorder of fatty acid oxidation. Its frequency has been estimated at 1 in 6–10,000 Caucasians. Most patients have the same mutation, an A985G change which makes for a protein containing glutamic acid where a lysine is found in the normal enzyme. So this simple DNA-diagnostic approach can be expected to yield a rapid diagnosis in a large number of the patients with this group of disorders. The acylcarnitine profile obtained by MS/MS can also detect MCAD deficiency; in this instance, octanoylcarnitine is the key compound; hexanoylcarnitine may be present as well. In a patient negative for the p.Ala985Gly mutation and positive in the acylcarnitine assay, enzyme analysis will document MCAD deficiency. In that case, it might be useful to test for the 4 bp deletion, for which there is a rapid test, as there is for p.Ala985Gly and which with p.Ala985Gly accounts for 93 % of the MCAD mutations seen in patients presenting with illness.

Patients detected by newborn screening also have another common mutation p.Thr199Cys, and these patients seldom develop clinical symptomatology.

Acylcarnitine assay may also point to enzyme assay for carnitine palmitoyltransferase (CPT) I and II deficiency, very long-chain acyl-CoA dehydrogenase (VLCAD) deficiency, or multiple acyl-CoA dehydrogenase (MAD) deficiency. Acylcarnitine profiles are obtained by MS/MS, which can be done on as little as a drop of dry blood.

Organic acid analysis can be expected to reveal the presence of 3-hydroxy-3-methylglutarate (HMG) in the presence of HMG-CoA lyase deficiency. This compound is abundant in the urine of affected patients even after recovery from the acute hypoglycemic episode. It has already been indicated that in most of the other disorders organic acid analysis is more often normal than abnormal in intervals between episodes of acute illness.

In some patients, essentially all of those not elucidated by the tests of the last three paragraphs, a controlled prolonged fast is necessary to document that the hypoglycemia really is hypoketotic and to elucidate the nature of the defect. As fasting is not only unpleasant but can be very dangerous in disorders of fatty acid oxidation, it is mandatory that carnitine status and acylcarnitine profile are reliably negative before planning a fasting study. In response to this long fast, the body's first step is lipolysis which releases free fatty acids. In patients with disorders of fatty acid oxidation, concentrations of free fatty acids are higher than those of 3-hydroxybutyrate in the blood when hypoglycemia develops. In addition fatty acids that accumulate in the presence of defective oxidation undergo Ω-oxidation to dicarboxylic acids giving an elevated ratio of dicarboxylic acids to 3-hydroxybutyrate in the analysis of organic acids of the urine. The nature of the dicarboxylic aciduria at the time the hypoglycemia develops may indicate the site of the defect. Thus, C8- to C10-dicarboxylic aciduria is seen in MCAD deficiency and 3-hydroxy long-chain acids in LCHAD deficiency.

Patients during the long fast must be monitored closely so that symptomatic hypoglycemia is avoided. Testing is best done in units where the staff has experience with the protocol. An intravenous line is placed to ensure access for therapeutic glucose, and bedside monitoring of blood concentrations of glucose is done at regular intervals. In abnormalities of fatty acid oxidation, fasting must be long enough to exhaust stores of glycogen and require the mobilization of fat and its oxidation.

Study of the concentrations of carnitine in the plasma and the urine and its esterification may point to the answer, particularly if a low level of free carnitine is documented in the blood and large amounts of esters are being excreted in the urine. Transport of long-chain fatty acids into the mitochondria, where β-oxidation takes place, requires carnitine, and the entry of carnitine into cells such as muscle requires a specific transporter which may be deficient as a cause of hypoketotic hypoglycemia. Assay of carnitine in the blood and urine reveals very low levels of free and esterified carnitine in these patients.

In patients in whom the blood and urine carnitine is normal or increased, a long-chain triglyceride (LCT) load may reveal abnormal ketogenesis, and MCT load normal ketogenesis. In such patients, MCT administration may even reverse fasting-induced hypoglycemia. In such patients, enzyme assay reveals the deficiency of carnitine palmitoyltransferase (CPT I). Esterification of carnitine with fatty acyl-CoA esters is catalyzed by acyltransferases, such as CPT I. The transport of acylcarnitines across the mitochondrial membrane is catalyzed by carnitine acylcarnitine translocase, and then hydrolysis, releasing free carnitine and the fatty acid acyl-CoA, is catalyzed by a second acyltransferase, CPT II. Inborn errors are known for each of these three enzymatic steps.

When the carnitine ester level of the urine is high and the free carnitine level of the blood is low, the basic problem is one in which the metabolic block causes the accumulation of acyl-CoA compounds which are esterified with carnitine and excreted in the urine, particularly in disorders of β-oxidation.

Low plasma-free carnitine may be seen in defective carnitine transporter or may be secondary to any condition in which acyl-CoA esters accumulate. These include the disorders of fatty acid oxidation and the organic acidurias (see Chap. 13). Elevation in the level of urinary esterified carnitine is also seen in both latter sets of conditions. Especially patients with 3-hydroxy-3-

methylglutaryl-CoA synthase deficiency can be difficult to diagnose as in addition to hypoketotic hypoglycemia there are no abnormal acylcarnitines and no specific elevations of organic acids. Molecular analysis then confirms the diagnosis.

Remember

In β-oxidation, the fatty acid is successively shortened by two carbons, releasing acetyl-CoA. Specific dehydrogenases with overlapping specificities for chain length include short-chain acyl-CoA dehydrogenase (SCAD), MCAD, and VLCAD. In addition, a trifunctional enzyme catalyzes long-chain 3-hydroxyacyl-CoA dehydrogenation (LCHAD), long- and medium-chain 2-enoyl-CoA hydration, and 3-oxoacyl-CoA thiolysis. Diseases involving defects in each of these steps have been defined. The last two known defects leading to hypoketotic hypoglycemia are HMG-CoA synthetase and HMG-CoA lyase deficiencies, the enzymes producing ketone bodies from acetyl-CoA. Whereas the latter usually constantly shows a characteristic organic aciduria and often elevated C5-OH-acylcarnitine, the first one is very difficult to spot as organic acid analysis is nonspecific and acylcarnitines normal. In this constellation, enzyme analysis of the liver or direct mutation analysis will provide the diagnosis. All of these patients would be expected to have abnormal ketogenesis following an LCT load. When these patients are tested with MCT, MCAD patients display abnormal ketogenesis, while those with LCAD, LCHAD and VLCAD deficiency have normal ketogenesis. The follow-up of this testing is via assay for the specific enzyme or enzymes or molecular testing as suggested in Fig. 16.1.

The specific enzyme assays for specific disorders are technically demanding and not generally available. A reasonable step following the fast, if a specific disease is not identified, is to pursue a more general study of metabolism in cultured cells in which oxidation of fatty acids of varying chain length is studied in vitro, and carnitine esters are separated and identified after incubation with ^{14}C- or ^{13} C-labeled long-chain fatty acids such as hexadecanoate. Impaired oxidation of long-chain fatty acids such as palmitate in vitro may also be seen in patients with mitochondrial disorders (see Chap. 14). Such patients may also have hypoketotic hypoglycemia with increased levels of 3-hydroxydicarboxylic acids because of failure to oxidize the NADH produced in the 3-hydroxyacyl-CoA dehydrogenase step via the electron transport chain. Such patients usually display lactic acidemia.

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Work-Up of the Patient with Hyperammonemia

17

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Key Facts

- Routine clinical chemistry is helpful in pointing the direction of the work-up of a patient with hyperammonemia. Patients with urea cycle defects are alkalotic or normal; those with organic acidemias or disorders of fatty acid oxidation are acidotic. The presence or absence of ketosis distinguishes the latter two.
- Urea cycle defects are elucidated by analysis of the amino acids of the plasma (glutamine, citrulline, arginine), urine (argininosuccinic acid, orotic acid), or dried blood (argininosuccinic acid).
- Urinary orotic aciduria distinguishes ornithine transcarbamylase deficiency from carbamoyl phosphate I synthetase and *N*-acetylglutamate synthase deficiencies.

Elevated concentrations of ammonia occur episodically in a variety of inherited diseases of metabolism. These include not only the disorders of the urea cycle but also organic acidurias and disorders of fatty acid oxidation. Effective management is predicated on a precise diagnosis and understanding of the nature of the pathophysiology. A systematic progression from routine clinical chemistry to more specific analyses of amino acids, organic acids, and acylcarnitines will lead the clinician to the diagnosis. Liver biopsy has been required for enzymatic diagnosis of carbamoyl phosphate synthetase I deficiency and ornithine transcarbamylase (OTC) deficiency, as well as *N*-acetylglutamate synthase deficiency. Meanwhile, however, mutational analysis has usually obviated the need for this invasive approach.

Deficiencies of enzymes of the urea cycle and some other disorders, such as the organic acidurias and disorders of fatty acid oxidation, present with hyperammonemia. Normally, values of NH_3 are <110 μmol/L (190 μg/dL) in newborns and below 80 μmol/L (140 μg/dL) in older infants to adults. In the newborn period, a diagnostic work-up for hyperammonemia is warranted at values >150 μmol/L (260 μg/dL) and in older infants to adults at values >100 μmol/L (175 μg/dL). The classic onset of urea cycle defects is with sudden potentially lethal neonatal coma. The male with OTC deficiency exemplifies the classic presentation, but distinct disorders result from deficient activity of each of the enzymes of the urea cycle (Fig. 17.1).

The work-up of an infant in hyperammonemic coma is shown (Fig. 17.2). The differential diagnosis is very important because different disorders require different treatments. It must proceed with dispatch if the correct diagnosis is to be

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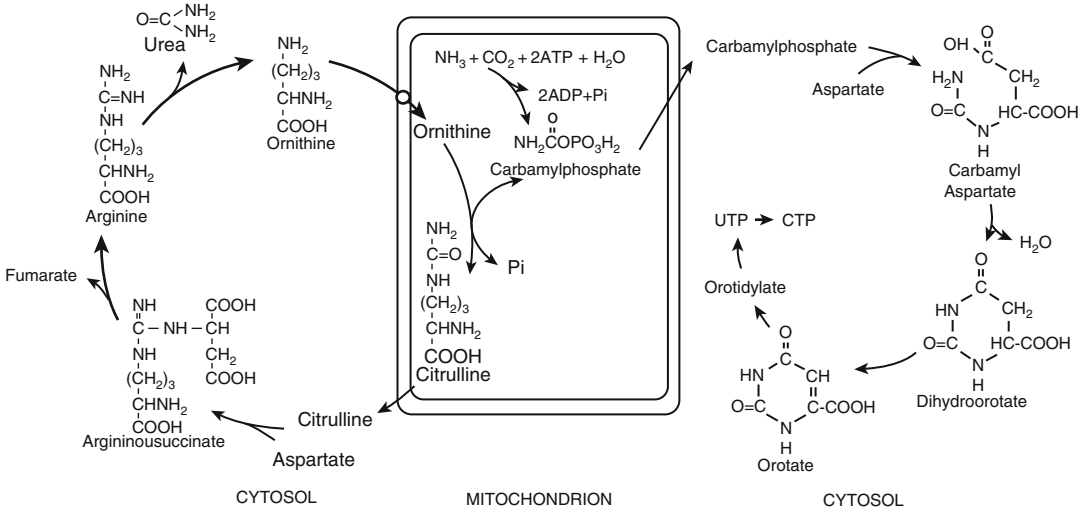

Fig. 17.1 Urea cycle

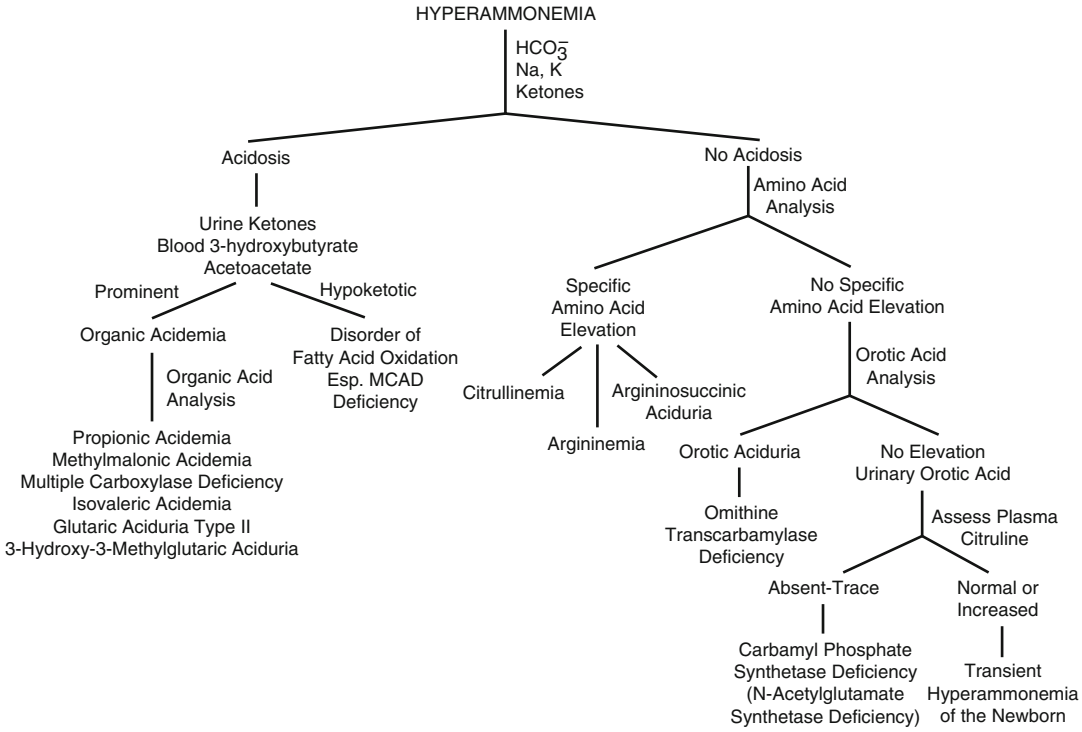

Fig. 17.2 An approach to the stepwise evaluation of a patient with hyperammonemia

made and appropriate therapy instituted to prevent death or permanent damage to the brain be done. The initial steps in the algorithm are available in the routine clinical chemistry laboratory.

The first step in the evaluation of a patient, especially an infant in coma, is the measurement of the concentration of ammonia in the blood. Since ammonia analysis is very sensitive to various mistakes (e.g., use of test tubes containing ammonium heparin, use of a tourniquet, lack of cooling in ice water, vigorous shaking, delayed start of the analysis), a correct pre-analytical process is indispensable to obtain reliable test results. Very good and reliable readings can be obtained

with bedside ammonia checkers. However, some machines have an upper reading fixed at below 300 μmol/L ammonia. Then the sample must be diluted and reanalyzed. We have repeatedly received clinically deteriorating children with supposedly this level where ammonia had in fact already raised >5,000 μmol/L. The next step after verifying hyperammonemia is the quantification of serum concentrations of bicarbonate, sodium, chloride, and the anion gap and testing of the urine for ketones. Metabolic acidosis and/or increased anion gap lowers the likelihood of a urea cycle disorders, which tend to present with respiratory alkalosis, but does not necessarily exclude this diagnosis. The acidotic patient with massive ketosis has an organic aciduria, such as propionic aciduria, methylmalonic aciduria, isovaleric aciduria, glutaric aciduria type II, or multiple carboxylase deficiency (see Chap. 13). A specific diagnosis is made by quantitative analysis of the organic acids of the urine or of the acylcarnitines of the blood. The disorders of fatty acid oxidation, which may present with hyperammonemia, are characteristically hypoketotic (see Chap. 16). However, testing of the urine for ketones may be misleading. We have observed impressively positive urinary KetoStix tests in patients with disorders of fatty acid oxidation. Quantification of concentrations of free fatty acids together with acetoacetic and 3-hydroxybutyric acid in the blood of such patients reliably indicate them to have defective ketogenesis. The acute crises in these patients often display hypoglycemia, and diagnoses of Reye syndrome have been made. A patient with hyperammonemic coma resulting from a urea cycle defect may develop hypoxia, leading to lactic acidosis. Adequate oxygenation and perfusion should be assured before a urea cycle defect is conceptually excluded and a diagnosis of organic aciduria pursued.

Remember

Hyperammonemia occurs not only in disorders of the urea cycle but also in organic acidemias and disorders of fatty acid oxidation. Testing for organic acids in urine and acylcarnitine profiles lead to the correct diagnosis.

The definitive diagnosis of a urea cycle abnormality is initiated by the quantitative assay of the concentrations of amino acids in the blood and urine. The plasma concentrations of amino acids provide the diagnosis in patients with argininemia and citrullinemia. Study of the urine is required in argininosuccinic aciduria.

If hyperammonemic patients are found not to have a diagnostic abnormality in the concentration of an amino acid, the urine should be tested for the excretion of orotic acid. This is not reliably performed as a part of organic acid analysis by GCMS, and a specific assay for the compound should be employed. Orotic aciduria is found in patients with OTC deficiency. It is also found in citrullinemia and in argininemia. In a patient without an elevation of a specific amino acid and without orotic aciduria, the usual diagnosis is carbamoyl phosphate synthetase (CPS) I deficiency. *N*-acetylglutamate synthase deficiency will present with an indistinguishable clinical and biochemical constellation but is much rarer. Very similar is also the mitochondrial carbonic anhydrase VA deficiency which is also associated with low-normal orotic acid excretion and hyperlactatemia. Carbonic anhydrase VA provides bicarbonate to CPS, pyruvate carboxylase, propionyl-CoA carboxylase, and 3-methylcrotonyl-CoA carboxylase. Transient hyperammonemia of the newborn may also present this picture, but for reasons that are not clear, this disorder is nowhere near as commonly encountered as it was 30 years ago. Failure of immediate closure of the ductus venosus after birth is thought to result in (transient) hyperammonemia of the newborn because portal blood bypasses the liver. The definitive diagnoses of CPS, OTC, *N*-acetylglutamate synthase and mitochondrial carbonic anhydrase VA deficiencies are made by mutation analysis instead of a liver biopsy. If a liver biopsy was planned, it would be well to bring the patient into control of the blood concentration of ammonia and to normalize clotting. The levels of arginine and citrulline in the blood may be helpful in distinguishing CPS I deficiency from transient hyperammonemia of the newborn, in

which it is usually normal or elevated. In neonatal CPS, OTC or *N*-acetylglutamate synthase deficiencies, citrulline is barely detectable. In citrullinemia, concentrations of citrulline in plasma usually exceed 1,000 μmol/L. They are elevated to levels of 150–250 μmol/L in argininosuccinic aciduria, and to 54 ± 22 μmol/L in transient hyperammonemia in the newborn. The normal range is 6–20 μmol/L. Persistent hypocitrullinemia can, in general, be viewed as a marker for disorders of mitochondrial urea cycle enzymes (*N*-acetylglutamate synthase, CPS I, and ornithine carbamoyltransferase) as well as for deficient pyrroline-5-carboxylate synthetase. Citrulline synthesis is directly coupled to ATP concentration. Consequently, hypocitrullinemia can also be observed in patients with respiratory chain disorders, especially as caused by NARP mutation.

Amino acid analysis also reveals concentrations of glutamine to be regularly elevated in patients with hyperammonemia except for those having classic organic acidurias. Concentrations of alanine are usually elevated, while concentrations of aspartic acid are elevated in some patients. These are nonspecific findings. They are not helpful in the differentiation of the different causes of hyperammonemia. They are potentially helpful in diagnosis, as sometimes an elevated level of glutamine is found in a patient that had not been expected to have hyperammonemia, and while concentrations of ammonia may vary from hour to hour, the elevated concentration of glutamine signifies a state in which there has been more chronic overabundance of ammonia. The transamination of pyruvic acid to alanine and oxaloacetic acid to aspartic acid, as well as 2-oxoglutaric acid to glutamic acid and its subsequent amidation to glutamine are detoxification responses to the presence of excessive quantities of ammonia. Since in patients with classic organic acidurias various enzymes in the tricarboxylic acid cycle are inhibited by toxic metabolic products (e.g., propionyl-CoA, methylcitrate), the availability of 2-oxoglutarate and thus glutamine synthesis is impaired.

Amino acid analysis may also reveal elevations of tyrosine, phenylalanine, and the branched-chain amino acids. If these are substantial, a primary liver disease should be carefully sought.

Some patients with defects of urea cycle and residual enzyme activity, especially many females with OTC deficiency, display completely unremarkable values of ammonia, amino acids, and orotate in between the crises. In female patients with OTC deficiency, ammonia may be normal even during crisis, whereas plasma and cerebral glutamine concentrations are high. It is indispensable that the cause of an unexplained symptomatic episode of hyperammonemia should always be investigated in detail even after the patient recovers, even in adults or aged adults. In those instances, in which the amino acids are normal, an allopurinol loading test may reveal the diagnostic direction.

The differential diagnosis of hyperammonemia also includes the HHH syndrome, which results from deficiency of the ornithine transporter in the mitochondrial membrane, lysinuric protein intolerance, resulting from a (re)absorption defect of the dibasic amino acids or by deficiency of the amino acid transporter citrin which occurs predominantly in east Asians. HHH signifies hyperammonemia, hyperornithinemia, and homocitrullinuria. It is usually suspected first by the identification of large amounts of homocitrulline in the urine. The diagnosis of lysinuric protein intolerance is best made by finding very low levels of lysine in the blood. These patients fail to thrive, and when body stores of lysine are much depleted, the characteristic amino aciduria may not be present; it returns when the diagnosis is made, and blood concentrations of amino acid are brought to normal. The metabolic abnormalities become more obvious by calculating the fractional clearances of lysine and other dibasic amino acids. The concentration of citrulline in the blood may be high. Citrin deficiency presents usually between the second and fourth decade of life as recurrent hyperammonemia with neuropsychiatric symptoms. Onset of symptoms can be rapidly precipitated by medications, surgery, and alcohol consumption.

In older children and adults, a number of acquired disorders can also present with hyper-

ammonemia, especially liver disease, Reye syndrome, drug toxicity, e.g., chemotherapeutics, and hepatotoxins. History, prothrombin time, a urinary toxic screen, and plasma amino acid pattern should help to differentiate these disorders. Symptomatic hyperammonemia may also result from a urinary tract infection or postsurgical superinfection of large hematomas in which the infecting *Proteus mirabilis* has urease activity, which produces ammonia from urea.

General Suggestions for Reading

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Work-Up of the Patient with Acute Neurological or Psychiatric Manifestations

18

William L. Nyhan, Stefan Kölker, and Georg F. Hoffmann

Key Facts

- Many inherited metabolic diseases present with acute encephalopathy to emergency departments where the emergency doctor/neurologist is confronted with coma, seizures, acute ataxia, extrapyramidal symptoms, stroke, and/or psychosis.
- Metabolic disorders must be included in the diagnostic work-up of acute encephalopathy/psychosis from the beginning.
- Acute metabolic cerebral edema must be especially quickly recognized and treated in order to prevent herniation and death. All too often, only a diagnosis of encephalitis is initially pursued.
- The initial diagnostic approach is based on a few metabolic screening tests. It is important that biologic fluids are collected during the acute attack.
- In addition to diagnostic constellations of metabolites in body fluids, important diagnostic clues, such as white matter abnormalities, cortical or cerebellar atrophy, and injury of the basal ganglia can be derived from cranial magnetic resonance imaging (MRI).

Acute or recurrent attacks of neurological or psychiatric features such as coma, ataxia, or abnormal behavior are major presenting features including several late-onset, inborn errors of metabolism. The initial diagnostic approach to these disorders is based on a few metabolic screening tests. It is important that biologic fluids are collected during the acute attack, and at the same time, it is also useful to take specimens both before and after treatment. Some of the most significant metabolic manifestations, such as acidosis and ketosis, may be moderate or transient, dependent on symptomatic treatment. On the other hand, at an advanced state of organ dysfunction, many laboratory abnormalities such as metabolic acidosis, lactic acidosis, hyperammonemia, and signs of liver failure may be secondary consequences of hemodynamic shock and multisystem failure. In organic aciduria such as methylmalonic aciduria, propionic

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aciduria, or isovaleric aciduria, moderate to severe hematological manifestations are common. In addition, these disorders are often characterized by neutropenia and, especially in infancy, thrombocytopenia. Recurrent infections, particularly mucocutaneous candidiasis, may be a common finding.

Flow charts for the differential diagnosis and diagnostic approach to acute neurological or psychiatric manifestations are presented in Figs. 18.1, 18.2, and 18.3.

Acute attacks of neurological or psychiatric manifestations, such as coma, intractable sei-

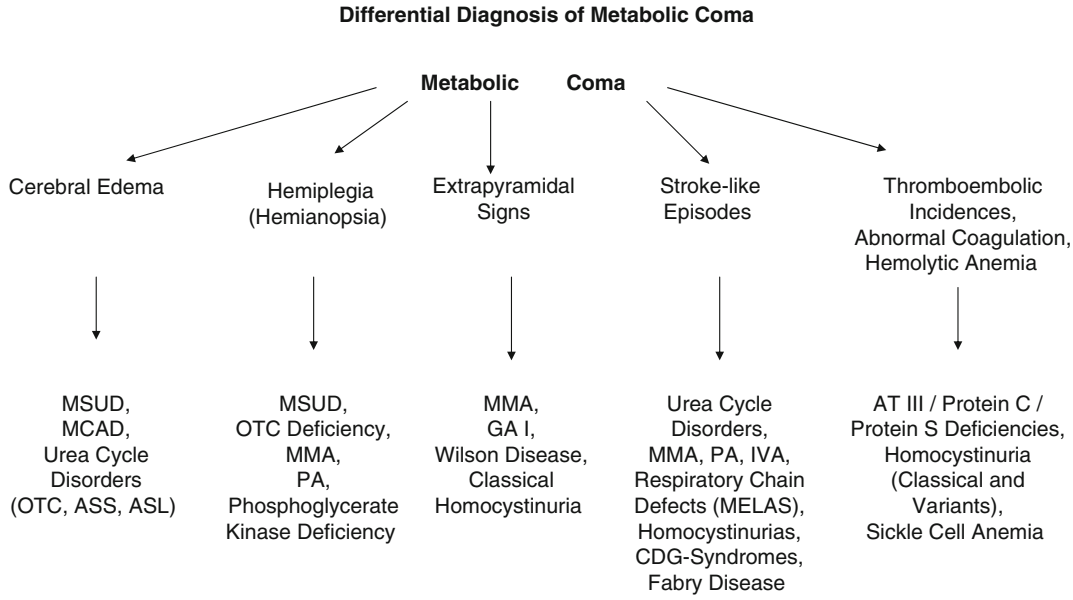

Fig. 18.1 Differential diagnosis of metabolic coma. *ASS* argininosuccinate synthetase deficiency, citrullinemia, *ASL* argininosuccinate lyase deficiency, argininosuccinic aciduria, *AT* antithrombin, *CDG* congenital disorders of glycosylation, *GAI* glutaric aciduria type I, *IVA* isovaleric aciduria, *MCAD* medium-chain acyl-CoA dehydrogenase, *MELAS* (mitochondrial encephalopathy, lactic acidosis, and stroke-like episodes), *MMA* methylmalonic aciduria, *MSUD* maple syrup urine disease, *OTC* ornithine transcarbamylase deficiency, *PA* propionic aciduria

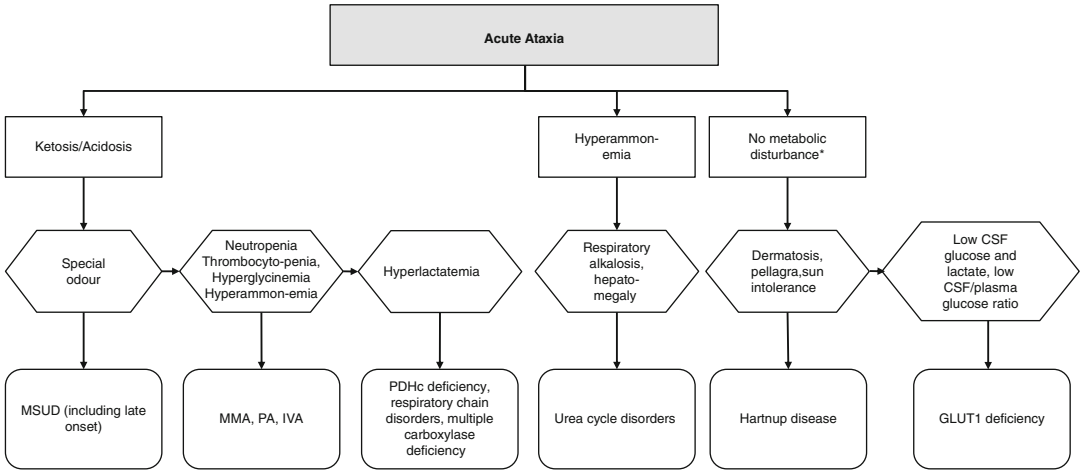

Fig. 18.2 Differential diagnosis of metabolic causes of acute ataxia. *IVA* isovaleric aciduria, *MMA* methylmalonic aciduria, *MSUD* maple syrup urine disease, *PA* propionic aciduria

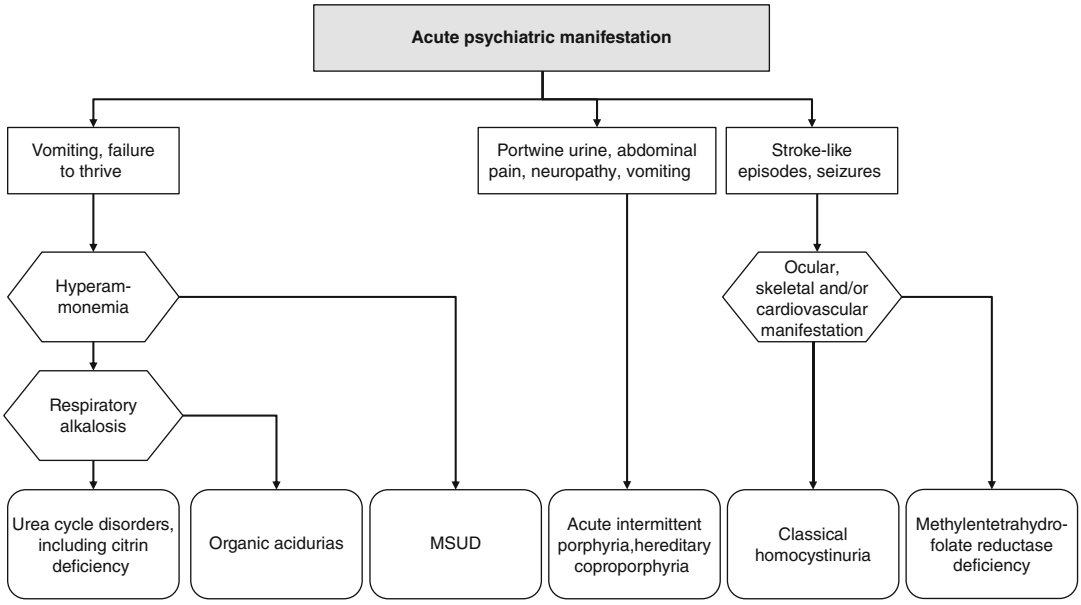

Fig. 18.3 Differential diagnosis of metabolic causes of acute psychiatric manifestations coma

zures, stroke, ataxia, or abnormal behavior, resulting from inherited metabolic diseases require prompt and appropriate diagnostic and therapeutic measures (see also Chap. 27). Acute metabolic cerebral edema must be especially quickly recognized and treated in order to prevent herniation and death. All too often, only a diagnosis of encephalitis is initially pursued. Cerebral edema is observed particularly in the acute urea cycle disorders [most frequently ornithine transcarbamylase (OTC) deficiency] and maple syrup urine disease (MSUD). Seizures can be observed in all acutely deteriorating metabolic disorders but again are especially prominent in urea cycle disorders. In contrast to mitochondriopathies or organic acidurias that involve the mitochondrial metabolism of CoA-activated compounds, the metabolic blocks in urea cycle disorders and MSUD do not directly interfere with mitochondrial energy production, and the typical signs of acute mitochondrial decompensation such as lactic acidosis may be lacking. In urea cycle disorders, however, enhanced astrocytic glutamine production is energy demanding, and brain edema and arginine depletion results in inadequate supply with energy substrates. Lactic acidosis may develop later as the condition of the patient deteriorates. Acute hemiplegia may be a presenting symptom; it has also been reported in patients with organic acidurias, in particular propionic aciduria and methylmalonic aciduria as well as mitochondriopathies.

Remember

Acute or recurrent attacks of neurological or psychiatric symptoms such as coma, ataxia, or abnormal behavior are major presenting features of several inborn errors of metabolism, especially in late-onset disease. The initial diagnostic approach is based on a few metabolic screening tests, in particular blood ammonia, lactate, amino acids, and urinary organic acids. It is important that the biologic fluids are collected simultaneously at the time of the acute attack. Flow charts for the differential diagnosis and diagnostic approach to acute neurological manifestations such as coma, ataxia, and psychiatric aberrations are presented in Figs. 18.1, 18.2, and 18.3. Coma can develop and progress quickly in sequel with any of the other neurological manifestations.

An acute onset of extrapyramidal signs during the course of a nonspecific intercurrent

illness, minor surgery, accident, or even immunization may initially be misinterpreted as encephalitis but represent a conspicuous feature of several metabolic disorders. In glutaric aciduria type I (GA1), a dystonic dyskinetic movement disorder is caused by the acute destruction of the basal ganglia, specifically the striatum. The acute encephalopathic crisis in GA1 typically occurs between the ages 6 and 18 months; affected children may have had mild neurological abnormalities prior to the acute episode and frequently are macrocephalic. Almost half of the patients with Wilson's disease present with neurological symptoms, usually after the age of 6 years. Dysarthria, incoordination of voluntary movements, and tremor are the most common signs. There may be involuntary choreiform movements, and gait may be affected. Patients with Segawa disease develop dystonia in the same age range, often deteriorating in the afternoon or after prolonged exercise. Some patients with propionic acidemia present a basal ganglia picture indistinguishable from those of GA1 or Lesch–Nyhan disease.

Table 18.1 Metabolic causes of stroke and stroke-like episodes

Homocystinuria, methylenetetrahydrofolate reductase deficiency
Mitochondrial encephalomyopathy
MELAS
Pyruvate dehydrogenase deficiency
Pyruvate carboxylase deficiency
Respiratory chain disorders
Leigh disease
Urea cycle disorders
Carbamoyl phosphate synthetase I deficiency
Ornithine transcarbamylase deficiency
Argininosuccinate synthase deficiency
Argininosuccinate lyase deficiency
Arginase deficiency
Methylmalonic aciduria
Propionic aciduria
Congenital disorders of glycosylation
Fabry disease
Menkes disease
Sulfite oxidase deficiency
Purine nucleotide phosphorylase deficiency
Vanishing white matter disease

Acute hemiplegia may be another presenting symptom; it has been reported in patients with organic acidurias, in particular propionic aciduria and methylmalonic aciduria, mitochondriopathies, and urea cycle disorders.

Acute stroke-like episodes and strokes occur in several metabolic disorders (Table 18.1). They are the hallmark feature of the mitochondrial encephalopathy, lactic acidosis, and stroke-like episodes (MELAS) syndrome, a disorder caused by mutations in the $tRNA^{Leu(UUR)}$ gene in the mitochondrial DNA. In total, 80 % of patients carry the mutation c.3243A>G. Acute episodes may present initially with vomiting, headache, convulsions, or visual abnormalities and may be followed by hemiplegia or hemianopia. The morphological correlates are true cerebral infarctions, but there is no evidence of vascular obstruction or atherosclerosis, and they do not correspond to vascular distribution. However, effective treatment with high arginine in MELAS patients and the finding of low plasma citrulline and arginine concentrations in classic organic acidurias challenge the notion that vascular dysfunction might not be involved, since arginine is the precursor for endothelium-derived nitric oxide. Mitochondrial diseases manifest in the CNS primarily in high-energy-consuming structures such as basal ganglia, capillary endothelium, and the cerebellum. Whereas disturbed function of the cerebellum and the basal ganglia leads to characteristic disorders of movement, disturbed capillary function results in fluctuating neurological signs from stroke-like episodes to nonspecific epileptic encephalopathy, mental deterioration, and progressively accumulating damage of both grey and white matter. Stroke-like episodes (metabolic stroke) are also observed in other metabolic disorders such as the congenital disorders of glycosylation, urea cycle disorders, and methylmalonic and propionic acidurias. Strokes may be absent in affected members of families with the MELAS syndrome; some have migraine-like headaches as the only manifestation of the disease, while others manifest only diabetes or are asymptomatic. Classic cerebral strokes as well as cardiovascular

accidents may also be caused by various metabolic disorders that cause vascular disease such as classical homocystinuria, the thiamine responsive megaloblastic anemia syndrome, and Fabry disease. Vascular changes resulting from altered elastic fibers are the cause of ischemic cerebral infarctions in Menkes disease. True thromboembolic events may be caused by defects in the anticoagulant systems, such as antithrombin III, protein C, or protein S deficiencies.

Acute ataxia (Fig. 18.2) occurs in organic acidurias, late-onset MSUD, and mitochondrial disorders, most specifically in association with peripheral neuropathy in defects of the pyruvate dehydrogenase complex. Moderate or substantial elevation of lactate with a normal lactate/pyruvate ratio and absence of ketosis supports this diagnosis. Acute ataxia associated with a high lactate/pyruvate ratio is suggestive of multiple carboxylase deficiency or respiratory chain defects. In the latter, thrombocytosis is often observed in contrast to the thrombocytopenia associated with organic acidurias. In late-onset MSUD, a special odor may be noticed.

Some inherited metabolic diseases, like Hartnup disease and glucose transporter 1 (GLUT1) deficiency, may present with clinical symptoms of recurrent acute ataxia without causing general metabolic abnormalities (in plasma and urine). Other typical symptoms such as skin rashes, pellagra, and sun intolerance may lead to analysis of plasma and urinary amino acids which provides the specific diagnosis. The characteristic pattern is of an excess of neutral monoaminomonocarboxylic acids in urine with (low) normal concentrations in plasma. The diagnosis of GLUT1 deficiency requires a lumbar puncture with analysis of plasma and CSF glucose-matched samples.

Acute and/or fluctuating psychiatric manifestations (Fig. 18.3) are most suspicious for disorders of the urea cycle but also occur in organic acidurias. In the latter, the metabolic derangement is most often associated with ketoacidosis (see also Chap. 13).

Urea cycle disorders do not often give any other hints to diagnosis but the neurological symptomatology, and ammonia should be measured in any patient with unexplained acute neurological or psychiatric symptomatology. Urea cycle disorders, such as OTC deficiency, argininosuccinic aciduria, citrullinemia, arginase deficiency, lysinuric protein intolerance, or citrin deficiency may present at any age with acute or recurrent episodes of hyperammonemia (Chap. 17). Clinical features may include acute ataxia or psychiatric symptoms such as hallucinations, delirium, dizziness, aggressiveness, anxiety, or schizophrenia-like behavior. Women with late-onset urea cycle disorders often present during the postpartum period (specifically postpartum day 3–12) with postpartum psychosis, a severe mood disorder, or delirium. Enhanced protein breakdown during uterine involution is thought to trigger these episodes. In addition there may be hepatomegaly, acute, and/or chronic liver dysfunction. The correct diagnosis will be missed unless ammonia levels are determined in plasma at the time of acute symptoms. Hyperammonemia may be moderate or mild (150–250 μmol/L) even in the presence of deep coma, and especially the late-onset forms of urea cycle disorders such as OTC deficiency, the diagnosis can be easily missed and the patient thought to have schizophrenia, encephalitis, or even intoxication from alcohol or abused drugs. Diagnostic procedures should include analyses of plasma amino acids, urinary organic acids, and orotic acid but most importantly ammonia during the acute episode.

Acute intermittent porphyria and hereditary coproporphyria usually present with recurrent attacks of vomiting, abdominal pain, unspecific neuropathy, and psychiatric symptoms. These disorders have to be excluded in the differential diagnosis of suspected psychogenic complaints and hysteria. Diagnosis can be made by specific analyses of porphyrins. Patients affected with disorders of the cellular methylation pathway such as methylenetetrahydrofolate reductase deficiency may also present with psychiatric symptoms. These often resemble acute schizophrenic episodes, but they may respond to folate therapy. Homocysteine is elevated in plasma, and a positive Brand reaction in the urine may be the first abnormal laboratory finding. Other neurological features include stroke-like episodes, seizures, and myelopathy. Diagnosis is made by

analysis of amino acids and homocysteine in plasma and CSF. Methyltetrahydrofolate in CSF is greatly reduced.

General Suggestions for Reading

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Emergency Treatment of Inherited Metabolic Diseases

19

William L. Nyhan, Stefan Kölker, and Georg F. Hoffmann

Key Facts

- Timely and correct intervention during the initial presentation of metabolic imbalance and during later episodes precipitated by dietary indiscretion or intercurrent illnesses are the most important determinants of outcome in inherited metabolic diseases in patients at risk for acute metabolic decompensation.
- Patients should be supplied with an emergency letter, card, or bracelet containing instructions for emergency measures and phone numbers.
- Logistics of therapeutic measures should be repeatedly evaluated by the specialist team with the family and the primary care physician(s).

The most critical challenge in many inherited metabolic diseases is the timely and correct intervention during acute metabolic decompensation in the neonatal period or in later recurrent episodes. Fortunately, there is only a limited repertoire of pathophysiological sequences in the response of infant to illness, and consequently, a limited number of therapeutic measures need to be taken. Three major groups of disorders at risk for acute metabolic decompensation that require specific therapeutic approaches in emergency situations were outlined by Prietsch and colleagues (see also Fig. 4.1):

- Disorders of intermediary metabolism that cause *acute intoxication* through the accumulation of toxic molecules
- Disorders in which there is *reduced fasting tolerance*
- Disorders in which there is *disturbed energy metabolism*

A preliminary differentiation of these three groups should be possible with the help of basic investigations, available in every hospital setting, namely, the determination of acid–base balance, glucose, lactate, ammonia, and ketones; see Chap. 12, especially Table 12.1. With this information, appropriate therapy can be initiated even before a precise diagnosis is known. In instances, especially during the initial manifestation of a metabolic disease, i.e., when the exact diagnosis is not yet known, measures must be quick, precise, and not halfhearted, and every effort must be undertaken to obtain the relevant diagnostic information within 24 h or sooner, i.e., results of acylcarnitines in dried blood spots or plasma, amino acids in plasma, and organic acids in urine.

In all instances, provision of ample quantities of fluid and electrolytes is indispensable.

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Table 19.1 Emergency treatment: energy needs in infants

Disorders requiring anabolism (*acute intoxication*): *60–100 kcal/kg/day*
Maple syrup urine disease, urea cycle disorders, glutaric aciduria type I ⇒ glucose (10–)15(–20) g/kg/day + fat 2 g/kg/day
Classic organic acidurias: start with IV glucose of 10 g/kg/d, increase the glucose intake step by step (to 15 or 20 g/kg/day) while carefully monitoring serum lactate; introduce fat (starting with 1 g/kg/day) after ketoacidosis has been treated successfully
Always: + insulin, starting with 0.05 U/kg/h; adjustments are made depending on blood glucose (useful combination is 1 U/8 g glucose)
Early: central venous catheterization
Disorders requiring glucose stabilization (*reduced fasting tolerance*):
Fatty acid oxidation defects; HMG-CoA lyase deficiency; HMG-CoA synthase deficiency glycogen storage diseases types I, III, and IX; disorders of gluconeogenesis; galactosemia; fructose intolerance; tyrosinemia I ⇒ glucose 10 g/kg/day
Disorders requiring restriction of energy turnover (*disturbed energy metabolism*):
PDHC deficiency ⇒ reduce glucose supply: glucose 5–7 g/kg/day (carefully monitor lactate)
Add fat: 2–3 g/kg/day
Test response to pharmacological doses of thiamin: 3×50–100 mg/day
Electron transport chain (OXPHOS) disorders ⇒ glucose 10(–15) g/kg/day
Consider cofactor treatment (thiamin, riboflavin, coenzyme Q_{10}) and carnitine (100 mg/kg/day) to improve aerobic energy production

Differences in the therapeutic approach relate primarily to energy requirements and methods of detoxification (Table 19.1).

Remember

Emergency treatment must start without delay.

19.1 Acute Intoxication (Fig. 4.1)

In diseases, in which symptoms develop because of *acute intoxication*, rapid reduction of toxic molecules is a cornerstone of treatment. In disorders of amino acid catabolism, such as maple syrup urine disease, classical organic acidurias, or urea cycle defects, the toxic compounds may be derived from exogenous as well as endogenous sources. In addition to stopping the intake of natural protein until the crisis is over but no longer than 12–48 h, reversal of catabolism, promotion of anabolism, and consequently reversal of the breakdown of endogenous protein are the major goals.

Table 19.2 Oral administration of fluid and energy for episodic acute intercurrent illness in patients with metabolic disorders

Age (years)	Glucose polymer/maltodextrin solution		
	(%)	(kcal/100 mL)	Daily amount
0–1	10	40	150–200 mL/kg
1–2	15	60	95 mL/kg
2–6	20	80	1,200–1,500 mL
6–10	20	80	1,500–2,000 mL
>10	25	100	2,000 mL

Adapted from Dixon and Leonard (1992). Oral substitution should only be given during minor intercurrent illnesses and for a limited time of 2–3 days. Intake of some salt should also be provided. The actual amounts given have to be individually adjusted, e.g., according to a reduced body weight in an older patient with failure to thrive

In patients, known to have a disorder of amino acid catabolism, who develop an intercurrent illness and manifestations of metabolic imbalance, such as ketonuria in a patient with an organic aciduria, primary emergency measures at home for 24–48 h consist of frequent feedings with a high carbohydrate content and some salt (Table 19.2) and reduction, even to zero, of the intake of natural protein. In diseases such as maple syrup urine disease, the individual amino acid mixture devoid of the amino acids of the defective pathway is continued. Detoxifying medication, such as carnitine in the organic acidurias, benzoate, phenylacetate, and arginine or citrulline in the hyperammonemias, is employed. During this time period, patients should be reassessed regularly regarding state of consciousness, fever, and food tolerance. If the intercurrent illness continues into the third day, symptomatology worsens, or if vomiting compromises external feedings, admission to hospital is mandatory.

In the hospital, therapy must be continued without interruption. In most instances, an

intravenous line is essential, but nasogastric administration, for instance, of amino acid mixtures in maple syrup urine disease is very useful. The management of many of these infants is greatly simplified by the early placement of a gastrostomy tube. This can usually be discontinued after infancy.

Remember

Calculations for maltodextrin/dextrose, fluid, and protein intake should be based on the expected and not on the actual weight!

Large amounts of energy are needed to achieve anabolism, e.g., in neonates >100 kcal/kg bodyweight/day. In a sick baby, this can usually be accomplished only by hyperosmolar infusions of glucose together with fat through a central venous line. Insulin should be started early, especially in the presence of significant ketosis or in maple syrup urine disease, to enhance anabolism. One approach is to use a fixed ratio of insulin to glucose (Table 19.1). The administration of lipids intravenously can often be increased to 3 g/kg if serum levels of triglycerides are monitored.

Acute episodes of massive ketoacidosis, as seen in methylmalonic or propionic aciduria, require especially vigorous supportive therapy. Adequate rehydration, correction of electrolyte imbalances, and high doses of intravenous carnitine must be infused together with glucose. Blood concentrations of electrolytes and bicarbonate are determined and an intravenous infusion started before taking time for history and physical examination. Rehydration should be initiated with a bolus of 20 mL/kg of ringer lactate or physiologic (0.9 %) saline. Buffering should be performed carefully. In patients with severe ketoacidosis, buffering with sodium bicarbonate might be ineffective and even increase the risk of brain edema. Buffering with TRIS/HCl via a central line or – as ultima ratio – extracorporeal detoxification using hemodialysis/hemofiltration should be considered instead. In patients with less severe metabolic acidosis, sodium bicarbonate is usually effective and safe. Carnitine supplementation should be given at 100 mg/kg intravenously. To enhance intracellular detoxification, the doses might be doubled or tripled in patients with propionic or methylmalonic aciduria. Electrolytes and acid–base balance are checked q6h, in severe metabolic decompensations more often. Serum sodium should be ≥138 mmol/L. Some children with these disorders benefit from implantation of a venous port to facilitate blood sampling and intravenous infusions.

Forced diuresis with large amounts of fluids and furosemide is especially useful in the methylmalonic acidurias in removing methylmalonate from the body. However, this intervention is only effective and safe after careful rehydration.

Detoxification in the organic acidurias depends on the ability to form esters such as propionylcarnitine, which are preferentially excreted in the urine, thus removing toxic intermediates. In these disorders, tissue stores of carnitine are depleted. Carnitine is given to restore tissue supply, but its major utility is to promote detoxification by the formation and excretion of carnitine esters. It is given intravenously in doses of 100–300 mg/kg/day. Carnitine is less well tolerated and much less reabsorbed enterally. Oral doses of >100 mg/kg are employed, but the dose may have to be reduced in some patients.

In isovaleric aciduria it is useful to add glycine to the regimen to promote the excretion of isovalerylglycine (up to 500 mg/kg/day).

In maple syrup urine disease, the major element of therapy is to harness the forces of anabolism to lay down accumulated leucine and other branched-chain amino acids into protein. This is done by the provision of mixtures of amino acids lacking leucine, isoleucine, and valine. The use of intravenous mixtures is very efficient and essential in a patient with intractable vomiting, but these are not generally available. Enteral amino acid supplements are dissolved in minimal fluid volume and dripped over 24 h in doses of 2 g/kg of amino acids. Often even a vomiting patient will tolerate a slow drip. Since concentrations of isoleucine are much lower than those of leucine, concentrations of amino acids must be measured at least daily, and when the concentrations of isoleucine become low, isoleucine is

added to the enteral mixture. In many patients valine must also be added before the leucine concentration is lowered adequately.

The pharmacological approach to the detoxification of ammonia in urea cycle defects, and also in those organic acidurias that present with hyperammonemia, is the provision of alternative methods of waste nitrogen excretion (Fig. 19.1). Sodium benzoate is effectively conjugated with glycine to form hippurate, which is then excreted in the urine. Similarly, sodium phenylacetate is conjugated with glutamine to form phenylacetylglutamine, which is efficiently excreted. Note that there is only one licensed drug for the combination of sodium benzoate and sodium phenylacetate. Administered orally, sodium or glycerol phenylbutyrate is converted to phenylacetate. In a metabolic emergency with an as yet unknown diagnosis and a documented hyperammonemia ≥200 μmol/L (350 μg/dL) and in relapses of known patients, benzoate and phenylacetate should be given intravenously (Table 19.3), along with arginine. In infants with citrullinemia, intravenous arginine is especially effective as long as the episode is treated promptly and the level of ammonia is not too high. During combined intravenous supplementation of benzoate and phenylacetate, electrolytes must be checked regularly to avoid hypernatremia and hypokalemia. A dose of 400 mg sodium benzoate corresponds to 2.77 mmol and therefore to 2.77 mmol of sodium. In some situations, enteral phenylbutyrate or benzoate along with arginine may be adequate. In urea cycle defects, carnitine administration is also recommended (50–100 mg/kg). Zofran (0.15 mg/kg i.v.) appears to be especially effective against hyperemesis, which can accompany hyperammonemia. Since phenylacetate stimulates the breakdown of branched-chain amino acids, regular amino acid analyses are indispensable to avoid protein catabolism resulting from the depletion of these essential amino acids.

The response of newborns and infants with suspected deficiency of N-acetylglutamate or carbamoyl phosphate I to carbamoyl glutamate (100–250 mg/kg/day) should be tested. For patients with propionic, methylmalonic, and isovaleric acidurias, oral treatment with this drug can also be used during hyperammonemic episodes.

Hyperammonemias may require extracorporeal dialysis for detoxification. This should be started at ammonia levels > 500 μmol/L (850 μg/dL) in infants during clinical deterioration and if pharmacotherapy fails to normalize plasma ammonia. Older children and adults are far more sensitive to high ammonia levels and may require hemofiltration or hemodialysis at levels > 200 μmol/L (350 μg/dL). Hemodialysis/hemofiltration has been repeatedly shown to be more effective than exchange transfusions, peritoneal dialysis, or arteriovenous hemofiltration. This is an argument for anticipatory transport of such an infant if the necessary logistics cannot be promptly organized locally.

Long-term management of disorders of urea formation has involved oral benzoate and phenylbutyrate. In some patients, this has been limited by the unpalatability of the latter compound. Glycerol phenylbutyrate has been developed to

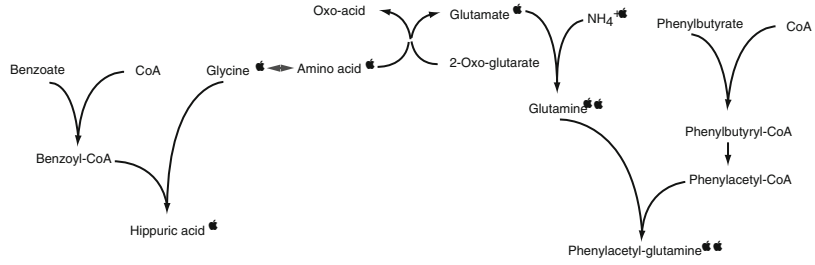

Fig. 19.1 Pharmacological detoxification of ammonia. The apple symbols represent nitrogens. Conjugation of glycine with benzoate yields hippurate; conjugation of phenylacetate with glutamine yields phenylacetylglutamine, and these are the end products excreted. One nitrogen is excreted with each molecule of hippuric acid and two with phenylacetylglutamine

Table 19.3 Emergency treatment of hyperammonemic crises

Drug	Loading dose over 1.5–2 h (mg/kg)	Maintenance dose	Preparation
Sodium benzoate	250	250(–500) mg/kg	1 g in 50 mL 5–10 % of glucose
Sodium phenylacetate[a]	250	250(–500) mg/kg	1 g in 50 mL 5–10 % of glucose
L-arginine HCl	Undiagnosed patient: 250(–400)	Undiagnosed patient: 250 mg/kg	1 g in 50 mL 5–10 % of glucose
	NAGS, CPS, OTC, ASS: 250	NAGS, CPS, OTC, ASS: 250	
	ASL: 250–400	ASL: 250–400	
	ARG1: avoid	ARG1: avoid	
Carbamoyl glutamate	NAGS: 100 (per nasogastric tube)	25–62.5 mg/kg every 6 h	

Dosages are according to the guideline by Häberle et al. (2012)
NAGS N-acetylglutamate synthase deficiency, *OTC* ornithine transcarbamylase deficiency, *CPS* carbamoyl phosphate synthetase deficiency, *ARG1* arginase deficiency (hyperargininemia), *ASS* argininosuccinate synthetase deficiency (citrullinemia type I), *ASL* argininosuccinate lyase deficiency
[a]In a known patient, not vomiting, it may be possible to employ oral or gastric sodium phenylbutyrate

minimize this. Furthermore, this drug is absorbed more slowly which helps to maintain plasma concentrations.

In aminoacidopathies, other than maple syrup urine disease, such as phenylketonuria, the homocystinurias or the tyrosinemias, the toxic metabolites lead primarily to chronic organ damage rather than a metabolic emergency. Hepatorenal tyrosinemia (tyrosinemia type I) may lead to a crisis of hepatic insufficiency. The rationale and principles of therapy remain the same as described earlier in the metabolic emergencies, but hypercaloric treatment through central catheters is seldom indicated. Severe vomiting in pregnant females with PKU may necessitate treatment following these principles. Glucose should be administered in accordance with endogenous glucose production rates together with fat. Oral therapy should be resumed as soon as possible including medication and the appropriate amino acid mixture. Patients with tyrosinemia type I should be treated with nitisinone (NTBC) as soon as possible. NTBC is a potent inhibitor of *p*-hydroxyphenylpyruvate dioxygenase; it blocks the production of the highly toxic fumarylacetoacetate and its derivatives (see Chap. 24).

In patients with galactosemia or fructose intolerance, toxic metabolites derive predominantly from exogenous sources. Once the diagnosis is suspected or made, therapy consists of the elimination of the intake of galactose and fructose. However, if intravenous alimentation devoid of galactose and fructose is begun without suspicion of the underlying metabolic disorder, gradual reintroduction of oral feeding will lead to protracted disease courses with varying and complicated symptomatology until the diagnosis is made. Patients with galactosemia or fructose intolerance require energy to stabilize and maintain blood glucose.

19.2 Reduced Fasting Tolerance (Fig. 4.1)

Patients with defects of fatty acid oxidation and gluconeogenesis, such as medium-chain acyl-CoA dehydrogenase deficiency and glycogen storage disease type I, require vigorous administration of glucose in amounts sufficient to restore and maintain euglycemia. Frequent monitoring of blood glucose is essential if symptomatic hypoglycemia is to be avoided. The patient seen first in an emergency room following a convulsion and found to have little or no measurable glucose in the blood is usually treated first with

enough hypertonic glucose to restore euglycemia. It is acceptable to follow that with infusion of 5 % glucose and water, but glucose levels should be obtained promptly and the concentration changed to 10 % or higher as required to keep the sugar elevated.

Supplementation of carnitine in suspected or proven defects of fatty acid oxidation is currently controversial. At least, restoration of levels of free carnitine appears indicated. A dose of 50 mg/kg is usually adequate. Disorders of carbohydrate metabolism do not need detoxifying treatment.

Long-term management of patients with disorders of fatty acid oxidation is best served by avoidance of fasting. Diagnosed patients should have written instructions that if anorexia or vomiting precludes oral intake, the patient must be brought to the hospital for the intravenous administration of glucose. Supplemental cornstarch is a useful adjunct to chronic therapy in disorders of carbohydrate metabolism and of fatty acid oxidation.

19.3 Disturbed Energy Metabolism (Fig. 4.1)

The *disorders of disturbed energy metabolism* include defects of the pyruvate dehydrogenase complex (PDHC), the Krebs cycle, and the respiratory electron transport chain. These disorders are characterized by chronic multisystemic disease rather than acute metabolic emergency. The situation calling for emergency treatment is the occasional occurrence of life-threatening acidosis and lactic acidemia. Therapy in this situation calls for vigorous treatment of the acid–base balance as outlined earlier. An issue is the fact that patients with PDHC deficiency are glucose sensitive. Glucose infusions can result in a further increase in lactate. In fact it is advisable to test all patients with lactic acidemia for the lactate response of lactate to glucose. In sensitive patients, intravenous glucose may be employed at rates well below the endogenous glucose production rate (Table 19.1). The correction of metabolic acidosis may require high amounts of sodium bicarbonate. In as many as 20 % of children with mitochondrial disease, the acute decompensation may be complicated by proximal renal tubular acidosis, and this may increase the requirement for sodium bicarbonate. Regardless of the cause, levels of lactate can be lowered by dialysis or the administration of dichloroacetate. Dichloroacetate activates the PDHC in the brain, liver, and muscle. Although levels of lactate have been shown to improve, the overall outcome may not be altered.

In patients with mitochondrial disease, replacement of cofactors is commonly undertaken. Evidence in support of the argument is a positive response to biotin in multiple carboxylase deficiency and to riboflavin in some patients with multiple acyl-CoA dehydrogenase deficiency. It is common practice to prescribe a combination of coenzyme Q_{10}, vitamin E, and a balanced B-vitamin supplement called "B50." B50 contains a combination of thiamine, riboflavin, niacin, pyridoxine, biotin, folate, B_{12}, and pantothenic acid. If a measured deficiency in blood carnitine is found, or if urinary excretion of carnitine esters is high, the patient is treated with L-carnitine.

The ultimate goal of therapy is not simply to reverse the metabolic emergency but to prevent irreversible damage to the patient's brain. The diseases leading to acute intoxication, such as maple syrup urine disease, the classical organic acidurias, and the urea cycle defects, carry the greatest risk of major sequelae. Additional supportive therapeutic measures are used informally in some centers to enhance this goal. These include mannitol for the treatment of cerebral edema, which may also enhance detoxification through increased diuresis. Increased intracranial pressure may be monitored neurosurgically. New trends include the use of hypothermia in neonatal hyperammonemia due to organic acidurias or urea cycle disorders. Mild systemic hypothermia is used with the rational of lowering the ammonia production. Overall supportive care is critical in patients in intensive care units, with special vigilance for the detection and prompt treatment of infection.

In summary, the metabolic emergency calls for prompt diagnostic and therapeutic measures, which follow the principles of adequate energy

supply, the promotion of anabolism, and the use of pharmacological and, if necessary, extracorporeal detoxification. These are the determinants of success in handling the metabolic emergencies of inborn errors of metabolism. It is important to provide patients after diagnosis with an emergency card or bracelet containing essential information and phone numbers in a letter with instructions on emergency measures. The logistics of rational therapeutic measures should be repeatedly evaluated by the specialist team with the family and primary care physicians.

General Suggestions for Reading

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Anesthesia and Metabolic Disease 20

William L. Nyhan

Key Facts

- In general elective surgery should only be carried out in centers experienced with the respective inherited metabolic disease, especially if prone to acute metabolic decompensation.
- Succinylcholine is persistent in patients with butyrylcholinesterase variants; they may fail to breathe long after the surgery is completed. Artificial ventilation must continue.
- Instability of the atlantoaxial joint in patients with mucopolysaccharidoses may lead to disaster during anesthesia. These patients should have surgical procedures in centers which have anesthesiologists who have experience in dealing with these patients, and preoperative evaluation of the cervical spine and cord is mandatory.
- (Long) fasting must be avoided in disorders of fatty acid oxidation and in Refsum syndrome. The provision of ample amounts of glucose in these disorders prevents hypoglycemia, rhabdomyolysis, and the crises of Refsum syndrome.
- Catabolism is inherent in surgery; it is magnified by fasting. It must be minimized in patients with organic acidemia or urea cycle defects by the provision of parenteral glucose. After surgery, close monitoring of the clinical status and laboratory values and shifting to oral medications and diet are required.
- Urea cycle defects may also be amenable to the provision of intravenous arginine during the procedure. Intravenous benzoate/phenylacetate provides an alternate mode of waste nitrogen excretion.
- Mitochondrial complex I deficiency leads to sensitivity to volatile anesthetics.
- Preoperative preparation of patients with homocystinuria, especially those who are B_6 responsive, is designed to minimize levels of homocystine during the procedure.
- Malignant hyperthermia is seen in response to inhalation anesthetics and succinylcholine. It is usually the result of mutations in the RYR1 gene. Rhabdomyolysis in disorders of fatty acid oxidation may mimic malignant hyperthermia. Malignant hyperthermia may follow anesthesia in a variety of myopathies, especially central core disease and King–Denborough syndrome.
- Patients with cystinosis have reduced volume of sweating which may lead to hyperthermia and heat intolerance. Intraoperative hyperthermia has been reported. These patients should not be anesthetized without venous access and the ability to meet their high renal losses during a procedure.

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20.1 General Remarks

Special considerations for anesthesia and surgery were dramatized by the recognition, more than 50 years ago, that genetically determined variants in *butyrylcholinesterase*, the cholinesterase found in serum, lead to prolongation of the action of *succinylcholine*, the agent used in surgery for relaxation of muscles. Patients with deficient activity of this enzyme remain paralyzed and unable to breathe for long after the surgery is completed.

Testing for these variants, of which there are a number, is done spectrophotometrically in the presence of the inhibitor, dibucaine, and the percentage of inhibition is called the dibucaine number. Management of the patient with this problem is simply to continue assisted ventilation until the succinylcholine is broken down. All carboxylic acid esters will ultimately be hydrolyzed despite the absence of specific esterase activity.

Remember

Ideal presurgical procedure is to obtain the butyrylcholinesterase dibucaine number. Then the anesthesiologist can plan to continue ventilation until the succinylcholine has broken down as a result of the action of other esterases.

The issue of neurotoxicity in infants and children has been raised for most of the available general anesthetic agents based predominately on studies in experimental animals. Lower scores on cognitive testing were recorded on a sizable sample of children exposed to anesthesia versus controls. Of course, it is impossible in this experience to separate effects of the drugs from those of the surgery itself.

20.2 Mucopolysaccharidoses

The great risk of anesthesia in mucopolysaccharidosis (MPS) is the result of instability of the atlantoaxial joint. This is a particular problem in Morquio disease, but patients with MPS I, MPS II, and MPS VI are also at risk. Deaths have been recorded as complications of anesthesia. Careful positioning is required and hyperextension of the neck must be avoided. General anesthesia should be undertaken in these patients only in centers in which anesthesiologists have had experience with patients with these diseases. In preparation for surgery, the patient or parents should be asked about previous problems with anesthesia, obstructive sleep apnea, or transient paralysis that might be an index of cervical instability. The patient should be examined for evidence of cord compression, kyphoscoliosis, and excessive upper respiratory secretions. The blood pressure should be determined, and an EKG and echocardiogram obtained. Recent roentgenograms of the chest and of the cervical spine should be reviewed. Those with kyphoscoliosis should have pulmonary function studies. Sleep studies may be useful. Those with evidence or history of cord compression should have an MRI of the spine. Intubation and induction of anesthesia may be difficult because of limited space; smaller tubes than usual may be required. Visualization may be limited by macroglossia, micrognathia, and immobility of the neck. It may be necessary to immobilize the neck with a halo brace or plaster to avoid damage to the cervical cord. Thick secretions may lead to postoperative pulmonary problems. Recovery from anesthesia may be slow, and postoperative obstruction of the airway has been observed. Wherever possible, local anesthesia is preferable, but in young or uncooperative patients such as those with Hunter or Sanfilippo diseases, this may not be possible. General anesthesia is preferable to sedation because of the need to control the airway.

Remember

The instability of the atlantoaxial joint is the major anesthetic issue in MPS, but macroglossia and micrognathia may interfere with intubation. Thick secretions may cause postoperative problems.

20.3 Avoidance of Hypoglycemia

Patients with many metabolic diseases are at risk for the development of hypoglycemia. For these patients, the usual fasting prior to general

anesthesia and surgery could be disastrous. The objective of management is the maintenance of euglycemia: a concentration of glucose in the blood above 4 mmol/L. Relevant disorders include the disorders of fatty acid oxidation (Chap. 16), glycogen storage diseases, and disorders of gluconeogenesis, such as fructose 1,6-diphosphatase deficiency, hyperinsulinism, and ketotic hypoglycemia (Chap. 15). In each instance, the patient's history and tolerance of fasting should be known prior to making plans for surgery. Most patients with disorders of fatty acid oxidation do not become hypoglycemic until they have fasted more than 12 h, while some patients with glycogenosis or hyperinsulinism cannot tolerate a 4-h fast.

Patients undergoing short or minor procedures can be scheduled for noon or later and given glucose. Patients receiving overnight nasogastric glucose should have intravenous glucose started before the nasogastric administration is discontinued. Every patient should be receiving 10% glucose intravenously well prior to the time that hypoglycemia would be expected to begin, and intravenous glucose should be discontinued only after the patient has demonstrated an ability to eat and retain sources of oral sugar. The cannula should not be removed until the possibility of vomiting has been excluded. In general, 10% glucose should be employed at rates approximating 2,500 mL/m^2/24 h. This would be equivalent to 150 mL/kg in infants under 1 year; 100 mL/kg, 1–2 years; 1,200–1,500 mL, 2–6 years; and 1,500–2,000 mL, over 6 years of age. Rates must be readjusted on the basis of determined levels of glucose in the blood.

20.4 Rhabdomyolysis and Myoglobinuria in Disorders of Fatty Acid Oxidation/Refsum Disease

General anesthesia and the stress of surgery have each been thought responsible for the acute breakdown of muscle that has been observed in patients with abnormalities of fatty acid oxidation. These triggers of the acute attack are particularly notable for the myopathic form of carnitine palmitoyltransferase (CPT) II deficiency. They may do the same in any disorder of fatty acid oxidation, especially long-chain hydroxyacyl-CoA dehydrogenase (LCHAD) deficiency (Chap. 28). Renal failure may be a complication of myoglobinuria. The best answer to preventive anesthesia and surgery in these patients is an ample supply of glucose and water and the avoidance of fasting. This is accomplished by early placement of an intravenous line so that the patient is fasting not more than 6 h. In the presence of myoglobinuria, intravenous glucose should be 10% or higher; adjunctive insulin may be helpful in maintaining euglycemia. Ringer's lactate should be avoided because of lactic acidosis. Metabolic acidosis should be corrected. Drugs stimulating lipolysis and fatty acid oxidation, like epinephrine and other beta-agonists, theoretically might pose a hazard for patients with fatty acid oxidation disorders. Enflurane was reported to increase free fatty acids during perioperative stress caused by minor elective surgery. Premedication with morphine, flunitrazepam, and promethazine had no effect on plasma concentrations of free fatty acids. Propofol infusion syndrome, a rare but frequently fatal complication in critically ill children given long-term propofol infusions, results in an impaired fatty acid oxidation and an inhibition of the respiratory chain at several points. It should not be used.

Surgery and anesthesia may also induce a metabolic crisis in Refsum disease via mobilization of phytanic acid in fat stores. The same preventive approaches apply.

Remember

The avoidance of fasting and the provision of parenteral glucose are essential for the prevention of myoglobinuria in disorders of fatty acid oxidation. These precepts will also prevent crises in Refsum syndrome.

20.5 Organic Acidemias and Maple Syrup Urine Disease

The objective in the management of anesthesia and surgery in patients with organic academia or maple syrup urine disease (MSUD) is the minimization of catabolism. This objective is met

best by avoiding anesthesia and surgery, if at all possible, until the patient is in an optimal metabolic state and well over any infections. In preparation for the procedure, metabolic balance should be ascertained by checking the urine for ketones; the blood for ammonia, pH, and electrolytes; and in the case of MSUD, the plasma concentrations of amino acids. Catabolism is minimized by the administration of glucose and water in the regimen employed for hypoglycemia. In MSUD, a dose of the amino acid supplement, either one-third of his/her daily dose or at least 0.25 g/kg, is given as late prior to anesthesia as feasible. This would be a place for intravenous preparations of amino acids designed for the treatment of MSUD. Following the procedure, intravenous glucose should be continued until the oral route is clearly feasible and the electrolytes are stable. A patient with MSUD may be maintained for a while with intravenous amino acids or in their absence with a nasogastric drip of a mixture of amino acids containing no isoleucine, leucine, or valine; blood concentrations of amino acids must be monitored, and it may become necessary to add isoleucine and valine. Mixtures of amino acids containing no sugar, fat, or minerals that can be made in minimal volume and dripped so slowly that they are tolerated by patients usually thought of as requiring nothing by mouth are available.

Remember

Catabolism can be minimized by the provision of parenteral glucose. Patients with MSUD can best avoid catabolic elevation of leucine as a consequence of surgery by the provision of a mixture of amino acids, which do not contain the offending branched-chain amino acids. This is quite successfully done with intravenous mixtures, but these are seldom widely available.

20.6 Urea Cycle Defects

In disorders of the urea cycle, the objective is the avoidance of hyperammonemia by the minimization of catabolism. The approach is as outlined for organic acidemias, except that it is the ammonia that must be carefully monitored.

In addition, patients whose usual medication includes arginine or citrulline should be given intravenous arginine. The patient's usual dose is employed, diluted 2.5 g in 50 mL 10 % glucose, and piggybacked via a syringe pump to the glucose infusion. In patients receiving sodium benzoate, phenylbutyrate, or both, the intravenous mixture of benzoate and phenylacetate is employed, again in a dilution of at least 2.5 g of each per 50 mL, and given by a piggyback pump. For short procedures, these medications can be begun in the postoperative period. For a longer procedure or one particularly likely to induce catabolism, and certainly in the presence of hyperammonemia, they can be given intraoperatively. It is prudent to use anesthetics with low toxicity to the liver. In one child with argininosuccinic aciduria, hyperammonemia was induced by enflurane.

20.7 Mitochondrial Disease

Patients with mitochondrial disease are candidates for anesthesia for imaging studies, muscle biopsy, or placement of a gastric tube. An intravenous line should be placed before induction of anesthesia. Any patient with mitochondrial disease may have lactic acidemia. This can be exacerbated by fasting. Preoperative use of glucose is prudent. The avoidance of Ringer lactate solutions seems reasonable.

Patients with deficiency of complex I have sensitivity to volatile anesthetics. Sevoflurane has been recommended for patients with mitochondrial disease. Very low doses of local anesthetic and lidocaine have been added at the site.

Propofol inhibits complex I and uncouples complex III and acylcarnitine transferase. Ketamine inhibits complex I and uncouples complex II in experimental animals. Short-term use and lower doses have been successfully employed in mitochondrial patients. Barbiturates inhibit complex I. Etomidate inhibits complexes I and III and uncouples complex II. Succinylcholine may induce rhabdomyolysis (v.i.).

Among opioids, remifentanil is preferred over fentanyl, which is preferable to morphine.

Among regional anesthetics, which inhibit complex I, lidocaine is preferred. Clonidine appears not to affect mitochondria.

Remember, avoid volatile anesthetic agents in patients with complex I deficiency.

20.8 Homocystinuria

Patients with cystathionine synthase deficiency are predisposed to the development of thrombosis. This may create an added risk for general anesthesia. Those who are pyridoxine responsive can be prepared for surgery by titrating values for homocysteine and homocystine with added B_6 until an optimal preoperative level is achieved. Nonresponders may be treated with betaine in quantities to achieve minimal concentrations.

20.9 Malignant Hyperthermia

Malignant hyperthermia is a genetically determined response to inhalation anesthetics or succinylcholine in which rapidly escalating fever and generalized muscle spasm may be fatal. Prognosis has improved with preanesthetic identification and the discovery that dantrolene is specifically therapeutic. Hyperthermia is often a late sign. The earliest manifestation is an increase in end-tidal CO_2. Extreme spasm of the masseter may make insertion of a laryngoscope impossible (jaws of steel). Muscle rigidity is generalized. The skin becomes hot to the touch.

Lactic acid accumulates, and there is a mixed metabolic and respiratory acidosis. Hypoxia, hypercarbia, and metabolic acidosis accompany rhabdomyolysis. Ventricular tachycardia, pulmonary edema, or disseminated intravascular coagulation may ensue, as well as cerebral edema and renal failure.

The RYR1 gene on chromosome 19q 13.1 is mutated in a heterozygous fashion in a majority of patients. Very many different mutations have been identified over the 106 exons. The RYR1 protein is an integral part of the structure in the sarcolemma/reticulum involved in the voltage-dependent Ca++ channel. Rows of RYR1 bind to tetrads of the dihydropyridine receptor (DHPR), and mutations in DHPR genes have also been found in patients with malignant hyperthermia.

Malignant hyperthermia has also been encountered following anesthesia in a variety of muscular dystrophies, including Duchenne. It is particularly found in central core disease and King–Denborough syndrome (KDS). Central core disease gene mutations have been linked to RYR1.

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Principles of Dietary Management

21

Anita MacDonald

Key Messages

- Prescribed diets for IMDs are individualised and need to consider the patient's clinical condition, individual tolerances, metabolic stability, age, developmental ability and prognostic outcome.
- In IMDs associated with acute metabolic decompensation, stringent use of dietary emergency regimens is imperative.
- Feeding problems, malnutrition and growth failure are common complications of many IMD conditions.
- The nutritional follow-up requires systematic assessment of nutrient intake, anthropometry, clinical signs of nutrient deficiency and biological biomarkers to detect subclinical micronutrient excess or deficiency.
- Good family and patient education is essential in ensuring successful dietary management and is fundamental in bringing about change in eating behaviour and family lifestyle.

21.1 General Remarks

Common inherited metabolic disorders (IMD) treated by life-long diet therapy are responsible for a collection of diverse clinical conditions. The overall goal of nutritional treatment is to correct the metabolic imbalance whilst providing adequate nutritional support for normal growth and development. Each condition may present at different ages with a spectrum of disease severity and outcome, so designing a diet that has the 'right balance' between maintaining metabolic stability but is not unnecessarily 'over-restrictive' is challenging. Diet may be the sole form of therapy or used in combination with other treatments such as pharmacological chaperones, vitamin cofactors and nitrogen-scavenging drugs.

Prescribed diets for IMDs are individualised and need to consider the patient's clinical condition, tolerances, metabolic stability, age, developmental ability and likely prognosis. Dietary treatments require vigilance and skilled care. Overall the delivery of dietary treatment is best managed by a multidisciplinary team but with a dietitian operating as a key member. Dietitians should receive extensive post-registration training in IMD, developing treatment skills and attaining essential knowledge. As each condition is rare, it may take months or years to develop the necessary skill set to treat a wide range of conditions competently.

21.2 Core Elements of Dietary Treatment

There are wide-ranging dietary treatment approaches for many conditions, commonly based on historical and national practice. Recently, this has improved with the publication of evidenced-based guidelines for several IMD conditions. Diet therapies can be divided into chronic and acute.

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21.2.1 Chronic Dietary Treatment

The main forms of nutritional therapy, either used on their own or in combination, are:

Substrate reduction/removal: This aims to decrease the tissue and plasma concentrations of toxic substrates by reducing the intake of nutrients/substrates that produce toxic metabolites, e.g. phenylalanine in phenylketonuria (PKU), branched-chain amino acids in maple syrup urine disease (MSUD), galactose in galactosaemia and long-chain fat in long-chain hydroxyacyl-CoA dehydrogenase deficiency (LCHADD).

Provision of '*conditionally*' *essential or essential nutrients*: *This is* necessary as a consequence of the enzyme block, e.g. tyrosine in PKU, arginine/citrulline in urea cycle disorders and phenylalanine in tyrosinaemias, because of the dietary restriction.

Provision of alternative energy substrates, e.g. use of medium-chain triglycerides (MCT) in long-chain fatty acid oxidation disorders (LC-FAODs), or provision of glucose and uncooked cornstarch (UCCS) in glycogen storage disease (GSD).

Avoidance of fasting/prolonged fasting to avoid accumulation of toxic metabolites or deficiency of substrate, e.g. increased acylcarnitines/free fatty acids in LC-FAOD, increased odd-chain fatty acids in propionic acidaemia (PA) and lack of glucose in GSD.

See Sect. 21.2 for more detailed discussion of individual conditions.

21.2.2 Acute Dietary Management

Many patients with an IMD are at risk of acute metabolic decompensation not only at initial neonatal presentation but throughout life with infections, poor appetite, surgery, trauma and protracted fasting. Illness depresses appetite, but enhances energy requirement and increases fluid losses from sweating, vomiting and diarrhoea. An emergency regimen provides an exogenous energy source (primarily from oral glucose polymer solutions or IV glucose) to reduce production of potentially toxic metabolites or to prevent hypoglycaemia and promote anabolism (see also Chap. 19).

The composition of the regimen is important. Substrates (fat/protein) of toxic metabolites are temporarily reduced or removed from emergency feeds with high energy intake from carbohydrate sources (glucose polymer). Carbohydrate is effective in helping restore nitrogen balance and limiting nitrogen excretion. It also reduces amino acid catabolism during the postprandial phase, both directly and via

Table 21.1 Emergency feeds (containing glucose polymer and fat emulsion) for protein-free feeding in infants and children

Age	Glucose polymer concentration % carbohydrate	Fat emulsion % fat	Energy kcal per 100 ml from CHO + fat	Energy kJ per 100 ml	Suggested daily intake	Feeding frequency
Up to 12 m	10	3.5	72	302	120–150 ml/kg	Either administered every 2–3 h orally day and night or via continuous tube feeding
1–2 years	15	5	105	441	1,200 ml/day	
2–9 years	20	5	125	525	Estimated as indicated	
>10 years	25	5	145	609	Estimated as indicated	

For children >10 kg emergency regimen fluid requirements can be calculated as: 11–20 kg: 100 ml/kg for the first 10 kg plus 50 ml/kg for the next 10 kg >20 kg: 100 ml/kg for the first 10 kg plus 50 ml/kg for the next 10 kg plus 25 ml/kg thereafter up to a maximum of 2,500 ml/day (adapted from E-IMD UCD (Häberle et al. 2012) and MMA/PA (Baumgartner et al. 2014) guidelines)

NB: If fat is not tolerated/contraindicated, glucose polymer only should be administered in the same concentrations

their effect on insulin stimulation. However, emergency feeds based on glucose polymer, using concentrations recommended in Table 21.1, will provide infants and young children with <100 kcal/kg/day. Therefore, if fat is able to be tolerated (and not contraindicated, e.g. in FAOD), it should be added to increase energy intake (see Table 21.1). In GA1 and MSUD, a source of substrate-free L-amino acids is an essential component to minimise cerebral accumulation of the offending amino acids.

Delays in application of the emergency management plan or inadequate consumption of the emergency feeds increases the risk of irreversible neurological damage in some conditions. In mild illness only, emergency feeding can be commenced at home (delivered orally or by enteral tube feeds), but because clinical condition may deteriorate rapidly, caregivers require regular contact with the IMD team for assessment of symptoms, energy and fluid intake. If there is any clinical deterioration, non-tolerance or inadequate intake of emergency feeds, patients should be assessed and treated in hospital immediately.

Patients/parents should keep a letter explaining their diagnosis, an 'updated' emergency management plan and 24 h contact details to access their metabolic team. They should be instructed on how to prepare their emergency feeds and have an emergency 'kit' of emergency feed ingredients, medications and equipment to take with them to their hospital during illness episodes.

Remember

All patients with an IMD at risk of metabolic decompensation should have clear written instructions about their emergency feeding regimen, keep 'in-date' emergency feed ingredients and plan for its administration.

Remember

The E-IMD (www.e-imd.org) gives emergency guidelines for acute management in UCD, MMA, PA and IVA. The British Inherited Metabolic Disease Group (BIMDG) (www.bimdg.org.uk) also provides emergency guidelines (oral and IV) for metabolic disorders.

21.2.3 Nutritional Support and Feeding Problems

Feeding problems, malnutrition and growth failure are common complications of many IMDs. Achieving the correct balance between providing essential requirements of 'dietary substrates' of toxic metabolites but avoiding their deficiency is difficult. In IMD, nutritional supplementation and enteral tube feeding (nasogastric/gastrostomy) is utilised to deliver nutritional support to some patients. Tube feeding may supply a sole source of nutrition to children with dysphagia as a consequence of their IMD or supply nocturnal enteral nutrition in patients with GSD, PA and severe forms of LC-FAOD. Enteral feeding should also be used in hospital or at home to provide emergency feeds or for medication administration only. Tube feeding is not without risks and accidental nasogastric tube removal or equipment failure commonly occurs. For long-term feeding, early gastrostomy placement is encouraged in most conditions, except where wound healing might be impaired such as GSD 1b.

Remember

A reliable enteral feeding pump that accurately controls the flow rate and has a comprehensive alarm system, safety interlocks to prevent child tampering and battery backup in case of mains failure is essential in IMD.

The aetiology of feeding problems is extensive in IMD. Neurological impairment is common particularly in urea cycle disorders (UCD) and organic acidaemias (OA). There may be difficulties with chewing and swallowing, gastro-esophageal reflux (GOR), vomiting, slow feeding and anorexia. Poor appetite may be associated with elevated levels of toxic metabolites, i.e. glutamine and ammonia in UCD, altered serotonin metabolism in OA and UCD and deficiency of essential amino acids. A dislike of protein-containing foods is common in UCD. Problems

with administering strong tasting protein substitutes may create additional anxiety for some families, contributing to food neophobia and poor feeding experiences, and so acceptance of new tastes may be slow. It is also particularly challenging to introduce new and restrictive diet therapy in late-treated patients.

21.2.4 Monitoring of Nutritional Status

Careful monitoring of nutritional status is necessary in the chronic management of many of these disorders. The nutritional follow-up requires systematic assessment of nutrient intake, anthropometry, clinical signs of nutrient deficiency and biological biomarkers to detect subclinical micronutrient excess or deficiency. Protein/amino acid deficiencies are common and infants are at risk due to their higher requirements and increased requirements particularly post recovery from illness.

Remember

Protein/amino acid deficiencies may result in hair loss, skin problems, poor weight gain, poor growth, decreased muscle mass, poor wound healing, amino acid imbalance, osteopaenia and developmental delay/impairment.

Subnormal plasma concentrations of isoleucine and valine are common in OA; low isoleucine concentrations are associated with an acrodermatitis enteropathica-like syndrome. Patients with amino acid disorders, OA and galactosaemia are at risk of bone diseases, especially osteopaenia and osteoporosis. There are reports of other nutritional deficiencies such as selenium and zinc. The following should be conducted at each clinic visit:

- *Dietary assessment*: either using a 3 day recorded food record, 24 h recall or food frequency diaries to check overall food intake and amounts/presence of any restricted/excluded 'substrate' foods. Ingestion of restricted 'substrate' foods, e.g. phenylalanine sources, may be less than prescribed amounts, leaving patients at risk of deficiency. Adherence with L-amino acid supplements (± added vitamin and mineral supplements) taken with each meal or with separate vitamin and mineral supplements should be monitored.
- *Anthropometry*: including weight, height (length) and BMI. Preferably children should be weighed on the same scales, under the same conditions at each clinic visit. Weight gain and linear growth within the normal range are good indicators of adequate nutrition. Prevention of obesity is also important.
- *Clinical examination*: with particular reference to hair, skin and nails.

For patients on protein-restrictive diets, biochemical monitoring of vitamin and mineral status is essential including haemoglobin, iron, ferritin, zinc, selenium, vitamin D and vitamin B_{12} at least annually in addition to quantitative plasma amino acids and pre-albumin. Blood samples should be collected fasted, providing there is no fasting intolerance. Fatty acid status and fat soluble vitamin status should be monitored in patients on severe fat restrictions. Bone mineral density should be monitored formally in galactosaemia and homocystinuria. Bisphosphonate treatment may be indicated especially in the presence of fractures.

Remember

Attentive nutritional support with the provision of macronutrients and micronutrients to meet dietary reference values/requirements is essential with all diet therapy.

21.2.5 Monitoring of Biochemical Control

In amino acid disorders, small frequent changes in the diet prescription are commonly required and serial monitoring of substrate amino acids is essential to assess metabolic control. The frequency of blood samples and effect on dietary adherence has not been adequately assessed but

regular patient/parent feedback on blood substrate concentrations is essential in order to aid adherence and give appropriate guidance. Blood substrate amino acid samples should be routinely obtained under the same conditions. In order to measure the highest value of the day, samples should be collected in the morning after fasting overnight. Increases in blood substrate amino acids may be associated with poor adherence, overprescription of substrate amino acids, inadequate intake of L-amino acid supplements, energy and catabolic stress associated with infections or trauma. In MSUD, valine or isoleucine deficiency may cause blood leucine concentrations to rise associated with decreased protein synthesis or muscle catabolism. Regular blood monitoring and appropriate supplementation should avoid deficiency.

Remember

In MSUD, additional supplementation with valine and isoleucine supplements is commonly necessary, particularly with emergency feeds.

Continuous glucose-monitoring systems (CGMS), allowing glucose levels to be monitored over a period of time, help individualise and improve 24 h blood glucose profiles in GSD. CGMS provide information on the extent, timing and duration of fluctuations of blood glucose allowing the IMD team to compare this with dietary intake and usual activity.

21.2.6 Educational/Training Needs of Patients/Family

Good family and patient education is essential in ensuring successful dietary management and is fundamental in bringing about change in eating behaviour and family lifestyle. Education should always be delivered in a method that meets the cultural, language and educational diversity of the family (see also Chap. 7). Families should understand the basic information about the condition and any consequences if untreated, the practicalities of diet therapy and how to apply this on a day-to-day basis to their individual schedules. Parents/caregivers require practical training on safe feed preparation, storage of feeds and safe delivery of enteral tube feeds. They should be taught core 'special cookery' skills, understand how to interpret food labels and know how to prepare and administer any L-amino acid supplements. Home support workers can help assist families in day-to-day care. Parents/patients should be guided to professional Internet website information sites in order to gain reliable dietary information.

21.3 Dietary Management of Common IMD Conditions

21.3.1 Disorders of Amino Acid Metabolism

Dietary treatment is the core therapy for PKU (phenylalanine), homocystinuria (HCU) (methionine) and MSUD (valine, leucine and isoleucine). In tyrosinaemia type 1 (HT1) (tyrosine and phenylalanine), NTBC is the principle treatment, but adjunct dietary treatment is necessary particularly as a consequence of NTBC increasing blood tyrosine concentrations. Overall, diet involves restricting one or more essential substrate amino acids to individual patient tolerance.

Dietary treatment principles involve:

- Avoidance of foods high in natural protein to prevent excess accumulation of the 'substrate' amino acid(s). Foods such as meat, fish, eggs, cheese, nuts and seeds are not permitted unless it is a very mild disorder phenotype.
- A limited amount of natural protein is given to maintain 'substrate' blood amino acids within the target treatment range. The quantity of natural protein will vary according to the type and severity of each condition and the amount tolerated is defined as the quantity that maintains blood substrate amino acids consistently within target range. For classical phenotypes for all conditions, natural protein tolerance is usually below 10 g/day, but over-restriction may affect growth and lead to amino acid

deficiency (guidelines are given in Table 21.2). In infants, natural protein allowance is supplied by breast milk or standard infant formula and in children foods such as cereal, potato, some vegetables and milk. Systems for allocating substrate amino acids vary across Europe. Detailed food lists may be provided to assist in estimating substrate amino acid intake. Alternatively families may be encouraged to use a diet exchange system for their daily allocation. The allowance is given evenly during the day and no more than 50% of the natural protein should be given at any one meal in order to reduce blood amino acid fluctuations over 24 h.

- Provision of L-amino acid supplements (protein substitutes/medical foods) that are free of 'substrate' amino acids are essential. The prescribed dose should meet at least safe levels of protein/nitrogen requirements with an additional amount added to compensate for the inefficiency of L-amino acid utilisation. In the majority of patients with 'classical' phenotypes, it is likely that substrate-free L-amino acids will supply >75% of the daily total protein intake. The optimal dose of L-amino acid for amino acid disorders is not agreed but because L-amino acids are absorbed and oxidised more rapidly than amino acids derived from digestion of whole protein, recommended protein intakes are higher than safe levels of protein intake (WHO/FAO/UNU 2007). It is best to give L-amino acid supplements in small frequent doses, three to four times, spread evenly throughout the day rather than only once or twice daily and ideally together with a natural protein and carbohydrate source. Age-appropriate L-amino acid supplements are available in a variety of formats including ready-to-drink pouches, gels and powders.

Remember

L-amino acid supplements have a high osmolality and may cause abdominal cramping, distension, diarrhoea or even constipation if not given with water.

Remember

L-amino acid supplements could cause metabolic instability, encephalopathy and even death if accidentally/incorrectly given to patient with a different condition. Dietitians and IMD treatment centres need to establish

Table 21.2 Tolerance of individual substrate amino acids

	PKU	MSUD			HT1	HCU
Age	Phe	Leucine	Valine	Isoleucine	Tyrosine	Methionine
	mg/kg/day					
0–6 m	25–60[a]	80–110[b]	Give supplements until plasma concentrations are between 200 and 400 μmol/l		40–50[d]	15–60[e]
7–12 m	25–40[a]	40–50[c]			40–50[d]	12–43[e]
	mg/day					
1–10 years	200–700[a]	400–600[c]	Give supplements until plasma concentrations are between 200 and 400 μmol/l		150–500[d]	Median 230[f]
11–16 years	220–1,000[a]	400–600[c] 500–700[b]			250–750[d]	

From MacDonald and White, *Clinical Paediatric Dietetics* (2015)

[a]MacDonald A. Unpublished clinical data (maintaining plasma phenylalanine 110–360 μmol/L in 1–10 years and 110–700 μmol/L in 11–16 years)

[b]De Baulny et al. 2012 (maintaining leucine concentrations 80–200 μmol/L)

[c]Dixon M. *Clinical Paediatric Dietetics* 2007 (maintaining leucine concentrations 200–400 μmol/L)

[d]Daly A. Unpublished clinical data (maintaining plasma tyrosine 200–400 μmol/L)

[e]Van Calcar S 2010. Nutrition management of patients with inherited metabolic disorders (maintaining total homocysteine <50 μmol/L)

[f]White F. *Clinical Paediatric Dietetics 2007* (maintaining free homocysteine <10 μmol/L)

safe systems that ensure their patients receive correctly prescribed L-amino acid supplements (e.g. education for GPs, pharmacists, use of home delivery).

- Most L-amino acid supplements contain additional vitamins, minerals and trace minerals. Many also contain essential fatty acids and omega-3 LC-PUFAs such as docosahexaenoic acid. If L-amino acid supplements do not contain micronutrients, vitamins, minerals and trace elements should be administered separately, although adherence with extra prescriptions may be suboptimal.
- Essentially/conditionally indispensable amino acids that may become deficient as a result of the enzyme block or dietary treatment (e.g. phenylalanine in HT1, cystine in HCU) should be given. In MSUD, the amount of natural protein intake that provides leucine requirements often leads to deficiency of valine and isoleucine. Any lack of valine/isoleucine may become rate limiting for protein synthesis and supplementation with these amino acids is commonly necessary. Valine supplementation is particularly important because it has a low affinity for the blood brain barrier LAT1 transporter and it is especially vulnerable to competitive inhibition by leucine. The dosage should be titrated to maintain blood valine and isoleucine levels between 200 and 400 μmol/l, although some recommend that plasma valine concentration should be at least twofold plasma leucine concentration. High intakes of supplementary tyrosine is added to L-amino acid supplements formulated for PKU and so additional tyrosine should be unnecessary providing adherence with L-amino acid supplement is acceptable. Cystine is added to L-amino acid supplements in HCU but it is unclear if the amount is adequate and how much additional supplementation is necessary. (Dixon et al. 2015a)
- Maintenance of a normal energy intake is achieved by encouraging (1) the use of foods naturally low in protein and (2) specially manufactured low-protein foods such as bread and pasta (Table 21.3). Adequate energy from non-protein sources is essential for growth and to minimise tissue catabolism that can lead to poor metabolic control. Foods such as fruits, low-protein vegetables and low-protein cereals (e.g. tapioca) are supplemented with sugar and fats. Special foods that have been modified/devised so they are low in protein such as pasta, breads and baked items also provide energy and variety in the diet without significantly increasing protein intake. Inadequate energy intake may result from over-restrictive food choices, unpleasant taste of L-amino acid supplements, poor appetite

Table 21.3 Foods that can be eaten without measurement/restriction in low-protein diets (Dixon et al. 2015a)

Food groups	Examples of suitable foods that can be eaten without measurement
Fruits and vegetables	Fruits and vegetables containing less than 1 g/100 g protein should be allowed without measurement. Studies indicate in PKU, fruit and vegetables containing ≤75 mg/100 g phenylalanine (but this does not include potato) can be incorporated with no adverse impact on phenylalanine control
Fats	Butter, margarine, ghee, lard, dripping and vegetable oils
Starches	Cassava flour, arrowroot, cornflour, custard powder, potato starch, sago, tapioca and tapioca starch. Some protein-free low-protein cheese replacements based on food starches and oils are available
Sugars	Sugar, glucose, jam, honey, marmalade, golden syrup, maple syrup, fruit sorbets, ice lollies, sweets containing <0.5 g protein/100 g
Drinks	Water, squash, lemonade, cola drinks and fruit juice, black tea, fruit tea, green tea, coffee, tonic water, soda water and mineral water, providing all are aspartame free (in PKU only). Rice drink. Other plant drinks, e.g. almond or coconut contain some protein and require calculation in the diet
Low-protein special foods	A selection of low-protein breads, flour mixes, pizza bases, sausage mixes, pasta, biscuits, egg replacers, cheese and milk replacements are available

and limited access to special low-protein foods.
- Catabolism and avoidance of metabolic decompensation during illness/trauma, particularly in MSUD, should be prevented. Although in other amino acid conditions (PKU, HCU, HT1) there is no acute risk of decompensation, blood substrate amino acid concentrations are likely to remain high until symptoms have abated. Therefore, it is important L-amino acids and high-carbohydrate drinks be administered during infection helping decrease muscle protein loss.

In mild or moderate PKU, adjunct therapy in the form of tetrahydrobiopterin (BH4), a coenzyme in the hydroxylation reaction of phenylalanine to tyrosine, may help to enhance natural protein tolerance or improve blood phenylalanine control. It is considered to have a chaperone-like effect on a misfolding enzyme subunit and thereby increases the activity of the defective enzyme. In non-pyridoxine-responsive HCU, low-methionine diet may be used in combination with betaine.

21.3.2 Organic Acidaemias

The main principle of nutritional management in OA, where dietary treatment is indicated, is to reduce toxic tissue metabolites of organic compounds whilst supporting anabolism, normal growth and nutritional status. In these disorders (methylmalonic aciduria [MMA], PA, isovaleric acidaemia [IVA] and GA1), excessive protein intake or catabolic stress, such as infection or prolonged fasting, leads to an accumulation of organic acids.

21.3.2.1 Propionic Acidaemia and Methylmalonic Acidaemia (Affecting Catabolism of Methionine, Threonine, Valine and Isoleucine)

Treatment strategies include (1) natural protein restriction of substrate amino acids (aiming to provide (WHO/UNU/FAO 2007) safe levels of protein intake), (2) maintaining an optimal energy intake (Table 21.4), (3) use of adjunctive compounds to dispose of toxic metabolites (e.g. carnitine) or to increase activity of deficient enzymes (e.g. vitamin B_{12} in MMA) and (4) adequate hydration. Many IMD centres prescribe substrate-free amino acids to supplement natural protein intake although their long-term effect requires further research to delineate their benefit. There is little evidence to suggest they improve metabolic control or long-term outcome, and they may be mainly broken down and excreted as urea and are associated with deficiencies of valine and isoleucine. In severe MMA and PA, to reduce the production of propionate, it is also necessary to avoid prolonged fasting (with the use of overnight tube feeding) in order to limit oxidation of odd-chain fatty acids liberated from triglyceride stores during lipolysis.

Metabolic decompensation caused by catabolic stress (e.g. from vomiting and decreased oral intake) requires prompt intervention with an emergency regimen. Increased risk of basal ganglia stroke is associated with acute metabolic decompensation.

Remember

Some patients with variant forms of MMA respond to pharmacological doses of vitamin B_{12} and should tolerate a relaxed or even normal protein intake.

21.3.2.2 IVA (Affecting Leucine Catabolism)

For symptomatic patients, the goal of treatment is to reduce production of isovaleryl-CoA from leucine through protein restriction and enhance alternative metabolic pathways using the conjugating agent's carnitine and glycine that produce non-toxic compounds that are readily excreted. There are wide differences in dietary practices, but the E-IMD-proposed guidelines on IVA recommend only moderate protein restriction with natural protein intake supplying at least the safe levels of protein intake (WHO/UNU/FAO 2007; EIMD 2015). The routine use of leucine-free L-amino acid supplements should be unnecessary as

Table 21.4 WHO/FAO/UNU 2007 safe levels of protein and energy intake for different age groups

Energy requirements					Protein requirements[a]	
	kJ/kg/day		kcal/kg/day			
Age	WHO/FAO/UNU 2007		Converted from WHO/FAO/UNU 2007		Age	g/kg/day
Infants (y)	Males	Females	Males	Females	Infants (y)	
0.5	335	340	80.0	81.2	0.1	1.77
					0.2	1.5
					0.25	1.36
					0.5–1	1.31
Children (y)					Children (y)	
2.5	348	334	83.1	79.8	1–10	0.84–0.90
5.0	315	305	75.2	72.8		
10	275	248	65.7	59.2		
15	230	193	54.9	46.1	11–16	0.92–1.14
Adults (y) (moderate activity, 70 kg)					Adults (y)	
18–29	183	159	43.7	38.0	>16	0.84–0.87
30–59	175	148	41.8	35.3		
Adults (y) (moderate activity, 50 kg)						
18–29	212	180	50.6	43.0		
30–59	212	183	50.6	43.7		

[a]The FAO/WHO/UNU (2007) have set safe levels of protein intake titrated as an age adjusted mean + 2 SD. Values for safe levels of protein intake apply to males and females

reported case studies suggest that patients maintain long-term metabolic stability on a dietary protein restricted diet only. It is important that individual energy requirements are met as protein catabolism contributes significantly to isovaleric acidaemia production of isovaleric acid.

21.3.2.3 Glutaric Aciduria Type 1 (Affecting Lysine and Tryptophan Catabolism)

A low-lysine diet supplemented with lysine-free/low-tryptophan L-amino acids aims to reduce brain concentrations of glutaric acid and 3-hydroxyglutaric acid to prevent neurological crisis. In presymptomatic patients, diet with meticulous emergency management prevents neurological damage in the majority of patients (Kolker et al 2012). Guidelines on the dosage of L-amino acid supplement and prescription of dietary lysine intake are given in Table 21.5. Lysine-free/low-tryptophan L-amino acids are not advocated after 6 years of age (neurological crisis has not been reported after this age). After the onset of neurological damage, initiation of dietary treatment does not reverse the neurological damage in symptomatic patients but might prevent further encephalopathic crisis or progression of neurological damage.

21.3.3 Disorders of Urea Cycle Disorders

UCDs are rare defects in waste nitrogen metabolism associated with the breakdown of protein and other nitrogen-containing molecules. The long-term goals of nutritional therapy are to reduce ammonia concentrations to normal by restricting protein intake, providing sufficient nitrogen for optimal growth and providing adequate protein-free energy to minimise protein catabolism. Dietary treatment is used in combination with

Table 21.5 Summary of dietary management

	Age				
Diet	0–6 months	7–12 months	1–3 years	4–6 years	>6 years
Lysine-free, low-tryptophan L-amino acids: g/kg/day of amino acids	0.8–1.3	0.8–1	0.8	0.8	No recommendation
Lysine intake mg/kg/day	100	90	60–80	50–60	Avoid excess intake of protein but meet safe levels of protein intake (WHO/FAO/UNU 2007)
Energy intake Kcal/kg/day	80–115	80–95	80–95	80–90	Meet energy requirements for age

Adapted from guidelines for the diagnosis and management of glutaric aciduria type 1 revised recommendations (Kolker et al. 2011)

ammonia-scavenging drugs and arginine/citrulline (except in arginase deficiency); the latter increases flux through the urea cycle and acts as substrate for production of arginine metabolites. There is little consensus on the amount of natural protein that should be recommended but the European UCD guidelines suggested that the FAO/WHO/UNU (2007) 'safe levels of protein intake' could be used as a guide and the amount titrated to individual metabolic control, age and growth. Generally protein consumption should be high enough to meet cellular requirements for physiological functioning whilst low enough to prevent hyperammonaemia and maintain metabolic stability. Protein tolerance is at its highest during the first 6 months of life when infants grow rapidly and excrete lower amounts of dietary nitrogen as urea nitrogen. Excessive protein restriction may be associated with amino acid imbalance, including branched-chain amino acid deficiency, and could lead to catabolism and hyperammonaemia. It is important that at least 50% of natural protein should be encouraged from higher biological value sources and that natural protein is evenly distributed throughout the day.

The E-IMD-proposed guidelines on UCD recommended essential amino acid supplementation when natural protein intakes fall below safe levels of protein intake. All EAA supplements contain between 8.5 and 13.7% of nitrogen, compared with an average of 16% nitrogen from natural protein sources. EAA supplements also contain higher amounts of branched-chain amino acids than natural protein sources. In UCD, some European countries routinely supply part of the total protein allocation from essential amino acid supplements at diagnosis but this may be unnecessary if natural protein intake meets requirements and glutamine and ammonia concentrations are well controlled. It should also be unnecessary in patients with milder phenotypes. Vitamin and mineral supplements are recommended as patients may have low vitamin B_{12} and zinc intakes. Energy modules such as glucose polymers and fat emulsions may be required to supplement energy intake to ensure 'normal' population energy requirements are met.

21.3.4 Disorders of Carbohydrate Metabolism

The disorders of carbohydrate metabolism display a wide range of clinical features: Symptoms may be caused by toxicity (e.g. galactosaemia and hereditary fructosaemia) or lack of glucose causing hypoglycaemia in disorders of gluconeogenesis (GSD).

21.3.4.1 Galactosaemia

Galactosaemia is treated with a life-long galactose-restricted diet. From the time of suspecting the diagnosis of galactosaemia, breastfeeding or infant formula containing lactose is

Table 21.6 Milk, milk products and milk derivatives avoided in galactosaemia

Milk and milk products
Cow's milk, goat's milk, sheep's milk
Cheese, cream, butter
Ice cream, yoghurt, fromage frais, crème fraiche
Chocolate
Milk derivatives
Skimmed milk powder, milk solids, milk protein, non-fat milk solids, separate milk solids
Whey, hydrolysed whey protein, margarine or shortening containing whey, whey syrup sweetener, casein, hydrolysed casein, lactose
Buttermilk, butterfat, butter oil, milk fat, animal fat (may be butter), ghee, artificial cream
Cheese powder
Lactose as a filler may be used in:
Flavourings
Tabletop or tablet artificial sweeteners
Medicines

stopped and replaced with a lactose-free formula. Soya infant formula is usually advocated but a lactose-free infant formula based on L-amino acids is also appropriate. Avoiding cow's milk and milk products is universally recommended (Table 21.6). Labels on all processed foods and medications should be checked to avoid ingredients such as whey, non-fat dry milk, milk solids, lactoglobulin, lactalbumin and hydrolysed protein in addition to lactose. Lactose is widely used by the food industry as an anticaking agent, coating, browning enhancer, filler, flowing agent and carrier. Some mature cheese types have high levels of casein (which contains no more than 1 % lactose) and low levels of whey and when analysed for lactose and galactose content contain only trace/undetectable amounts. In the UK, the following low-lactose cheeses are allowed in galactosaemia: Emmental, Gruyere, Jarlsberg, some mature cheddar cheese, Parmigiano Reggiano and Grana Padano Italian parmesans. All other dairy cheeses are not permitted.

It is also well established that fruit, vegetables and legumes contain free or bound sources of galactose, and these foods are still avoided by some IMD treatment centres. However, these foods' sources contribute less than 60 mg/day of galactose, and this amount is negligible compared with the quantity produced via endogenous production. Thereby any restriction of fruit, vegetables and legumes is considered unnecessary.

Remember

In galactosaemia, low-lactose milks aimed at the lactose-intolerant population are contraindicated as they contain galactose. In their production, lactose is hydrolysed to glucose and galactose by lactase.

Long-term complications are common in galactosaemia and appear to be independent of the type of diet therapy or dietary adherence, and there is debate about the need for stringent galactose restriction in later life.

Reduced calcium intake from elimination of dairy products may lead to decreased bone density. Lactose-free calcium and vitamin D supplements are usually required if intake from milk replacements are inadequate.

21.3.4.2 Hereditary Fructosaemia (HFI)

In HFI, accumulation of fructose-1-phosphate causes inhibition of glycogen breakdown and glucose synthesis, thereby resulting in severe hypoglycaemia following fructose ingestion. It is treated by a very strict exclusion of dietary fructose, sucrose and sorbitol, but it is not possible to exclude these sugars completely. Different thresholds of fructose intake leading to the development of symptoms vary from 40 to 250 mg/kg/day. Some have suggested it should be as little as 1,500 mg/day. The average amount of fructose consumed in a 'normal' Western society's diet is 1–2 g/kg/day (Dixon et al. 2015 b).

Fructose is a naturally occurring sugar and is found in all fruits. Vegetables containing highest amounts of fructose and sucrose include beetroot, Brussels sprouts, carrots, green beans, okra, onion, peas, pepper, plantain, sweetcorn, sweet potato and tomato. Generally only vegetables with a very low fructose/sucrose (e.g. old potatoes, mushrooms, spinach, celery) are permitted in controlled and limited amounts. Cooking of vegetables causes a loss of free sugars, and they

therefore have a lower fructose content than raw vegetables.

Sucrose and fructose may be used as a carrier for flavourings. Polyols, derived from carbohydrates, are potential sources of sorbitol or derived from fructose. It is important to avoid maltitol, mannitol, isomalt, lycasin and sorbitol. Polyols are versatile ingredients and act as sweeteners, bulking agents, emulsifiers, stabilisers, humectants, thickeners, glazing agents or anticaking agents. Sucrose, sorbitol and artificial sweeteners may be also added to medications and ingredients should be checked (Dixon et al. 2015 b).

Starch, glucose and lactose are included in the diet. Glucose may be used as an alternative sweetener, although many individuals with HFI dislike the taste and avoid sweet-tasting foods.

Individuals with HFI are at risk of vitamin C and possible folic acid deficiency due to the exclusion of the major sources of these vitamins (fruits and vegetables). Therefore suitable supplementation of these vitamins is important.

Remember

Intravenous fructose and sorbitol are potential sources of fructose. Several lethal episodes of HFI following sorbitol and fructose infusion have been reported.

21.3.4.3 Hepatic Glycogen Storage Disease (GSD's Type I and Type III)

The aim of dietary treatment is to maintain normoglycaemia by the provision of exogenous carbohydrates to compensate for defective endogenous glucose production and prevent secondary biochemical abnormalities. Attaining normal growth with healthy body mass index is another important goal.

21.3.4.4 GSD Type Ia

With infants, normoglycaemia is maintained through frequent feeds (typically every 2 h) with a lactose-free formula, supplemented with maltodextrin if necessary to meet carbohydrate requirements. At around 4–6 months of age, the glucose requirements are around 0.5 g/kg/day but carbohydrate requirements decrease with age.

In some countries continuous overnight tube feeding is used to deliver glucose supply. Alternatively, UCCS can be introduced from 6 months of age, although the tolerance may be reduced as a consequence of lower pancreatic amylase activity. For all patients, UCCS can prevent the need for two hourly feeding and extends fasting tolerance to typically every 4–6 h, dependent on the results of UCCS loading tests. It is given in doses which approximate the basal glucose production rate (doses, 2 g/kg/ body weight/ day in young children decreasing to 1 g/kg/day in adolescents). The recent introduction of a modified form of high-amylopectin cornstarch (produced by a controlled heat moisture process) has been associated with longer periods of normoglycaemia, better short-term biochemical control and relatively less colonic fermentation in some individuals. Both UCCS and continuous overnight tube feeding are associated with disadvantages (Table 21.7).

Medium-chain triglycerides (MCT) have been given as an energy source in a small number of case reports in GSD type I. The use of MCT as a fuel may lower carbohydrate requirements by increasing ketone bodies and decreasing triglyceride concentrations.

21.3.4.5 GSD Type Ib

Dietary management is similar to GSD type Ia but is more challenging as complications such as

Table 21.7 Issues with uncooked cornstarch and nocturnal tube feeding in hepatic glycogen storage disease

UCCS	Nocturnal tube feeding
Poor tolerability, gastrointestinal symptoms	Safety: risk of technical failure, tube dislodgement
Limited normoglycaemia	Invasiveness of treatment
Night administration/sleep disturbance	Rebound hypoglycaemia
Reduced appetite	Reduced appetite
High carbohydrate intake and nutritional imbalance, poor nutritional quality	High carbohydrate intake and nutritional imbalance, poor nutritional quality
Excess energy intake and obesity	Excess energy intake and obesity

chronic inflammatory bowel disease causing perioral and anal ulcers, and diarrhoea may lead to intolerance of both UCCS and enteral feeds leading to poor nutritional status. Due to neutropaenia and increased infections, gastrostomy insertion may be contraindicated.

21.3.4.6 GSD Type III

The main principles of diet are to maintain normoglycaemia by provision of regular carbohydrate-containing meals, UCCS and a high-protein intake. Protein supplementation acts as a substrate for gluconeogenesis during fasting conditions and improves myopathy and growth failure. There is no consensus on the amount of protein (although approximately 3 g/kg/day has been suggested) or the quantity of UCCS that should be given (Derks et al. 2015). There is concern that surplus carbohydrate or UCCS can contribute to cardiomyopathy, as excess accumulation can cause deposition and accumulation of abnormal glycogen in the heart. Patients with GSD type III can utilise fructose and galactose so no special infant formula is required.

21.3.5 Disorders of Fatty Acid Oxidation

LC-FAODs (e.g. LCHADD, mitochondrial trifunctional protein deficiency [mTFPD], very-long-chain acyl-CoA dehydrogenase deficiency [VLCADD] and carnitine palmitoyl-CoA transferase 2 deficiency [CPT2D]) are caused by defects in the pathway that converts stored long-chain fats into energy resulting in deficiency of mitochondrial energy during exercise and fasting.

Treatment is primarily by diet and symptoms are mainly reversed by provision of regular dietary energy supply. The aims of dietary treatment are to minimise lipolysis, reduce accumulation of hydroxyacylcarnitines, provide adequate carbohydrate and MCT as energy substrates, ensure normal growth and avoid nutritional deficiencies. However, the severity of these disorders varies, with mild and severe phenotypes for each disorder, and this will determine dietary management. For example, for late-presenting patients with VLCADD who develop myopathic symptoms with exercise only, the use of MCT prior to exercise may be the only treatment necessary. In contrast, in disorders of mitochondrial trifunctional protein complex, progression of retinopathy is linked with hydroxyl acylcarnitine accretion, so strict low-fat diet with MCT supplementation is recommended, although retinopathy and neuropathy may still occur in strictly treated patients.

21.3.5.1 Fasting Guidelines

Avoidance of prolonged fasting is the cornerstone of therapy for all LC-FAOD, but acceptable fasting times vary between conditions, age and individual disorder severity. Patients with severe LC-FAOD, with fasting tolerance of less than 8 h, are likely to require nocturnal enteral tube feeds. There are no guidelines regarding the use of UCCS for LC-FAOD. Although it is recommended by some, its efficacy has not been established.

21.3.5.2 LCT

The degree of LCT restriction should be adapted to the severity and type of disorder. In LCHADD or mTFPD, fat restriction (<10% of energy intake) is essential to slow progression of chorioretinopathy. In symptomatic VLCADD patients, LCT restriction recommendations vary from 10% to 25–30% of total energy.

21.3.5.3 MCT

MCT supplementation is an essential part of therapy. MCT preparations consist mainly of caprylic acid (C8) and capric acid (C10). Substituting MCT for long-chain fat is associated with resolution of the cardiomyopathy seen in some infants with LC-FAOD. MCT transport into the mitochondria is carnitine independent and, as it is less likely to require esterification into chylomicrons, is rapidly released into the serum. Furthermore, because MCT is able to bypass the regulatory step for fatty acid oxidation, it is able to form ketone bodies. MCT supplementation decreases hydroxylated long-chain

acylcarnitine production from LCHADD or TFP-deficient cultured skin fibroblasts (Spiekerkoetter et al 2010). It also lowers plasma hydroxycarnitines at rest and during acute metabolic crisis in children with LCHADD. Although the dose has not been clearly defined, it is suggested that the energy percentage supplied by MCT should be 20–25 % in LCHADD and between 15 and 25 % of energy intake in symptomatic patients with VLCADD. The timing of dosage should be adapted to the activity of the patient. MCT oil may cause gastrointestinal symptoms, and it requires titrated introduction when given in older patients.

21.3.5.4 Triheptanoin

An oily substance consisting of glycerol bound to three molecules of heptanoic acid, is a C7 odd-chain fatty acid and has been advocated for disorders of the TFP complex including LCHADD. It has an anaplerotic effect; heptanoate metabolism provides substrate for the citric acid cycle and the electron transport chain that bypasses the deficient fatty acid oxidation enzyme in order to enhance ATP production. It is associated with a lower number of patient hospitalizations, improved cardiomyopathy and improved muscle strength in small patient numbers, but does not prevent the decline in retinal function. It is suggested that 30–35 % of energy intake is supplied by triheptanoin, i.e. 60 mg/day in children weighing <20 kg and a dose of 120 mg per day in children >20 kg body weight. It is not without side effects and may cause gastrointestinal symptoms and excessive weight gain (if energy intake from other foods is not adjusted).

21.3.5.5 Carbohydrate

Generally no additional carbohydrate is recommended in LC-FAOD unless clinically indicated. Probably the regularity of carbohydrate intake is more important than the absolute amount. However, if fat intake is severely restricted without adequate MCT, extra carbohydrate may be added to provide the energy deficit, inhibit mobilisation of fatty acids and prevent hypoglycaemia (though always a late event). Increased weight gain is documented in children with LCHADD and this may be due to excessive carbohydrate intake.

21.3.5.6 Micronutrient Supplementation

Over-restriction of long-chain fat may potentially lead to essential fatty acid deficiency and supplementary fatty acids, and LCPs are essential in all patients on severe fat restrictions. Fat-soluble vitamins (vitamins A, D and K) are also necessary.

21.3.5.7 Infants

Treatment depends on each LC-FAOD condition and presence of symptoms with abnormal biochemistry. In all LCHADD infants, breast milk or normal infant formula should stop and be replaced with a high-MCT infant formula, providing carbohydrate equivalent to at least standard normal infant formula, essential fatty acids and, ideally, a source of LCPs. It should be given regularly with avoidance of fasting. In VLCADD infants diagnosed by newborn screening, treatment depends on the presence of symptoms and abnormal biochemistry (elevated transaminases or creatinine kinase). With symptoms and abnormal biochemistry, MCT formula should be given and fasting avoided. Without symptoms or abnormal biochemistry, the use of breast milk is commonly supported.

21.3.5.8 Exercise Management

With prolonged exercise, fatty acid oxidation is increased, causing a probable lack of energy for the exercising muscles, and a common complication is rhabdomyolysis. Routine supplementation with MCT and carbohydrate given immediately before exercise may improve exercise tolerance. MCT supplements (0.5 g/kg lean body mass) given prior to exercise improves post-exercise metabolites and produces a lower steady-state heart rate.

21.3.5.9 MCADD (Medium Acyl-CoA Dehydrogenase Deficiency)

No fat restriction is necessary (except avoidance of dietary products high in MCT). Treatment consists of avoidance of fasting and use of glucose polymer-based emergency regimen for

Table 21.8 MCADD UK (2007) guidelines with MCADD for 'maximum safe fasting times for the well child (access via BIMDG website)

Age	Time in hours
From time of positive screening test result to 4 months of age	6
From 4 months	8
From 8 months	10
From 12 months onwards	12

illness, surgery or trauma. It is important that infants are fed regularly (Table 21.8 for fasting guidelines). On diagnosis, breastfed infants may be vulnerable if feeding is not well established, and top-up feeds with expressed breast milk may be necessary. Over the age of 1 year, safe fasting times may be extended to 12–14 h.

Take-Home Messages

- Diet therapy should be always customised to meet the specific needs of each patient considering their disorder severity and dietary tolerance.
- Systematic and observant nutritional follow-up, with regular monitoring of biochemical control, is essential to avoid nutritional deficiencies and identify early signs of adherence issues. Early counteractive measures can improve diet quality, metabolic control and long-term outcome.

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Gene and Cell Therapy for Inborn Errors of Metabolism

22

Cary O. Harding

22.1 Introduction

Contemporary therapy for many inborn errors of metabolism (IEM) is associated with significant shortcomings in terms of overall treatment efficacy and quality of life. For example, current recommendations for the treatment of phenylketonuria (PKU) due to recessively inherited phenylalanine hydroxylase (PAH) deficiency dictate that dietary phenylalanine restriction be maintained for life (Singh et al. 2014; Vockley et al. 2014). Adherence to this complicated and unpalatable diet is difficult for most adolescents and adults (Walter and White 2004), and even with therapy, many hyperphenylalaninemic adults suffer symptoms of anxiety and depression and exhibit significant impairments in executive function (VanZutphen et al. 2007; Christ et al. 2010). Treatment outcomes for other more life-threatening disorders such as urea cycle disorders or organic acidemias are even more problematic. For this reason, a search for novel treatments and even potentially permanent cures is highly desired. Cell transplantation therapy, namely, hematopoietic stem cell transplantation, has become a widely accepted treatment modality for genetic disorders affecting bone marrow function, such as hereditary immune deficiencies and thalassemia, but also for certain lysosomal storage disorders in which therapeutic levels of the relevant enzyme can be achieved in target tissues through transfer from donor bone marrow-derived cells. For other disorders, the therapeutic efficacy of hematopoietic stem cell transplantation (HSCT) has been limited. Liver transplantation has also been applied to the treatment of several IEM, but donor organ availability is limited, and lifelong immune suppression is required. Perhaps, rather than whole-organ transplantation, cell transplantation or gene therapy for these diseases will in the future become standard of care. A comprehensive review of cell transplantation and gene therapy technologies could fill complete textbooks on their own. The interested reader should consult the excellent textbook edited by Nancy Smyth Templeton for up-to-date and in-depth discussions regarding the greater field of cell and gene therapy (Smyth Templeton 2015). This chapter will focus only upon key aspects of cell transplantation or gene therapy directed toward the treatment of select inborn errors of metabolism with emphasis upon applications that have already entered or are nearing clinical trial.

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22.2 Critical Issues in the Design of Cell and Gene Therapy for IEM

22.2.1 Fundamentals

Cell therapy is defined as the transplantation of living cells from another individual to the patient for the purpose of treating disease. Propagation and transplantation of a truly pluripotent stem cell that can differentiate into any cell type are the holy grail of the cell therapy field, but in reality this feat has yet to be fully accomplished. Fully differentiated cells (such as hepatocytes) or of tissue-specific stem cells that have a restricted differentiation repertoire (such as hematopoietic stem cells (HSC)) are the cell types currently available for clinical use in transplantation protocols. In gene therapy, the goal is to alter gene expression in the treated individual; this is typically accomplished through transfer of a nucleic acid-based (DNA or RNA) vector system. Two general gene therapy approaches exist: direct gene therapy and indirect or ex vivo gene therapy. Direct gene therapy is relatively self-explanatory; a DNA- or RNA-based vector is introduced directly into the patient. In indirect gene therapy, cell and gene therapies are combined. A population of cells (bone marrow, for instance) is removed from the patient, treated ex vivo with a gene transfer vector, and then reintroduced back into the patient. The specific choice of one of these potential treatment strategies is dependent upon the specific organ and disease to be treated.

The sound design of a rational cell or gene therapy treatment strategy for a specific disease must be based upon a thorough understanding of disease pathophysiology. This knowledge is used to answer several fundamental questions regarding trial design (Table 22.1). First, since no single cell type or currently available gene transfer system can be used to treat all tissue types simultaneously, a specific target tissue must be selected. The choice may be seemingly obvious for many disorders; for instance, targeting the liver is the natural choice for treatment of many inborn errors of intermediate metabolism such as aminoacidopathies and organic acidemias, but fully understanding the disease pathophysiology may reveal the possibility of effectively expanding the choice of target. Why might this be desirable? The regenerative capacity of hepatocytes complicates the ability to achieve permanent liver transduction; gene transfer is more stable in quiescent tissues. For many IEM, accumulation of specific toxins in the circulation is the proximate cause of pathology, and removal of those toxins through any effective method will ameliorate the phenotype. For example, PKU is not associated with any liver pathology but rather causes elevated blood and consequently brain phenylalanine. Chronic hyperphenylalaninemia is associated with brain dysfunction and in juveniles' impaired neuronal development, but correction of hyperphenylalaninemia through diet manipulation prevents the major manifestations of the disease even without restoring liver PAH activity. Liver-directed gene

Table 22.1 Fundamental decisions in the design of a cell or gene therapy trial

Question	Parameter influenced
What target tissue?	Cell or gene transfer vector type chosen
Is disease pathophysiology due to insufficiency of a gene product or to a dominant negative effect from aberrant protein?	Gene addition vs gene correction
Is the disease pathophysiology cell autonomous?	Gene transfer vector choice Need for cell selection
How many corrected cells are required to influence the phenotype?	Gene transfer vector choice Need for cell selection
Permanent or temporary gene expression?	Gene transfer vector choice
Level of gene expression per cell required?	Selection of cell donor Choice of promoter in gene transfer vector
Potential for immune or inflammatory reactions?	Gene transfer vector choice Need for immunomodulation
Potential for genotoxicity or oncogenesis?	Cell or gene transfer vector type chosen Design of gene transfer vector

therapy can correct hyperphenylalaninemia in mouse models (Fang et al. 1994; Mochizuki et al. 2004; Ding et al. 2006; Harding et al. 2006), but expression of a phenylalanine-metabolizing system in a heterologous tissue such as skeletal muscle can also lead to permanent correction of hyperphenylalaninemia (Harding et al. 1998; Ding et al. 2008). In another example, CNS pathology is a hallmark of many lysosomal storage diseases, but because transcytosis of lysosomal enzymes from one cell to another occurs naturally, enzyme-expressing donor cells from a tissue other than the brain can be effectively used to deliver therapeutic enzyme to the CNS. HSC transplantation for Hurler syndrome is one specific example of this treatment approach. In summary, disease pathophysiology determines the choice of target tissue.

The pathology of many IEM is due to deficiency of a specific gene product such as an enzyme or transport protein; these disorders are likely amenable to gene addition strategies in which the gene transfer vector expresses the missing protein but does not alter the genome of the target cell. However, in many diseases, including most dominantly inherited neurologic disorders, the production of an aberrant protein from the mutated allele is directly responsible for the disease-associated pathology. For these diseases, simple gene addition will likely not affect the phenotype, but successful treatment would require a gene correction approach (gene editing) in which the mutant gene is replaced or inactivated to prevent expression of the aberrant gene product. Again, the specific need for either gene addition or gene correction will influence the choice of DNA transfer vector.

The cell autonomous nature of the pathophysiology in a specific disease also greatly influences the choice of treatment approach. For some disorders, such as for the PKU example above, correction of an inherited enzyme deficiency in only a portion of the hepatocytes in the liver may correct the disease phenotype. However, for other diseases, pathology occurs in all genotypically mutant cells, and correction of a few neighboring cells will not rescue cells that have not undergone genetic correction. For example, gene therapy in *Fah*$^{\Delta exon5}$ mice (Grompe et al. 1995), a model of human tyrosinemia type 1, will rescue a portion of FAH-deficient hepatocytes, but the remaining uncorrected cells will still undergo apoptosis without treatment (Overturf et al. 1996). Successful therapy of similar disorders likely will require a gene transfer method that can effectively transduce all or at least a majority of target cells.

The proportion of cells in a target organ that must be replaced (in a cell therapy protocol) or corrected (through gene therapy) in order to effectively alter the disease phenotype will differ between different disorders. Successful treatment of hemophilia due to inherited deficiency of either coagulation factor VIII or factor IX (FIX) likely requires successful transduction of only a few percent of hepatocytes in order to produce sufficient circulating levels of the needed factor, but most disorders of intermediate metabolism will likely require transduction of a greater proportion of hepatocytes in order to sufficiently clear pathogenic metabolites. Hepatocyte transplant experiments have clearly demonstrated a therapeutic threshold of approximately 10 % PAH-expressing hepatocytes needed to completely correct blood phenylalanine concentrations in *Pah*^{enu2} mice, a model of human PKU (Hamman et al. 2005). Other diseases will likely require even greater proportions of cell correction. In the dominantly inherited acute porphyrias, for example, 50 % enzyme activity in all hepatocytes is already insufficient to prevent symptoms under certain physiologic conditions; how much more enzyme activity and spread among how many hepatocytes will be necessary to prevent disease? Additionally, the proportion of successfully corrected hepatocytes is likely as much or perhaps even more important than the amount of enzyme produced per cell. In *Pah*^{enu2} mice, correction of blood phenylalanine concentration occurs at approximately 10 % liver repopulation with PAH-expressing cells regardless of whether the donor cells were wild type and therefore expressing 100 % normal levels of PAH activity or heterozygous *Pah*$^{enu2/+}$ cells with only partial PAH activity (Hamman et al. 2011). This result demonstrates that phenylalanine clearance

was limited by cell number rather than the amount of PAH activity per cell at this low level of cell repopulation and suggests that even supraphysiologic PAH expression from a gene transfer vector would not successfully treat PKU unless PAH expression had been induced in at least 10 % of hepatocytes. The therapeutic thresholds in terms of the number of corrected cells and amount of therapeutic gene expression per cell needed to correct a disease phenotype will likely vary among different disorders.

Permanent production of a therapeutic protein is desired for most IEM, and therefore cell or gene therapy methods that lead to permanent treatment are sought. However, for some conditions, only temporary gene expression might be sufficient. Anticancer gene therapy or gene therapy approaches to specific infectious diseases may require therapeutic gene expression for only a limited period. The needed duration of therapy will influence the choice of gene transfer vector. Gene transfer vectors based upon the Moloney murine leukemia virus (MMLV) (so-called retroviral vectors) or human immunodeficiency virus (so-called lentiviral vectors) often lead to permanent cell transduction because the vector genome is permanently integrated into the genome of the host cell. The difficulty then is to achieve permanent transduction in a sufficient number of cells to positively influence the disease phenotype. Recombinant adeno-associated virus (rAAV) vector genomes seldom integrate into the host genome but remain as pseudostable circular concatemers within the nuclei of treated cells. rAAV-mediated gene expression remains stable in mitotically quiescent tissues but is quickly lost in tissues with rapid cell division, such as occurs in the liver following toxin-induced hepatocellular damage or partial hepatectomy. On the other hand, random integration of a vector genome, although leading to permanent gene expression, can also be associated with deleterious effects of oncogene activation or tumor suppressor disruption. The need for permanent gene expression must be balanced against the risk of genotoxicity.

Some vector systems induce substantial immediate inflammatory or delayed immune reactions. Gene transfer vectors based upon human adenovirus (Ad) are now notorious for their association with acute inflammatory reactions as this was the cause of death for an 18-year-old male enrolled in a recombinant adenovirus gene therapy trial for ornithine transcarbamylase (OTC) deficiency (Raper et al. 2003). However, for certain applications such as antitumor therapy, inflammation directed at vector-transduced tissues could yield enhanced therapeutic benefit. The first gene transfer vector ever licensed for clinical use (licensed by the government of China) was a recombinant Ad vector designed to treat head and neck squamous cell carcinoma. This vector (ONYX-015) was designed to replicate in and lyse p53-deficient tumor cells; inflammation triggered by the Ad virus and tumor cell necrosis could have enhanced the antitumor effect of the vector.

22.2.2 Available Methods for Gene Transfer

Many different methods for transferring DNA into cells are available (Table 22.2), but these differ significantly in their ability to transduce cells in culture or in a whole organism. Recombinantly engineered viral vectors exploit the robust native

Table 22.2 Common reagents for gene transfer

DNA-mediated gene transfer
Endocytosis-mediated methods
Calcium phosphate precipitation
Protein-DNA conjugates
In vivo intramuscular injection
In vivo hydrodynamic vein injection
Lipofection
Electroporation
Particle bombardment
Recombinant virus-mediated gene transfer
Moloney murine leukemia virus (MMLV) (colloquially known as “retrovirus”)
Human immunodeficiency virus (colloquially known as “lentivirus”)
Adenovirus
Adeno-associated virus (AAV)
Herpes simplex virus (HSV)

ability of viruses to penetrate living cells and are therefore commonly employed for in vivo trials. As described above, MMLV and lentivirus vectors typically are associated with permanent integration of the vector genome into the host cell genome allowing for vector genome replication and sustained expression even in the face of host cell division. With Ad or AAV vectors, vector genomes do not integrate into the host genome but reside as independent episomes within host cell nuclei. Typically, vector genome copy number in any single host cell nucleus is much greater following infection with a nonintegrating vector than with an integrating MMLV or lentiviral vector. This may lead to greater levels of therapeutic gene expression per cell in host cells infected with a nonintegrating viral vector, but expression may be lost following host cell division because the viral episome cannot be replicated or retained during nuclear division. Other drawbacks of viral vectors include inflammatory and immune-mediated reactions typically against the virus capsid leading to unwanted symptoms and elimination of vector-transduced cells. Furthermore, viral biology firmly limits the size of the foreign DNA that can be accommodated in a recombinant viral genome. Viral vectors are often also difficult to produce in great quantity and with high purity. Non-viral or so-called DNA-mediated gene transfer vectors generally avoid any immune complications and can accommodate very large therapeutic expression cassettes, and the necessary reagents are relatively easy to produce in large quantity, but their transduction frequency, especially in whole organisms, is generally significantly inferior to that of viral vectors. The non-viral approaches are however very useful for gene transfer into cultured cells, and methods designed to improve their in vivo performance continue to be developed (Viecelli et al. 2014).

Regardless of the gene transfer vehicle chosen, all vector genomes designed to accomplish gene addition require specific DNA elements in their construction. As an example, the genome of prototypic adeno-associated virus (AAV) vector is displayed in Fig. 22.1. The expression cassette must contain the gene of interest, typically its complementary DNA (cDNA) rather than the full-length genomic sequence, a promoter sequence to drive expression, and a polyadenylation (polyA) signal (either the native polyA signal associated with the gene of interest or more typically a standard mammalian polyA signal appended 3′ to the cDNA). Many different promoter sequences are available for use, and most include specific enhancer elements alongside the RNA polymerase binding site to increase gene expression. Promoter/enhancer sequences adapted from viral genomes, such as cytomegalovirus (CMV), typically yield high levels of gene expression, are initially ubiquitously active in most tissues and cell types, but are subject to inactivation in certain tissues, especially the liver. Promoter/enhancer combinations derived from native human genes are very useful for supporting tissue-specific gene expression (for instance, the promoter from the human albumin gene is active only in hepatocytes), but the amount of gene expression from these native promoters is frequently much less robust than from viral promoters. In recent years however, significant progress has been made in the development of tissue-specific promoters that drive physiologically relevant levels of gene expression through

Fig. 22.1 Anatomy of an expression cassette for gene addition. A model of an expression cassette within an adeno-associated virus (AAV) vector genome is presented. Expression of a therapeutic complementary DNA (cDNA) is driven by either an ubiquitously active or tissue-specific promoter/enhancer combination (promoter). A polyadenylation signal (*pA*) must be included for successful transcription. In many constructs, a hybrid intron sequence is included in the expression cassette. Splicing of intronic sequences from pre-mRNA is coupled to nuclear mRNA export; inclusion of an intron sequence in the expression cassette often enhances therapeutic gene expression. All therapeutic gene transfer vectors must contain these minimal elements. For this putative recombinant AAV vector, the viral intermediate terminal repeats (ITR) are the only viral sequences remaining in the vector genome; these are necessary to direct packaging of the recombinant genome into functional AAV particles and for appropriate processing of the single-stranded genome once it has penetrated the target cell nucleus

directed recombination of enhancers and promoters from various human genes; this approach has led to the development of significantly improved liver-specific promoters, for instance (Nair et al. 2014).

22.2.3 Gene Editing

Gene therapy for most dominantly inherited disorders will require gene correction rather than simple gene addition to influence the disease phenotype. Gene correction is also preferred over gene addition for treatment of tissues in which gene addition is not stable. The goal is to edit the mutant allele back to the normal wild-type sequence and restore normal gene product expression in the target tissue. Several DNA endonuclease systems have been developed for site-specific gene editing including zinc-finger nucleases (ZFN) (Porteus 2006), transcription activator-like effector nucleases (TALEN) (Cermak et al. 2011), and most recently and most successfully, clustered regularly interspaced short palindromic repeats (CRISPR)/Cas9 endonuclease (Hsu et al. 2014). In each of these systems, a site-specific DNA-binding motif is coupled with a DNA endonuclease to create a double-strand break (DSB) in the target genomic sequence. In the cases of ZFN or TALEN, the DNA-binding motifs are DNA-binding proteins (zinc-finger proteins or transcription activator-like effector proteins) derived from naturally occurring bacterial systems that have been engineered to bind to specific mammalian DNA sequences. These binding proteins are coupled to an endonuclease (typically the *Fok*I nuclease) to create site-specific endonucleases. The primary limitation to either strategy is restricted ability to engineer the DNA-binding protein to target a specific desired genomic sequence in the target cell. For the CRISPR/Cas9 system, the Cas9 endonuclease is drawn to the target cleavage site by a single-stranded guide RNA; alteration of the target site is readily accomplished through synthesis of a guide RNA that is complementary to the desired DNA target.

Gene editing using CRISPR/Cas9 technology has enjoyed rapid development and widespread adoption, because of the facile ease in altering its targeting site, since the first reports of using these reagents to cleave specific DNA sequences in mammalian cells in 2013 (Cong et al. 2013; Mali et al. 2013). The naturally occurring CRISPR/Cas system is a key component of microbial adaptive immunity which bacteria uses to protect themselves from viral invasion. The natural system employs an RNA-guided endonuclease (Cas9) to cleave specific CRISPR sequences in and to eliminate bacteriophage genomes. However, engineering of the guide RNA to be complementary to a specific genomic target also allows Cas9 to induce DSB in DNA from any eukaryotic species. The site of the DSB in the genomic target is directed by the presence of so-called protospacer adjacent motifs (PAMs) occurring natively in the target DNA sequence. Once Cas9 induces a double-strand break at a specific PAM-containing genomic site, the break can be repaired through one of two DNA repair mechanisms operating endogenously within the host mammalian cell, either nonhomologous end joining (NHEJ) or homology-directed recombination (HDR). NHEJ often results in a small deletion at the site of the initial DSB and causes the target gene to remain disrupted even after gene repair. This technique has been used to interrogate specific gene function in cultured cells and to develop targeted knockout animal models. For instance, CRISPR/Cas9 reagents expressed from rAAV8 vectors delivered to mouse liver have caused targeted deletion of the cholesterol regulatory gene *Pcks9* (Ran et al. 2015) or when expressed from adenovirus vectors to knockout *Pten* (Wang et al. 2015). Alternatively, if an intact copy of genomic DNA with homology to sequences flanking the DSB is supplied along with the CRISPR/Cas9 machinery, this repair template can be integrated through HDR into the region of the DSB leading to gene correction. This latter mechanism has been used to correct the missense mutation causing tyrosinemia type 1 in $Fah^{\Delta exon5}$ mice (Yin et al. 2014). The CRISPR/Cas9 system has also been utilized to introduce a foreign marker gene (green fluorescent protein or

GFP) into a specific genomic site in *C. elegans* worms (Dickinson et al. 2013).

The requirements for successful application of a gene-editing method to the treatment of a disease phenotype include (1) the ability to induce permanent gene correction in a proportion of target cells that is sufficient to affect the disease phenotype, (2) the ability to fully correct the target gene to its wild-type sequence without any residual mutation introduced by the DNA repair process, and importantly, (3) the ability to not cause DNA strand breaks in regions other than the desired target gene (so-called off-target effects). Each of the available gene-editing methods and, even within a given method, each of the reagents designed for a specific target gene or mutation vary significantly in their ability to fully meet these requirements. To date, only ZFN technology has been employed in a human clinical trial (ZFN has been used to disrupt the CCR5 gene in hematopoietic stem cells of HIV-infected patients in an attempt to attenuate the infection), but many investigators are actively pursuing therapeutic gene correction for future clinical application.

22.2.4 Required Elements for a Successful Cell Therapy Protocol

The success of cell transplantation in achieving a therapeutic threshold is dependent upon two factors: there must be a stimulus for cell proliferation in the target organ at the time of donor cell transplantation, and the donor cells must have a selective growth advantage over the native cells. Unless these two factors are met at the time of transplant, the extent of repopulation of the target organ with donor cells will likely be insufficient to affect the disease phenotype. In HSC transplantation, for example, administration of chemotherapy or radiation to the recipient initiates a stimulus for cell growth and proliferation in bone marrow and also impairs cell division of the native HSC in the recipient. Under these conditions, donor HSC enjoys a tremendous growth advantage and will readily repopulate the depleted marrow compartment. Similar factors must be satisfied for successful therapeutic liver repopulation following hepatocyte transplantation (Laconi and Laconi 2002). In some disorders, such as tyrosinemia, a selective growth advantage for donor cells over native deficient cells occurs naturally (Overturf et al. 1996). This advantage can significantly amplify the effects of liver-directed gene therapy as well, for even a small population of transduced hepatocytes will expand to repopulate the diseased liver if the corrected cells enjoy a growth advantage. For many disorders however, no such selective growth advantage exists, and the extent of liver repopulation is limited even if a growth stimulus, for instance, partial hepatectomy, is provided prior to donor cell transplant. Mathematically, the number of donor cells transplanted will firmly limit the final extent of liver repopulation if no selective growth advantage exists (Fig. 22.2). Investigators in the field have attempted to overcome this barrier through either repeated cell transplants or by treating the native liver with some toxin or insult (such as irradiation to both induce a growth stimulus but also slow the replication of native hepatocytes). A current clinical trial of hepatocyte transplantation in PKU at the University of Pittsburgh utilizes focused gamma irradiation of a single liver lobe prior to hepatocyte transplant (Zhou et al. 2012) in an attempt to block native hepatocyte regeneration and allow effective repopulation with donor cells (Ira Fox, personal communication).

22.3 Specific Clinical Applications of Cell and Gene Therapy in IEM

22.3.1 Bone Marrow-Directed Gene and Cell Therapy for IEM

Hematopoietic stem cell transplantation (HSCT) has become standard treatment for many diseases affecting bone marrow but has also found utility in the treatment of select IEM. HSCT performed prior to 2 years age has been shown to significantly delay CNS neurodegeneration in Hurler

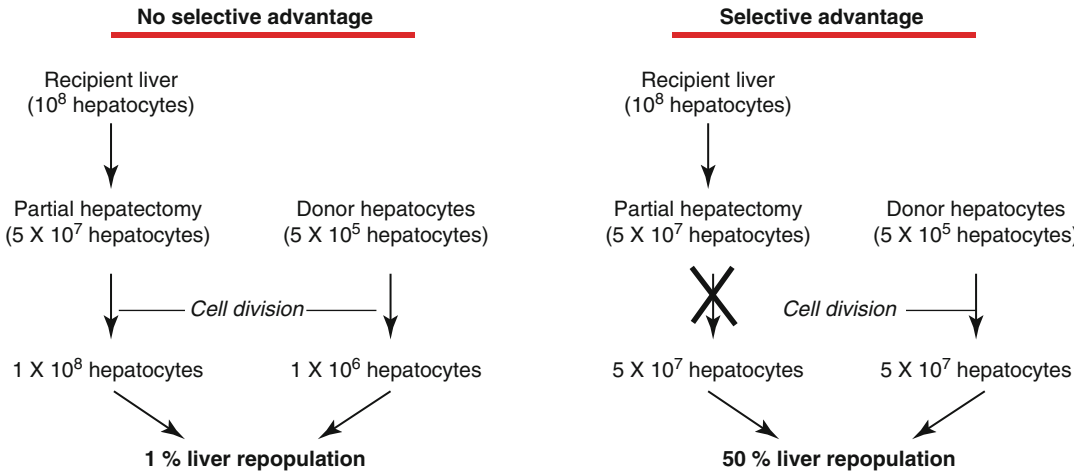

Fig. 22.2 Selective cell growth advantage and therapeutic liver repopulation. Therapeutic liver repopulation following hepatocyte transplantation can only be successful if donor hepatocytes enjoy a selective growth advantage over cells in the recipient liver, as illustrated by this model of therapeutic liver repopulation in mice. In this thought experiment, hepatocyte regeneration is stimulated through a partial (50 %) hepatectomy. The adult mouse liver contains approximately 1×10^8 hepatocytes; following partial hepatectomy, this number is reduced to 5×10^7 hepatocytes. 5×10^5 hepatocytes isolated from a congenic donor liver are then delivered to the portal circulation via intrasplenic injection. Donor hepatocytes migrate to and repopulate the liver. The regeneration stimulus continues until the hepatocyte content of the liver has been restored to 1×10^8 cells. Without a selective growth advantage for the donor cells, both the recipient and the donor hepatocytes go through a single cell division before the liver is fully repopulated and the regeneration stimulus ceases; ultimately donor hepatocytes make up only 1 % of the repopulated liver. Under conditions in which regeneration of hepatocytes from the recipient liver is blocked (such as may occur naturally in some IEM or that may be induced through liver irradiation or other insults), donor hepatocytes will continue to proliferate until the regeneration stimulus ceases. At that point, the donor cells will make up 50 % of the repopulated liver if regeneration of recipient hepatocytes has been completely blocked

syndrome (Cartier and Aubourg 2008) and will also arrest early inflammatory cerebral degeneration that occurs in boys with the cerebral form of X-linked adrenoleukodystrophy (X-ALD) (Shapiro et al. 2000). Presumably in the former case, donor-derived reticuloendothelial cells penetrate into the CNS and allow for enzymatic cross correction of glial cells and neurons of the recipient with Hurler syndrome. The mechanism of how HSCT arrests cerebral disease in X-ALD is not precisely known; circulating very long-chain fatty acid levels are little affected by HSCT, but the CNS inflammatory process that triggers neuronal degeneration is typically arrested following HSCT, if the procedure is performed early in the course of degeneration. HSCT is not effective in children with Hurler syndrome who are greater than 2 years age presumably because fewer donor marrow-derived cells penetrate across the blood-brain barrier (BBB) after this age. Likewise, HSCT has little effect upon advanced cerebral degeneration in X-ALD. HSCT has not demonstrated similar efficacy in a variety of other lysosomal storage diseases probably because the amount of enzyme that is transferred to the brain by donor-derived reticuloendothelial cells is insufficient to correct those phenotypes.

Hematopoietic cell-directed gene therapy for severe combined immunodeficiency (SCID) due to recessively inherited adenosine deaminase (ADA) deficiency (Blaese et al. 1995) was the first clinical attempt at therapeutic gene transfer, performed some 25 years ago now. In this trial, peripheral blood lymphocytes collected from two different young girls with ADA-SCID were transduced ex vivo with a recombinant Moloney murine leukemia retroviral (MMLR) vector engineered to express ADA and then reinfused into the patients. This resulted in improved

peripheral blood lymphocyte survival, but the girls were never trialed off PEG-ADA enzyme replacement therapy to determine the full extent of immune reconstitution. Because peripheral blood lymphocytes and not bone marrow-derived hematopoietic stem cells had been the treatment target, the number of transduced lymphocytes waned with time and repeated infusions of vector-treated lymphocytes were required. Subsequent ongoing clinical trials employ ex vivo transduction of patient-derived HSC with ADA-expressing vector. In this system, the recombinant viral genome integrates permanently into the host hematopoietic stem cell, and, if exogenous PEG-ADA infusions are discontinued, ADA-positive progeny cells enjoy a significant growth advantage over untransduced cells leading to repopulation of bone marrow and the peripheral blood with ADA-expressing cells. This procedure has nearly become the standard of care for these rare patients if they lack an HLA-matched HSC donor suitable for HSC transplantation.

Similar treatment protocols using MMLR vector (Hacein-Bey-Abina et al. 2002) to permanently transduce HSC ex vivo have been successfully used to treat X-linked immunodeficiency due to deficiency of the cytokine receptor common gamma (c) chain. However, in a subset of treated boys, integration of the vector genome upstream of the LMO2 oncogene led to activation of LMO2 expression and the development of acute leukemia (Hacein-Bey-Abina et al. 2003). Ultimately, nine males with gamma (c) chain deficiency underwent retrovirus-mediated gene therapy with eight achieving substantial immune reconstitution. Four of the treated patients however suffered acute leukemia and one of these died (Hacein-Bey-Abina et al. 2010). In order to lessen the risk of oncogenesis, current trials utilize novel retroviral vectors, so-called self-inactivating (SIN) vectors, which have been further engineered to truncate vector-derived RNA transcription before expression can extend beyond the confines of the vector genome and incorporate any open reading frames from neighboring host genome sequences. This approach does not alter the frequency of vector genome integration but should dramatically reduce the risk of oncogenesis.

For lysosomal storage disorders and for other diseases where a therapeutic gene product can be supplied to the brain via circulating reticuloendothelial cells, bone marrow-directed ex vivo gene transfer utilizing either recombinant MMLR or lentiviral vectors is poised to develop into a true treatment revolution if recent experimental success continues. Lentiviral-mediated HSC transduction and expression of arylsulfatase have been reported to arrest and even reverse neuropathology associated with metachromatic leukodystrophy (Biffi et al. 2013). Similar astounding results were seen in boys with advanced cerebral X-linked adrenoleukodystrophy (X-ALD) following ex vivo transduction of marrow with a lentiviral vector expressing the ABCD1 transport protein (Cartier et al. 2009). In this first trial, the two trial subjects, lacking any family history of the disease, had presented with neurologic deterioration as the initial disease manifestation. HSCT would have been the treatment of choice (Shapiro et al. 2000), but no HLA-matched marrow donors were found for the boys. Autologous CD34+ cells were then harvested from the patients, infected with the lentiviral vector, and then reinfused back into the subjects following myeloablation. 24–30 months after treatment, ABCD1 protein was detected in 9–14 % of circulating white blood cells. By 14–16 months after treatment, the neurologic disease, as assessed by symptoms and cranial MRI changes, had been completely arrested. Subsequent oral presentations described a third boy so treated in which the white matter abnormalities associated with the cerebral degenerative form of X-ALD had actually partially reversed over several months after therapy.

This treatment approach has two primary advantages over HSCT from an allogeneic donor. First, the patient's own marrow is used in the procedure so there is little risk of rejection or graft-vs-host disease. There will always be the small possibility of an immune reaction against the expressed therapeutic gene product, particularly if the patient carries null mutations associated

with complete lack of the protein in question. Secondly, transduction of HSC with lentiviral vectors may lead to supraphysiologic expression of the therapeutic gene. This would be advantageous for the treatment of lysosomal storage disorders as the lentivirus-transduced marrow-derived cells would deliver a greater quantity per cell of the required lysosomal enzyme to target tissues in comparison with non-transduced cells from an allogeneic marrow donor. This treatment approach holds great promise for the treatment of disorders such as Hurler syndrome or globoid cell leukodystrophy in which HSCT is not uniformly curative (Biffi et al. 2011).

22.3.2 Liver-Directed Cell and Gene Therapy

Many inborn errors of intermediate metabolism are due to inherited deficiency of key enzymes typically expressed in the liver. As discussed above, for these diseases, the liver is the prime therapeutic target. Successful repopulation of the liver with donor hepatocytes or correction of liver enzyme deficiency through gene therapy has been in intensive areas of research for many investigators. A complete recounting of this long adventure is beyond the scope of this chapter, but the highlights of these attempts will be presented here.

Any disease that can be successfully treated by orthotopic liver transplantation is theoretically a candidate for either liver-directed cell transplantation or gene therapy. Whole-organ transplantation, although increasingly employed in the treatment of IEM, is associated with significant surgical risk and the need for lifelong immunosuppression. Importantly, the supply of donor organs is extremely limited. Cell transplantation in most forms will still require immunosuppression to maintain the donor graft, but cells, such as hepatocytes, from a single donor organ may be able to repopulate several recipients and thereby somewhat alleviate limited donor availability. Perhaps in the future, transplantation of hepatocytes differentiated from cultured stem cells could further reduce the need for cadaveric organ donors. Gene therapy of course is not dependent upon the availability of donor cells and likely would not require ongoing immunosuppression, although immune reactions against the delivery vehicle are possible.

22.3.2.1 Therapeutic Liver Repopulation Through Hepatocyte Transplantation

The history of hepatocyte transplantation development and the application of hepatocyte transplantation in the treatment of human disease have been reviewed (Hansel et al. 2014). The first transplants of allogeneic human hepatocytes occurred as an attempt to bridge individuals with liver failure to whole liver transplant (Strom et al. 1997). The first use of human hepatocyte transplantation to treat an IEM occurred in an individual with Crigler-Najjar syndrome type 1 (UDP-glucuronosyltransferase 1A1 (UGT1A1) deficiency) (Fox et al. 1998). In this experiment, a single hepatocyte transplantation resulted in reconstitution of 5 % of normal liver UGT activity and a 50 % reduction in serum bilirubin. Subsequent reported clinical trials of hepatocyte transplantation have included treatment of further individuals with Crigler-Najjar syndrome (Darwish et al. 2004; Ambrosino et al. 2005; Dhawan et al. 2006; Allen et al. 2008; Khan et al. 2008; Meyburg et al. 2010), urea cycle disorders including ornithine transcarbamylase deficiency (Horslen et al. 2003; Stephenne et al. 2005; Puppi et al. 2008; Meyburg et al. 2009; Meyburg and Hoffmann 2010) and argininosuccinate lyase deficiency (Stephenne et al. 2006; Newnham et al. 2008), alpha-1 antitrypsin deficiency (Strom et al. 1997), glycogen storage disease (Muraca et al. 2002; Muraca and Burlina 2005; Lee et al. 2007), and infantile Refsum disease (Sokal et al. 2003). To date, approximately 100 individuals have now undergone human hepatocyte transplantation. Achieving physiologically relevant degrees of therapeutic liver repopulation with healthy hepatocytes remains the greatest single challenge to the field.

22.3.2.2 Liver-Directed Gene Therapy

Liver-directed gene therapy uses DNA transfer methods to either directly correct or compensate for the effects of a mutant gene in the liver. For most recessively inherited diseases, the addition of a functional gene through gene addition should be sufficient to correct deficiency of the critical gene product. Years of animal studies by many investigators form the experimental basis for gene therapy studies in humans. This chapter will however concentrate upon actual clinical liver gene therapy trials. Attempts to treat familial hypercholesterolemia due to recessively inherited low density lipoprotein (LDL) receptor deficiency were among the first human liver gene therapy trials (Grossman et al. 1995). In this study, primary hepatocytes were cultured following partial hepatectomy from five individuals with LDL receptor deficiency. The cultured cells were transduced ex vivo with a Moloney murine leukemia retrovirus vector that expressed LDL receptor, and then transduced hepatocytes were reinfused back into the patients via portal vein catheter. Significant reduction of serum LDL cholesterol was demonstrated in three of five treated individuals; the variable reconstitution between subjects caused by differences in transduction frequencies however precluded general use of this approach.

Recombinant human adenovirus vectors directly injected into the circulation of multiple different animal models showed early promise in liver-directed gene therapy due to the high transduction frequency and robust gene expression. For instance, administration of a recombinant adenovirus vector that expressed the human phenylalanine hydroxylase (PAH) cDNA was associated with the first complete correction of hyperphenylalaninemia in *Pah*^{enu2} mice, a model of human PKU (Fang et al. 1994). However, eventual development of an anti-vector immune response including the production of anti-adenoviral capsid antibodies caused the elimination of transduced hepatocytes and reemergence of hyperphenylalaninemia about 2 weeks after initial vector administration. Antiviral antibodies rapidly eliminated a second vector dose preventing any effect upon liver PAH deficiency. These results presaged the outcome of a dose escalation trial of an adenoviral vector in adults with ornithine transcarbamylase (OTC) deficiency (Raper et al. 2003). In this trial, an 18-year-old male with partial OTC deficiency, who received the highest vector dose delivered by intrahepatic artery infusion, died from a systemic inflammatory response against the vector. Adenoviral vectors have for the most part been abandoned for the treatment of inherited disease, although they continue to be employed in specific anticancer treatment protocols that are augmented by the antiviral immune response.

Recombinant gene transfer vectors based upon adeno-associated virus (AAV), a non-pathogenic single-stranded DNA parvovirus, have become a favorite vector for many applications. The history of the development of this versatile vector system from discovery and characterization in the laboratory to clinical application has been reviewed (Flotte 2013). The relatively simple biology of the AAV genome, the lack of known pathogenicity from the virus, and the ability of the wild-type genome to latently and safely integrate into a specific site on human chromosome 19 initially made this vector platform extremely attractive for clinical use. Ultimately, it has been shown that the spontaneous integration frequency of recombinant AAV genomes is quite low (<0.5 % of hepatocytes) and that AAV-associated gene expression is large mediated through head-to-tail concatemers of AAV genomes residing independently of native chromatin as episomes in the nucleus of the transduced cell. Therefore, the time course of AAV-mediated therapeutic gene expression is limited in proliferating tissues due to the loss of the vector episomes with nuclear division. This limitation is most dramatically demonstrated following administration of AAV vector to juvenile animals; rapid hepatocyte proliferation in the developing liver is associated with rapid clearance of AAV episomes (Nakai et al. 1998; Dane et al. 2009; Dong et al. 2010; Wang et al. 2012). Permanent gene expression following AAV administration to juvenile animals occurs only after rare random integration of the

AAV genome into the host genome that subsequently conveys a selective growth advantage to transduced cells (Inagaki et al. 2008). Another limitation of the AAV system is the relatively small carrying capacity of the AAV genome. The native AAV genome is approximately 4.6 kb in length. To package and produce an infectious recombinant viral particle, only the 154 bp intermediate terminal repeats (ITR) from each end of the AAV genome must be retained; the remainder of the genome can be replaced by a therapeutic expression cassette (Fig. 22.1), but the total capacity of the AAV particle is biologically limited to 4.6 kb. This can sometimes be stretched to as much as 5.5 kb, but the recovery of functional viral particles suffers significantly when the typical limits on packaging capacity are exceeded (Dong et al. 2010). Packaging of the replication-defective recombinant AAV genome into infectious particles is accomplished by transfecting a bacterial plasmid, encoding the recombinant AAV genome into cultured mammalian cells (typically HEK293 cells), and providing all other replication and packaging functions including a plasmid encoding the viral capsid proteins *in trans*. Functional viral particles are then purified from the culture media using various different techniques depending upon the specific viral serotype. Following administration of AAV vector to cells or to an animal, the rate-limiting step in achieving AAV-mediated therapeutic gene expression is the time necessary to convert the single-stranded AAV (ssAAV) genome into a double-stranded episome, either through second-strand DNA synthesis or through capture and annealing of the + and – DNA strands introduced through separate AAV particles (Nakai et al. 2000). A novel wrinkle to this system is mutation of the second ITR in the viral genome to develop a so-called self-complementary AAV (scAAV) vector. During production of typical recombinant single-stranded AAV (ssAAV), the + and – vector genome strands are replicated and packaged individually into separate viral particles; mutating the 3′ ITR in an scAAV genome causes a hairpin loop that prevents separation of the newly replicated + and – strands, allowing the genome to fold back upon itself and the + and – strands to spontaneously anneal and form an already double-stranded vector genome. These scAAV vectors typically demonstrate more rapid therapeutic gene expression and more extensive cell transduction than ssAAV (Nathwani et al. 2006), but because the packaging capacity of the viral particle is still only 4.6 kb of the total DNA, the available space for a therapeutic gene expression cassette is reduced by half in an scAAV vector. This has led to the search for and development of shortened promoter/enhancer sequences that can fit into an scAAV genome with the therapeutic cDNA and still direct robust tissue-specific expression (Nair et al. 2014).

Even with all the limitations cited above, the AAV vector system has enjoyed remarkable application throughout the field of gene therapy and recent outstanding clinical success. In one of the first dramatic successes, administration of an AAV serotype 2 vector expressing the gene RPE65 by subretinal injection led to either arrest of disease or even improvement in vision in individuals with Leber's hereditary amaurosis due to RPE65 mutation (Maguire et al. 2008). The positive effects upon the disease have been stable out to at least 3 years after a single vector injection (Testa et al. 2013). In the initial trial, only a single eye was treated in case there were any severe adverse events that threatened the viability of the treated eye. Finding none, the investigators administered the same vector to the contralateral eye at 1.7–3.3 years after the initial injection and found an equally positive treatment signal (Bennett et al. 2012). This likely was possible because the eye is an immune privileged organ. Typically, systemic administration of any AAV results in anti-AAV capsid antibody production that prevents efficacy from a second vector administration.

AAV vectors are capable of transducing virtually every tissue and cell type, but the various naturally occurring serotypes differ greatly in their tropism for specific tissues. The initial vector system based upon human AAV sero-

type 2 was shown to direct robust gene expression in cultured cells (Hermonat and Muzyczka 1984; Tratschin et al. 1984; Flotte et al. 1992), but the transduction frequency following AAV2 administration to the liver, although detectable, was too low to affect the phenotype of most IEM (Koeberl et al. 1997; Snyder et al. 1997; Nakai et al. 1998). The isolation from humans and other nonhuman primates of further AAV serotypes differing predominantly by the amino acid sequences of capsid protein promoted the development of novel recombinant AAV vectors that demonstrated vastly different tissue-specific tropism. In most of these systems, an AAV2-based genome that includes only the viral ITRs and carrying a therapeutic gene expression cassette is packaged and pseudotyped with capsid from another AAV serotype. For liver-directed gene transfer in particular, AAV serotype 8, derived initially from rhesus macaque, has proven to yield extremely robust liver-specific transduction (Gao et al. 2005), and AAV8 vectors have now been successfully employed to treat several different liver-based enzyme deficiencies in a variety of animal models including models of hemophilia (Sarkar et al. 2004; Wang et al. 2005), ornithine transcarbamylase deficiency (Moscioni et al. 2006), phenylketonuria (Ding et al. 2006; Harding et al. 2006), glycogen storage disease type 1 (Ghosh et al. 2006; Koeberl et al. 2006), glycogen storage disease type 2 (Sun et al. 2005), and methylmalonic acidemia (Carrillo-Carrasco et al. 2010). AAV9, AAVrh10, and a hybrid serotype AAVrh32/33 have also proven particularly useful in directing liver-directed gene transfer but also with significant tropism to a variety of tissues. AAV9 is particularly adept at crossing the blood-brain barrier and transducing brain (Dayton et al. 2012).

To date, the most impressive results from a liver-directed gene therapy trial have occurred following AAV administration to males with hemophilia A disease due to X-linked coagulation factor IX (FIX) deficiency. In an initial trial, a human FIX-expressing ssAAV2 vector was administered intramuscularly to adult males with FIX deficiency with the goal of expressing human FIX (hFIX) from skeletal muscle into circulation. Although no severe safety issues were documented, the amount of hFIX produced was insufficient to correct the coagulation deficit (Kay et al. 2000; Manno et al. 2003). Subsequently, escalating doses of the hFIX AAV2 vector administered by hepatic artery injection directly into the liver were safely tolerated, and in men who had received the highest vector dose (2×10^{12} vector genomes/kg body weight), plasma hFIX concentrations increased significantly, reaching therapeutic levels (Manno et al. 2006). However, hFIX expression ceased at approximately 8 weeks following vector injection. This loss of efficacy was associated with a transient transaminitis that had not been previously seen in response to the AAV2 vector administered to either mice or dogs with FIX deficiency. Subsequent evidence suggests that the transduced hepatocytes had been eliminated by a T-cell-mediated immune response against cells displaying AAV2 capsid protein (Mingozzi and High 2007).

More recently, in a collaborative clinical trial between St. Jude Children's Research Hospital who produced the vector and University College London who administered the vector to the study subjects, single-dose peripheral intravenous administration of an hFIX-expressing scAAV8 vector to adult males with hemophilia A has been associated with therapeutically relevant hFIX levels in sera and a marked decrease in the need for prophylactic exogenous clotting factor administration (Nathwani et al. 2011). This publication reported on the initial six subjects, but a further four subjects have subsequently been treated with hFIX AAV8. As was seen in the hFIX AAV2 trial mentioned above, four of six participants that received the highest AAV8 dose exhibited transient elevation of liver transaminases at 1–2 weeks after vector injection. When this occurred, the subjects were treated with a short course of oral prednisolone in an attempt to suppress the likely immune response against the vector. Seemingly the transaminitis resolved with the steroid therapy, and although the amount of circulating hFIX decreased, the residual FIX levels

were thereafter stable and still sufficient to deliver positive benefit in terms of decreased need for exogenous clotting factor infusions. Therapeutically relevant circulating hFIX levels have been maintained for at least 3 years in the entire study cohort without any significant safety issues being revealed (Nathwani et al. 2014).

Based upon these exciting results, further clinical trials of liver-directed gene therapy for both hemophilia A and hemophilia B utilizing various different promoter/cDNA combinations and AAV capsids are currently underway or are being planned. AAV clinical trials, currently in early planning stages, have also been announced for OTC deficiency, familial hypercholesterolemia due to LDL receptor deficiency, and glycogen storage disease type 1a.

22.3.3 Brain-Directed AAV-Mediated Gene Therapy

Administration of AAV vectors expressing aromatic amino acid decarboxylase (AADC), a key enzyme in the dopamine and serotonin synthetic pathways, to adult individuals with Parkinson disease by direct stereotactic injection into the putamina has demonstrated promising results in influencing the motor symptoms of the disorder (Christine et al. 2009; Muramatsu et al. 2010). Inherited AADC deficiency is common in Taiwan due to a prevalent founder mutation. Children with this disorder present with severe hypotonia and recurrent oculogyric crises prior to 1 year age that have not typically responded to drug therapy. Bilateral intraputaminal injection of an AAV2 vector encoding human AADC in four AADC-deficient children ranging from 4 to 6 years age resulted in improved growth and dramatically improved motor performance (Hwu et al. 2012) in all four children. CSF concentrations of dopamine and serotonin metabolites increased significantly after gene transfer indicating restoration of dopamine and serotonin synthesis with restored putaminal AADC activity. The long-term benefits of this promising treatment approach continue to be studied. Similar applications of AAV-mediated brain-directed gene therapy are being explored for a variety of primary neurologic disorders.

22.3.4 Glybera™: The First Licensed Gene Therapy Reagent for an Inborn Error of Metabolism

Lipoprotein lipase (LPL) deficiency is a recessively inherited disorder of lipoprotein metabolism associated with severely impaired chylomicron lipolysis and clearance of chylomicrons and triglyceride from the circulation. The primary clinical manifestation is recurrent pancreatitis. Typical treatment includes consumption of a fat-restricted diet in order to reduce blood triglyceride content; this treatment approach is only partly successful at controlling serum triglycerides and preventing episodes of pancreatitis. Alipogene tiparvovec (Glybera™, uniQure, Amsterdam, the Netherlands) is a human LPL-expressing AAV serotype 1 vector that was successfully licensed in Europe in 2012 for the treatment of inherited LPL deficiency. The vector is injected at multiple sites into the skeletal muscles of the lower limbs under spinal anesthesia, and subsequent muscle LPL expression leads to improved chylomicron lipolysis and triglyceride clearance. This positive benefit persisted to beyond 26 weeks after vector injection and was associated with a significant decrease in the incidence of pancreatitis episodes in the first five subjects studied.

Conclusion

The diagnosis and treatment of rare IEM, from the advent of newborn screening to the development of dietary and pharmaceutical therapies, have always provided valuable paradigms for the broader field of medicine. Successes in the treatment of IEM using either cell or gene therapy will ultimately spread to the development of novel treatments for many other diseases, both inherited and not. The keys to success for both cell and gene therapy reside in the ability to stably and

safely provide sufficient and physiologically relevant numbers of non-disease cells in a target tissue to significantly affect the disease phenotype. After decades of gradual improvements in methods and several therapeutic disappointments along the way, both cell and gene therapies are on the verge of living up to years of hyperbole and finally providing truly revolutionary treatment options for IEM. Certainly, continued improvements in these methods will be needed and ultimately will come, but the promise of a complete cure for a specific disease from cell or gene therapy is seemingly now within reach.

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Part III

Organ Systems in Metabolic Disease

Approach to the Patient with Cardiovascular Disease

23

Joachim Kreuder and Stephen G. Kahler

Key Facts

- Metabolic disorders are associated with a wide variety of cardiovascular manifestations, including cardiomyopathy, dysrhythmias and conduction disturbances, valvular heart disease, vascular disorders, and pulmonary hypertension.
- Most metabolic cardiomyopathies result from disorders of energy production (most of which also involve other organs, particularly the skeletal muscle, liver, and brain) and storage diseases.
- In some metabolic disorders, the cardiac manifestations may be late, subtle, or secondary to metabolic derangements in other organs.
- Valvular dysfunction and infiltrative cardiomyopathy occur as a late complication in many lysosomal storage disorders.
- Pulmonary hypertension may be a quite rare complication in metabolic diseases, especially glycogen storage disease type I.
- Myocardial dysfunction is common in hemochromatosis, a metabolic cardiomyopathy most easily prevented.
- Symptomatic coronary heart and cerebrovascular disease during childhood is restricted to severe defects of low-density lipoprotein metabolism.
- Peripheral vascular disease is prominent in homocystinuria and CDG syndromes.
- Disorders of lipoprotein metabolism possess a significant long-term, but modifiable burden of premature atherosclerosis to a large number of children.

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23.1 General Remarks

A substantial number of metabolic disorders significantly contribute to cardiovascular morbidity because of a direct relationship between the inborn error and outcome or a genetic predisposition resulting from interrelations between gene variants and environmental factors.

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Clinical manifestations of metabolic cardiovascular disease are mainly determined by the site of involvement. Cardiomyopathies may present with reduced exercise capacity, edema, tachypnea and dyspnea or increased frequency of respiratory infections. Palpitations and syncopes are typical clinical features of cardiac rhythm disorders. In some cases, sudden cardiac death due to ventricular tachycardia or fibrillation may be the first manifestation. Metabolic vascular disorders may present as stroke-like episodes, coronary heart disease with angina pectoris or myocardial infarction, peripheral thromboembolism, or tuberous xanthoma. In pulmonary hypertension, reduced exercise capacity, exercise-induced cyanosis, and syncope are the main clinical features.

As in all metabolic disorders, the family history reflects the various patterns of inheritance, including differing penetrance, maternal inheritance of some mitochondrial disorders, and acceleration from one generation to the other. Family examination by echocardiography and electrocardiography is especially important in cardiomyopathies and cardiac channelopathies.

The age at presentation may be another important key to diagnosis in cardiac metabolic disorders. Overt cardiomyopathy within the first year of life has a much higher association with inborn metabolic diseases than in older age groups.

23.2 Cardiomyopathy and Cardiac Failure

In epidemiological studies, 5–10 % of cardiomyopathies in children result from identified or suspected inborn errors of metabolism. However, in one study a comprehensive diagnostic approach including biochemical analysis of skeletal muscle and cardiac biopsies revealed a metabolic disease in up to 22 % of affected children. Inborn errors of metabolism constitute a less frequent proportion of cardiomyopathy among adults, where ischemic heart disease and diabetic cardiomyopathy have become two of the major causes in the developed countries. During infancy and early childhood, metabolic disorders represent a more frequent cause of cardiomyopathy than in the older children and adolescents. Children with metabolic cardiomyopathy present with more severe symptoms and a poorer prognosis than patients with other causes of cardiomyopathy.

Approximately, 5 % of the inborn metabolic disorders are associated with cardiomyopathy. These disorders can be conveniently divided into those in which the cardiac manifestations are primary or prominent (Table 23.1) and those in which they are less common or less significant (Table 23.2). Rarely, the heart is the only affected organ, as described in cardiac phosphorylase kinase deficiency and certain mitochondrial disorders.

The type of metabolic involvement may determine the age at clinical presentation. Pompe disease [severe lysosomal type II glycogen storage disease (GSD)] usually presents in infancy, whereas cardiomyopathy in other lysosomal storage diseases becomes symptomatic during later childhood. Defects of fatty acid oxidation are likely to present in infancy; mitochondrial disorders may manifest at any age.

23.2.1 Special Aspects of Cardiac Metabolism

The major fuels for the heart are glucose and fatty acids. The heart, like skeletal muscle, maintains a reserve of high-energy phosphate compounds (e.g., phosphocreatine) as well as glycogen. Before birth, the heart uses less fatty acids and more glycolysis and tolerates anaerobic metabolism more readily than after the neonatal period. This transition period after birth sometimes leads to the appearance of a cardiomyopathy that was clinically silent before then. After a few weeks, the metabolism of the heart relies on both the fatty acids and glucose for fuel; if glucose becomes limiting (during hypoglycemia), the heart can function well, whereas hypoglycemia in the newborn period may lead to significant impairment of cardiac function and dilatation of the heart. During times of increased energy demand and greater cardiac output, there is increased utilization of fatty acids and glucose. Long-chain fatty acids,

Table 23.1 Metabolic disorders with cardiomyopathy as a presenting or early symptom

Disorder	Hypertrophic type	Dilated type	Mixed type	Noncompaction type	Tachyarrhythmias	Conduction disorders	Organic acids	Carnitine level	Acylcarnitine profile	Diagnostic tissues	Specific treatment	Comments
Disorders of fatty acid oxidation and the carnitine cycle												
Carnitine transporter deficiency	+	++	+		+	+	+	↓↓	+	F, M, W, D	Carnitine	Sudden death in adults
Carnitine-acylcarnitine translocase deficiency	+	+	++		++		+	↓	++	F, W, D	Low-fat, high-carbohydrate diet, MCT. Carnitine, if low	
Carnitine palmitoyl-CoA transferase II deficiency	++	+	++		++		+	↓	++	F, M, L, W, D	Low-fat, high-carbohydrate diet, MCT. Carnitine, if low	Heterozygotes may have symptoms, be vulnerable to malignant hyperthermia
Very-long-chain acyl-CoA dehydrogenase deficiency	++	+	+		+		++	↓	++	F, W, D	Low-fat, high-carbohydrate diet, MCT	
Long-chain hydroxyacyl-CoA dehydrogenase/ trifunctional enzyme deficiency	++		+		++		++	↓	++	F, W, D	Low-fat, high-carbohydrate diet, MCT	HELLP syndrome in pregnant heterozygotes

(continued)

Table 23.1 (continued)

Disorder	Hypertrophic type	Dilated type	Mixed type	Noncompaction type	Tachyarrhythmias	Conduction disorders	Organic acids	Carnitine level	Acylcarnitine profile	Diagnostic tissues	Specific treatment	Comments
Multiple acyl-CoA dehydrogenase deficiency	++		+				++	↓	++	F, M, D	Low-fat, high-carbohydrate diet, riboflavin, D,C-3-hydroxybutyrate, carnitine	
Mitochondrial disorders												
Complex I–V deficiencies	++	+	++	+	+	+	+			F, H, L, M, D	High-fat, low-carbohydrate diet, vitamins, antioxidants, cofactors	Heart block or sudden death may occur. May have lactic acidosis and increased lactate/pyruvate ratio
Kearns-Sayre syndrome	+				+	++				F, M, D		Typical heteroplasmic deletions of mitochondrial DNA
Barth syndrome	+		+	++	+		++			B, D	Pantothenic acid (case report)	Specific isolated left ventricular noncompaction
												Increased monolysocardiolipin as a biochemical marker suitable for screening

Disorder	Hypertrophic type	Dilated type	Mixed type	Noncompaction type	Tachyarrhythmias	Conduction disorders	Organic acids	Carnitine level	Acylcarnitine profile	Diagnostic tissues	Specific treatment	Comments
Disorders of amino and organic acid metabolism												
Propionic aciduria, methylmalonic aciduria		+	+		+	+	++	↓↓	++	F, L, W, D	Protein-modified diet carnitine, OH-cobalamin	Cardiomyopathy unrelated to metabolic decompensation or nutritional status; may be presenting symptom. QT prolongation may be the first sign
β-Ketothiolase deficiency, malonic aciduria	+		+				++	↓	++	F, W, D	Protein-modified diet, carnitine, bicarbonate. High-carbohydrate, low-fat diet, carnitine	
Lysosomal storage disorders												
Lysosomal glycogen storage disease (severe form – Pompe)	++		++		+					W, F, M, D	Enzyme replacement	Macroglossia, severecardiomyopathy, and skeletal myopathy. Characteristic ECG

(continued)

Table 23.1 (continued)

Disorder	Hypertrophic type	Dilated type	Mixed type	Noncompaction type	Tachyarrhythmias	Conduction disorders	Organic acids	Carnitine level	Acylcarnitine profile	Diagnostic tissues	Specific treatment	Comments
MPS I (Hurler type)		+	+							W, F, U, D	Enzyme replacement, hematopoietic stem cell transplantation	Early cardiac manifestation due to coronary vascular involvement
Disorders of cytoplasmic glycogen metabolism												
GSD type III (debrancher deficiency)	++		+							F, M, L, D		
GSD type IV (brancher deficiency)	++		+							W, F, M, L, D		Severe liver disease. Neuropathy, dementia in adult form
GSD type IXb (phosphorylase b kinase deficiency)	++		+							H, D		Cardiac involvement rare; can be rapidly fatal
Disorders of glycoprotein metabolism (CDG)	+	+	+							B, F, D		Abnormal subcutaneous fat distribution, psychomotor retardation, pericardial effusion
Hemochromatosis	+	+	+		+	+				P, D	Phlebotomy	Restrictive cardiomyopathy occasionally
Nutrient deficiency												
Secondary carnitine deficiency	+	+	+				+	↓↓		P, M, U	Carnitine	Underlying causes should be clarified

Disorder	Hypertrophic type	Dilated type	Mixed type	Noncompaction type	Tachyarrhythmias	Conduction disorders	Organic acids	Carnitine level	Acylcarnitine profile	Diagnostic tissues	Specific treatment	Comments
Selenium deficiency		+			+	+				E	Selen	Additional pancreatic insufficiency
Thiamine deficiency/ dependency		+								P, E, and U	Thiamine	Lactic acidosis

Diagnostic tissues commonly used. *D* DNA (leukocytes and other tissues), *E* erythrocytes, *F* fibroblasts, *H* heart, *L* liver, *M* skeletal muscle, *P* plasma, *U* urine, *W* white blood cells (leukocytes)

++ Usually and prominently abnormal; + mildly abnormal

Table 23.2 Disorders in which cardiovascular involvement is usually mild or late – symptoms are recognized after systemic illness or involvement of other organs

Disorder	Comments and special issues
MAD deficiency (mild, including SCHAD deficiency)	Biochemically similar to severe forms, but later onset and symptoms milder or intermittent. Cardiomyopathy similar to fatty acid oxidation defects (Table 23.1)
Friedreich ataxia	Mitochondrial damage may be due to iron accumulation. Diabetes common
Mucopolysaccharidoses – general pathology	Myocardial thickening, especially septum and LV wall. Aortic and mitral valve thickening and regurgitation, narrowing of aorta, and coronary, pulmonary, renal arteries
MPS I-S, I-H	Infiltration, thickening of septum, LV wall, severe systolic dysfunction (10% of patients)
MPS II	EFE, MS, AS, AR, MR, infarction
MPS VI	MV, AV calcification, stenosis
Gaucher disease	Myocardial thickening due to infiltration; pulmonary hypertension
Fabry disease	Infiltration leading to LVH; MV prolapse, thickening; CAD
Fucosidosis	Myocardial thickening due to infiltration
Sialidosis	MR, CHF, pericardial effusion
Mucolipidosis II, III	Aortic regurgitation, myocardial thickening due to infiltration, CHF; pulmonary hypertension (II)
Aspartylglycosaminuria	MR, aortic thickening
Homocystinuria	Hypercoagulability, strokes. MV prolapse

AR aortic regurgitation, *AS* aortic stenosis, *CAD* coronary artery disease, *CHF* congestive heart failure, *EFE* endocardial fibroelastosis, *LV* left ventricle, *LVH* left ventricular hypertrophy, *MR* mitral regurgitation, *MS* mitral stenosis, *MV* mitral valve

which require carnitine for transport into the mitochondria, are the usual forms present in the blood. Medium-chain fatty acids, which enter the mitochondria directly, can be used to provide fuel to the heart and other organs, if there is a problem with long-chain fatty acid oxidation. Furthermore, during times of metabolic stress, the myocardium switches to use more glucose than at other times, and so provision of continuous glucose during times of cardiac dysfunction can be beneficial.

The heart, because it relies more than the other tissues on fatty acid oxidation, may be particularly vulnerable to disturbances of this major energy source. Disturbances of cellular function affecting a small group of cells can have a devastating effect if the region affected is part of the conduction pathways or is able to influence them.

23.2.2 Patterns and Pathophysiology of Metabolic Cardiac Disease

From a functional point of view, cardiomyopathies during infancy and childhood may be categorized as a hypertrophic, dilated, hypertrophic-hypocontractile (mixed type), restrictive, or noncompaction type.

With the exception of mitochondrial disorders, each metabolic disease is preferentially associated with a functional type of cardiomyopathy by echocardiography, which helps to focus the differential diagnosis. Hypertrophy can be the result of accumulated material stored in the myocardium (e.g., glycogen), the heart's response to poor functioning of the contractile apparatus (abnormalities of structural proteins), or impaired energetics (mitochondrial disorders). Dilation results when the

abnormal or poorly functioning heart begins to stretch out of shape because it is unable to contract adequately due to impaired energy production or toxic intermediates such as acids or oxidizing mitochondrial components. This type preferentially occurs in the disorders of carnitine availability and in some of the organic acidurias. The hypertrophic-hypocontractile (mixed) type presents the transition from the hypertrophic to the dilated type and may occur in the late stages of disorders of fatty acid oxidation, oxidative phosphorylation, and storage disorders.

Remember

In general, the most adaptive response of the heart in metabolic disorders is hypertrophy, with or without dilation and systolic dysfunction.

Pure restrictive cardiomyopathy is extremely rare in children with metabolic cardiomyopathy, but restrictive ventricular performance is observed in endomyocardial fibroelastosis. The noncompaction type mimicking the morphological appearance of the embryonic myocardium represents a nonspecific myocardial response to a variety of stimuli. From a metabolic view, noncompaction appearance is suggestive for Barth syndrome or defects of oxidative phosphorylation.

Beyond direct damage of myocardial cells, coronary vasculopathy due to mucopolysaccharidoses, oligosaccharidoses, or lipoprotein disorders may induce dilated cardiomyopathy.

23.2.2.1 Endocardial Fibroelastosis

Endocardial fibroelastosis describes a condition peculiar to infants and young children, in which there are significant thickening and stiffening of the endocardium. The basis for this response is not yet known. It can occur in disparate conditions ranging from viral myocarditis to carnitine transporter deficiency, Barth syndrome, severe mucopolysaccharidosis I (Hurler syndrome), and mucopolysaccharidosis VI (Maroteaux-Lamy syndrome). It is a predictor of poor outcome.

23.2.3 Inborn Errors of Metabolism That Cause Cardiomyopathy

23.2.3.1 Disorders of Fatty Acid Oxidation and the Carnitine Cycle

Disorders of fatty acid oxidation involving long-chain fatty acids often present as cardiomyopathy. Sometimes the onset is abrupt or overwhelming particularly in the newborn period. Sudden death may occur, presumably reflecting arrhythmia or apnea. Liver involvement (manifest as fasting hypoketotic hypoglycemia, hyperammonemia, or a Reye-like syndrome) and skeletal muscle involvement are common. Very-long-chain acyl-CoA dehydrogenase (VLCAD) and carnitine palmitoyl-CoA transferase II (CPT II) deficiencies are probably the most common; long-chain hydroxyacyl-CoA dehydrogenase (LCHAD)/trifunctional protein and carnitine-acylcarnitine translocase (CACT) deficiencies present similarly. The metabolic pathways and skeletal muscle aspects of these conditions are discussed in Chap. 28. MCAD deficiency, the most common disorder of fatty acid oxidation (and a cause of carnitine depletion), is unusual for a fatty acid oxidation disorder, in that cardiomyopathy is an extremely uncommon feature, but dysrhythmias and sudden death are real concerns.

Although the majority of fatty acid oxidation defects are now included in newborn screening programs and diagnosed before symptoms occur, ECG and echocardiography should be included in routine surveillance of these patients.

Disease Info: Defects of Long-Chain Fatty Acid Oxidation

VLCAD Deficiency

Very-long-chain acyl-CoA dehydrogenation is the first step of β-oxidation (Figure in Chap. 28) in the mitochondria, after synthesis of the acyl-CoA (e.g., palmitoyl-CoA) catalyzed by CPT II. It is easy to understand why impairment of this step can lead to severe organ dysfunction, especially in heart, liver, and skeletal muscle.

Cardiac symptoms (congestive failure with feeding difficulties and tachypnea), if they occur, are likely to occur in infants and young children, and there may be concurrent liver dysfunction. Cardiac hypertrophy, especially of the left ventricle, is common. ECG may show increased voltages. Urinary organic acids may show increased dicarboxylic acids; plasma or blood spot acylcarnitine analysis shows elevation in C14:1 species. Enzyme assay on fibroblasts and lymphocytes and mutation analysis will confirm the diagnosis. Treatment involves avoiding fasting, limiting essential long-chain fats to the amount needed for growth, and providing medium-chain lipids and other foods as calorie sources in place of long-chain fatty acids. Although the total plasma carnitine level is often low, chronic carnitine supplementation has not led to documented improvement and may increase the generation of arrhythmogenic long-chain acylcarnitines.

LCHAD/Trifunctional Enzyme Deficiency

Many infants with long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency present with cardiomyopathy, sometimes in the newborn period. This is one of the common disorders of cardiac fatty acid oxidation. Liver dysfunction may be severe, including cirrhosis, and there may be significant skeletal muscle involvement. Urine organic acids reveal increased saturated and unsaturated dicarboxylic and hydroxyl species. Plasma carnitine level may be low; acylcarnitine analysis shows increased hydroxy forms of C16:0, C18:1, and C18:2. Nearly all patients are homozygous for the common mutation c.1528G > C (p.E510Q). Additional findings that are unusual for other disorders of fatty acid oxidation are lactic acidosis, pigmentary retinopathy (in older children and adults), and peripheral neuropathy. Absence of deep tendon reflexes and toe walking have been reported.

In the late-onset form, dominated by intermittent muscle symptoms, there may be asymptomatic ventricular hypertrophy. Treatment is similar to that for VLCAD deficiency.

Multiple Acyl-CoA Dehydrogenase Deficiency (Glutaric Acidemia Type 2)

Infants with the severe form of multiple acyl-CoA dehydrogenase deficiency (MADD), especially the subgroup without malformations, often have cardiomyopathy. Hypotonia, encephalopathy, overwhelming acidosis, and metabolic derangements resulting from impairment of multiple pathways for the degradation of fatty acids and amino acids are common features. The diagnosis is usually established by analysis of urinary organic acids (dicarboxylic aciduria, including ethymalonic, adipic, and glutaric acids) or plasma/blood acylcarnitines (increased median- and long-chain species) and confirmed by enzyme analysis or mutation analysis. Carnitine depletion is common. Severely affected patients are treated with a low-fat, high-carbohydrate diet, avoidance of fasting, provision of abundant carnitine, and riboflavin (50–100 mg/day), but response to therapy is often limited. Administration of D,L-3-hydroxy-butyrate (100–800, up to 2,600 mg/kg/day) has been shown to induce regression of myocardial systolic dysfunction and hypertrophy as well as improvement of neurological deficits.

Disease Info: Defects of the Carnitine Cycle

CACT Deficiency

Most patients with translocase deficiency have had severe disease in early infancy, including acute cardiac events (shock, heart failure, and cardiac arrest). Arrhythmias can be prominent. There may be ventricular hypertrophy. There is significant hepatic and skeletal muscle

dysfunction (hyperammonemia and weakness). Total blood carnitine level may be low, especially the free fraction. Acylcarnitine analysis shows mainly long-chain species C16:0, C18:1, and C18:2, reflecting their synthesis by CPT I and accumulation. Emergency management includes provision of glucose, restriction of long-chain fats, and supplementation with medium-chain lipids and carnitine (see also Chap. 19). Despite this, neonatal fatality is common.

CPT II Deficiency

The severe neonatal/infantile form of CPT II deficiency leads to severe cardiac, skeletal muscle, and liver dysfunction, resulting in circulatory shock, heart failure, hypoketotic hypoglycemia, hyperammonemia, and coma. Dysmorphic features and renal dysgenesis may be present, reminiscent of the cystic malformations that occur in glutaric aciduria type II (MAD deficiency) or pyruvate dehydrogenase deficiency.

Plasma and tissue carnitine levels are low, especially free carnitine. Acylcarnitine analysis shows long-chain species, as in translocase deficiency.

Arrhythmias represent a common feature of CPT II deficiency. Long-chain acylcarnitines, long-chain acyl-CoAs, and long-chain free fatty acids all have detergent effects on membranes, so an accumulation might have disruptive effects. On the other hand, the relative deficiency of free carnitine adversely affects the ratio of free CoASH to acyl-CoA, and the plasma and tissue deficiency of carnitine would impair fatty acid oxidation. The true magnitude of the contribution of long-chain acylcarnitines to arrhythmias in CPT II deficiency (and related disorders) is neither not known nor is the true risk of carnitine administration in a desperately ill child with this disorder. Therapy like this for CACT deficiency is somehow effective in the infantile form, whereas patients with the neonatal type respond poorly to therapy.

Mild CPT II deficiency, among the most common fatty acid oxidation disorders, is typified by recurrent rhabdomyolysis and does not present with significant cardiac involvement. Most of these patients harbor at least one copy of a mild CPT II mutation allowing higher residual activity than in the severe infantile form.

Disease Info: Carnitine Transporter Deficiency

A deficiency of the plasma membrane carnitine transporter leads to severe carnitine depletion by a few months to several years of age. In the most extreme cases, it can present as neonatal hydrops. There are hypertrophy of the left ventricle, hepatomegaly with hepatic dysfunction (hypoglycemia, hyperammonemia, and increased transaminases), hypotonia, and developmental delay. The ECG may show high, peaked T waves and left ventricular hypertrophy. Total plasma carnitine is exceedingly low, often less than 5 μmol/L, and the acylcarnitine profile shows no abnormal species. In contrast to specific elevations in other disorders of fatty acid oxidation, the quantities of all acylcarnitines may be very low, suggesting the diagnosis. Dicarboxylic aciduria is rarely observed. Endomyocardial biopsy (if done – not needed for diagnosis) can show lipid infiltration and, in some cases, endocardial fibroelastosis.

The milder, later-onset form may present with cardiac dilation, abnormal ECG, and hepatomegaly. Skeletal muscle strength may appear to be normal on static testing, but endurance is poor. Muscle biopsy shows lipid storage. Urinary organic acids are usually unremarkable. Diagnosis is confirmed by demonstrating markedly reduced (<10 % of controls) carnitine transport in fibroblasts. Heterogeneous mutations have

been found in the *SLC22A5* gene encoding the OCTN2 carnitine transporter.

In this autosomal recessive condition, the fractional excretion of carnitine in the urine approaches 100 %, as the renal carnitine reabsorptive transport system is the same as that for most other tissues. Accordingly, carnitine must be provided frequently. Intravenous carnitine (during an acute crisis) can be given in a dose of 300 mg/kg/days, as a continuous infusion. Oral carnitine should be given four times daily. The oral dose for children is 100–200 mg/kg/days and for adults 2–4 g/days, but some patients have required much more to maintain satisfactory plasma levels. The maximal oral dose is usually set by intestinal tolerance, while diarrhea does not occur with parenteral use. If treatment started before irreversible organ damage occurs, the cardiomyopathy improves dramatically, and the heart size returns to normal. Skeletal weakness may also improve, although muscle carnitine levels remain low (2–4 % of normal). The long-term prognosis is favorable as long as children remain on carnitine supplements. However, recurrence of cardiomyopathy or sudden death from arrhythmia even without cardiomyopathy has been reported in patients discontinuing carnitine supplementation. Sudden death in adults may be the initial symptom.

Secondary Carnitine Deficiency

Secondary carnitine deficiency occurs in a large number of settings, discussed in Chap. 28. Even with low plasma levels, the cardiac uptake of carnitine is usually sufficient to avoid symptoms. However, there are occasional premature infants receiving long-term total parenteral nutrition (TPN) without carnitine supplementation who become profoundly depleted after a few months. The need for carnitine in infants may exceed their synthetic capability, as an infant needs sufficient carnitine for the increasing mass of muscle and other tissues, whereas adults need only to replace what is lost. A lower renal threshold for carnitine in a sick infant may also become pathophysiologically relevant.

In some cases, infants with severe carnitine depletion may have poor cardiac output and dilated cardiomyopathy. The plasma carnitine level may be less than 10 μmol/L. One would expect hypoglycemia from liver carnitine depletion, but this does not occur because of the continuous high-dose glucose of the TPN. Carnitine supplementation (15–30 mg/kg/days IV, given with the TPN infusion) leads to rapid improvement in blood levels and cardiac function.

Severe carnitine depletion can occur in the setting of renal Fanconi syndrome (as in cystinosis and mitochondrial dysfunction) or treatment with the antibiotic pivampicillin. Although very low plasma carnitine levels are found for a short time during episodes of illness with MCAD deficiency and glutaric aciduria type I, they are not usually associated with cardiomyopathy.

23.2.3.2 Mitochondrial Disorders

The mitochondrial disorders are discussed in general terms in the Chaps. 14 and 42. About 40 % of patients with mitochondrial disorders suffer from cardiac involvement in terms of cardiomyopathy or dysrhythmias, leading to earlier presentation and much worse prognosis for survival compared to patients without cardiac involvement. Involvement of other organs is common, especially the brain, eye (and eye movements), skeletal muscle, liver, and kidney. However, isolated cardiac deficiency of oxidative phosphorylation represents a substantial part of mitochondrial cardiomyopathies. Of those with cardiac manifestations, about 50 % show the hypertrophic pattern, followed by the mixed hypertrophic-dilated, the

dilated, and the noncompaction patterns. Disturbances of cardiac rhythm such as complete heart block or bundle branch block affect about 15–25 % of these patients (with mitochondrial cardiomyopathy), and sudden death may occur, sometimes as the first indication of a mitochondrial disorder. A diagnosis of cardiac mitochondrial dysfunction leading to sudden death may be possible if tissues for mitochondrial studies (especially heart, skeletal muscle, and skin) are acquired rapidly after death.

The most common defects of the respiratory chain complexes are complex I, complex IV, and combined deficiencies. An apparent correlation between specific defects of the respiratory chain complexes and cardiac involvement is not possible. Lactic acidosis with an increased ratio of lactate/pyruvate is common, but may not be found. Mutations of mitochondrial protein-encoding genes, tRNA and rRNA, and deletions/duplications are found in only a minority of pediatric patients with mitochondrial disorders. Next-generation sequencing is now rapidly unraveling numerous nuclear molecular defects, e.g., defects of aminoacyl tRNA synthetases proving that mutations of nuclear genes are the leading cause for these diseases (for details of distinct genetic lesions in mitochondrial disorders, see www.mitomap.org).

The Kearns-Sayre syndrome is the prototypical mitochondrial disorder in which there is progressive disturbance of cardiac conduction. Most patients represent nonfamilial heteroplasmic deletions of mitochondrial DNA. Symptoms begin before age 20. Cardiac symptoms include hypertrophic or dilated cardiomyopathy, atrial or ventricular arrhythmias, Wolff-Parkinson-White syndrome, and other preexcitation syndromes. Progressive heart block may necessitate pacemaker placement. Major noncardiac symptoms include retinitis pigmentosa, ophthalmoplegia, ataxia, myopathy, and increased CSF protein. Many other manifestations may occur, including deafness, seizures, diabetes or other endocrinopathies, renal and gastrointestinal symptoms, and lactic acidosis. Similar manifestations with later onset are typical of chronic progressive external ophthalmoplegia-plus (CPEO-plus).

Other clinical entities due to mitochondrial DNA disorders are likely to have cardiac involvement including Leigh syndrome, MERFF, and MELAS. Mutations in nuclear genes which are also associated with the appearance of cardiomyopathy may affect both the structural proteins (part of the respiratory chain complexes, e.g., NDUFS2 and NDUFV2 in complex I) and nonstructural proteins responsible for the assembly of respiratory chain complexes (e.g., SCO2 and SURF1), mtDNA stability (e.g., POLG), iron homeostasis (e.g., frataxin), mitochondrial integrity (e.g., tafazzin = G4.5), or mitochondrial metabolism (e.g., PDH E1-α-subunit).

Sengers syndrome, Barth syndrome, and Friedreich ataxia represent other nuclear encoded diseases of the oxidative energy production with neuromuscular involvement. Sengers syndrome (autosomal recessive) is characterized by hypertrophic cardiomyopathy, congenital cataracts, and exercise-induced lactic academia. Deficiency of acylglycerol kinase (AGK) is the cause of Sengers syndrome. Other disorders of mitochondrial DNA depletion can also cause cardiomyopathy.

Disorders of oxidative phosphorylation are often treated with a high-fat, low-carbohydrate diet and supplemental vitamins (especially coenzyme Q, carnitine, and riboflavin in complex 1 deficiency), antioxidants, electron acceptors, cofactors, and metabolic intermediates (e.g., creatine, arginine in acute MELAS manifestation; see Chap. 36). Response to metabolic therapy is highly variable and a matter of continuing debate.

Remember

Serial echocardiography, 24 h-ECG, and measurement of BNP/NT-proBNP are valuable tools to supervise metabolic therapy.

In some cases, metabolic therapy can be directed to the pathobiochemical substrate like ubiquinol for inherited primary coenzyme Q deficiency or copper-histidine supplementation in SCO2 deficiency.

Disease Info: Barth Syndrome

Barth syndrome is an X-linked recessive condition that is similar to many mitochondrial oxidative phosphorylation diseases. Neutropenia, cardiomyopathy, and increased urinary excretion of 3-methylglutaconic acid, 3-methylglutarate, and 2-ethylhydracrylate – compounds suggestive of mitochondrial dysfunction – occur. Noncompaction pattern is typical, but not exclusive for the cardiac involvement in Barth syndrome, and may occur independent of the other features. The onset is typically in infancy; there may be spontaneous improvement in later childhood. Biopsy of skeletal muscle or heart shows abnormal mitochondria, which may be abnormal in shape and contain inclusion bodies and dense, tightly packed concentric cristae. Lactic acidosis may be prominent. The molecular defect in Barth syndrome is attributed to the G4.5 or tafazzin (TAZ) gene, which encodes a mitochondrial transacylase involved in the remodeling of cardiolipin. Deficiency of the TAZ protein results in reduced levels of cardiolipin and increased monolysocardiolipin content, which can serve as a biochemical marker for this disease.

At least one patient has had a dramatic and sustained response to treatment with pantothenic acid, a precursor of coenzyme A. Pantothenol was ineffective.

A syndrome of dilated cardiomyopathy with ataxia (DCMA) has been described in association with mutations in *DNAJC19* gene encoding an inner mitochondrial membrane protein (3-methylglutaconic aciduria type V). The clinical presentation initially resembles Barth syndrome (3-methylglutaconic aciduria type II) with early-onset severe dilated (or noncompaction) cardiomyopathy with conduction defects.

Disease Info: Friedreich Ataxia (See also Chap. 40)

This disorder presents with slowly progressive spinocerebellar dysfunction in children and young adults. Cardiomyopathy, cardiac dysrhythmias, and diabetes are common. The genetic defect is usually a trinucleotide expansion in the frataxin gene leading to reduced levels of this mitochondrial protein. The mechanism of cellular injury appears to be related to iron accumulation within mitochondria. Beyond deficient energy production, cellular damage leading to apoptosis may be due to peroxidative damage to mitochondrial membranes.

In roughly half the patients, idebenone, a coenzyme Q analog, is beneficial to reduce myocardial hypertrophy and rhythm disturbances, but neurological benefit did not occur.

23.2.3.3 Disorders of Amino and Organic Acid Metabolism

Two common organic acid disorders, propionic aciduria and methylmalonic aciduria, in the branched-chain amino acid catabolic pathway, may present with a cardiomyopathy, from birth to a few years of age. The onset may be subtle or sudden. Cardiac failure with dilatation, poor contractility, and rhythm disturbances including ventricular electric instability or bradycardia may occur.

Remember

Prolongation of normalized QT (QTc) time may be the first sign of cardiac involvement in organic acidurias and should initiate regular cardiac surveillance by echocardiography and measurement of BNP/NT-proBNP blood levels.

Cardiomyopathy may also occur in β-ketothiolase deficiency (isoleucine degradation) and malonic aciduria.

The basis for this cardiomyopathy is not known. In some cases it has occurred in children being treated successfully for the metabolic disorder, and in a few it has been the presenting symptom. Suggested causes include primary toxicity of metabolites accumulating as a result of the underlying enzymopathy, nutritional deficiency of carnitine, or deficiency of some other nutrients. It is possible that there are several causes.

Treatment is directed at correcting the metabolic derangement (e.g., diet and carnitine) and providing appropriate supportive care.

23.2.3.4 Lysosomal Storage Disorders (Infiltrative Cardiomyopathies)

In this subgroup, the myocardium may become thickened because of accumulated material, particularly within lysosomes. Because of mechanical interference, accumulation initially results in isolated diastolic dysfunction but may progress to severe systolic failure. In most cases, echocardiography shows global concentric ventricular hypertrophy.

Disease Info: GSD II (Pompe Disease and Lysosomal Acid α-Glucosidase Deficiency)

Pompe disease is among the most common primary metabolic cardiomyopathies and the leading lysosomal disorder in which there are severe cardiac manifestations. In the classical infantile form, symptoms appear in early infancy, and the disorder progresses rapidly. Weakness, floppiness, and macroglossia may be apparent within a few months. Cardiac manifestations include shortness of breath and poor feeding. Chest X-ray shows cardiomegaly. Echocardiography shows concentric symmetric hypertrophy with or without outflow tract obstruction. ECG shows left axis deviation, short PR interval, high QRS voltages, and inverted T waves. Peripheral blood leukocytes may show vacuoles, and there is often pathological excretion of oligosaccharides, especially glucose tetrasaccharide, in the urine. Enzyme assay can be performed on mixed leukocytes or purified lymphocytes. Dried blood spot on Guthrie cards is available in some laboratories and may be applied to newborn screening programs. Reduced α-glucosidase activity in a blood sample should be confirmed by enzyme measurement in fibroblasts, muscle biopsy, or by DNA analysis. Muscle biopsy shows a major accumulation of PAS-positive material and membrane-bound (i.e., in lysosomes) accumulation of glycogen in electron microscopy.

Infants with infantile Pompe disease often succumb in a few months due to cardiac failure or arrhythmias but may survive for 2 or 3 years. Patients with the late-onset form have higher residual activity of α-glucosidase. They present beyond the first year of life with progressive weakness, whereas cardiomyopathy is rare and much less severe (see Chap. 28). Enzyme replacement therapy has recently become available with encouraging results for both forms.

Cardiomyopathy with lysosomal glycogen storage, but normal α-glucosidase activity, is observed in X-linked Danon disease, which is caused by mutations of the gene encoding lysosome-associated membrane protein 2 (LAMP2).

Other Infiltrative Cardiomyopathies

In men with Fabry disease, there may be thickening of the left ventricle. This can also occur in the heterozygous women of this X-linked disorder, perhaps reflecting skewed lyonization. Coronary artery disease is also common in Fabry disease. The mucopolysaccharidoses and fucosidosis may be complicated by storage in the septum and posterior wall of the left ventricle. Lysosomal storage in the heart may also occur in Gaucher disease.

23.2.3.5 Disorders of Glycogen Metabolism

Glycogen Debrancher Deficiency (Cori or Forbes Disease, GSD III)

In type IIIa GSD, there is involvement of the liver and skeletal or cardiac muscle; in the less common type IIIb, there is only hepatic disease, manifest as fasting hypoglycemia, increased transaminases, and rarely mild lactic acidosis. The cardiomyopathy in type IIIa usually does not cause symptoms before adulthood; outflow tract obstruction and heart failure occur occasionally. During childhood, there may be ventricular hypertrophy on ECG and echocardiography.

Glycogen Brancher Deficiency (Anderson Disease, GSD IV)

The usual form of this disorder manifests hepatomegaly and cirrhosis. The deficiency of the branching enzyme activity leads to the accumulation of an amylopectin-like glycogen (also known as polyglucosan bodies), which incites an inflammatory response. Rarely, severely affected patients may have heart involvement in childhood with ventricular hypertrophy and secondary dilatation. Liver transplantation is curative for the liver disease. The heart condition may be ameliorated as well, as shown by improved cardiac function and the lessening of storage.

Intramyocardial polyglucosan bodies leading to myocardial thickening and dysfunction may also be observed in phosphofructokinase deficiency (GSD VII), mutations of the PRKAG2 gene encoding the α-2 subunit of AMP-activated protein kinase, and unclassified polysaccharidoses of the heart and skeletal muscle.

23.2.3.6 Disorders of Glycoprotein Metabolism

The heart is quiet frequently involved in organ dysfunction in patients with congenital disorders of glycosylation (CDG) leading to early death within the first year of life in a substantial number of patients. Hypertrophic cardiomyopathy is associated with phosphomannomutase 2 deficiency (PMM2-CDG, previously labeled CDG-Ia). Dilated cardiomyopathy is found in several forms of defective N- and O-linked or multiple pathway glycosylation (e.g., ALG6-CDG, DK1-CDG, DPM3-CDG). Specific therapy is not available.

Disease Info: Hemochromatosis

Hereditary hemochromatosis (HH) characterized by iron deposition and tissue injury in multiple organs is among the most common genetic metabolic diseases in many parts of the world, occurring as often as in 1 in 300 persons (white Australians). In all types of HH, iron overload results from impairment of the hepcidin-ferroportin regulatory pathway. The most common of the inherited forms (>95 %) is autosomal recessive type 1 or *HFE*-related HH. Two mutant alleles of the HFE gene that regulates the expression of the iron-regulatory hormone hepcidin account for essentially all cases. Homozygotes for C282Y are at highest risk; the risk is lower for compound heterozygotes C282Y/H63D; and homozygotes for H63D are at minimal risk for iron overload. The clinical penetrance of C282Y homozygosity for iron overload-related diseases is up to 30 % in men and 2 % in women in the fourth to sixth decades; overt illness is very rare before adulthood. In total, 25–35 % of C282Y homozygotes may not develop iron overload. Carrier (heterozygote) frequency of the severe C282Y allele is as high as 10 % in some populations.

HH is difficult to recognize clinically, because its early symptoms of fatigue and depression do not point to iron overload. Hepatic dysfunction and hepatomegaly are common, manifested by mild elevation of transaminases (see also Chap. 24). Pancreatic dysfunction may lead to diabetes and adrenal dysfunction to Addison disease. Darkening of the skin from iron may be mistaken

for the effects of Addison disease. Arthropathy is common.

Cardiomyopathy has an insidious onset and may be attributed to diabetes. Compared to the normal population, cardiomyopathy is 306-fold more frequent in patients with HH. The cardiomyopathy usually results in biventricular dilatation. Ventricular thickening is an early finding; a restrictive form occurs occasionally. Arrhythmias (ventricular ectopic beats, tachycardias, ventricular fibrillation, and heart block) may occur.

Raised transferrin-iron saturation (>45 %) and ferritin levels are the usual biochemical markers for iron overload; definitive diagnosis, formerly done by liver biopsy, is now generally achieved by molecular testing, although biopsy may be needed to assess the degree of liver injury.

As hemochromatosis is a recessive disease, all siblings of probands should be tested. Parents should also be tested because of the possibility that they might carry a second mutation. Molecular genetic testing to identify C282Y (and H63D) mutants is the first step of cascade testing, followed by iron studies in C282Y homozygotes and C282Y/H63D heterozygotes.

A distinct, but rare disorder is juvenile or type 2 hemochromatosis (JH), in which there are similar findings, but the onset is in childhood. Autosomal recessive JH is caused by mutations in the gene of hepcidin-regulating protein hemojuvelin or the hepcidin gene itself.

Type 3 and 4 HH are caused by mutations in the transferring receptor 2 gene or the ferroportin gene. Symptoms in these subtypes also manifest in the fourth to fifth decades.

HH is treated by phlebotomies whose frequency is targeted by serum ferritin levels (<300 ng/mL in men und 200 ng/mL in women). Tissue damage, such as cardiomyopathy and hepatic fibrosis, may not be reversible, so early diagnosis is essential.

23.2.3.7 Nutrient-Deficient Cardiomyopathies

Thiamine

Thiamine (vitamin B_1), as thiamine pyrophosphate, is essential for the function of several decarboxylases of 2-oxoacids, including pyruvate, the branched-chain oxoacids, and 2-oxoglutarate. Severe thiamine deficiency leads to beriberi, a form of cardiac and peripheral vessel dysfunction characterized by loss of peripheral vascular tone, edema, tachycardia, cardiac dilatation, and heart failure. Brain damage (Wernicke's encephalopathy) reminiscent of Leigh disease may occur, and other organs may become damaged. Thiamine deficiency can occur in patients with organic acidurias receiving limited diets. In this setting, lactic acidosis has been the presenting feature, reflecting impaired activity of pyruvate decarboxylase, the first step in the pyruvate dehydrogenase complex. Deficiency of thiamine resulting from thiamine transport defects (autosomal recessive) typically results in anemia (usually megaloblastic but may be sideroblastic or aplastic), sensorineural deafness, and diabetes; tachycardia and edema may occur in the most severely affected infants. Diagnosis is made by measuring blood or urine thiamine content.

Selenium

Selenium in the amino acid selenomethionine is a component of several proteins and cofactor, particularly, for glutathione peroxidase. Selenium deficiency, especially when it co-occurs with vitamin E deficiency, impairs the antioxidant function of various pathways. There may also be increased vulnerability to cardiotropic enteroviruses. Selenium deficiency is a common cause of cardiomyopathy in the Keshan district of China, where there is insufficient selenium in soil and foods. Manifestations include cardiac dilatation, dysrhythmias, and ultrastructural changes. The condition may be fatal unless treated. Pancreatic insufficiency may also develop.

Selenium deficiency may also occur in developed countries. It has been observed following prolonged parenteral nutrition and in patients with small bowel disease. Patients with many forms of heart disease, including ischemic heart disease and HIV cardiomyopathy, have lower selenium levels than controls, raising the possibility that selenium depletion may play a role.

Selenium deficiency is diagnosed by the measurement of erythrocyte selenium, glutathione (total and reduced), and glutathione peroxidase; it is treated with sodium selenite, 60–100 μg/days.

23.2.4 Investigations for Metabolic Cardiomyopathies

Laboratory tests to investigate a suspected metabolic cardiomyopathy should focus initially on obtaining information rapidly to determine if there is a specific treatment for the disorder. At the same time, supportive care to ease myocardial workload should be instituted and preparations made for invasive testing if needed.

Remember

Because of complex etiology, diagnosis of cardiomyopathy requires a systematic and integrated approach including careful clinical examination, echocardiography, laboratory investigations, and search for additional organ dysfunctions (Fig. 23.1).

Cardiomyopathy may be the presenting or dominant clinical feature, but careful searching often reveals other signs of a multisystemic disease as well as abnormal metabolites in blood or urine. Clinical features may include dysmorphic features, neurological and muscular symptoms, skeletal findings, and signs of liver dysfunction. In inborn errors of metabolism that impair energy production or produce toxic metabolites, congestive heart failure often occurs in the setting of an acute metabolic decompensation triggered by intercurrent infections, surgery, fasting, or physical exertion. Since these inciting events may suggest an alternative etiology, e.g., viral myocarditis; it is important to include laboratory testing of blood and urine as part of the initial evaluation.

Initial laboratory tests include complete blood count, glucose, blood gas analysis, plasma lactate, amino acids, carnitine, acylcarnitine profile, electrolytes, transaminases, creatine kinase, urea, creatinine, and uric acid. Troponin I and BNP or proNT-BNP levels reflect the extent of myocardial damage, ischemia, and overload, independent from their etiology (Table 23.3).

Lactate is often elevated in patients with cardiac failure simply reflecting poor perfusion. In such a setting, the lactate/pyruvate ratio is likely to be elevated and not necessarily suggestive of disturbed oxidative phosphorylation (see Chap. 14). Renal tubular function can be assessed by checking for wasting of amino acids, phosphate, and bicarbonate, resulting in a Fanconi syndrome or isolated renal tubular acidosis. Urinary organic acids with increased Krebs cycle intermediates and lactate may be suggestive of mitochondrial dysfunction, while prominent 3-methylglutaconic acid suggests Barth syndrome (methylglutaconic aciduria, or MGA, types II or V) or defective oxidative phosphorylation, especially complex V deficiencies (MGA type IV). Distinct organic acidurias may present with their typical excretion pattern. Hepatic dysfunction may be reflected by increases in transaminases, bilirubin, and ammonia, hypoglycemia, an abnormal amino acid profile, and reduced coagulation factors. Consideration should be given to determine thiamine, selenium, vitamin E, and glutathione levels when nutritional deficiencies are suspected.

Abdominal ultrasound and eye examination should be included in the initial diagnostic workup.

Encephalopathy, hypoglycemia, metabolic acidosis, and neuromuscular symptoms have been proposed as key entry points to diagnostic algorithms and second-order tests in suspected metabolic cardiomyopathy [for detailed algorithms, see Cox (2007)].

Second-order investigations (Table 23.4) include analysis of additional metabolites, targeted genetic analyses, and enzyme studies (e.g., fatty acid oxidation and lysosomal enzymes) in fibroblasts, lymphocytes, or tissue. The echocardiographic type of cardiomyopathy and some ECG findings may allow one to narrow the diagnostic approach. However, the substantial heterogeneity of echocardiographic and ECG findings has to be considered.

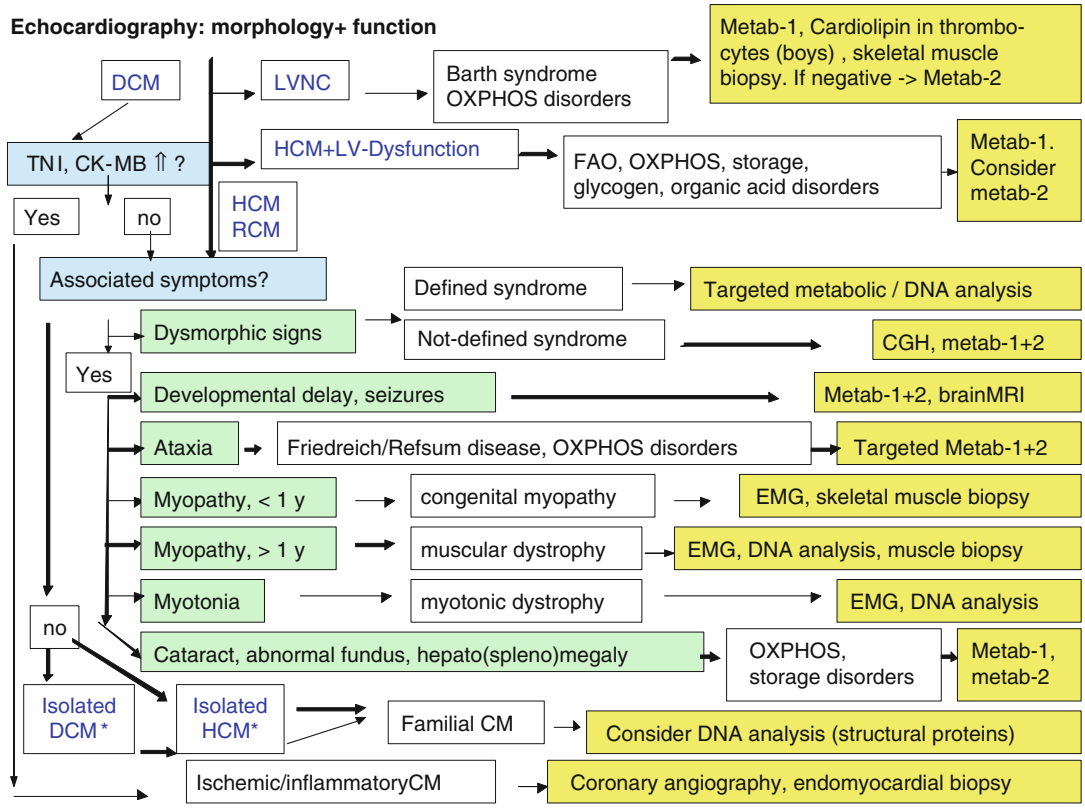

Fig. 23.1 Algorithm for the diagnostic approach to cardiomyopathies. *DCM* dilated cardiomyopathy, *HCM* hypertrophic cardiomyopathy, *HDCM* hypertrophic-hypocontractile (mixed type) cardiomyopathy, *LVNC* left ventricular noncompaction, *OXPHOS* mitochondrial, *RCM* restrictive cardiomyopathy, *TNI* troponin I. *Metab-1* first-line investigations (Table 23.3), *Metab-2* second-line investigations (see Table 23.4), *Blue type* echocardiographic types of CM, *Blue background* early routine examinations, *Green background* potentially associated symptoms, *Red frame* suspected etiology, *Yellow background* recommended investigations. *In the case of isolated CM, complete first-line metabolic investigations should be performed in all children younger than 3 years. Otherwise well-defined secondary cardiomyopathies with recognizable etiology (e.g., hypertensive CM and anthracycline-induced CM) were not included in this algorithm

Morphological studies including ultrastructural examination as well as biochemical investigations of endomyocardial and skeletal muscle biopsies should be performed in all cases, whose etiology is unresolved after the noninvasive workup.

Remember

Skeletal muscle biopsy should be considered in all infants with hypertrophic or hypertrophic-hypocontractile phenotype, due to the high probability of defective oxidative phosphorylation, even in the absence of lactic academia.

Defects of glycogen metabolism or oxidative phosphorylation confined to the heart may only be detected by direct endomyocardial analysis (see Chaps. 14 and 42).

23.2.5 Principles of Treatment in Metabolic Cardiomyopathy

Treatment should be directed to the specific metabolic defect as well as to the functional state of the myocardium. Signs of acute metabolic disturbance like hypoglycemia and metabolic

Table 23.3 First-line investigations in suspected metabolic cardiomyopathy

Examination	Criteria/suspected disorder (examples)
Additional clinical symptoms	
Dysmorphic features	Coarse facial appearance, cloudy corneas, slow growth
Muscular symptoms	Weakness, hypotonia, cramps, myotonia
Neurological findings	Encephalopathy, developmental delay, seizures
Liver and spleen involvement	Organ size, ascites, liver skin signs
Skeletal findings	Dysostosis multiplex
Blood	
Complete blood count	Vacuoles, lysosomal storage disease
Blood gas analysis, lactate	Defects in oxidative phosphorylation
Sodium, potassium, calcium, phosphate	Renal involvement (tubular)
Urea, creatinine, uric acid	Renal involvement (glomerular)
Creatine kinase	Myopathy
Alanine and aspartate aminotransferases	Liver involvement
Carnitine (free and total)	Carnitine transporter deficiency
Acylcarnitine profile	Defects in fatty acid oxidation, organic aciduria
Amino acids	Organic aciduria, tyrosinemia
Transferrin isoelectric focusing	CDG syndromes
Acid α-glucosidase	Pompe disease
Cholesterol, triglycerides	Lipoprotein disorders
Troponin I	Myocardial ischemia
BNP/pro-NT-BNP	Ventricular overload/dysfunction
Urine	
Protein, electrolytes	Proteinuria, electrolyte loss (renal involvement)
Organic acids	Organic acidurias
Glycosaminoglycans	Mucopolysaccharidoses
Oligosaccharides	Oligosaccharidoses
Eye	
Lens	Cataract, defects in oxidative phosphorylation
Retina	Storage disease, defects in oxidative phosphorylation
Other organs	
Abdominal ultrasound	Hepatic or renal involvement

BNP brain-type natriuretic peptide, *NT* N-terminal

acidosis should be corrected as soon as possible. In metabolic acidosis, carnitine supplementation may help to restore intermediate metabolism by replenishing low free carnitine levels, binding acid compounds, and liberating coenzyme A from acyl-CoA species. For disorders of the intermediary metabolism, there are several guiding principles for treatment. Diet should reduce the intake of nonmetabolizable substrates and offer alternative, metabolizable energy sources and nutrients (e.g., substitution of long-chain by medium-chain fatty acids in VLCAD deficiency, D,L-3-hydroxy-butyrate in MADD). Fasting should be avoided by use of night feeding or continuous tube feedings during infancy. Cornstarch at bedtime may provide sufficient carbohydrate supply through the night in older children. Dietary supplements can reduce secondary toxicity (e.g., carnitine in organic acidurias and antioxidants in defects of oxidative phosphorylation). In some cases, residual enzyme activity can be enhanced by the use of pharmacologic doses of vitamin cofactors (e.g., riboflavin in MADD). Enzyme replacement therapy has emerged as a new therapeutic option, at least in terms of myocardial function and

Table 23.4 Second-line investigations in suspected metabolic cardiomyopathy

Abnormalities	Second-line investigation
Clinical symptoms	
Dysmorphic features	
Blood	Acylcarnitines (if not done before), chromosome analysis, CGH array
Urine	Organic acids, mucopolysaccharides, oligosaccharides (if not done before), free sialic acid
Ataxia	
Blood	Phytanic acid
DNA	Frataxin gene mutations
Developmental delay	
Blood	Lactate/pyruvate ratio (L/P) (preferentially before and after a glucose load), NH_3
CSF	Lactate + pyruvate, amino acids
Urine	Free sialic acid
Cranial MRI, MR spectroscopy	
Muscular symptoms	See below
ECG	
AV block	mtDNA analysis
Preexcitation	mtDNA analysis
Blood	
High lactate + hypoglycemia	
Blood	Lactate/pyruvate ratio (L/P), NH_3
During acute hypoglycemia	
Blood	Insulin, GH, free fatty acids, β-OH-butyrate, amino acids, acylcarnitines
Urine	Ketones, organic acids
With high ketones and uric acid	Amylo-1,6-glucosidase (debrancher enzyme) (GSD III) (liver, muscle, fibroblasts)
Persistent high lactate	
Blood	Lactate/pyruvate ratio (L/P), acetoacetate/β-OH-butyrate ratio
L/P > 25:1	Assays for respiratory chain enzymes (skeletal muscle biopsy, fibroblasts) mtDNA analysis (blood, fibroblasts), next-generation sequencing
L/P < 15:1	Pyruvate dehydrogenase activity (fibroblasts)
CSF	Lactate + pyruvate, amino acids, protein
Cranial MRI, MR spectroscopy	
Moderate creatine kinase ⇑ (<10× of normal), proximal or generalized muscle weakness	
Blood	Lactate + pyruvate + L/P ratio (preferentially after glucose load)
Urine	Lactate, organic acids, oligosaccharides (if not done before)
Electrophysiology	EMG, NCV, EEG
Skeletal muscle biopsy	(Ultrastructural) morphology, histochemistry, enzyme studies
DNA	According to biopsy studies (mtDNA, nDNA)
High creatine kinase ⇑ (>10× normal), proximal and limb girdle muscle weakness	
Man: DNA, if negative: skeletal muscle biopsy	Dystrophin gene mutation analysis (ultrastructural) morphology, immunohistology, histochemistry, enzyme studies
Woman: skeletal muscle biopsy	See above
Moderate creatine kinase ⇑ (<10× normal), scapulo-peroneal muscle weakness + contractures (w/o AV block)	
DNA	Lamin A/C (autosomal dominant), emerin (X-chromosomal)

Table 23.4 (continued)

Abnormalities	Second-line investigation
If negative: skeletal muscle biopsy	See above
Moderate creatine kinase ⇑ (<10× normal), myotonia	
DNA	Number of CTG repeats in DMPK gene
Liver enzymes ⇑ (w/o hepatomegaly)	
Blood	Lactate/pyruvate ratio (L/P) (preferentially after glucose load), NH_3, ferritin
Liver biopsy	(Ultrastructural) morphology, immunohistology, histochemistry, enzyme studies

CGH comparative genomic hybridization, *mtDNA* mitochondrial DNA, *nDNA* nuclear DNA, *EMG* electromyogram, *NCV* nerve conduction velocity, *EEG* electroencephalogram, *w/o* with or without

morphology, in several lysosomal storage diseases like Gaucher disease, Pompe disease, Fabry disease, and MPS I, II, IVA, and VI. Beneficial effects of hematopoietic stem cell transplantation have been observed in MPS I and VI. In certain cases of severe cardiomyopathy, enzyme replacement may be transiently needed to support the patient during hematopoietic stem cell transplantation.

In systolic myocardial failure, conventional therapy with ACE inhibitors, β-adrenergic receptor blockers, spironolactone, and other diuretics should be instituted as soon as possible. Digoxin may be introduced in the presence of atrial flutter or fibrillation but may also be beneficial in pure dilated forms. Independent from the morphologic pattern, efficient reduction of β-adrenergic sympathetic activity may be crucial for the long-term prognosis especially in cardiomyopathies due to defective energy production. In hypertrophic cardiomyopathy with isolated diastolic dysfunction, β-blocking agents or calcium antagonists may be helpful when outflow tract obstruction exists.

In the case of severe myocardial failure, extracorporal membrane oxygenation or ventricular assist devices offer the potential for survival during diagnostic workup and initiation of specific metabolic therapy. Reducing afterload can facilitate myocardial recovery and bridge the time until specific and nonspecific treatments have taken effect. If advanced heart failure is accompanied by ventricular dyssynchrony, implantation of a biventricular pacemaker may be considered. Heart transplantation may be an option in cardiac-restricted metabolic cardiomyopathies or well-controlled generalized metabolic diseases with irreparable heart damage. (for review see Lipshultz et al. (2011, 2014 + 2015)).

23.3 Dysrhythmias and Conduction Disturbances

Disturbances of the cardiac rhythm due to metabolic disorders are often combined with overt cardiomyopathy but may also proceed morphological and functional abnormalities of the myocardium and may be the first, in some cases even fatal clinical manifestation. Routine ECG may show preexcitation in respiratory chain disorders, Pompe and Danon disease. Prolongation of QTc interval which increased with age has been observed in about 70 % with propionic acidemia. Prolonged repolarization of the myocardium may predispose propionic acidemia patients to ventricular fibrillation or *torsades de pointes* and may be the cause of the increased rate of sudden death in these patients.

All FAO disorders that affect cardiac metabolism are another disease group in which rapid ventricular dysrhythmias (e.g., fibrillation, *torsades de pointes*) can occur. LCHAD, VLCAD, and CPTII deficiencies possess the greatest risk for ventricular tachycardia, probably due to the accumulation of potentially arrhythmogenic long-chain acylcarnitines. Even in MCAD deficiency, which usually does not affect the myocardium, ventricular tachycardia has been observed in adulthood.

In addition to the specific metabolic therapy, tachyarrhythmias should be treated by β-receptor blockers, propafenone, sotalol, or amiodarone as indicated. In some cases, implantable converters

may be indicated due to sustained ventricular tachycardia or symptomatic arrhythmias.

AV and ventricular conduction disturbances and bradycardia are typical sign of the Kearns-Sayre syndrome and other mitochondrial disorders with cardiac manifestation.

Remember

Because of the potentially rapid progression, pacemaker implantation should be considered early in patients with neuromuscular disorders such as Kearns-Sayre syndrome who have second-degree AV block or fascicular block, even when they are asymptomatic.

AV block may also occur in MPS II and VI and Fabry disease.

23.4 Metabolic Disorders and Valvular Abnormalities

Several metabolic disorders are associated with valvular dysfunction late in the course of the disorder. Thickening of the valves, sometimes with calcification, especially the aortic and mitral, occurs in several lysosomal storage disorders, including Hurler (MPS I-H), Scheie (MPS I-S), Hunter (MPS II), Sanfilippo (MPS III), Morquio (MPS IV), and Maroteaux-Lamy (MPS VI) syndromes. Mitral and aortic regurgitation can be observed in more than 50 % of MPS patients, especially in MPS I, II, and VI. Aortic and mitral stenosis and mitral valve prolapse are less frequent. As a rule, prevalence of valvular abnormalities largely depends on the age of the patients. In some cases, postcapillary pulmonary hypertension may also develop. Therefore, serial color Doppler echocardiography in regular intervals is required in patients with MPS to assess ventricular function and the progression of cardiac abnormalities with age.

Remember

In contrast to their benefit for ventricular function, long-term enzyme replacement therapy, now available in MPS I, MPS II, MPS IVA, and MPS VI, and allogeneic hematopoietic stem cell transplantation seem to have no or low benefit for the preexisting valvular lesions but may slow the progression of valvular changes.

The mucolipidoses, especially mucolipidosis I (sialidosis), can be complicated by mitral regurgitation. The lipidoses, especially Gaucher disease and Farber disease, may infrequently show storage in the connective tissues of the valves, causing thickening and subsequent calcification of the aortic and mitral valves and the development of nodules. The chordae tendineae may develop similar deposits. Functionally, there may be aortic or mitral stenosis or regurgitation. Stiffening and calcification of the aortic and mitral valves occur in alkaptonuria, a disorder of tyrosine catabolism that causes darkening (ochronosis) and stiffening of cartilaginous tissues.

23.5 Thromboembolic and Vascular Disorders

Morphological and functional abnormalities of blood vessels occur in several metabolic disorders. Tortuosity of vessels is particularly prominent in Menkes syndrome. Angiokeratomata of the umbilicus, buttocks, and genitalia are a feature of X-linked Fabry disease, where they appear in adolescence. In fucosidosis (autosomal recessive), they appear in the first few years.

Hypercoagulability is a prominent aspect of homocystinuria and the CDG syndromes (PMM2-CDG, MPI-CDG, and ALG1-CDG) which can lead to major occlusions of cerebral vessels or multiple small cerebral infarcts. Strokes are also a frequent feature in mitochondrial disorders, perhaps as a consequence of endothelial dysfunction and gradual vascular occlusion. Migraine with stroke and stroke-like episodes without residua or with persistent hemiplegia may all occur in mitochondrial disorders, particularly MELAS.

Beyond lipoprotein disorders, coronary atherosclerosis is accelerated in Fabry disease (including in some heterozygous females) and in some mucopolysaccharidoses. In MPS I, infiltration of small and precapillary arteries by storage

material may result in severe ischemic cardiomyopathy in infants and young children.

23.5.1 Lipoprotein Disorders

The long-term sequelae of lipoprotein disorders (Table 23.5) lead to atherosclerotic vascular disease in all the arterial beds. Plasma elevation of low-density lipoprotein cholesterol (LDL-C), very low-density lipoproteins, lipoprotein (a), and reduced levels of high-density lipoprotein cholesterol (HDL-C) are well-known risk factors for coronary heart and cerebrovascular disease. LDL metabolism may be impaired by mutations affecting the hepatic cell surface LDL receptor (familial hypercholesterolemia), the receptor that recognizes apolipoprotein B100 protein on the surface of LDL particles (familial defective apo B-100) or proprotein convertase subtilisin kexin type 9 (PCSK9), involved in LDL receptor degradation. Affected children may present with myocardial infarction and cerebrovascular disease even during the early second decade in untreated homozygotes or severe compound heterozygotes. Therefore, screening and treatment of these children have to take into account the severity of the phenotype, the long-term risk of developing vascular disease, and available evidence of clinical benefit in a group of diseases that are mostly asymptomatic within childhood and adolescence.

Fasting lipid profile (LDL-C and HDL-C) should be determined between the ages of 2 and 8 years in children with a positive family history for premature cardiovascular events in parents or grandparents below the age of 55 years in males and 65 years in females or with high risk-burden diseases, e.g., diabetes mellitus, chronic kidney disease, after orthotopic heart transplantation, or after Kawasaki disease with current aneurysms. Universal lipid screening has recently been recommended in all children between the age of 9 and 11 years using non-fasting non-HDL-C measurement (total cholesterol minus HDL-C) or fasting lipid profile. Age-dependent normal levels of LDL-C and HDL-C, stratification by underlying diseases, and the assessment of additional risk factors [hypertension, overweight, impaired glucose tolerance, smoking, reduced HDL-C, and elevated lipoprotein(a)] may promote gradual treatment goals.

Remember

Lifestyle modification, especially of diet and exercise, should be initiated in children with LDL-C persistently ≥ 130 mg/dl. Lipid-lowering medication is recommended for levels of LDL-C ≥130–159 mg/dL in the high-risk group, ≥160–189 mg/dL in patients with moderate risk, and ≥190 mg/dL in patients with no additional risk factors.

Statins as HMG-CoA-reductase inhibitors are the first-line drugs to lower increased LDL-C to target levels (<130 mg/dl or ≥50 % reduction of LDL-C) in children ≥8 years (only pravastatin) and 10 years (all statins). In younger children, pharmacological treatment is limited to homozygous/compound heterozygous familial hypercholesterolemia and those who have overt cardiovascular disease. Ezetimibe inhibiting intestinal cholesterol absorption via the membranous Niemann-Pick C1-like 1 protein and LDL apheresis are additional options in severe hyperlipoproteinemia. (Expert Panel on Integrated Guidelines for Cardiovascular Health and Risk Reduction in Children and Adolescents; National Heart, Lung, and Blood Institute (2011)).

23.6 Pulmonary Hypertension

Pulmonary hypertension (PH), a chronic vasculoproliferative disorder of the pulmonary arterial vasculature, has been associated with an increasing number of inborn metabolic disorders (group 5.3. in the current classification of PH). PH is defined as a mean arterial pressure ≥25 mmHg at rest, measured by right heart catheterization. In GSD Ia, PH may be a late complication observed in about 10 % of these patients during adolescence and adulthood. Abnormal production of serotonin and its release from thrombocytes have been suggested to be the most probable cause for pulmonary vasoconstriction and remodeling of the vessel wall in GSD Ia. Several cases of unexplained PAH have been reported in association

Table 23.5 Genetic lipoprotein disorders with significantly increased cardiovascular risk

Disorder	Inheritance	Prevalence	LDL-C (mg/dL)	HDL-C (mg/dL)	TG (mg/dL)	Genetic lesion	Risk of atherosclerosis (age at clinical presentation)
Familial hypercholesterolemia							
Heterozygous	AD	1:200–1:500	>135	n-↓	Normal	LDL receptor	↑–↑↑ (Early to mid adulthood)
Homozygous	AD	1:1,000,000	>425	<35	Normal	LDL receptor	↑↑↑ (Starting in first decade)
Familial defective apo B-100							
Heterozygous	AD	1:500–1:700	>135	n-↓	Normal	Apo B-100	↑ (Mid to late
Homozygous	AD	1:1,000,000	>320	<35	Normal	Apo B-100	↑↑–↑↑↑ (Starting in second decade)
Familial combined hyperlipidemia[a]	AD	1:20–1:50	150–250	n-↓	150–400	Polygenic	↑–↑↑ (Mid to late adulthood)
Familial dysbetalipoproteinemia	AR	1:10,000	>130	n-↓	>270	ApoE-2/-2	↑↑ (Early to mid adulthood)
Apolipoprotein A-I deficiency	AR	<1: 100,000	N	<10	n	Apo A-I	↑–↑↑ (Early to mid adulthood)
Sitosterolemia	AR	1:1,000,000 (homozygous)	Sitosterol.↑, campesterol↑	N	Normal	ABCG5, ABCG8	↑↑–↑↑↑ (Starting in first decade)

AD autosomal dominant, *AR* autosomal recessive, *LDL-C* LDL cholesterol, *TG* triglycerides, HDL-C HDL cholesterol

[a]Prevalence is age dependent

with Gaucher disease. Liver disease, splenectomy, capillary plugging by Gaucher cells, and enzyme replacement therapy could all play a role in the development of PH.

PH may also be a manifestation of multisystemic mitochondrial respiratory chain disorders, especially in defects affecting the integrity of the mitochondrial iron-sulfur clusters. Preceding liver disease and young age have been consistently found in these patients. Recurrent episodes of cyanosis or oxygen dependency due to pulmonary vasoconstriction result from the marked muscular thickening and intimal proliferation of the medium and small pulmonary arteries. Accordingly, mitochondrial dysfunction in pulmonary vascular cells has recently been identified as a typical cellular feature of PH.

PH has also been reported as a main presenting symptom in preschool children with cobalamin C deficiency and in patients with mucolipidosis II. In the latter ones, impaired endothelin degradation due to lysosomal dysfunction has been suggested as a major cause of the pulmonary vascular disorder.

Early detection of increased pulmonary pressure is crucial to initiate medical therapy (e.g., endothelin antagonists, phosphodiesterase inhibitors, systemic or inhaled prostacyclin analogs) (Ivy et al. 2013).

Remember

Routine echocardiographic assessment of right ventricular performance and pulmonary pressure should be included in the follow-up of patients with metabolic disorders at risk for PAH.

Suspicion for PAH should be verified by right heart catheterization and measurement of pulmonary artery pressures including vasodilator studies. Assessment of pulmonary function and additional risk factors and exercise testing and echocardiographic grading of pulmonary pressures and right ventricular function complete the diagnostic spectrum (Table 23.6).

Table 23.6 Investigation in pulmonary hypertension

Aim/methods	Parameter	Value
Screening of PH		
Echocardiography	Tricuspid valve regurgitation	Normal <2.5 m/s
		Borderline 2.5–2.8 m/s
		Abnormal >2.8 m/s
Verification of suspected pulmonary hypertension		
Right heart catheterization	Pulmonary artery pressure pulmonary vasodilator studies including oxygen, nitric oxide, inhaled iloprost	Baseline hemodynamics changes of pulmonary arterial pressure and resistance (especially Rp/Rs ratio)
Main differential diagnosis		
Disorders of the respiratory system	Spirometry, oxygen diffusion capacity, chest HR-CT, sleep studies	
Grading of pulmonary hypertension		
Echocardiography	Right atrium area, TAPSE, Tei index, left ventricular eccentricity index	
Exercise testing	6-min walk test, spiroergometry	
Assessment of additional risk factors	Homocysteine	>12 µmol/L
	Lipoprotein (a)	>30 mg/dL
	Genetic thrombophilic factors	FV-Leiden mutation (1691G > A), prothrombin variation 20210G > A, MTHFR variation 677C > T

Rp/Rs ratio pulmonary arterial resistance/systemic arterial resistance, *HR-CT* high-resolution computer tomography, *TAPSE* tricuspid annular plane systolic excursion

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Liver Disease

24

Georg F. Hoffmann and Patrick McKiernan

Key Facts

- Liver disease is a common and important sequel of inherited metabolic diseases.
- Defects of the following major pathways of intermediary metabolism can lead to significant liver disease: degradation of fatty acids, fructose, galactose, and glycogen as well as of gluconeogenesis, ketogenesis, urea cycle including citrin deficiency, or oxidative phosphorylation.
- Hints to diagnosis can be specific symptoms of these disorders reflecting the demand on the pathway affected; a detailed history as well as pathological alterations of blood ammonia, glucose, lactate, ketone bodies, and pH can lead the path.
- Specific metabolic symptoms can be obscured by consequences of rather nonspecific responses of the liver to hepatocellular damage, decreased liver function, cholestasis, and hepatomegaly.
- Inherited diseases interfering primarily with hepatic cell integrity are Wilson disease, tyrosinemia type I, transaldolase deficiency, RALF syndrome caused by mutations in NBAS, cystic fibrosis, α-1-antitrypsin deficiency, and deficiencies of biosynthetic pathways, such as of cholesterol biosynthesis, bile acid synthesis, peroxisomal disorders, and CDG syndromes. Very striking physical involvement of the liver with relatively little functional derangement is seen in lysosomal storage disorders.
- The diagnostic laboratory evaluation of liver disease must be broad, especially in neonates, and initiated early. Successful outcome depends very much on early institution of specific therapy. In addition to the increasing therapeutic options for individual metabolic disorders, pediatric liver transplantation has developed into a well-established procedure, with the best outcome rates of ≥90 % in liver-based metabolic disorders.

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24.1 General Remarks

Despite the complex interrelated functions of the liver in intermediary metabolism, the hepatic phenotype resulting in inherited metabolic disease is limited and often indistinguishable from that resulting in acquired causes, such as infections or intoxications. The corollary is that acquired liver diseases affect many metabolic processes. The diagnostic laboratory evaluation of liver disease must therefore be broad. Careful evaluation of the history and clinical presentation should include a detailed dietary history as well as a list of all medications, the general appearance of the patient, somatic and psychomotor development, signs of organomegaly, neurological signs, and an ophthalmologic examination. In combination with the results of routine laboratory investigations (Table 24.1), this should lead to the suspicion of an inborn error of metabolism, the initiation of specific metabolic tests (Table 24.2), and a provisional grouping into one of the four main clinical presentations (A, jaundice/cholestatic liver disease; B ,liver failure/hepatocellular necrosis; C, cirrhosis; or D, hepatomegaly).

The family and personal history of the patient may be informative in either acquired or inherited liver disease. Routes of infection may become obvious. Linkage of symptoms to oral intake of food or drugs may almost be diagnostic in fructose intolerance, paracetamol intoxication, or accidental poisoning.

Another important key to diagnosis is the age at presentation. During the first 3 months of life, the majority of patients with liver disease, including those with inherited metabolic diseases, present with conjugated hyperbilirubinemia. Later in infancy, the presentation becomes broader (Fig. 24.1).

Only a few well-defined inherited diseases cause unconjugated hyperbilirubinemia: hemolytic anemias or impaired conjugation of bilirubin resulting in recessive deficiency of UDP-glucuronyl transferase in Crigler–Najjar syndrome types 1 and 2 or in the milder *Gilbert syndrome* (bilirubin, 1–6 mg/dL). The latter is a benign condition manifesting in neonates only if they are afflicted by a second hemolytic disorder, such as glucose-6-phosphatase deficiency. In later life, mild jaundice aggravated by fasting or intercurrent illness is the only symptom, and the condition is often discovered accidentally. *Crigler–Najjar syndrome type 1*, usually defined as bilirubin >20 mg/dL or >360 μmol/L that does not respond to phenobarbitone therapy, leads to severe nonhemolytic jaundice. Severe neurological damage and death due to kernicterus are a common sequel. Patients with *Crigler–Najjar type 2* have bilirubin levels of up to 20 mg/dL. Both orthotopic and auxiliary liver transplant have proven highly successful. Crigler–Najjar syndrome type 1 has been ameliorated by

Table 24.1 First-line investigations in disease of the liver

Alanine and aspartate aminotransferases (transaminases)
Lactate dehydrogenase
Cholinesterase
Alkaline phosphatase
γ-Glutamyl transpeptidase
Bilirubin, conjugated and unconjugated
Bile acids
Coagulation studies: INR, PT, and PTT, and factors V, VII, and XI
Albumin, prealbumin
Urea nitrogen, creatinine, uric acid, CK
Glucose
Ammonia
Hepatitis A, B, C, E
Cytomegalovirus, EBV, herpes simplex, toxoplasmosis, HIV
Viral cultures of stool and urine
Abdominal ultrasound (gall bladder before and after meal, hepatic tumor)
Ultrasound of the heart (vitium cordis, peripheral pulmonary stenosis)
In infancy
Rubella, parvovirus B19, echovirus, and a variety of enterovirus subtypes
Bacterial cultures of blood and urine

INR international normalized ratio, *PT* prothrombin time, *PTT* partial thromboplastin time, *EBV* Epstein–Barr virus, *HIV* human immunodeficiency virus

Table 24.2 Second-line investigations in suspected metabolic liver disease

Plasma/serum
Copper, ceruloplasmin
α-Fetoprotein
Cholesterol, triglycerides
Serum iron and ferritin, transferrin, and transferrin saturation
Free fatty acids, lactate, pyruvate, 3-hydroxybutyrate, acetoacetate
Chitotriosidase
Amino acids
Free and total carnitine, acylcarnitines profile
α-1-Antitrypsin activity and PI phenotyping
Lysosomal enzymes
Blood spots
Acylcarnitines profile
Acid lipase
Urine
Amino acids
Ketones
Reducing substances/sugars (galactosemia, fructose intolerance)
Organic acids (incl. specific assays for orotic acid and succinylacetone)
Individual bile acids (bile acid synthesis defects)
Copper in 24-h urine
Sweat chloride(cystic fibrosis)
In infancy
Galactose-1-phosphate uridyl transferase (galactosemia)
Chromosomes (especially when liver disease is accompanied by malformations suggesting trisomy 13 or 18)
Free T_4 and TSH (hypothyroidism)
Transferrin isoelectric focusing (CDG syndromes)
Very long-chain fatty acids (peroxisomal disorders)

hepatocyte transplantation in a few cases and is an attractive candidate for gene therapy (Table 24.3).

Pediatric liver transplantation has developed into a well-established procedure. Patients with cholestatic or liver-based metabolic diseases have the best outcome with 5-year survival ≥90% and high-quality long-term survival. Auxiliary transplantation is an option when there is no significant fibrosis, and the objective of treatment is the replacement of the missing enzyme, e.g., in Crigler–Najjar syndrome type 1 or urea cycle disorders.

24.2 Cholestatic Liver Disease

24.2.1 Cholestatic Liver Disease in Early Infancy

Cholestatic liver disease may aggravate or prolong physiological neonatal jaundice. It becomes obviously pathological when conjugated hyperbilirubinemia is recognized (conjugated bilirubin >15% of total). Normal infants pass colorless urine. An important early warning sign is finding colored urine which may vary from only faintly yellow to distinctly yellow or even brown. The significance of this finding is often missed, as this color is similar to adult urine. Of course, by this time the sclerae are yellow – with chronic direct hyperbilirubinemia, the skin becomes yellow and may have a greenish hue. Cholestasis frequently causes pale or acholic stool. A colored stool, however, does not exclude cholestasis.

Remember

A stool specimen should always be looked at in the first visit of jaundiced neonates with conjugated hyperbilirubinemia.

Transient conjugated hyperbilirubinemia can be observed in neonates, especially premature infants, after moderate perinatal asphyxia. It is associated with a high hematocrit and a tendency to hypoglycemia and has an excellent prognosis, if the pathological conditions described below have been excluded.

Remember

Cholestatic liver disease in infancy may be the initial presentation of cystic fibrosis, Niemann–Pick disease type C, and tyrosinemia type I. In East Asians citrin deficiency has to be considered.

Biliary obstruction. Infants with cholestatic liver disease often appear well. Early differentiation between biliary atresia, a choledochal cyst, and a "neonatal hepatitis syndrome" is most important. Biliary atresia accounts for 20–30% of neonatal cholestasis syndromes. Structural cholestasis can also be due to intrahepatic bile

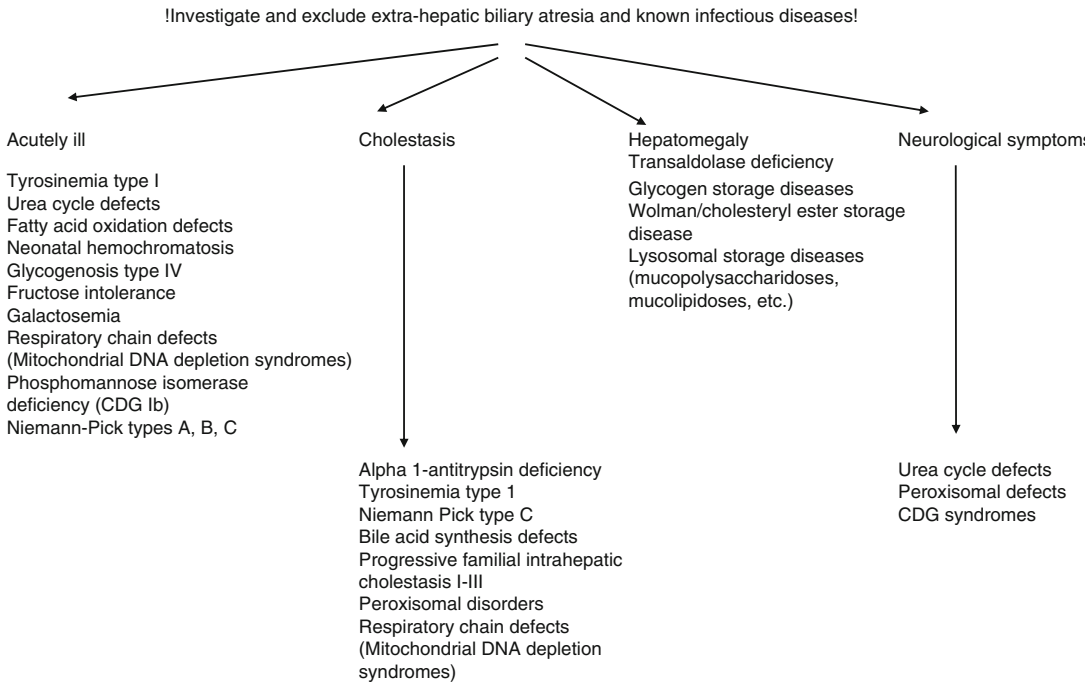

Fig. 24.1 Differential diagnosis of metabolic liver diseases in infants

duct paucity. The syndromal form is *Alagille syndrome*, in which a dominantly inherited dysplasia of the pulmonary artery occurs with distinctive dysmorphic features and paucity of intrahepatic bile ducts resulting in cholestatic liver disease. Intrahepatic bile duct paucity can be an isolated finding. Routine clinical chemical investigations can seldom differentiate between biliary obstruction and neonatal hepatitis syndrome, although low or normal γ-GT suggests a disorder of bile acid synthesis or transport. Impaired coagulation unresponsive to vitamin K points to the development of liver failure and should prompt referral for consideration of liver transplantation.

Remember

The differentiation between biliary obstruction and neonatal hepatitis syndrome must be vigorously pursued by a structured protocol so that biliary atresia, or other surgical causes, can be confirmed within 1 week as late surgery affects survival.

Disease Info: α-1-Antitrypsin Deficiency

α-1-Antitrypsin is one of the most important inhibitors of proteases (e.g., elastase, trypsin, chymotrypsin, thrombin, and bacterial proteases) in plasma. Different protein variants of α-1-antitrypsin are differentiated by isoelectric focusing. The Z variant is characterized by a glutamine to lysine exchange at position 342 in the protein. It alters the charge and tertiary structure of the molecule and is associated with reduced enzyme activity. The frequency of this particular allele is very high in Caucasians; 5 % of the population of Sweden are MZ heterozygotes and 2 % of the United States. The frequency of the homozygous Pi-ZZ phenotype is 1 in 1,600 in Sweden and 1 in 6,000 in the United States. Normal serum levels of α-1-antitrypsin are from 20 to 50 μmol/L and, in the ZZ phenotype, 3 to 6 μmol/L. Patients with values below 20 μmol/L should have PI phenotyping.

Table 24.3 Hepatic diseases that may be cured by liver transplantation

Disease	Timing
Acute Wilson disease	If acute liver failure or if no response to therapy
Neonatal hemochromatosis	Soon if no response to antioxidant cocktail
α-1 Antitrypsin deficiency	In early infancy if cholestasis deteriorates
Urea cycle disorders	Early in the course to prevent further crises
Tyrosinemia type I	Early in the course if no response to therapy or if hepatocellular carcinoma is suspected
PFIC I–III	If pruritus and portal hypertension develops
Primary hyperoxaluria	Liver transplantation before development of renal impairment
	Combined liver/kidney TX when GFR <20
Crigler–Najjar type 1	Before school age, consider liver cell or auxiliary transplantation
Cystic fibrosis	If cholestasis deteriorates or portal hypertension is resistant to therapy
Glycogenosis type I	In childhood if poor metabolic control (non-A) or later if risk of malignancy
Glycogenosis type IV	If decompensated liver disease
Maple syrup urine disease	If poor metabolic control

Cholestatic liver disease occurs in 10–20 % of infants with the Pi-ZZ α-1-antitrypsin phenotype, which in turn accounts for 14–29 % of the neonatal hepatitis syndrome, once infectious and toxic causes have been excluded. Bleeding may occur as a result of deficiency of vitamin K with prompt response to intravenous vitamin K. The stool may be acholic. Low serum α-1-antitrypsin and genetic phenotyping usually lead to the diagnosis. In the acute stages, liver biopsies of infants with α-1-antitrypsin deficiency may show giant cell hepatitis but may mimic biliary atresia.

Some patients with α-1-antitrypsin deficiency, who had no history of neonatal cholestasis, develop cirrhosis eventually and may present with unexplained liver failure. These patients have had the Pi types ZZ, MZ, and M (Malton). In addition, hepatocellular carcinomas have been described in ZZ and MZ phenotypes.

A major proportion of patients with α-1-antitrypsin deficiency remain free of liver disease throughout their life. In adulthood, 80–90 % of patients with the Pi-ZZ phenotype develop destructive pulmonary emphysema. α-1-Antitrypsin is protective of the lung, because it is an effective inhibitor of elastase and other proteolytic enzymes, which are released from neutrophils and macrophages during inflammatory processes. Children with the rare Pi null variant may have severe emphysema early in their life. Intravenous infusions of α-1-antitrypsin have been shown to impede the development of emphysema but are not indicated for liver disease.

In *dysmorphic infants with cholestasis*, very long-chain fatty acids, as well as chromosomes, should be investigated as peroxisomal diseases, and trisomies 13 and 18 are associated with the neonatal hepatitis syndrome and biliary atresia (trisomy 18). Alagille syndrome has been discussed earlier.

A group of inherited metabolic diseases characterized by *progressive familial intrahepatic cholestasis* (PFIC) starting in infancy are increasingly recognized. These include *defects in bile acid biosynthesis*. Unfortunately the necessary laboratory facilities to diagnose these latter treatable disorders are only available in a few laboratories worldwide. Multiple enzyme defects in the modification of the steroid nucleus of bile acids and in conjugation of bile acids have been elucidated. Malabsorption of fat-soluble vitamins is pronounced and results in spontaneous bleeding and rickets. The level of alkaline phosphatase is often highly elevated and γ-GT disproportionally low. Determination of total bile acids is not helpful. More widespread availability of fast atom bombardment mass spectrometry for rapid analysis

of urine samples will hopefully result in a quicker recognition of these disorders in the future. After diagnosis, there is a good clinical and biochemical response to supplementation with bile acids such as cholic or chenodeoxycholic acids. Ursodeoxycholic acid may have some nonspecific benefits in cholestasis but does not address the underlying metabolic defect.

Remember

After exclusion of other diseases, infants with cholestatic liver disease should be examined for the treatable defects of bile acid biosynthesis, which requires determination of individual bile acid metabolites.

24.2.2 Cholestatic Liver Disease in Later Infancy and Childhood

After 3 months of age, the initial clinical and biochemical presentation usually allows a clearer suspicion and differentiation of inherited metabolic liver disease than in neonates. In patients with α-1-antitrypsin deficiency, cholestatic liver disease gradually subsides before 6 months of age. In later infancy, three distinct metabolic diseases present with cholestatic liver disease (Table 24.4).

Disease Info: Progressive Familial Intrahepatic Cholestasis (PFIC) (Byler Disease)

PFIC is a genetically heterogeneous group of autosomal recessive liver disorders, characterized by cholestasis that frequently progresses to cirrhosis and liver failure before adulthood. There are at least four types of PFIC recognized and in approximately 30 % of cases no genetic cause has been identified. The term Byler disease is generally used for PFIC type 1 caused by a deficiency of a P-type ATPase that is required for ATP-dependent amino phospholipid transport and encoded by the *ATP8B1* gene. Other PFIC types are linked to the *ABCB11* gene (PFIC2), the *ABCB4* gene (PFIC3), and the *TJP2* gene. Symptoms may start anytime in infancy with jaundice, pruritus, growth failure, and conjugated hyperbilirubinemia. Liver function slowly deteriorates, and terminal liver failure usually occurs before 15 years. All types except PFIC3 have a low/normal γ-glutamyltransferase (γ-GT). Partial biliary diversion can have a dramatic effect in some types if used early, with liver transplantation as the treatment of choice for advanced liver disease. Transplantation

Table 24.4 Differential diagnosis of metabolic cholestatic liver disease (conjugated hyperbilirubinemia)

Age of presentation	Diseases to be considered	Additional findings
<3 months	α-1-Antitrypsin deficiency	⇓ α-1-Globulin, ⇓ α-1-antitrypsin
	Cystic fibrosis	⇑ Sweat chloride
	Tyrosinemia type I	⇑ AFP
	Niemann–Pick type	Foam cells in marrow
	Peroxisomal diseases	Encephalopathy
	Bile acid synthesis defects	Prominent malabsorption
	Citrin deficiency	Failure to thrive, ⇑ alpha-fetoprotein
>3 months	Progressive familial intrahepatic cholestasis (e.g., Byler disease)	Progressive cirrhosis
	Rotor syndrome	Normal liver function
	Dubin–Johnson syndrome	Normal liver function

Known infectious diseases should have been ruled out by laboratory tests listed in Table 24.1 and extrahepatic biliary disease by imaging techniques

may be complicated by postoperative intractable diarrhea, progressive liver disease in the graft and pancreatitis in PFIC1, and, more rarely, immune-mediated recurrence in PFIC2.

PFIC3 is due to mutations in *ABCB4* which encodes for MDR3 and can be distinguished from the other disorders by high-serum γ-GT activity and liver histology that shows portal inflammation and ductular proliferation at an early stage. MDR3 acts to translocate phosphatidylcholine across the canalicular membrane where it neutralizes the detergent effect of bile acids. In the absence of phosphatidylcholine, canalicular bile acids cause a chemical cholangitis. Heterozygous mutations in *ABCB4* are associated with some types of intrahepatic cholestasis of pregnancy and with gallstone disease.

Disease Info: Dubin–Johnson and Rotor Syndromes

Dubin–Johnson and Rotor syndromes are both autosomal recessively inherited disorders characterized by isolated conjugated hyperbilirubinemia. Patients are usually asymptomatic except for jaundice. Bilirubin levels can range from 2 to 25 mg/dL (34–428 μmol/L). Both the conditions are rare and can be differentiated by urinary porphyrins and appearance of the liver, which is deeply pigmented in Dubin–Johnson syndrome and unremarkable in Rotor syndrome. Excretion of conjugated bilirubin is impaired in both the disorders. Rotor syndrome requires mutations in both of the adjacent genes *SLCO1B1* and *SLCO1B3*, encoding organic anion-transporting polypeptides OATP1B1 and OATP1B3, respectively. Dubin–Johnson syndrome is due to mutations in *ABCC2* which encodes the canalicular multispecific organic anion transporter.

24.3 Liver Failure

24.3.1 Fulminant Liver Failure in Early Infancy

The differential diagnosis in this age group is wide, ranging from toxic or infectious causes to several inherited metabolic diseases (Table 24.5). Mortality is high. The age of presentation (Table 24.6) as well as associated features (Table 24.7) may be helpful in directing the investigations. Jaundice is usually present, but more important and characteristic features are elevated liver transaminases and markers of hepatic insufficiency, such as hypoglycemia, hyperammonemia, hypoalbuminemia, and vitamin K-unresponsive coagulopathy. Failure to thrive is usually present. Deranged liver function may result in spontaneous bleeding or neonatal ascites, indicating end-stage liver disease.

Remember

Encephalopathy associated with severe liver failure may not be obvious in neonates and young infants.

Acute disease may progress rapidly to hepatic failure. In severely compromised infants, routine clinical chemical measurements often do not distinguish acutely presenting inherited metabolic diseases from severe viral hepatitis or septicemia, although disproportionate hypoglycemia, lactic acidosis, and/or hyperammonemia all point to a primary metabolic disease. Furthermore, it is not uncommon for septicemia to complicate and aggravate inherited metabolic diseases. Help in the differential diagnosis may come from the judgment of liver size. Decompensated inherited metabolic diseases are often accompanied by significant hepatomegaly due to edema, whereas rapid atrophy can develop in fulminant viral hepatitis or toxic injury.

In babies with acute hepatocellular necrosis, rapid diagnosis of the inherited metabolic diseases listed in Tables 24.5 and 24.7 is essential as specific therapy is available for most and must be initiated as soon as possible. Metabolites accumulating in galactosemia

(galactose-1-phosphate) and hereditary fructose intolerance (fructose-1-phosphate) have a similar toxicity particularly for the liver, kidneys, and brain but are usually differentiated by different clinical settings (different age groups) in which first symptoms occur. Determination of amino acids in plasma and urine and analysis of organic acids in urine (particularly succinylacetone, dicarboxylic acids, and orotic acid) should elucidate the presence of hepatorenal tyrosinemia, fatty acid oxidation defects, and urea cycle disorders.

Disease Info: Galactosemia

Galactosemia is caused by a deficiency of galactose-1-phosphate uridyltransferase (GALT). Clinical symptoms usually start after the onset of milk feeds on the third or fourth day of life and include vomiting, diarrhea, jaundice, disturbances of liver function, or sepsis and if untreated may progress to death from hepatic and renal failure. Whenever galactosemia is suspected (and in all neonates with liver failure), adequate blood and urine tests should be initiated (galactose and galactose-1-phosphate in serum, erythrocytes, or dried blood spots; enzyme studies in erythrocytes) and a lactose-free diet should be started immediately. Galactose in urine is not detected by standard stix tests based on the glucose oxidase method (Clinistix® and Tes-tape®), and there is a strong argument for the continued use of the older methods of screening urine for reducing substances (Benedict or Fehling test and Clinitest®), which also detect galactose. Urinary excretion of galactose depends on the dietary intake and will not be detectable 24–48 h after discontinuation of milk feedings. On the other hand, babies with severe liver disease from any cause may have impaired galactose metabolism and gross secondary galactosuria. After discontinuing galactose for 2–3 days, a baby with galactosemia begins to recover. Cataracts may have developed in only a few days (Fig. 30.2) and slowly clear in early infancy after removal of the toxic sugar.

Remember

In any baby who has received milk and developed liver disease, the investigation should include determination of the enzymatic activity of galactose-1-phosphate uridyl transferase in erythrocytes, regardless of the results of newborn screening.

Remember

Elevations of succinylacetone in urine may be small in young infants, and repeated analyses with special requests for specific determination by stable isotope dilution may be warranted in patients in whom clinical suspicion is strong.

Genetic defects of fatty acid oxidation and of the respiratory electron transport chain have become recognized as causes of rapidly progressive hepatocellular necrosis in infancy. Defects of fatty acid oxidation are suggested in prolonged intermittent or subacute presentations by myopathy, cardiomyopathy, hypoketotic hypoglycemia, hyperuricemia, elevation of CK, lactic acidosis, and dicarboxylic aciduria (see also Chap. 16). Defects of the respiratory chain causing hepatocellular necrosis are characterized by additional variable multiorgan involvement, especially, of the bone marrow, pancreas, and brain, moderate to severe lactic acidosis, and ketosis (see also Chaps. 14 and 42). During acute hepatocellular necrosis in infancy, these differentiating features may be masked by generalized metabolic derangement. Repeated determinations of metabolites such as lactate, pyruvate, 3-hydroxybutyrate, acetoacetate, free and total carnitine, and acylcarnitines in addition to determinations of amino acids in blood and organic acids in urine should be performed in any baby with progressive hepatocellular

Table 24.5 Differential diagnosis of liver failure (acute or subacute hepatocellular necrosis)

Age of presentation	Diseases to be considered	Additional findings
<3 months	Neonatal hemochromatosis	⇑ ⇑ ⇑ Ferritin, ⇑ ⇑ ⇑ AFP
	Galactosemia	Cataracts, urinary reducing substance
	Tyrosinemia type I	⇑ ⇑ AFP
	Urea cycle defects	⇑ ⇑ ⇑ Ammonia
	Respiratory chain defects	⇑ ⇑ Lactate
	Long-chain fatty acid oxidation defects	⇑ Lactate, ⇑ urate, ⇑ CK, ⇑ ammonia, (cardio)myopathy, myoglobinuria, abnormal acylcarnitines
	Niemann–Pick types A, B, C	Foam cells in marrow
	Phosphomannose isomerase deficiency (MPI-CDG, formally type Ib)	°Pattern of transferrin isoforms
3 months–2 years	Fructose intolerance	⇓ Glucose
	Tyrosinemia type I	⇑ ⇑ AFP
	Fatty acid oxidation defects	⇓ Glucose, ⇑ uric acid, ⇑ CK, ⇓ ketones, ⇑ lactate, ⇑ ammonia, abnormal acylcarnitines
	Respiratory chain defects	⇑ Lactate
	Urea cycle defects	⇑ ⇑ ⇑ Ammonia
	Wolcott–Rallison syndrome	Diabetes
	RALF syndrome	Recurrent episodes precipitated by fever
>2 years	Wilson disease	Corneal ring, hemolysis, renal tubular abnormalities, neurologic degeneration
	α-1-Antitrypsin deficiency	⇓ α-1-Globulin, ⇓ α-1-antitrypsin
	Respiratory chain defects	⇑ ⇑ Lactate
	Fatty acid oxidation defects	⇓ Glucose, ⇑ uric acid, ⇑ CK, ⇓ ketones, ⇑ lactate, ⇑ ammonia
	Urea cycle defects	⇑ ⇑ ⇑ Ammonia
	Glycogenoses type VI/IX	± ⇓ Glucose, ⇑ transaminases, ⇑ lactate, ⇑ CK
	Phosphoglucomutase 1 deficiency or PGM1-CDG	Bifid uvula ± ⇓ Glucose, ⇑ transaminases, ⇑ CK
	RALF syndrome	Recurrent episodes precipitated by fever
	Urea cycle defects	⇑ ⇑ ⇑ Ammonia

Known infectious diseases should have been ruled out by laboratory tests listed in Table 24.1 and extrahepatic biliary disease by imaging techniques

necrosis. If the clinical and biochemical presentation is suggestive of a defect of fatty acid oxidation or of the respiratory chain, appropriate enzymatic confirmation in the muscle and liver, or molecular studies of nuclear or mitochondrial DNA, should be sought (see also Chaps. 14, 16, and 42). If results are negative, those suspected of fatty acid oxidation should then have a defect of the respiratory chain excluded and vice versa. Small infants with primary defects of fatty acid oxidation may present with overwhelming lactic acidosis and infants with severe liver disease due to defects of the respiratory chain with hypoketotic hypoglycemia and dicarboxylic aciduria.

Table 24.6 Age at presentation as a clue to the cause of hepatic failure in infancy

0–7 days	Herpes simplex types 1 and 2
	mtDNA depletion
	Neonatal hemochromatosis
1–4 weeks	Infections including enteroviruses
	Galactosemia
	Tyrosinemia
4–8 weeks	Hepatitis B (vertical transmission)
	Familial hemophagocytic lymphohistiocytosis
2–6 months	Bile acid synthesis defects
0.5–1 year	Hereditary fructose intolerance
	Ralf syndrome
	mtDNA depletion
	Wolcott–Rallison syndrome
	Viral hepatitis
	Autoimmune disease

Table 24.7 Neonatal liver failure

Disorder	Additional clinical features
Mitochondrial hepatopathy, often mtDNA depletion	Muscular hypotonia, multisystem disease, encephalopathy, ↑↑ lactate
Neonatal hemochromatosis	Hepatocellular necrosis, cirrhosis, ↑↑ ferritin, ↑↑ AFP, transaminases may be low
Galactosemia	Onset after milk feeds, jaundice, renal disease, cataracts
Fatty acid oxidation disorders	(Cardio)myopathy, hypoketotic, hypoglycemia, ↑↑ lactate
Urea cycle disorders	↑↑ Ammonia, encephalopathy
Niemann–Pick type C	Jaundice, hypotonia, hepatosplenomegaly
Glycosylation disorders (CDG, especially MPI-CDG, formally type Ib)	Hepatomegaly, hepatocellular dysfunction, protein losing enteropathy, multisystem disease

Rarely: α_1-antitrypsin deficiency, bile acid synthesis disorders

Remember

When cardiomyopathy is present, lactic acidosis may be due to heart failure and poor perfusion.

Disease Info: Neonatal Hemochromatosis

Neonatal hemochromatosis is the commonest cause of rapidly progressive hepatocellular necrosis in infancy. While neonatal hemochromatosis may be a phenotype rather than a single entity, in the majority of cases, it appears to be alloimmune in origin. Maternal antibodies to an uncharacterized fetal antibody have been detected, the recurrence pattern is characteristic of other gestational alloimmune diseases, and early (<18 weeks of gestation) prenatal maternal immunoglobulin treatment modifies the disease. Diagnosis is by exclusion of other causes and demonstration of increased concentrations of serum iron, ferritin (>2,000 μg/L), and α-fetoprotein as well as decreased concentrations of transferrin with complete or near-complete saturation of iron-binding capacity. Demonstration of extrahepatic siderosis is pathognomic. This can be assessed by minor salivary gland biopsy or abdominal MRI. Exchange transfusion in combination with immunoglobulin treatment is the first-line treatment, but liver transplantation is often required and all cases should be discussed with a transplant center (Table 24.3). Iron storage is not permanently disturbed as survivors with and without transplantation do not develop permanent iron storage disease.

24.3.2 Hepatic Failure in Later Infancy and Childhood

Fulminant hepatic failure in later infancy or early childhood may present in a similar fashion to that in neonates with elevated transaminases, hypoglycemia, hyperammonemia, decrease of coagulation factors, spontaneous bleeding, hypoalbuminemia, and ascites. Mortality is high and most will die without liver transplantation (Table 24.3). On liver biopsy hepatocellular necrosis is obvious. Renal tubular dysfunction or rickets is indicative of an

inherited metabolic disease. In combination with early infancy insulin-dependent diabetes mellitus, *Wolcott–Rallison syndrom*e (OMIM #226980) should be looked for by mutation analysis. The association of acute hepatocellular necrosis with a prominent noninflammatory encephalopathy suggests a diagnosis of Reye syndrome; most patients with this syndrome are now found to have inherited metabolic disease.

Most cases of acute hepatocellular necrosis in older children are unexplained and labeled as seronegative hepatitis. A small or rapidly decreasing liver size and deep jaundice is a strong argument against an inherited metabolic disease. Autoimmune disease must also be taken into consideration; autoantibodies are present in the majority of affected children, and there usually is an increase in IgG.

Disorders of fatty acid oxidation and urea cycle defects should be high on the list of differential diagnosis of children presenting with acute hepatocellular necrosis. If disproportionate hyperammonemia or hypoketotic hypoglycemia has been observed, vigorous emergency measures should be promptly initiated and diagnostic confirmation sought by specialized metabolic investigations (Chaps. 16 and 17). Both are potentially lethal in the acute episode.

The recently described recurrent acute liver failure (RALF) syndrome is caused by mutations in NBAS which encodes for a protein involved in transport between endoplasmic reticulum and Golgi apparatus. Haack et al. (2015) Children present with bouts of liver failure consisting of very high transaminases, severe coagulopathy, and encephalopathy starting in infancy. Bouts are self-limiting, and recovery may be hastened by antipyresis, aggressive support, and the use of intralipid infusion. There is complete recovery between episodes, which persist throughout childhood but diminish in adulthood.

Tyrosinemia type I and fructose intolerance are usually diagnosed in infancy or early childhood; urea cycle and fatty acid oxidation defects can cause acute hepatic dysfunction at any age.

Hepatopathy with increased liver transaminases, intermittent hypoglycemia, short stature, and bifid uvula with or without cleft palate are characteristics of phosphoglucomutase 1 deficiency or PGM1-CDG. Patients with this disorder, which is eminently treatable with oral galactose supplementation, often develop exercise intolerance, increased muscle glycogen content, and increased serum creatine kinase.

In later childhood, Wilson disease and α-1-antitrypsin deficiency are important metabolic causes of severe hepatocellular necrosis. Courses may be subacute or chronic, and initial presentation is variable, ranging from isolated hepatomegaly, jaundice, or ascites to a chronic active hepatitis-like picture or acute liver failure. Hemolysis, when present, may be an important clue to Wilson disease.

Disease Info: Tyrosinemia Type I

Tyrosinemia type I (fumarylacetoacetase deficiency) usually presents with pronounced acute or subacute hepatocellular damage and only occasionally with cholestatic liver disease in infancy. Patients who have an acute onset of symptoms very quickly develop hepatic decompensation. They may have jaundice and ascites along with hepatomegaly. There may be gastrointestinal bleeding. Several infants have been noted by their mothers to have a peculiar sweet cabbage-like odor. Generalized renal tubular dysfunction occurs, leading to glucosuria, aminoaciduria, and hyperphosphaturia. Very low levels of phosphate in serum are common findings, as are hypoglycemia and hypokalemia. In some patients the diagnosis of tyrosinemia type I can be difficult, as increases of tyrosine and methionine occur in many forms of liver disease but may be missing in tyrosinemia type I. A highly elevated α-fetoprotein is sensitive but not specific for tyrosinemia. Very high elevations of α-fetoprotein are also seen in neonatal hemochromatosis, which should be differentiated on the basis of gross elevations of iron and ferritin. Diagnostic proof of tyrosinemia type I comes from the demonstration of succinylacetone in urine and subsequently by detection of pathogenic mutations.

If tyrosinemia type I does not become symptomatic until later in infancy, patients usually follow a less rapid course. Vomiting, anorexia, abdominal distension and failure to thrive, rickets, and easy bruising may be the presenting features, and there is usually hepatomegaly. Although transaminases may be normal or only slightly elevated, prothrombin time and partial thromboplastin time are usually markedly elevated, as is α-fetoprotein, which may range from 100,000 to 400,000 ng/mL. Individual patients may present with different clinical pictures, such as with acute liver disease and hypoglycemia as a Reye-like syndrome. They may present with isolated bleeding and undergo initial investigation for coagulation disorders before hepatic disease is identified.

Renal tubular disease in tyrosinemia type I is that of a renal Fanconi syndrome with phosphaturia, glucosuria, and aminoaciduria (Chap. 26). There may be proteinuria and excessive carnitine loss. Renal tubular loss of bicarbonate leads to systemic metabolic acidosis. The affected infants have been observed to develop vitamin D-resistant rickets at less than 4 months of age.

Beyond infancy, neurological crises very similar to those of acute intermittent porphyria are a more common cause of admission to the hospital than hepatic decompensation. Succinylacetone inhibits porphobilinogen synthase (the enzyme affected in acute intermittent porphyria); increased urinary excretion of delta-aminolevulinate and porphobilinogen occurs during neurological crises. About half of the patients experience such crises, starting with pains in the lower extremities, followed by abdominal pains, muscular weakness, or paresis and paresthesias. The head and trunk may be positioned in extreme hyperextension, suggesting opisthotonus or meningismus. Systemic signs include hypertension, tachycardia, and ileus. Symptoms can continue for up to a week and slowly resolve. Intellectual function has been thought to be normal in tyrosinemia type I but recent reports suggest that educational difficulties are relatively common.

In the natural disease course, most children with tyrosinemia type I die in early life, most of them before 1 year of age. Survivors have chronic liver disease with macronodular cirrhosis. Splenomegaly and esophageal varices develop and are complicated by bleeding. A common complication is hepatocellular carcinoma, which may first be suspected because of a secondary rise in the level of α-fetoprotein. Liver or combined liver–kidney transplantation was the only promising option of treatment until the advent of 2(2-nitro-4-trifluoromethylbenzoyl)-1,3-cyclohexanedione (NTBC). Currently, liver transplantation in tyrosinemia type 1 is only necessary in nonresponders to NTBC therapy. The drug is a potent inhibitor of *p*-hydroxyphenylpyruvate dioxygenase, thus preventing the formation of the highly toxic fumarylacetoacetate, and its products succinylacetoacetate and succinylacetone. Treatment is combined with tyrosine and phenylalanine restriction, and compliance is crucial as hepatic malignancy or neurological crises may develop rapidly after even a short interruption of or inconsistent treatment. If the treatment is instituted early, hepatic and renal function slowly improves to normal, and neurological crises are prevented. Concentrations of succinylacetone, α-fetoprotein, and delta-aminolevulinate gradually decrease to near-normal values. If treatment is started preemptively following newborn screening, significant liver disease can be avoided.

The differential diagnosis of the patient with elevated levels of tyrosine in the blood includes tyrosinemia types II and III and transient neonatal tyrosinemia. *Tyrosinemia type II* (tyrosine aminotransferase deficiency), known as the Richner–Hanhart syndrome, results in oculocutaneous lesions, including corneal erosion, opacity, and plaques. Pruritic or hyperkeratotic lesions may develop on the palms and soles. About half of the patients described have had low-normal to subnormal levels of intelligence, but this may reflect bias of ascertainment.

The phenotype of *tyrosinemia type III* (*p*-hydroxyphenylpyruvate dioxygenase deficiency) is less well defined. It may include neurological manifestations such as psychomotor

retardation and ataxia but also remain asymptomatic. As treatment with NTBC in patients with tyrosinemia type I shifts the metabolic block from fumarylacetoacetate hydrolase to *p*-hydroxyphenylpyruvate dioxygenase or from tyrosinemia type I to tyrosinemia type III, treatment with NTBC must be supplemented by dietary treatment with a phenylalanine- and tyrosine-reduced diet, which is the rational approach to treatment in tyrosinemia types II and III.

Metabolic investigations or newborn screening, particularly in premature infants, sometimes detects tyrosinemia and hyperphenylalaninemia not due to a defined inherited metabolic disease. The protein intake is often excessive, especially when an evaporated milk formula is being used. This form of tyrosinemia is thought to result from physiological immaturity of *p*-hydroxyphenylpyruvate dioxygenase and is a warning that protein intake should be moderate during the first weeks of life. Relative maternal vitamin C deficiency may play a role. This condition is sometimes associated with prolonged jaundice and feeding problems and can cause diagnostic confusion.

Disease Info: Hereditary Fructose Intolerance

Symptoms develop in hereditary fructose intolerance when fructose or sucrose is introduced into the diet. The recessively inherited deficiency of fructose-1-phosphate aldolase results in an inability to split fructose-1-phosphate into glyceraldehyde and dihydroxyacetone phosphate. Fructose-1-phosphate accumulates in the liver, kidney, and intestine. Pathophysiological consequences are hepatocellular necrosis and renal tubular dysfunction, similar to what occurs in galactosemia. There is an acute depletion of ATP caused by the sequestration of phosphate and direct toxic effects of fructose-1-phosphate.

Depending on the amount of fructose or sucrose ingested, infants may present with isolated asymptomatic jaundice or with rapidly progressive liver failure, jaundice, bleeding tendency, and ascites, suggesting septicemia or fulminant viral hepatitis; hepatosplenomegaly, if present, argues against the latter diagnosis. Postprandial hypoglycemia develops in 30–50 % of the patients affected with fructose intolerance and may progress to coma and sudden death. Most patients present subacutely with vomiting, poor feeding, diarrhea, or sometimes failure to thrive. Pyloric stenosis and gastroesophageal reflux are common initial diagnoses. The clinical picture may be more blurred in later life, and laboratory findings may be unrevealing. Hereditary fructose intolerance deserves consideration as a cause of renal calculi, polyuria, and periodic or progressive weakness or even paralysis. A major clue to diagnosis may be an accurate dietary history that will reveal an aversion to fruits and sweets.

Characteristic clinical chemical laboratory features include elevated transaminases, hyperbilirubinemia, hypoalbuminemia, hypocholesterolemia, and a decrease of vitamin K-dependent, liver-produced coagulation factors. There is an occasional pattern of consumptive coagulopathy. In addition, patients may have hypoglycemia, hypophosphatemia, hypomagnesemia, hyperuricemia, and metabolic acidosis in a renal Fanconi syndrome with proteinuria, glucosuria, aminoaciduria and loss of bicarbonate, and high urine pH despite acidosis. In these circumstances detecting fructose in the urine is virtually diagnostic of the disease, but it may be absent. Elevated plasma levels of tyrosine and methionine in combination with markedly elevated excretion of tyrosine and its metabolites in urine may misleadingly suggest a diagnosis of tyrosinemia type I.

The prognosis in hereditary fructose intolerance depends entirely upon the elimination of fructose from the diet. After withdrawal of fructose and sucrose, clinical symptoms and laboratory findings quickly reverse. Vomiting stops immediately and the bleeding tendency within

24 h. Most clinical and laboratory findings become normal within 2–3 weeks, but hepatomegaly takes longer to resolve.

The excellent response to treatment supports a presumptive diagnosis of fructose intolerance. Confirmation of diagnosis should first be attempted by molecular analysis. Several frequent mutations are known, such as A149P in Caucasians. An intravenous fructose tolerance test can usually not be performed any longer in diagnostically difficult cases as there are no i.v. preparations of fructose available. Demonstration of the enzyme defect in biopsied liver or intestine may be necessary in exceptional circumstances.

Mitochondrial DNA depletion syndromes are increasingly identified as causes of rapidly progressive liver disease. Forms known so far are caused by defects in the deoxyguanosine kinase (DGUOK), polymerase-γ (POLG), MPV17, recessive Twinkle helicase (PEO1) genes, as well as in the EIF2AK3 gene.

Disease Info: Wilson Disease

Wilson disease, or hepatolenticular degeneration, is characterized by the accumulation of copper in various organs and low serum levels of ceruloplasmin. Clinical manifestations are highly variable, but hepatic disease occurs in ≈80 % of affected individuals. This is usually manifested at school age, rarely before 4 years of age. About half of the patients with hepatic disease, if untreated, will develop neurological symptoms in adolescence or adulthood. In older patients with Wilson disease, neurological manifestations may be the presenting symptoms.

Liver transplantation is also usually contraindicated in liver failure due to mutations in *Twinkle* and *DGUOK* with neurological involvement. In contrast, older children with mutations in *MPV17* may have good quality long-term survival following liver transplantation. Disease due to mutations in *TRMU* is particularly important to identify as spontaneous recovery may occur.

Disease Info: Alpers Disease

In patients with Alpers disease, liver failure mostly occurs later in the course of disease, often triggered by the use of valproic acid. Mutations in the *POLG* gene result in defects in respiratory chain complexes I and IV. Despite fulminant liver failure, aminotransferases typically are only mildly elevated. Most patients with Alpers disease present at preschool or school age with neurological symptoms like seizures or even epileptic status and epilepsia partialis continua. Patients' history usually reveals mild and then progressive psychomotor development. However, liver failure may develop before significant neurological disease. If in an unclear situation a therapeutic liver transplantation is being considered, mutation analysis of the *POLG* gene should be pursued as soon as possible. Alpers syndrome due to mutations in the *POLG* gene is a contraindication for transplantation as the neurological disease progresses post transplantation.

One extreme of the clinical spectrum of Wilson disease is a rapid fulminant course in children over 4 years of age progressing within weeks to hepatic insufficiency manifested by jaundice, ascites, clotting abnormalities, and disseminated intravascular coagulation. This is followed by renal insufficiency, coma, and death, sometimes without diagnosis.

Wilson disease can also present with a picture of acute hepatitis. Nausea, vomiting, anorexia, and jaundice are common presenting complaints, and the episode may subside spontaneously. In the presence of splenomegaly, the diagnosis may mimic infectious mononucleosis. Recurrent bouts of hepatitis and a picture of chronic active hepatitis in children above the age of 4 years are suggestive of Wilson disease.

These patients experience anorexia and fatigue. Hepatosplenomegaly is prominent. In some patients isolated hepatosplenomegaly may be discovered accidentally. Any hepatic presentation of Wilson disease implies the development of cirrhosis; the disease may present as cirrhosis. The histological picture is indistinguishable from chronic active hepatitis. Terminally there may be hepatic coma or a hepatorenal syndrome.

Hemolytic anemia may be a prominent feature of Wilson disease in children, and its presence is very suggestive of the diagnosis. Renal tubular disease is subtle; a generalized aminoaciduria often displays an unusually high excretion of cystine, other sulfur-containing amino acids, and tyrosine. Later there may be a full-blown Fanconi syndrome, and patients may develop renal stones or diffuse nephrocalcinosis.

In adults, the onset of Wilson disease is classically neurological, predominantly with extrapyramidal signs. Choreoathetoid movements and dystonia reflect lenticular degeneration and are frequently associated with hepatic disease. This picture has been associated with poor prognosis. Progression tends to be much slower in patients presenting with parkinsonian and pseudosclerotic symptoms, such as drooling, rigidity of the face, and tremor. Speech or behavior disorders, and sometimes frank psychiatric presentations, can occur in children. The lack of overt liver disease can make diagnosis difficult. Dementia develops ultimately in untreated patients.

Wilson disease is treatable by copper-chelating agents making early diagnosis crucial. A key finding is the demonstration of Kayser–Fleischer rings around the outer margin of the cornea as gray–green to red–gold pigmented rings (Fig. 24.2). Slit lamp examination may be required. The rings are difficult to be seen in green–brown eyes. They are pathognomonic in neurological disease, but they take time to develop and are absent in most children who present with hepatic disease.

Biochemical diagnosis of Wilson disease may sometimes be difficult. The diagnosis is usually made on the basis of an abnormally low serum ceruloplasmin, liver copper content, and urine copper content plus mutation analysis. In 95 % of patients, ceruloplasmin is below 20 mg/dL (200 mg/L) (control children 25–45 mg/dL). However, intermediate and even normal values have been reported. Levels of copper in the serum are usually elevated, but measurement of urine copper is more reliable. Urinary excretion of copper is increased to >100 µg/day (1.6 µmol/day in about 65 % of patients) (control children <30 µg/day (0.5 µmol/day)). Further increase in urinary copper excretion can be provoked by loading with 500 mg of D-penicillamine 12 h apart while collecting urine for 24 h. Controls excrete less than 600 µg (9.4 µmol)/day, whereas in Wilson disease, excretion ranges from 1,600 µg (25 µmol) to 3,000 µg (47 µmol)/day. The most sensitive test is the measurement of the concentration of copper in the liver, and this test may be required for diagnosis. Disposable steel needles or Menghini needles should be used. Patients with Wilson disease usually have highly elevated concentrations of copper in the liver (>250 µg (4 mmol) of copper per gram of dry weight, heterozygotes 100–200 mg/g, controls <50 µg (0.8 µmol)). Elevated concentrations of copper in the liver may also be found in children with extrahepatic biliary obstruction or cholestatic liver disease.

Liver histology is not specific. Rhodamine or other staining techniques for copper are not sensitive in childhood Wilson and therefore do not

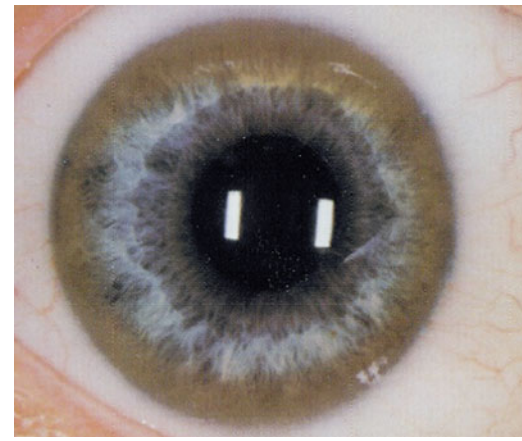

Fig. 24.2 Kayser–Fleischer ring in Wilson disease (Courtesy of Prof. Dr. Wolfgang Stremmel, Heidelberg, Germany)

contribute to the diagnosis. As 80 % of patients with Wilson disease show at least one of the 200 known mutations in the ATP7B gene, molecular diagnosis of Wilson disease is a helpful tool in confirming the diagnoses.

Once the diagnosis of Wilson disease is established, family members (particularly sibs) should be thoroughly examined. This is much easier when known diseases causing mutations have been recognized. Where the mutation status is unknown, liver biopsy may be necessary in the case where an asymptomatic sibling has a low ceruloplasmin level, elevated liver enzymes, and high copper urine, which can be consistent with heterozygosity as well as homozygosity. Stable isotopic ^{65}Cu studies may help to avoid liver biopsy in selected cases. Following oral administration ^{65}Cu enrichment peaks at 1–2 h. ^{65}Cu enrichment then falls to a minimum at about 6 h as ^{65}Cu is taken up by the liver. Thereafter there is a secondary rise over 72 h as ^{65}Cu is excreted from the liver in ceruloplasmin. In Wilson disease this secondary rise does not occur. Early diagnosis must be vigorously pursued, as the best prognosis has been demonstrated following early treatment of asymptomatic patients.

Remember

The association of liver disease with intravascular hemolysis and renal failure is highly suggestive of Wilson disease as are bouts of recurrent hepatitis and a picture of chronic active hepatitis in children above the age of 4 years.

24.4 Cirrhosis

Cirrhosis is the end stage of hepatocellular disease. The list of diseases that can result in cirrhosis and failure of the liver is extensive and includes infectious, inflammatory, and vascular diseases, biliary malformations, as well as toxic and finally metabolic disorders. Cirrhosis can result from most of the diseases discussed in different places of this chapter. Exceptions are primary defects of bilirubin conjugation and some of the storage disorders that lead to isolated hepatomegaly.

Glycogenosis type IV, branching enzyme deficiency, can cause primarily cirrhotic destruction of the liver. In Wilson disease and even more frequently in α-1-antitrypsin deficiency, a cirrhotic process may be quiescent for a long period with no evidence of signs or symptoms of liver disease. Patients may be recognized following family investigations, during investigations of an unrelated disease, or the disease may remain unrecognized until decompensation leads to full-blown liver failure. Specific metabolic diseases leading to cirrhosis are listed in Tables 24.8 and 24.9.

Table 24.8 Differential diagnosis of cirrhosis

Age of presentation	Diseases to be considered	Additional findings
<1 year	Glycogenosis type IV	Myopathy
	Galactosemia	Cataracts, urinary reducing substance
	Neonatal hemochromatosis	⇑⇑⇑ iron, ⇑⇑ ferritin, ⇑⇑ AFP, ⇓ transferrin
	Tyrosinemia type I	⇑⇑ AFP
	Transaldolase deficiency	Dysmorphic features, pancytopenia, cardiac defects
>1 year	α-1-Antitrypsin deficiency	⇓ α-1-Globulin, ⇓ α-1-antitrypsin
	Wilson disease	Corneal ring, hemolysis, renal tubular abnormalities, neurologic degeneration
	Tyrosinemia type I	⇑ AFP

Known infectious and autoimmune diseases should have been ruled out by laboratory tests listed in Table 24.1 and extrahepatic biliary disease by imaging techniques

Table 24.9 Chronic hepatitis or cirrhosis in older children

Disorder	Clinical features
Wilson disease	Neurological and renal disease, corneal ring
Hemochromatosis	Hepatomegaly, cardiomyopathy, diabetes mellitus, diabetes insipidus, hypogonadism
α_1-Antitrypsin deficiency	Failure to thrive, ↓ α_1-antitrypsin
Tyrosinemia type I	Coagulopathy, renal disease, failure to thrive, ↑ AFP
Hereditary fructose intolerance	Symptoms after fructose intake: hypoglycemia, renal disease, failure to thrive, ↑ urate
Transaldolase deficiency	Hepatosplenomegaly, dysmorphic features, pancytopenia
Cystic fibrosis	Failure to thrive, recurrent airway infections
Celiac disease	Failure to thrive, diarrhea, small stature

Table 24.10 Signs and symptoms of liver cirrhosis

General	Malnutrition, failure to thrive, muscle wasting, hypogonadism, elevated temperature, frequent infections
CNS	Lethargy progressing to coma, behavioral changes, depression, intellectual deterioration, pyramidal tract signs, asterixis
Gastrointestinal tract	Nausea and vomiting, splenomegaly, caput medusa, hemorrhoids, epistaxis hematemesis, abdominal distension, steatorrhea, ascites
Kidney	Fluid and electrolyte imbalance, progressive renal insufficiency
Skin	Jaundice, flushing, pruritus, palmar erythema, spider angiomata, digital clubbing

Remember

α-1-Antitrypsin should be quantified in any child, adolescent, or adult in the differential work-up of cirrhosis.

As cirrhosis progresses, signs and symptoms of decompensation eventually emerge. Regardless of the primary disease, patients develop weight loss, failure to thrive, muscle weakness, fatigue, pruritus, steatorrhea, ascites, or anasarca, as well as chronic jaundice, digital clubbing, spider angiomatoma and epistaxis, or other bleeding (Table 24.10). Complications of cirrhosis include portal hypertension, bleeding varices, splenomegaly, and encephalopathy. Terminally there may be hepatic coma. Liver transplantation provides the only realistic therapy. In some cases bridging to transplantation with albumin dialysis may be indicated.

Glycogenosis type IV is due to a deficiency of the branching enzyme α-1,4-glucan: α-1,4-glucan-6-glucosyl transferase that leads to a decrease in the number of branch points making for a straight chain of insoluble glycogen like starch or amylopectin. The content of glycogen in liver is not elevated, but the abnormal structure appears to act like a foreign body causing cirrhosis. The diagnosis can be established by enzyme assay of leucocytes, cultured fibroblasts, or the liver.

Disease Info: Glycogenosis Type IV

Infants with this rare form of glycogen storage disease often present around the first birthday with findings of hepatic cirrhosis, an enlarged nodular liver, and splenomegaly. Hypotonia and muscular atrophy are usually present and may be severe. Treatment is symptomatic and palliative. Transplantation of the liver is curative in predominant liver disease; untransplanted patients usually succumb to complications of cirrhosis before the age of 3 years. Cardiomyopathy and myopathy are often present.

α-1-Antitrypsin deficiency (see textbox Chap. 24 page 4) is an important cause of neonatal

cholestasis as well as of chronic active hepatitis (v. r.). In patients with α-1-antitrypsin deficiency manifesting cholestatic liver disease in infancy, cholestasis gradually subsides before 6 months of age and patients become clinically unremarkable. However, 20–40 % of these children go on to develop hepatic cirrhosis in childhood.

Remember

About 50 % of apparently healthy children with the homozygous Pi-ZZ phenotype have subclinical liver disease, as indicated by elevated levels of aminotransferases and γ-glutamyl transpeptidase.

In the presence or absence of a history of neonatal cholestasis or hepatitis, α-1-antitrypsin should be quantified in any child, adolescent, or adult with unexplained liver disease.

24.5 Hepatomegaly

Hepatomegaly is often the first clinical sign of liver disease. Two clinical aspects are helpful to the diagnostic evaluation of the patient: first, the presence or absence of splenomegaly and, second, the consistency and structure of the enlarged liver.

Remember

Splenomegaly, especially hepatosplenomegaly, is the hallmark of storage diseases.

Functional impairment of the liver, such as decreases of coagulation factors and serum albumin or impaired glucose homeostasis, is usually absent in lysosomal storage diseases, and aspects of liver cell integrity are unremarkable. Exceptions are Niemann–Pick diseases, both the types A/B and C (Table 24.11). In lysosomal storage diseases, the liver and spleen are firm but not hard on palpation. The surfaces are smooth and the edges easily palpated. The liver is not tender. There may be a protuberant abdomen and umbilical hernias. Hepatosplenomegaly may lead to late hematological complications of hypersplenism. A presumptive diagnosis of lysosomal storage disease is strengthened by involvement of the nervous system and/or mesenchymal structures resulting in coarsening of facial appearance and skeletal abnormalities. Macroglossia makes a storage disorder virtually certain. In addition, slow gradual progression is evident.

The diagnostic work-up for lysosomal storage disorders in a patient with hepatosplenomegaly may start with the investigation of mucopolysaccharides and oligosaccharides in urine. Positive results are followed up with confirmatory enzymatic studies. If mucopolysaccharides and oligosaccharides are negative, lymphocytes are investigated for vacuoles (D5 – Chap. 43, Pathology). If negative, bone marrow is investigated for storage cells. If storage cells are found, corneal clouding is sought with a slit lamp. If both are present, *N*-acetylglucosaminylphosphotransferase is determined to make a diagnosis of mucolipidoses II or III. If corneal clouding is not present, potential enzymes to be determined are sphingomyelinase (Niemann–Pick type I, A and B), acid lipase (Wolman), and cholesterol uptake and storage (Niemann–Pick type II or C). The demonstration of an elevated activity of chitotriosidase reinforces the suspicion of a lysosomal storage disorder in ambiguous cases. It is clearly elevated in Gaucher disease, Niemann–Pick types A and B and often in Niemann–Pick type C, and other lysosomal storage disorders. However, about 5 % of the population have very low and uninterpretable levels of chitotriosidase, and false-positive values may result from chronic inflammatory disease. If there are neither pathological urinary screening results nor storage cells but peripheral neuropathy, the activity of ceramidase is determined seeking a diagnosis of Farber disease. Histological, histochemical, electron microscopical, and chemical examinations may be required of biopsied liver (D5 – Chap. 43, Pathology).

Disease Info

Transaldolase deficiency is a disorder of the pentose phosphate pathway presenting in infancy with hepatosplenomegaly, pancytopenia, and bleeding tendency which can progress to liver failure. Growth

Table 24.11 Differential diagnosis of hepatomegaly

Age of presentation	Diseases to be considered	Additional findings
<3 months	Lysosomal storage diseases, specifically	Splenomegaly
	Wolman disease	Adrenal calcifications
	CDG syndromes (PMM2-CDG, MPI-CDG, ALG8-CDG)	Lipodystrophy, inverted nipples
	Defects of gluconeogenesis	⇓ Glucose, ⇑ lactate
	Transaldolase deficiency	Splenomegaly, dysmorphic features, wrinkly skin, pancytopenia, and abnormal urinary polyols
	Mevalonic aciduria	Severe failure to thrive, splenomegaly, anemia
3 months–2 years	Glycogen storage diseases	± ⇓ Glucose, ⇑ lactate, ⇑ lipids, myopathy
	Defects of gluconeogenesis	⇓ Glucose, ⇑ lactate
	Lysosomal storage diseases	Splenomegaly
	α-1-Antitrypsin deficiency	α-1-Globulin, ⇓ α-1-antitrypsin
>2 years	Hemochromatosis	Diabetes mellitus, hypogonadism
	Cystic fibrosis	Pulmonary involvement, malnutrition, ⇑ sweat chloride
	Lysosomal storage diseases, specifically Niemann–Pick, type B	Splenomegaly
	Niemann–Pick, type B	Pulmonary infiltrates
	Cholesterol ester storage disease	Hypercholesterolemia
	Glycogenosis type VI/IX	± ⇓ Glucose, ± ⇑ lactate, ± ⇑ CK
	Fanconi–Bickel syndrome	Fanconi syndrome, ± ⇓ glucose

retardation, dysmorphic features, cutis laxa, and congenital heart disease are also common. While liver involvement is prominent, other phenotypic manifestations are variable and the psychomotor development usually normal. Urine analysis for polyols identifies elevated excretions of erythritol, ribitol, arabitol, sedoheptitol, perseitol, sedoheptulose, mannoheptulose, and sedoheptulose-7-phosphate consistent with transaldolase deficiency. Diagnosis is confirmed by detection of mutations in *TALDO1*.

Any acutely developing liver disease due to infectious, inflammatory, toxic, or metabolic origin may cause hepatomegaly as a result of edema and/or inflammation. In these disorders, other manifestations of the disease have usually led to consultation, and hepatomegaly is discovered during physical examination. On palpation, the liver may feel firm but not hard and the surface is smooth. The liver may be tender. Clinical or routine clinical chemical studies (Table 24.1) are likely to reveal abnormalities which direct further diagnostic evaluation. Inherited metabolic diseases considered in this category have been discussed under acute or subacute hepatocellular necrosis (Table 24.5).

If the enlarged liver feels hard, is not tender, and has sharp or even irregular edges, a detailed evaluation of causes of cirrhosis should be performed even in the presence of unremarkable liver function tests. A hard irregular or nodular surface is virtually pathognomonic of cirrhosis. Metabolic causes of silent liver disease associated with hepatomegaly, which may lead to quiescent cirrhosis, are Wilson disease and α-1-antitrypsin deficiency. Another important metabolic disorder is hemochromatosis, in which

hepatomegaly may be the only manifestation in adolescence and young adulthood. Although this disease usually does not progress to hepatic failure, as does Wilson disease, early recognition and initiation of treatment allow the prevention of irreversible sequelae.

In patients with persistent isolated hepatomegaly, additional findings are helpful in the differential diagnosis and should be specifically sought (Table 24.12). Defects of gluconeogenesis result in severe recurrent fasting hypoglycemia and lactic acidosis (Table 24.13). Of the three enzymatic *defects of gluconeogenesis*, hepatomegaly is a consistent finding in fructose-1,6-diphosphatase deficiency, while patients with deficiency of pyruvate carboxylase or phosphoenolpyruvate carboxykinase tend to present with lactic acidemia and multisystem disease without hepatomegaly.

Table 24.12 Differential diagnosis of metabolic causes of hepatomegaly

Additional findings	Suggestive disorder
Cardiomyopathy	Glycogenosis III, hemochromatosis, phosphoenolpyruvate carboxykinase deficiency, disorders of fatty acid oxidation and of oxidative phosphorylation
Muscular weakness	Glycogenoses III, IV, VI, and IX, fructose intolerance, disorders of fatty acid oxidation and of oxidative phosphorylation
Enlarged kidneys	Glycogenosis I, Fanconi–Bickel syndrome
Renal Fanconi syndrome	Glycogenoses I and III, Wilson disease, fructose intolerance, tyrosinemia type I, Fanconi–Bickel syndrome, mitochondrial disorders
Hemolytic anemia	Wilson disease, fructose intolerance
Fasting intolerance, hypoglycemia	Glycogenoses I and III, fructose-1,6-diphosphatase deficiency, disorders of fatty acid oxidation and of oxidative phosphorylation
Diabetes mellitus, hypogonadism	Hemochromatosis
Neurologic deterioration (Kayser–Fleischer ring)	Wilson disease
Early onset emphysema	α-1-Antitrypsin deficiency
Susceptibility to infections	Glycogenoses I non-A
Malnutrition	Cystic fibrosis
Lipodystrophy, inverted nipples	CDG syndrome type I
Isolated	Glycogenosis type IX

Confronted with an infant or young child with a moderately enlarged smooth, soft liver and otherwise completely unremarkable history and physical examination, investigations may be postponed until confirmation of persistence of hepatomegaly on repeat clinical examinations a few weeks later. If unexplained hepatomegaly persists, first-line investigations should be undertaken as in Table 24.1. Subsequent second-line investigations should measure red blood cell glycogen, glycogen phosphorylase, phosphorylase kinase, and debranching enzyme as well as acid lipase, sphingomyelinase, and β-glucosidase. In hepatic glycogen storage disorders (types I, III, VI, and IX and Fanconi–Bickel syndrome), biotinidase activity is usually significantly elevated. Suspected glycogen storage disease can be confirmed with high accuracy by mutation analysis using next-generation screening. As a result, liver biopsy can be reserved for when no specific disorder can be confirmed or where there is evidence of structural liver disease. If liver biopsy is undertaken, histological and electron microscopic

Table 24.13 Hepatomegaly plus hypoglycemia

Disorder	Clinical features
Glycogen storage disease I	Hepatocellular dysfunction, large kidneys, ↑↑↑ triglycerides, ↑ urate, ↑ lactate
Glycogen storage disease III, IX	Short stature, skeletal myopathy
Fanconi–Bickel disease	Tubulopathy, glucose/galactose intolerance
Disorders of gluconeogenesis	↑ Lactate
Glycosylation disorders (CDG, e.g., PMM2-CDG)	Hepatomegaly, hepatocellular dysfunction, protein losing enteropathy, multisystem disease

analysis should be performed with additional tissue frozen at −80 °C for biochemical analyses. Patients with hepatomegalic glycogenoses may present with a moderately enlarged smooth, soft liver and otherwise completely unremarkable history and physical examination in infancy and early childhood.

Disease Info: Juvenile Hemochromatosis

Juvenile hemochromatosis is a rare multisystemic disease which usually presents in the second decade with nonspecific symptoms including abdominal pain, fatigue, and delayed puberty. Hepatomegaly is the most frequent early manifestation. Additional manifestations include diabetes mellitus, hypogonadism, skin pigmentation, cardiac arrhythmias, and congestive heart failure.

Laboratory investigations of symptomatic patients reveal increased serum iron and ferritin and markedly elevated saturation of transferrin of 77–100 %.

The disease is caused by mutations in *HJV* (90 %) and *HAMP* (10 %). Increasingly hepatic siderosis can be quantified and monitored by quantitative MRI. Liver biopsy is still necessary when chronic liver disease is suspected.

Management consists of regular phlebotomy which can be titrated by hemoglobin, ferritin, and liver iron content. Hormone replacement and symptomatic treatment of heart failure may be required.

Children with the much more common hereditary hemochromatosis due to mutations in *HFE* may be detected by family screening. There is rarely any clinical or significant laboratory abnormality during childhood although lifelong monitoring is required.

24.5.1 The Glycogenoses

Isolated hepatomegaly is found in several glycogen storage diseases. The combined frequency varies considerably according to the ethnic background and approximates 1:50,000–1:100,000 in Europe. The original description was by von Gierke in 1929, and hepatic glycogenoses were the first inborn errors of metabolism defined enzymatically by Cori and Cori in 1952. The current classification of the glycogenoses has been extended to fifteen entities. Glycogenosis type 0, also referred to as glycogenosis, is the deficiency of glycogen synthetase. As the glycogen content in the liver is actually reduced, it is not a storage disorder but a disorder of gluconeogenesis (see also Chap. 15). The symptoms of glycogenoses types II, V, VII, X, XI, XII, XIII, and XV and sometimes IV and XIV are primarily those of muscle disease.

The results of advances in enzymatic and molecular diagnosis mean liver biopsy is rarely necessary.

Disease Info: Glycogen Storage Disease Type I

Glycogen storage disease type I results in a deficiency of any of the proteins of the microsomal membrane-bound glucose-6-phosphatase complex. In classic Ia glycogen storage disease (von Gierke disease), glucose-6-phosphatase is deficient. Type I non-A is due to defective microsomal transport of glucose-6-phosphate. A variant type Ia results from a deficiency of the regulatory protein; this has so far only been reported in a single patient.

The hallmark of von Gierke disease is severe fasting hypoglycemia with concomitant lactic acidosis, elevation of free fatty acids, hyperlipidemia, elevated transaminases, hyperuricemia, and metabolic acidosis. Lactic acidosis may be further aggravated by ingestion of fructose and galactose, as the converted glucose is again trapped by the metabolic block in the liver. Affected patients may be symptomatic in the neonatal period, when there may be hypoglycemic convulsions and ketonuria but sometimes

no hepatomegaly yet. The condition often remains undiagnosed until hypoglycemic symptoms reappear in the course of intercurrent illnesses or, at about 3–6 months, when the infant begins to sleep longer at night. Infants are then chubby in appearance, but linear growth usually lags. The liver progresses slowly in size. An immense liver down to the iliac crest is generally found by the end of the first year when the serum triglycerides reach very high levels. Because of the accumulation of lipid, the liver is usually soft and the edges may be difficult to palpate. With increasing activity of the child at around the first birthday, the frequency of hypoglycemic symptoms tends to increase. As in any of the diseases that cause severe hypoglycemia, convulsions and permanent brain injury or even death may occur. However, many children are quite adapted to low glucose levels, and in the untreated state, the brain may be fuelled by ketone bodies and lactate. Unusual patients may remain clinically asymptomatic of hypoglycemia until up to 2 years of age. Increased bleeding tendency may result in severe epistaxis and multiple hematomas, and abnormal hemostasis and persistent oozing may complicate traumatic injuries or surgery.

Patients with glycogen storage disease type I non-A develop progressive neutropenia and impaired neutrophil function during the first year of life. Recurrent bacterial infections result including deep skin infections and abscesses, ulcerations of oral and intestinal mucosa, and diarrhea. In the second or third decade, inflammatory bowel disease may develop.

In the clinical setting, diagnosis is usually confirmed by detection of mutations with liver biopsy reserved for where uncertainty remains.

Several late complications have been observed in patients with type I glycogen storage diseases despite treatment. Most patients develop osteoporosis and some have spontaneous fractures. Hyperuricemia may result in symptomatic gout after adolescence. Xanthomas may develop. Pancreatitis is another consequence of hypertriglyceridemia. Multiple hepatic adenomas develop, sometimes to sizable tumors. They are usually benign; however, malignant transformation has occurred. Renal complications include Fanconi syndrome, hypercalciuria, nephrocalcinosis, and calculi. Microalbuminuria may be followed over time by proteinuria, focal segmental glomerulosclerosis, interstitial fibrosis, and renal failure. Pulmonary hypertension is a rare, although very serious, complication in adult patients.

Disease Info: Glycogen Storage Disease Type III

Glycogen storage disease type III results in a deficiency of the debranching enzyme, amylo-1,6-glucosidase. The physical and metabolic manifestations of liver disease are usually less severe than in type I glycogenosis, and fasting intolerance gradually diminishes over the years. The predominant long-term morbidity of this disease is myopathy. In infancy it may be impossible to distinguish types I and III on clinical grounds. Hypoglycemia and convulsions with fasting, cushingoid appearance, short stature, and nosebleeds characterize either disease. However, in contrast to type I glycogenosis, concentrations of uric acid and lactate are usually normal. Transaminases are elevated. Creatine phosphokinase level is elevated as well. This may be the earliest evidence of myopathy.

In glycogen storage disease type III, glycogen accumulates in muscle as well as in the liver. In approximately 85 % of patients, both the liver and muscle are affected, and this is referred to as glycogenosis type IIIa. When the deficiency is only found in the liver, it is referred to as IIIb.

With time in many patients, the major problem is a slowly progressive distal myopathy. It is characterized by hypotonia, weakness, and muscle atrophy. It is often notable in the interossei and over the thumb. Some patients have

muscle fasciculations, suggestive of motor neuron disease, and storage has been documented in peripheral nerves. Weakness tends to be slowly progressive. Ultimately the patient may be wheelchair bound. Rarely, the myocardium may be involved as well with left ventricular hypertrophy or even clinical cardiomyopathy.

Several functional tests have been designed to differentiate glycogen storage disease type I from type III, although in the era of rapid genetic diagnosis these are rarely necessary. Following a glucose load, the initially elevated blood lactate will decrease in glycogenosis type I. In type III, lactate levels are usually normal but rise postprandially. In type I gluconeogenesis is blocked and alanine concentration is increased. In type III, gluconeogenesis is overactive, resulting in significantly lowered concentrations of alanine. One of the most useful tests is a glucagon challenge 2–3 h after a meal, which will yield a good response in GSD type III (but no increase in glucose in GSD type I). After a 14-h fast, glucagon will not usually provoke a rise in blood glucose in GSD type III, as all the terminal glycogen branches have been catabolized (see Chap. 41) – Function Tests, Monitored Prolonged Fast, and Glucagon Stimulation). Finally, the diagnosis of glycogenosis type III is proven by demonstrating the deficiency of the debranching enzyme amylo-1,6-glucosidase in leukocytes, fibroblasts, the liver, or muscle. Prenatal diagnosis is possible through enzyme analysis in amniocytes or chorionic villi.

Defects of the phosphorylase system define three separate groups of glycogen storage diseases. Type VI describes primary defects of hepatic phosphorylase, type VIII impaired control of phosphorylase activation, and type IX deficient activity of the phosphorylase kinase complex. The phosphorylase kinase complex consists of four different tissue-specific subunits. By far the most common of these defects is an X-linked recessive defect of the α-subunit of phosphorylase kinase, which affects ≈75 % of all patients with defects of the phosphorylase system (type IXa).

Disease Info: Glycogen Storage Diseases, Types VI, VIII, and IX

The clinical symptoms of defects of the phosphorylase system, types VI, VIII, and IX glycogenoses, are similar to, but milder than in, type III or I. Hepatomegaly is a prominent finding and may be the only indication of a glycogen storage disease. Muscle hypotonia, tendency to fasting hypoglycemia, lactic acidosis, elevation of transaminases, and hypercholesterolemia are mild and may be normalize after childhood.

Following a glucose or a galactose load in glycogenoses types Vl, VIII, and IX, blood lactate will show pathological increase from normal or only moderately elevated levels. Overactive gluconeogenesis results in lowered concentrations of plasma alanine. The response to the administration of glucagon even after a 12–14-h fast is usually normal. Diagnosis of glycogenoses types Vl, VIII, and IX can be proven by demonstration of the enzyme deficiency in the affected tissue, liver, or muscle. Primary molecular diagnosis has greatly facilitated the diagnostic process.

The rare hepatic glycogenosis with renal Fanconi syndrome (Fanconi–Bickel syndrome) was shown to be due to a primary defect of the liver-type facilitated glucose transport. Hepatomegaly with glycogen storage, intolerance to galactose, failure to thrive, and consequences of full-blown Fanconi syndrome are usually obvious in early childhood.

Remember

Type I glycogenosis is the most serious of all hepatic glycogenoses because it leads to a complete blockage of glucose release from the liver, impairing both glucose production from glycogen and gluconeogenesis.

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Gastrointestinal and General Abdominal Symptoms

25

Stephen G. Kahler

Key Facts

- Vomiting is typical of the organic acidurias, the hyperammonemic syndromes, and fatty acid oxidation defects.
- Pain and constipation occur in the porphyrias and familial fever syndromes.
- Pancreatitis occurs with many organic acidurias, lipid and fatty acid disorders, and oxidative phosphorylation defects.
- Diarrhea and failure to thrive, often together with malabsorption, are very common and can be specifically due to disorders of digestive enzymes, especially disaccharidases, defects of carrier proteins, congenital disorders of glycosylation, disorders of mitochondrial function.
- Ascites occurs in many lysosomal disorders.

25.1 General Remarks

Gastrointestinal manifestations of metabolic disorders include vomiting, diffuse abdominal pain, pancreatitis, slowed transit time and constipation, maldigestion and malabsorption (which may result in diarrhea), and ascites. These symptoms may occur as part of a systemic disorder of intermediary metabolism in which other symptoms predominate or as the major or exclusive symptoms.

25.2 Vomiting

Vomiting is a characteristic feature of many disorders of intermediary metabolism, including the organic acidurias (usually characterized by acidosis), disorders of the urea cycle (with accompanying hyperammonemia), and fatty acid oxidation defects (Table 25.1). Initial laboratory investigations for vomiting, including acid–base balance, lactate, ammonia, and urinary organic acids, can point the way to a diagnosis.

Chronic or recurrent vomiting in infancy, leading to formula changes before the underlying acidosis is discovered, is a common event in the organic acidurias due to defects in the metabolism of branched-chain amino acids – propionic, methylmalonic, isovaleric acidurias, etc. (see also Chap. 13).

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Table 25.1 Important causes of vomiting in metabolic disorders

Neonatal/early infancy or later, with or without encephalopathy
Organic acidurias
Urea cycle defects/hyperammonemia syndromes
Fatty acid oxidation disorders
With severe abdominal pain
Porphyrias (acute intermittent porphyria, coproporphyria, variegate porphyria)
As a symptom of associated pancreatitis
With acidosis/ketoacidosis
Organic acidurias
With liver dysfunction
Organic acidurias (chronic or recurrent)
Urea cycle defects/hyperammonemic syndromes
Galactosemia
Fructose intolerance
Tyrosinemia type I
Fanconi–Bickel syndrome
Fatty acid oxidation disorders (acute)

The hyperammonemia of disorders of the urea cycle often provokes vomiting. In severe cases, this will be rapidly followed by deterioration of the level of consciousness, but in milder cases, vomiting can be intermittent. Hyperammonemia can also be prominent in the fatty acid oxidation disorders, especially MCAD deficiency. Symptoms in these disorders occur intermittently and are triggered by fasting or intercurrent infections. The appropriate investigations are discussed in more detail in Chap. 16.

Vomiting will lead to alkalosis, so the discovery of acidosis when investigating a vomiting infant or child should immediately raise the possibility of an underlying organic aciduria or loss of base (e.g., renal tubular acidosis).

Remember

Hyperammonemia can provoke hyperventilation, leading to respiratory alkalosis, so the discovery of alkalosis when acidosis is expected (i.e., during a work-up for suspected sepsis, especially in an infant) should immediately lead to measurement of the blood ammonia level.

25.2.1 Cyclic Vomiting of Childhood (Ketosis and Vomiting Syndrome)

Vomiting is a prominent feature of a relatively common and poorly understood condition characterized by ketosis and abdominal pain, and triggered by fasting, often in the setting of infection – e.g. otitis. In some children, episodes can also be provoked by strenuous exercise. Typically episodes begin in the second year and usually end the latest with puberty. The abdominal pain may be intense, similar to that which occurs in diabetic ketoacidosis. Urinary organic acid analysis shows prominent ketosis, but no pathological metabolites, and acylcarnitine analysis shows prominent acetylcarnitine. Treatment with intravenous glucose usually results in rapid resolution of symptoms. Phenothiazine antiemetics are of limited use, but ondansetron can be quite beneficial. Reassurance that this condition is difficult but not dangerous is helpful. Very few patients with this pattern of symptoms have been shown to have deficiencies of β-ketothiolase or succinyl-CoA:3-oxoacid CoA transferase, of monocarboxylate transporter 1 or a mitochondrial disorder (Rinaldo 1999). Abdominal migraine appears to be the explanation in others. However, there remain a substantial number of children with this syndrome for whom a coherent explanation has not yet been found (Li et al. 1998; Li and Fleisher 1999). Impaired uptake of ketones into peripheral tissues has been suspected. This disorder is sometimes confused with ketotic hypoglycemia, but the blood glucose level is not abnormally low, the patients do not have the slight body build of many children with ketotic hypoglycemia, and the treatments for ketotic hypoglycemia (avoiding fasting, cornstarch at bedtime, etc.) do not seem to be particularly beneficial in cyclic vomiting (Pfau et al. 1996).

25.3 Abdominal Pain

Crampy abdominal pain occurs with intestinal dysfunction – malabsorption, infectious diarrhea, etc. – or mechanical problems, while diffuse pain

is often due to an inflammatory response. Metabolic disorders are usually not considered until several episodes of abdominal pain have occurred without an obvious explanation. This section will address diffuse abdominal pain; pancreatitis is discussed separately. Abdominal pain due to the disorders discussed below is often intense and may lead to surgical exploration for suspected appendicitis. Recent advances in imaging may help in lessening unnecessary surgery. On the other hand, when the patient has a metabolic disorder associated with recurrent abdominal pain, the physician must be careful during each episode that appendicitis or another surgical problem is not being mistaken for the metabolic disorder.

25.3.1 The Porphyrias

The porphyrias are disorders of the synthesis of heme, a component of cytochromes as well as hemoglobin. Diffuse or colicky abdominal pain and constipation occur in three of the hepatic porphyrias – acute intermittent, variegate, and hereditary coproporphyria. All three show autosomal dominant inheritance, and enzyme activity is typically ~50% of normal. The dominant porphyrias are among the few dominantly inherited enzymopathies.

Symptoms are uncommon in childhood. Episodes of illness in all three of the dominant porphyrias with abdominal symptoms appear to be related to increased activity of the first step of porphyrin synthesis, δ-aminolevulinic acid synthase. Although many of the porphyrias intermittently have the characteristic red (or dark) urine, this feature is not always evident, especially in coproporphyria. Many of the porphyrias have prominent photodermatitis, with reddening and easy blistering after sun exposure; hypertrichosis can also occur. In unusual cases, an individual (doubly heterozygous) may have more than one form of porphyria and present with severe symptoms, even in childhood.

Acute intermittent porphyria (which does not have skin lesions) is the most common form in most populations. It is due to deficient activity of porphobilinogen (PBG) deaminase (also called hydroxymethylbilane synthase and previously known as uroporphyrinogen synthase). The incidence of carriers (heterozygote frequency) is generally thought to be 5–10/100,000, but it may be as high as 1 in 1,000 (Northern Sweden). A large proportion (50–90%) have no symptoms. Episodes of mental depression, abdominal pain, peripheral neuropathy, or demyelination may be triggered by ethanol, barbiturates, oral contraceptives or other drugs, or hormonal changes, but often no precipitating factor can be identified (Herrick and McColl 2005; Whatley and Badminton 1993).

Hereditary coproporphyria, due to defects in coproporphyrinogen III oxidase (coproporphyrin decarboxylase), is generally milder than acute intermittent porphyria and is less likely to have neurological symptoms. Onset is unusual in childhood. The rare homozygote may have the onset of symptoms in infancy, with persistent jaundice and hemolytic anemia (Bissell et al. 1993).

Variegate porphyria is due to protoporphyrinogen oxidase deficiency. The highest incidence is among Afrikaners in South Africa, where the heterozygote frequency is 3 per 1,000; about half will have symptoms, typically triggered by medications and made worse by iron overload (e.g., consider concurrent hemochromatosis). Heterozygotes rarely develop manifestations in childhood, but symptoms can begin as early as in infancy in the rare homozygote or compound heterozygote. Photosensitivity is more common in variegate porphyria than in hereditary coproporphyria (Singal and Anderson 1993).

The diagnosis of any of the porphyrias can be difficult. Sometimes a positive family history of porphyria or illness suggestive of porphyria (depression, recurrent abdominal pain, etc.) will be present. Dark or red urine can be a major clue. A positive urine screening test (the Watson–Schwartz test or similar reaction) may be present only during acute illness and cannot be relied upon at other times. Urine PBG is quite reliable during times of clinical illness. Comprehensive evaluation of blood, urine, and stool for porphyrins is the most reliable diagnostic approach (see

Table 25.2 Characteristic laboratory findings in porphyrias with abdominal symptoms

Disease	OMIM	Enzyme	Urine	Stool
Acute intermittent porphyria	176000	Hydroxymethylbilane synthase	ALA, PBG, ±coproporphyrin	±Protoporphyrin
Hereditary coproporphyria	121300	Coproporphyrinogen oxidase	ALA, PBG, coproporphyrin	Coproporphyrin
Variegate porphyria	176200	Protoporphyrinogen oxidase	ALA, PBG, ±coproporphyrin	Protoporphyrin, ±coproporphyrin

ALA delta-aminolevulinic acid, *PBG* porphobilinogen

Table 25.2), followed by molecular analysis of the relevant gene(s). Erythrocyte porphyrins are normal in the conditions with abdominal pain. Molecular analyses should not be the primary diagnostic measure; enzyme analysis has largely been replaced by DNA studies (Whatley and Badminton 2013).

Diffuse abdominal pain also occurs in the familial fever syndromes. *Familial Mediterranean fever* (FMF) is an autosomal recessive condition characterized by episodes of noninfective peritonitis, pericarditis, meningitis (Mollaret meningitis), orchitis, arthritis, and erysipelas-like erythroderma. Onset in childhood is common in severe forms. Amyloidosis leading to renal failure can develop. FMF is so common in some ethnic groups, including Sephardic and Armenian Jews, and some Arab communities, that pseudodominant pedigrees are regularly encountered. Defect in pyrin (marenostrin), a protein in the myelomonocytic-specific proinflammatory pathway, is the underlying cause. Pyrin downregulates several aspects of the inflammatory response. Diagnosis is based on molecular testing of the FMF gene called MEVF. Most patients respond well to colchicine; interferon-alpha, thalidomide, etanercept, infliximab, and anakinra have been helpful (Shohat and Halpern 2000).

Autosomal dominant familial periodic fever is a similar disorder, much less common than FMF. It was originally reported in an Irish/Scottish family and called familial Hibernian fever. It is also called TNF-receptor-associated periodic fever syndrome (TRAPS) and is caused by mutations in the tumor necrosis factor receptor-1 gene TNFRSF1A. Symptoms of inflammation without fever are an occasional manifestation.

Abdominal pain and fever also occur in mevalonic aciduria, in both forms, most commonly in the form known as hyper IgD with periodic fever, and in hemochromatosis.

25.4 Pancreatitis

Pancreatitis remains one of the most mysterious of acute life-threatening conditions. Except for mechanical obstruction of pancreatic secretion due to gallstones, the mechanisms of pancreatitis are not completely understood, even when a precipitating or proximate cause is known, such as chronic use of ethanol or certain medications. Gallstones in children are usually associated with hemolytic disorders; in adults, there is often no obvious cause (Lowe 2004).

The organic acidurias primarily associated with pancreatitis are in the catabolic pathways for branched-chain amino acids. They include maple syrup urine disease, isovaleric aciduria, 2-methylcrotonyl-CoA carboxylase deficiency, propionic aciduria, methylmalonic aciduria, and β-ketothiolase deficiency (Kahler et al. 1994). Acidosis, ketosis, vomiting, and abdominal pain are common during episodes of metabolic decompensation in these disorders, so pancreatitis may not be suspected. Conversely, pancreatitis may be the presenting illness in mild forms of these conditions, especially isovaleric acidemia. A search for an underlying organic aciduria (urine organic acids, plasma or blood acylcarnitines, and plasma amino acids) should therefore be part of the initial investigation of pancreatitis (Table 25.3). Valproic acid (valproate) is a branched-chain organic acid that is an iatrogenic cause of pancreatitis (Perucca 2002).

Table 25.3 First-line metabolic investigations for pancreatitis

Urinary organic acid analysis
Plasma amino acids
Plasma total homocysteine
Blood spot or plasma acylcarnitine analysis
Blood lipid profile (lipoprotein electrophoresis)
Selenium
Total glutathione
Zinc
Vitamins A, C, E
Calcium

Pancreatitis also occurs in disorders of oxidative phosphorylation, especially cytochrome c oxidase deficiency, MELAS syndrome due to mutations in the tRNA leucine gene, and carnitine-palmitoyl-CoA transferase (CPT) II (Kishnani et al. 1996; Debray et al. 2006). Lipid disorders, especially hyperlipidemia due to lipoprotein lipase deficiency and hypo-/abetalipoproteinemia, are also often associated with pancreatitis. Homocystinuria due to cystathionine β-synthase deficiency is the aminoacidopathy most commonly associated with pancreatitis (Simon et al. 2001; Makins et al. 2000). Depletion of antioxidants (glutathione, vitamin E, selenium, etc.) may play a role in the pathogenesis of pancreatitis (Braganza et al. 1995; Braganza 1996; Braganza et al. 2011). The contribution of heterozygous mutations in genes which can contribute to pancreatitis in other situations (e.g., CFTR, SPINK1, and PRSS1) is not yet known.

25.5 Constipation/Slowed Transit/ Pseudo-obstruction

Constipation and pseudo-obstruction occasionally are manifestations of systemic metabolic disease, although in most cases, they are due to diet, habit, or intestinal motility abnormalities associated with neuronal dysfunction. The porphyrias are noted for constipation, especially during times of crisis. The syndrome of hyper IgD with fever may have constipation (cf. FMF, in which diarrhea is more likely). Constipation may also be prominent in the Fanconi–Bickel syndrome of glycogen storage and renal tubular dysfunction and malonyl-CoA decarboxylase deficiency. Mitochondrial dysfunction can cause impaired peristalsis, especially in MNGIE (see also Chap. 42).

In the organic acidurias and hyperammonemias, constipation can be troublesome. Altered bowel function may lead to decompensation of the primary metabolic disorder, because of the accumulation of intermediate compounds (e.g., propionate and ammonia) produced by gut flora. Treatment of the constipation can lead to significant improvement in metabolic control. Metronidazole is often used to alter bowel flora, to diminish the production of propionate or methylmalonate in patients with disorders of propionate metabolism

25.6 Ascites

Ascites is rarely the sole presenting symptom of metabolic disease, but it accompanies several of them. Ascites or hydrops occurs in many lysosomal diseases, including sialidosis (mucolipidosis I), galactosialidosis, sialic acid storage disease, Farber lipogranulomatosis, Gaucher disease, Niemann–Pick disease, mannosidosis, and mucopolysaccharidoses IV-A and VI. In severe or early-onset disorders, the ascites may occur before birth as nonimmune hydrops fetalis. Hydrops is especially common in MPS VII (Sly disease–β-glucuronidase deficiency).

25.7 Diarrhea

Mitochondrial neurogastrointestinal encephalopathy (MNGIE) syndrome (myo-, neuro-, and gastrointestinal encephalopathy) is a generalized disorder of mitochondrial dysfunction. Onset of intestinal symptoms is in childhood or early mid-adult life and includes chronic diarrhea, stasis, nausea, and vomiting, resulting in impaired growth. Wasting and cachexia may occur. Skeletal growth may be retarded. There is eventual loss of longitudinal intestinal muscle, diverticuli (which may rupture), intestinal scleroderma,

Table 25.4 Extraintestinal manifestations in mitochondrial neurogastrointestinal encephalopathy syndrome

Organ	Findings
Growth	Slow, cachexia and wasting
Brain	Leukodystrophy
	Increased CSF protein
	Ataxia
Eye	Ophthalmoplegia, ptosis
Ear	Sensorineural deafness
Cranial nerves	Dysarthria, dysphonia, facial palsy
Heart	Heart block
Skeletal muscle	Ragged-red fibers, weakness
Peripheral nerves	Demyelinating neuropathy, axonal degeneration

and pseudoobstruction. Electrophysiologic studies have shown visceral neuropathy with conduction failure. Prokinetic drugs have been generally ineffective. Lactic acidosis is often present. The extraintestinal symptoms are variable but typical of a mitochondrial disorder (see Table 25.4).

In vitro analysis of mitochondrial function reveals a variety of impairments, especially deficiency of complex I or complex IV, or combined deficits. Mitochondrial DNA analysis (liver and muscle) shows depletion and multiple DNA deletions. Recurrences in sibs, high frequency of parental consanguinity, and lack of vertical transmission are consistent with an autosomal recessive inheritance. The genetic defect is in the gene for thymidine phosphorylase (TYMP), which has also been described as platelet-derived endothelial growth factor (PDECGF1), and gliostatin. Impaired function of this gene leads to impaired mitochondrial DNA synthesis. Mutations in POLG (DNA polymerase gamma) are another recessive cause of MNGIE, but without leukodystrophy.

A similar autosomal recessive condition, whose cause is not known, has been called *oculogastrointestinal myopathy* or familial visceral myopathy with external ophthalmoplegia. There is destruction of the gastrointestinal smooth muscle, whereas the myenteric plexus appears normal. Abdominal pain, diarrhea, diverticuli, and dilatation of the bowel occur. Patients also suffer from a demyelinating and axonal neuropathy, focal spongiform degeneration of the posterior columns, ptosis, and external ophthalmoplegia. The onset is in childhood or adolescence, with death by age 30 in many patients. Most reports of this condition are from the 1980s, and there may be a considerable overlap with MNGIE, which was generally not excluded by enzymatic or molecular testing.

Diarrhea and malabsorption due to intestinal infiltration with lipid occurs in lysosomal acid lipase deficiency. The severe infantile type is Wolman disease; the milder form is cholesteryl ester storage disease. Both have massive hepatomegaly and elevated cholesteryl esters. Sebelipase alfa is being studied for enzyme replacement therapy. It was approved for use in the European Union in 2015.

Chronic diarrhea also occurs in *Menkes disease* and its milder form, occipital horn syndrome, perhaps because of autonomic dysfunction.

25.8 Maldigestion

Generalized impairment of digestion due to pancreatic problems (e.g., cystic fibrosis) or liver disease is well known. Several disorders of digestion involve primary enzymes of carbohydrate metabolism (Table 25.5). A deficiency typically results in severe watery (osmotic) diarrhea when the affected substrate or its precursors are ingested. There may also be excessive gas production and bloating. All are autosomal recessive. The Pearson marrow–pancreas syndrome, usually caused by heteroplasmic deletion of mitochondrial DNA, has exocrine pancreatic failure. The same deletion (and pancreatic failure) can be found in Kearns–Sayre syndrome. Failure of the exocrine and/or endocrine pancreas and other endocrine organs may occur in a variety of other mitochondrial disorders (see Chap. 42).

25.8.1 Disaccharide Intolerance I: Sucrase/Isomaltase Deficiency

This deficiency is a rare cause of infantile diarrhea, which becomes evident with the introduction of sucrose in the diet. There are several

Table 25.5 Disorders of carbohydrate digestion

Disorder	OMIM	Enzyme	Major substrate
Disaccharide intolerance I	222900	Sucrase/isomaltase	Sucrose
Disaccharide intolerance II – congenital alactasia	223000	β-glycosidase complex – lactase, glycosylceramidase	Lactose
Disaccharide intolerance III – adult lactase deficiency	223100	β-glycosidase complex – lactase, glycosylceramidase	Lactose
Trehalase deficiency	275360	Trehalase	Trehalose (mushrooms)
Not recognized	154360	Glucoamylase (maltase) I and II	

different mechanisms for enzyme deficiency, including impaired secretion, abnormal folding, deficient catalytic activity, and enhanced destruction.

25.8.2 Disaccharide Intolerance II: Infantile Lactase Deficiency and Congenital Lactose Intolerance

Congenital lactase deficiency is extremely rare. In nearly all cases, lactase deficiency in infants and young children is *acquired* through infection and loss of the mature brush border.

Congenital lactose intolerance appears to be distinct from congenital lactase deficiency. In the former, there is excessive gastric absorption of lactose (leading to lactosemia and lactosuria), vomiting, failure to thrive, liver dysfunction, and renal Fanconi syndrome. This condition may be fatal if not recognized and treated by elimination of lactose from the diet. Interestingly, lactose becomes well tolerated after 6 months. The basis for this condition is not known.

25.8.3 Disaccharide Intolerance III: Adult Lactase Deficiency

Adult lactase "deficiency" is a common polymorphism. In fact, declining expression of intestinal lactase during childhood is the usual state, mirroring the declining importance of milk in mammalian nutrition after infancy. In a few regions, including Northern Europe, unfermented milk remains an important dietary component after infancy. The ability to digest milk is clearly an advantage, so among individuals from those regions, there is a minority with lactose intolerance. (It may be that the custom of drinking unfermented milk arose because of a chance mutation that interrupted the usual decline in lactase activity with age.) Intestinal lactase activity depends on the summed expression from both the alleles. Differences in lactase expression do not reside in the coding sequence itself but in a *cis*-acting regulatory element (Sibley 2004).

Generalized pancreatic insufficiency can lead to impaired digestion and therefore inadequate absorption of nutrients. Deficiency of a specific intestinal enzyme, enterokinase, will lead to impaired cleavage of pancreatic proenzymes (trypsinogen, chymotrypsinogen, procarboxypeptidase A), and their secondary targets. The result is impaired digestion, malabsorption, and poor weight gain.

25.9 Malabsorption

Malabsorption in metabolic disorders can be due to abnormalities in ion channels, transport molecules, carrier proteins for lipids, or cotransport molecules. Symptoms are attributable to the deficiency of the substance not being properly absorbed (i.e., essential fatty acids and fat-soluble vitamins) and the effects due to abnormally high amounts of the substance within the gut. Ion-channel defects distort the balance of water and electrolytes, leading to diarrhea; abnormalities of transport molecules lead to

diarrhea; and deficiency of a cotransport molecule (e.g., intrinsic factor) can have major consequences because of the resulting deficiency of an essential nutrient. First-line investigations for metabolic causes of malabsorption should include stool pH and analysis of reducing substances in stool. Characterization of stool sugars will confirm what has been suggested by the history. A breath hydrogen test after dietary challenge can confirm malabsorption. Cystic fibrosis is of course a common cause of malabsorption because of impaired secretion of pancreatic enzymes including lipase.

25.9.1 Glucose–Galactose Malabsorption

Glucose–galactose malabsorption is clinically similar to sucrase/isomaltase deficiency – severe life-threatening watery diarrhea from early infancy. Elimination of glucose and galactose from the diet is necessary. Fortunately, fructose is well tolerated so a fructose-based formula is effective. This condition is most often recognized in Middle Eastern Arab populations. The defect in the sodium–glucose cotransporter SGLT1 is due to mutations in the solute-carrier (SLC) gene SLC5A1 (Xin and Wang 2011).

25.9.2 Electrolytes

25.9.2.1 Chloride Diarrhea

In this recessively inherited form of diarrhea, there is voluminous watery diarrhea with high chloride content (greater than the sum of sodium and potassium), from an early age. Polyhydramnios is common, perhaps universal. The defect is in the brush-border chloride/bicarbonate exchange mechanism. Treatment with sodium and potassium chloride is effective. Mutations have been discovered in the gene SLC26A3.

Defects of the Na^+/H^+ exchange mechanism result in *sodium diarrhea* and metabolic acidosis. The presentation is similar to congenital chloride diarrhea, but the stool electrolyte composition shows high sodium and an alkaline pH. Treatment with oral Na–K–citrate will normalize the electrolyte status of the patient. Mutations have been found in the serine protease inhibitor gene SPINT2.

Microvillus inclusion disease is another recessive cause of intractable diarrhea in infancy and perhaps the most common noninfectious cause. Jejunal biopsy shows intracytoplasmic inclusions, consisting of brush-border microvilli, suggesting that this is a disorder of intracellular transport, impairing assembly. Mutations have been found in the myosin type 5 gene (MYO5B).

25.9.3 Protein-Losing Enteropathy

Protein-losing enteropathy, often due to infection or impaired function of intestinal lymphatics, is also a cardinal feature of the congenital disorder of glycosylation PMI-CDG (formally *CDG 1b*) due to phosphomannose isomerase deficiency. There may be liver disease and a bleeding diathesis. Unlike the other CDG syndromes, mental retardation and severe neurological problems do not occur. Diagnosis is based on quantifying glycosylation of transferrin by isoelectric focusing, capillary electrophoresis, or mass spectrometry, and gene sequencing. Treatment with oral mannose is effective. The CDG syndromes are discussed in greater detail in Chap. 33.

25.9.4 Amino Acids

The two main disorders of intestinal amino acid transport are those involving tryptophan and methionine. Tryptophan malabsorption (*Hartnup disease*) occurs as an autosomal recessive disorder that may be clinically silent. The transport of several neutral amino acids in the intestine and kidney are involved, but tryptophan is the one most likely to be limiting. In the absence of adequate niacin in the diet, there will be a deficiency of nicotinic acids and nicotinamide, resulting in a pellagra-like rash, light sensitivity, emotional instability, and ataxia. In severe cases, this can progress to an encephalopathy with delirium and

seizures. There may be an increase in stool indoles and urinary indican, reflecting the action of intestinal bacteria on the unabsorbed tryptophan. The diagnosis is suggested by finding high urinary levels of the neutral amino acids alanine, serine, threonine, asparagine, glutamine, valine, leucine, isoleucine, phenylalanine, tyrosine, tryptophan, histidine, and citrulline. Plasma levels of these amino acids are normal or slightly low. Mutations have been found in the gene SLC6A19.

A few infants have been reported to have *methionine malabsorption*. Clinical manifestations have included white hair, tachypnea, mental retardation, seizures, diarrhea, and a peculiar odor like that of hops drying in an oast house. The urine may show increased alpha-hydroxybutyric acid after a methionine load. The condition appears to be autosomal recessive. No new cases have been reported in several decades.

A single case of postulated deficient lysine transport was reported in 1976.

25.9.5 Vitamins and Other Small Molecules

25.9.5.1 Vitamin B_{12}

The intricate story of vitamin B_{12} illustrates the interface between diet, digestion, absorption, and intermediary metabolism. Vitamin B_{12}, synthesized by microbes, is the precursor of substances known as cobalamins. Two forms are essential for human metabolism: Methylcobalamin is a cofactor for the remethylation of homocysteine to methionine, and adenosylcobalamin is used by methylmalonyl-CoA mutase. Defects of the cobalamin pathway can therefore cause problems with either or both of these reactions.

Vitamin B_{12} in food (primarily meat and dairy products) is first released by digestion in the stomach. It binds to specific glycoproteins (r-proteins), especially transcobalamin I (TC I). In the duodenum, the pancreatic proteases act to release B_{12} again, and it now binds to intrinsic factor produced by gastric parietal cells. The intrinsic factor-B_{12} complex (B_{12} –IF) binds to a specific receptor (cubilin) on ileal enterocytes. The receptors internalize the B_{12} –IF, which then dissociates. The absorbed B_{12} is then transported to the bloodstream, coupled to transcobalamin II (TC II). The B_{12} –TC II complex is the main way of distributing newly absorbed B_{12} to the tissues. However, most of the B_{12} in the bloodstream is bound to TC I, as methylcobalamin.

Autosomal recessive genetic defects in intrinsic factor or the ileal receptor system typically lead to megaloblastic anemia with neurologic changes (developmental delay, hypotonia, hyporeflexia, and coma). Onset is usually after the first year. Infants in the first year apparently do not depend on the ileal receptor system for B_{12} uptake, which explains the lack of symptoms in young infants with problems in this system. The receptor defect is known as the Imerslund–Grasbeck syndrome (megaloblastic anemia I), which may have proteinuria as well as anemia. Defects in TC II, on the other hand, become apparent in the first few months with megaloblastic anemia, failure to thrive, and neurological delay. Congenital B_{12} deficiency can occur in the offspring of vegan/vegetarian mothers who have not had adequate intake of B_{12}. Deficiency of B_{12} will lead to mild methylmalonic aciduria and hyperhomocysteinemia. Infants with congenital B_{12} deficiency may be first recognized by increased propionylcarnitine on newborn screening. Late-onset B_{12} deficiency is usually due to the lack of intrinsic factor due to atrophic gastritis, other stomach problem, or an unexplained mechanism. The defects of B_{12} metabolism after uptake from the bloodstream lead to variant forms of homocystinuria, methylmalonic acidemia, or both (Carrillo-Carrasco et al. 1993; Manoli and Venditti 1993).

Investigation of suspected B_{12} disorders includes determining erythrocyte indices, plasma homocysteine, acylcarnitine analysis for propionylcarnitine, and plasma or urine methylmalonic acid. Serum cobalamin levels are low in defects involving intrinsic factor and gut uptake but are normal or close to it in TC II deficiency (see also Chap. 41).

Many transporters and their genes are now known – there are at least 395 members of the solute-carrier SLC system, organized into more than 52 groups (Hediger et al. 2013). Clinical deficiencies of only a few are known at present (Table 25.6).

Table 25.6 Representative disorders of transport molecules

Disorder	OMIM #	Gene/transporter	Nutrient
Glucose/galactose malabsorption (Wright 2013)	606824	SCL5A1	Glucose, galactose
Thiamine-responsive megaloblastic anemia (Zhao and Goldman 2013)	249270	SCL19A2/THTR1	Thiamine
Riboflavin deficiency (Nanbu et al. 1999)	615026	SLC52A1	Riboflavin
Brown–Vialetto–Van Laere syndrome-2	614707	SLC52A2	
Hereditary folate malabsorption	229050	SCL46A1/PCFT/HCP1	Folate
Imerslund–Grasbeck syndrome, Norwegian type megaloblastic anemia	261100	CBN/cubilin or AMN	B_{12}-intrinsic factor complex
Systemic primary carnitine deficiency, carnitine uptake defect	212140	SLC22A5/OCTN2	Carnitine
Intestinal hypomagnesemia 1 (HOMG1)	602014	TRPM6	Magnesium
Menkes syndrome/occipital horn syndrome	300011	ATP7A	Copper
Acrodermatitis enteropathica	201100	SLC39A4/ZIP4	Zinc
Primary bile acid malabsorption	613291	SLC10A2	Bile acids
Hartnup disease	234500	SLC6A19	Tryptophan, methionine
Lysinuric protein intolerance	222700	SLC7A7	Lysine

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Kidney Disease and Electrolyte Disturbances

26

William L. Nyhan

Key Facts

- Hypochloremic metabolic acidosis with increased anion gap points to classic metabolic disorders, e.g., methylmalonic acidemia.
- Renal tubular acidosis is hyperchloremic, and there is no increase in anion gap; differential diagnosis is diarrheal disease.
- Urinary tract stone disease in pediatric populations should suggest an inborn error of metabolism.
- Renal Fanconi syndrome is the common result of several inherited metabolic disorders that cause renal tubular dysfunction.
- Renal tubular alkalosis is characteristic of the Bartter and Gitelman syndromes. Calcium excretion is decreased in Gitelman syndrome; it is normal or increased in the presence of defective genes which cause Bartter syndrome.

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26.1 General Remarks

Kidney disease and disturbed fluid and electrolyte homeostasis are important sequelae of inherited metabolic disease. They may occur as presenting manifestations. Therapy must be addressed promptly and must be continued.

The most important renal manifestations of inherited metabolic disease are (recurrent) dehydration, renal tubular dysfunction, and/or urinary tract calculi and crystalluria (v.i).

Renal pathology may also lead to failure to thrive, myoglobinuria (Chap. 28), unusual odor (see Chap. 4 Table 4.1), and abnormal color (see Chap. 4 Table 4.2). Renal cystic disease is seen in Zellweger syndrome, carnitine palmitoyltransferase II deficiency, and multiple acyl-CoA dehydrogenase deficiency (glutaric aciduria type II) due to the deficiency of electron transfer flavoprotein (ETF) and ETF dehydrogenase. Multiple microcysts lead to congenital nephrotic syndrome in PMM2-CDG.

In methylmalonic aciduria, a major subset of patients develops a variety of renal manifestations leading to chronic renal failure. Isolated renal tubular dysfunction may lead to acidosis and hyperchloremia along with proximal renal tubular bicarbonate wasting. It may also be complicated by interstitial nephritis and renal glomerular failure. A hemolytic uremic syndrome has been observed in infants with cobalamin C

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disease, the combined methylmalonic aciduria and homocystinuria.

Renal disease is also a late complication of glycogenosis type I (von Gierke disease). These patients may develop proteinuria, increased blood pressure, urinary tract calculi, or nephrocalcinosis. Many of these manifestations have been attributed to hyperuricemia. However, there is also glomerulosclerosis and interstitial fibrosis leading to glomerular dysfunction that may end in renal failure.

Remember

Different types of renal pathology, but especially proximal tubular dysfunction (Fanconi syndrome) and focal segmental glomerulosclerosis, can be the result of a primary mitochondrial disorder (Table 26.1).

26.2 Dehydration

Renal and electrolyte abnormalities present frequently with acidosis and dehydration, and this may be indicative of a metabolic disease (Table 26.2). More commonly, this picture of abnormal clinical chemistry is caused by infectious diarrhea.

Rarely, chronic diarrhea is caused by an inherited disorder of intestinal absorption (Chap. 25). Patients with clinical manifestations of dehydration, decreased skin turgor, depressed fontanel, sunken eyes, decreased urine output, or documented acute loss of weight are fortunately regularly assessed by measuring the electrolyte concentrations, acid-base balance, urea nitrogen, and creatinine in the blood. Among the clinical signs of dehydration is poor skin turgor. Not so widely known is the fact that turgor is measured by the relative rate that a pinched up piece of skin becomes restored to its resting state. In fact, in rats, measured weight loss in experimentally induced dehydration had a straight-line correlation with the stop-watched time of the skin to flatten.

Electrolyte analysis in the dehydrated patient with renal disease often reveals hyperchloremic acidosis. The anion gap is not increased. Most commonly, this picture results from acute diarrhea, although chronic diarrhea can lead to chronic acidosis and chronic dehydration. Hyperchloremic acidosis also results from renal tubular acidosis (RTA) (v.i).

Table 26.1 Primary renal tubular disorders

Cystinuria: amino acid transport defect
Diabetes insipidus: usually vasopressin receptor defect
Bartter syndrome: electrolyte transport defects in the loop of Henle
Gitelman syndrome: Na/Cl cotransport defect in the distal tubule
Hypophosphatemic rickets: pathological activation of fibroblast growth factor 23
Renal tubular acidosis (RTA) type I: deficient distal tubular H^+ secretion
RTA type II: deficient proximal tubular HCO_3^- secretion
RTA type IV: reduced aldosterone effect, usually drug induced

Remember

Metabolic acidosis resulting from the classic inborn errors of intermediary metabolism is hypochloremic, and the anion gap is increased (Chap. 6).

Acute infectious diarrhea can precipitate an attack of metabolic imbalance in many inborn errors of metabolism, but in these situations, the metabolic abnormality predominates and there is an anion gap. Dehydration resulting from inherited disease is also characterized by recurrent or episodic attacks of dehydration.

Remember

Metabolic acidosis with increased anion gap indicates either ketosis (→check ketostix, measure ketones in blood), the presence of pathological organic acids (→examine urinary organic acids, acylcarnitines, blood lactate), or both.

A combination of the electrolyte pattern and the clinical manifestations permits a ready dissection of the heritable causes of recurrent dehydration. Actually, many of the chronic diarrheas, such as the disaccharidase deficiencies, do not often lead to dehydration. Patients are so accustomed to their problem that they

Table 26.2 Recurrent or episodic dehydration

Hyperchloremic acidosis	Renal tubular acidosis
Failure to thrive, rickets, polyuria	Cystinosis
Diarrhea	Lactase deficiency
	Sucrase, isomaltase deficiency, glucose–galactose malabsorption
	Acrodermatitis enteropathica
Hypochloremic alkalosis polyuria	Bartter and Gitelman syndromes
Diarrhea	Congenital chloride diarrhea
Diarrhea, hypoproteinemia, anemia	Cystic fibrosis
Vomiting	Pyloric stenosis, bulimia
Hypernatremia	Nephrogenic diabetes insipidus
	Diabetes insipidus
Hyponatremia, hyperkalemia	Congenital adrenal hyperplasias, adrenal aplasia, hypoplasia, pseudohypoaldosteronism
Hypochloremic acidosis	Propionic acidemia
Increased anion gap, ketoacidosis	Methylmalonic aciduria
	Diabetic ketoacidosis
	Isovaleric acidemia
	3-Oxothiolase deficiency

compensate with ample fluid intake. The water intake of infants and children with nephrogenic diabetes insipidus is enormous. These patients often get into trouble when exogenous forces such as intercurrent illness interfere with their ability to compensate. Admission to hospital and a requirement for parenteral fluids, even with fairly trivial surgery, can lead to major morbidity and mortality if physicians do not recognize and supply large quantities of water necessary to maintain these patients.

Hypochloremic alkalosis results from depletion of intracellular potassium resulting from intestinal losses. In small infants, it is the hallmark of pyloric stenosis. Infants with congenital chloride diarrhea and those with cystic fibrosis (CF) both have diarrhea, but those with CF are edematous from hypoproteinemia and pale from anemia. Those with Bartter syndrome and Gitelman syndrome do not have diarrhea. A teenager or adult with bulimia can mimic Bartter syndrome, so can one with chronic laxative abuse.

The adrenal hormone deficiency diseases are readily recognized in those females with ambiguous genitalia. They are often missed in those without, such as male infants with adrenal hyperplasia or infants with absent or hypoplastic adrenals or with pseudohypoaldosteronism. The hyponatremia and renal salt wasting and the hyperkalemia should be giveaways for the diagnosis.

26.3 Urinary Tract Calculi

Kidney stones or calculi occurring anywhere in the urinary tract represent a common affliction among adults; they are rare in children. In adults, the composition of the stone is usually a salt or a mixture of salts of calcium, calcium oxalate, and calcium phosphate.

Remember

In total, 60–70 % of calculi found in pediatric populations result from inborn errors of metabolism (Table 26.3) (Fig. 26.1).

Lithiasis in the urinary tract may present with pain, which is referred to as renal colic. This sudden onset of pain is of such extreme intensity that may lead to nausea and vomiting. The pain reflects the movement of a stone along the ureter and may be well localized by the patient to this curvilinear distribution. Pain may disappear on entry of the calculus into the

Table 26.3 Urinary tract calculi

Stone	Disorder	Enzyme
Cystine	Cystinuria	Cystine transporter
Uric acid	Lesch–Nyhan	HPRT
	PRPP synthetase superactivity	PRPP synthetase
	Glycogenosis I	Glucose-6-phosphatase
	Renal hypouricemia	URAT1 (*SLC22A12* gene)
2,8-Dihydroxyadenine	APRT deficiency	APRT
Xanthine	Xanthinuria	Xanthine oxidase
Oxalate	Oxaluria, glycolic aciduria	Alanine–glyoxylate aminotransferase
	Oxaluria, glyceric aciduria	D-glycerate dehydrogenase
Calcium salts	Hypercalciuria + uricosuria	Multifactorial
	Wilson disease	P-type ATPase transporter

HPRT hypoxanthine-guanine phosphoribosyl transferase, *PRPP* phosphoribosylpyrophosphate, *APRT* adenine phosphoribosyltransferase, *ATPase* adenine triphosphatase

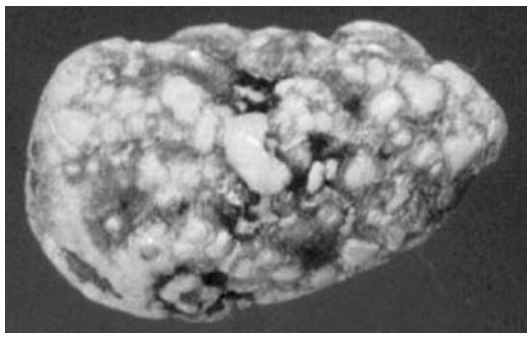

Fig. 26.1 Urinary calculus passed by a hyperuricemic 2-year-old boy with Lesch–Nyhan disease (Reprinted with permission from Nyhan et al. (2012))

bladder, only to reappear on entry into the urethra. There may be associated frequency or dysuria. Some patients may have these symptoms as a result of crystalluria as well as with discrete stones. Hematuria can also result from crystalluria or calculi.

Remember

Clinical symptoms in small children with urinary tract calculi are often nonspecific (abdominal pain, nausea, vomiting, and recurrent urinary tract infections). Urinary tract ultrasound is indicated in all children with microhematuria.

Urinary tract infection is a common complication of urinary tract calculi. It may present with pyuria, dysuria, and fever. Infection will usually not disappear until the stone is removed, by passage, lithotripsy, or surgery. Repeated episodes of pyelonephritis and obstruction may lead to renal failure.

Patients with symptoms should not only be examined for the possibility of stones, but also those with diseases in which calculi are common should be monitored. Stones containing calcium are radiopaque, but urate stones (Fig. 26.1) are not; cystine stones are radiopaque but may be difficult to visualize roentgenographically. Ultrasonography is useful in detecting hydronephrosis or hydroureter but may miss small stones. Intravenous urography is useful in delineating calculi and defining the presence or absence and degree of obstruction. Retrograde pyelography permits visualization without intravenous injection of the dye, but it requires cystoscopy. CT scan may permit detection of radiolucent stones not evident with other methods.

Cystinuria results from mutations in the gene, which is responsible for the cystine transporter of the kidney and intestine. It leads to increased urinary excretion of lysine, ornithine, and arginine, as well as cystine. Only cystinuria causes symptoms, all the consequence of its lack of solubility and consequent formation of calculi. Treatment is by ensuring ample fluid throughout the day and also at night to keep cystine soluble. Cystine crystallizes at concentrations above 1,250 μmol/l

at pH 7.5. The second approach, using pH adjustment as a tool, takes advantage of the rapid increase in cystine solubility above pH 7. By adjusting the urinary pH through ingestion of alkali, the excreted cystine thus is made more soluble. Treatment with penicillamine is effective by the formation of mixed disulfides with cysteine, which are soluble.

Uric acid stone disease is seen commonly in Lesch–Nyhan disease as well as the other variant hypoxanthine-guanine phosphoribosyltransferase deficiencies. Patients with phosphoribosyl-pyrophosphate (PRPP) synthetase superactivity and with glycogenosis type I also overproduce purine and excrete it as uric acid in large quantities. Deafness is common in patients with PRPP synthetase mutations. Other patients with uric acid calculi have uricosuria as a result of increased urate clearance by the kidney. Some well-defined kindreds have been reported. Most of them have normal blood concentrations of uric acid; some are hypouricemic and some, like the Dalmatian dog, are hyperuricemic. Mutations in the *SLC22A12* gene lead to abnormalities in URAT1, the predominant transporter of uric acid at the apical membrane of the renal tubule.

In interpreting values of uric acid in plasma or serum, it must be remembered that children have an especially high clearance capacity for uric acid and consequently can keep serum levels within normal ranges despite a pathologically increased endogenous production (Table 26.4). Urinary concentrations of uric acid give more reliable results. For diagnostic purposes, a random urine sample should be promptly analyzed for uric acid and creatinine. Age-related control ranges are summarized in Table 26.5.

Table 26.5 provides estimates of uric acid/creatinine ratios in morning urine samples (Kaufman et al. 1968), which can be taken as a possible hint to either elevated, or reduced, excretion of uric acid. A few facts have to be borne in mind when interpreting uric acid excretion in spot urine samples in general and using this table in particular.

Table 26.4 Investigations in children with renal stones

Ultrasound scan of renal tract (stones, dilatation)
Determine urinary oxalate, calcium, citrate, cystine (→ amino acids), uric acid (→ purines)
Urine microscopy
Consider intravenous urography, CT

Table 26.5 Uric acid excretion in control subjects

Age (years)	<2	2–4	4–8	8–10	10–12	>12
Upper limit Ua/Crea	<2[a]	<1.5	<1.3	<1.1	<1.0	<0.8
Lower limit Ua/Crea	<0.5[a]	<0.5	<0.4	<0.3	<0.3	<0.3

[a]In very young infants, especially, during the first week of life the standard deviation is extremely high (99th percentile between approximately 0.2 and 2.8)

The figures are estimated from a continuously falling curve with varying standard deviations at different ages (Kaufman et al. 1968). By that they represent "forced mean values" for the given age group and give an approximation of the normal range of uric acid excretion. Patients with Lesch–Nyhan syndrome (HPRT deficiency) seem to be always clearly above those upper limits.

In patients with either "borderline findings" with this proposed uric acid/creatinine ratio or high clinical suspicion of primary or secondary disturbances of uric acid metabolism, a determination of the 24 h excretion corrected for body surface is probably more accurate. $520 \pm 147/1.73\ m^2/24$ h (mean ± 1 SD) is given by Wilcox (1968) based on several previous publications. As an alternative, correction of uric acid excretion for creatinine clearance gives a constant value between 3 and 40 years of age: 0.34 ± 0.11 mg/dL of glomerular filtrate (mean ± 1 SD) according to Wilcox (1968). Glomerular filtrate was estimated from simultaneous measurement of serum creatinine (S_{cr}), urinary creatinine (U_{cr}), and urinary uric acid (U_{ua}) in overnight fasted patients. All the values are in mg/dL, using the following equation:

$$\left(U_{cr}\right) \times \left(S_{cr}\right) / \left(U_{cr}\right) = \text{uric acid excreted in mg / dL of glomerular filtrate.}$$

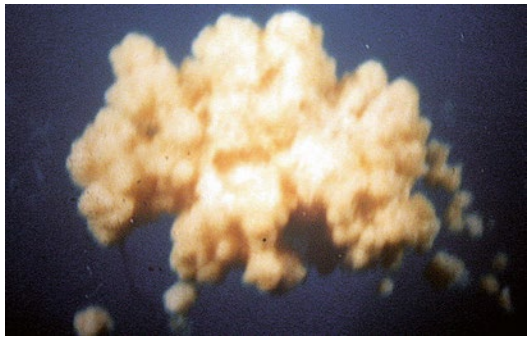

Fig. 26.2 Massive calcium oxalate material obtained from a 6-month-old patient with hyperoxaluria type II

Adenine phosphoribosyltransferase deficiency, common in Japanese, leads to increased excretion of the very insoluble dioxygenated derivative of adenine. Xanthine oxidase deficiency is associated with stones composed of the very insoluble xanthine and with hypouricemia. Xanthine stones are also observed in patients with hyperuricemia treated with allopurinol, but in many it is possible to find a dosage regimen that minimizes or avoids the propensity for stone formation, because hypoxanthine is very soluble.

The two forms of oxaluria, known as type I and type II, are characterized by very early onset renal stone disease (Fig. 26.2) and early renal failure. In type I, there is glycolic aciduria as well as hyperoxaluria; in type 2, there is glyceric aciduria. Successful treatment has combined transplantation of both the liver and kidney. Hyperoxaluria and calcium oxalate calculus formation have also been reported in cystic fibrosis.

26.4 Renal Tubular Dysfunction Fanconi Syndrome

The Fanconi syndrome represents a generalized disruption of renal tubular function in which the proximal tubular reabsorption of amino acids, glucose, phosphate, bicarbonate, and urate is impaired, leading to a generalized aminoaciduria, glycosuria, phosphaturia, uricosuria, and increased urinary pH. As a consequence, there is vitamin D-resistant rickets and osteomalacia.

Remember

Fanconi syndrome is characterized by polyuria and increased urinary excretion of amino acids, glucose, and phosphate (frequently also calcium, urea, and protein). Metabolic acidosis with increased urinary pH is common.

Obligatory polyuria in this syndrome can lead to clinically important dehydration, especially when fluid intake is restricted. Chronic hyperchloremic acidosis may worsen in acute situations. Losses of ions and metabolites in the urine may lead to symptomatic depletion. Thus, hypokalemia may lead to muscle weakness or even paralysis, constipation, and ileus, as well as disturbances of cardiac rhythm and function. Excretion of carnitine may deplete body stores and lead to disturbed fatty acid oxidation and hypoketotic hypoglycemia (Chap. 9), muscle weakness, or congestive cardiac failure. Losses of calcium may lead to tetany or convulsions. Hypomagnesemia may similarly develop. Shortness of stature or failure to thrive is seen regularly.

A number of genetically determined diseases lead to the Fanconi syndrome (Table 26.6). The most common of these is cystinosis (v.i).

Among these disorders, associated syndromic features lead to the diagnosis and the appropriate confirmatory test. Hepatic dysfunction is seen in hepatorenal tyrosinemia, usually as the major clinical feature, and the diagnosis is made by urinary organic acid analysis for succinylacetone. Hepatic dysfunction is also seen along with cataracts in galactosemia and with hypoglycemia in hereditary fructose intolerance. Confirmation of each is by enzyme assay; in the latter the liver is required; as an alternative mutational analysis may reveal the common Caucasian mutations. Fanconi syndrome has been reported in glycogenoses I and III and Fanconi–Bickel syndrome. Type I can be diagnosed clinically by glucagon testing (Chap. 43, glucagon stimulation), by enzyme assay of liver, or by mutation analysis. Currently, we reserve liver biopsy for

Table 26.6 Heritable causes of the Fanconi syndrome

Disorder	Molecular defect or basis	Distinguishing characteristics
Cystinosis	Lysosomal cystine transporter	Corneal deposits, shortness of stature
Hepatorenal tyrosinemia	Fumarylacetoacetate hydrolase	Hepatic dysfunction
Galactosemia	Galactose-1-phosphate uridyltransferase	Hepatic dysfunction, cataracts, mental retardation
Hereditary fructose intolerance	Fructose-1-phosphate aldolase	Hepatic dysfunction, hypoglycemia
Glycogenoses	Glucose-6-phosphatase, amylo-1,6-glucosidase, phosphorylase-b-kinase	Hepatomegaly, hypoglycemia, hyperlipidemia, hypercholesterolemia, lactic acidemia
Fanconi–Bickel syndrome	GLUT2 mutations	Hepatomegaly
Lowe syndrome	OCRL-1 gene on Xq25–26, phosphatidylinositol 4, 5-biphosphate-5-phosphatase	Cataracts, glaucoma, hypotonia, developmental retardation
Wilson disease	P-type ATPase transporter	Hepatic dysfunction, Kayser–Fleischer ring, neurologic dysfunction
Electron transport defects	mtDNA deletions (Pearson, Kearns–Sayre), mtDNA depletion (RRM2B), cytochrome c oxidase, complex III or IV	Lactic acidemia, encephalomyopathy
Drugs	Outdated tetracycline, gentamicin, valproic acid, 6-mercaptopurine, azathioprine	
Heavy metal poison	Cadmium, lead, manganese	
Primary (Fanconi renotubular syndromes 1–4)	E.g., SLC34A1 gene on chromosome 5q35 or EHHADH gene on chromosome 3q27 or HNF4A gene on chromosome 20q13 (FRTS4)	Dominant as well as recessive inheritance
		FRTS4 comprises both Fanconi renotubular syndrome and MODY

those in whom mutational analysis does not provide the diagnosis. The clinical chemistry is in characterized by hypoglycemia, lactic acidemia, hyperalaninemia, hyperlipidemia, and hypercholesterolemia. Other renal complications are common in glycogenosis I, including distal renal tubular disease, amyloidosis, nephrocalcinosis, and calculi (v.s.). Renal failure may be a late complication with proteinuria, focal glomerulosclerosis, and interstitial fibrosis. In Lowe syndrome and Wilson disease, the full Fanconi picture is often absent from the urine, but a generalized aminoaciduria is the rule. In Wilson disease, the pattern also includes increased cystine and methionine and indices of the liver dysfunction. In electron transport defects, a variety of patterns of RTA is seen, including the complete Fanconi syndrome. Raised levels of lactate should give the major clue. An early lethal mitochondrial DNA depletion syndrome caused by defects in the RRM2B gene characteristically results in the Fanconi syndrome.

In clearly genetic kindreds with idiopathic Fanconi syndrome, molecular defects are increasingly delineated; the rest could be classified as idiopathic. In addition, an acquired Fanconi syndrome has been observed with a variety of renal insults, including the ingestion of outdated tetracycline, 6-mercaptopurine, and heavy metal poisoning. Deficiency of vitamin D leads to a moderate generalized aminoaciduria, but not usually to the rest of the Fanconi syndrome.

26.4.1 Cystinosis

Cystinosis is one of the most important causes of the Fanconi syndrome and as such manifests all the features of the syndrome (v.s.). In addition, there are some clinical manifestations that are

unique to this disease. Patients generally have fair skin, hair, and irises. Ophthalmic abnormalities include photophobia that is caused by deposits of cystine in the cornea. These refractile crystalline bodies may be seen early by slit lamp. They may lead ultimately to thickened, hazy corneas and may be complicated by corneal ulcers. Crystalline cystine may also be identified in conjunctiva. In addition, there is a characteristic peripheral retinopathy visible as pigmentation and depigmentation. Some adults with the disease have been legally blind.

Hypothyroidism results late from deposition of cystine in the gland. Myopathy may follow cystine deposition in muscle or carnitine depletion because of losses in urine. Some patients have developed diabetes mellitus. Late neurologic abnormalities include tremor, seizures, or mental retardation. Hydrocephalus and cerebral atrophy have been documented. The diagnosis is usually made by the assay of cystine content in leukocytes or cultured fibroblasts. The molecular defect is in the transporter for cystine from lysosomes (CTNS, cystinosin) (Fig. 26.3). Therapy with cysteamine induces formation of the cysteine-cysteamine disulfide, which is soluble. The lysosomal lysine/arginine transporter (PQLC2) catalyzes the transport of the mixed disulfide out of lysosomes. Its function is relevant to the success of cysteamine therapy.

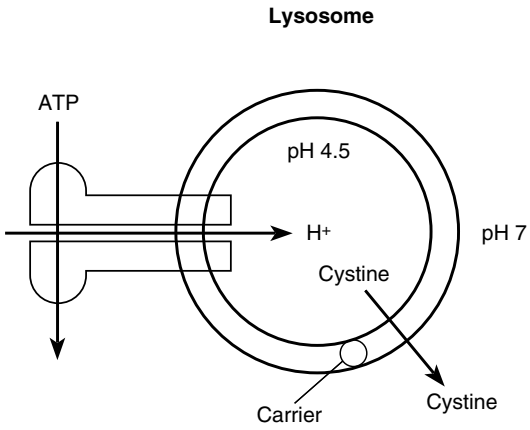

Fig. 26.3 The ATP-dependent lysosomal carrier-mediated efflux of cystine which is defective in patients with cystinosis (Reprinted with permission from Nyhan et al. (2012))

Remember

Cystinosis is a lysosomal transporter defect that causes increased intracellular deposition of cystine crystals. Typical clinical features include failure to thrive, short stature, and renal tubular disease. Early diagnosis and treatment with cysteamine has changed the prognosis of this disease.

26.4.2 Renal Tubular Acidosis

Renal tubular acidosis (RTA) includes the Fanconi syndrome and cystinosis (v.s.) (Table 26.7). A small population of patients have RTA without any of the features of the Fanconi syndrome. These patients fail to thrive as infants

Table 26.7 Renal tubular acidosis

Type I (distal)	Isolated
	With deafness
	Hypergammaglobulinemia (Sjögren)
	Nephrocalcinosis (e.g., hyperparathyroidism, vitamin D poisoning)
	Drugs: amphotericin B, lithium tubulointerstitial disease, renal transplantation, obstruction
Type II (proximal)	Isolated
	Pyruvate carboxylase deficiency
	Methylmalonic acidemia
	Carbonic anhydrase II deficiency (with osteopetrosis)
	Drugs (acetazolamide)
Combined type IV (distal hyperkalemic)	Isolated
	Carnitine palmitoyltransferase I deficiency
	Mineralocorticoid deficiency
	Pseudohypoaldosteronism
	Hyporeninemic, hypoaldosteronism (diabetes, NSAIDs)
	Drugs (spironolactone, amiloride, ACE inhibitors)

and display shortness of stature later. Vomiting is frequently encountered in infants, and many infants are anorexic. Infants are often described as irritable or apathetic. Metabolic bone disease manifests itself as rickets and osteomalacia, particularly, in proximal RTA. The diagnosis is often first suggested by roentgenographic examination of the bones of a patient with failure to thrive. Urolithiasis is sometimes seen in older patients with distal RTA.

A lowered serum bicarbonate, with hyperchloremia and a normal anion gap, is characteristic. Patients seldom have an HCO_3^- over 19 mEq/L, but values are usually over 10 mEq/L. The serum bicarbonate is the most reliable indicator, as the pH and pCO_2 may change in a crying infant. Relative alkalinity of the urine is often the key to the diagnosis. In distal RTA, the urine pH remains high, and net excretion of acid is low even when the serum bicarbonate is low. In some patients, it may not be readily evident that in an acidotic patient, a urinary pH of 6 may be relative alkalinity. Also, modern clinical chemistry laboratories have largely dispensed with pH meters to determine urinary pH, and dipstix may be less than precise.

Defective acidification of the urine leads to renal losses of sodium and potassium and polyuria. Bone resorption leads to hypercalciuria. The serum calcium is normal. Phosphaturia may lead to hypophosphatemia. Alkaline phosphatase may be increased. The excretion of citrate may be low, with hypercalciuria leading to nephrocalcinosis and nephrolithiasis. Renal ultrasonography is useful for early detection.

The distinction of the two major types of RTA, proximal RTA (sometimes called type II, mostly associated with a primary underlying disorder) and distal RTA (sometimes called type I), is best made by the assessment of urinary loss of bicarbonate and serum concentration during intravenous or oral loading with $NaHCO_3$ that causes a progressive increase in the serum level. This requires the collection of urine under oil and so maintained until analyzed. The fractional excretion of bicarbonate ($FEHCO_3$ %) is calculated as $UHCO_3 / UCreatinine(Cr) \times SCr / SHCO_3 \times 100$, where U and S indicate urine and serum, respectively. The percentage in patients with distal RTA is less than 10, whereas in proximal RTA, values of 10–15 % are seen when the serum level is 20 mEq/L, and there may be a massive bicarbonaturia, over 15 %. Patients with distal RTA and hyperkalemia may have values from 5 to 10 %, whereas those with classic hypokalemia usually have values less than 5 %. The hyperkalemic patients may represent a generalized tubular dysfunction and are sometimes referred to as type IV. These patients may have hypoaldosteronism, pseudohypoaldosteronism, or hyporeninemic hypoaldosteronism.

Some discrete syndromes are associated with RTA. Pyruvate carboxylase deficiency and methylmalonic aciduria are associated with proximal RTA. A mixed proximal and distal RTA is seen in patients with carbonic anhydrase deficiency in which there is a genetically determined syndrome of RTA, osteopetrosis, and cerebral calcifications. A mixed RTA is also seen in carnitine palmitoyltransferase I (CPTI) deficiency.

26.4.3 Bartter Syndrome

Bartter syndrome is an uncommon cause of salt wasting hypokalemic alkalosis in which calcium excretion is normal or increased. It is in this way distinguished from Gitelman syndrome, in which hypokalemic alkalosis is accompanied by hypocalciuria and hypomagnesemia.

Bartter syndrome (type 1) is caused by loss of function mutation in the Na–K–Cl cotransporter gene *SLC12A1*, previously called *NKCC2*, on chromosome 15q15–21.1, which codes for a protein that mediates electrolyte transport in the loop of Henle, at the site of action of the diuretics, furosemide, and bumetanide. Mutations in this gene have also been found in antenatal Bartter syndrome, in which ultrasonography has revealed fetal nephrocalcinosis in utero and polyhydramnios. Bartter syndrome is heterogeneous; patients with the typical syndrome have been found in whom the mutation was in the inwardly rectifying potassium channel, ROMK (*KCNJ1*), which recycles reabsorbed K^+ back into the tubule (type

2) and also causes antenatal Bartter syndrome. The classic syndrome as described by Bartter (type 3) is caused by mutations in the renal chloride channel B gene *CLCNKB*. Infantile Bartter syndrome with sensorineural deafness (type 4) may be caused by mutation in the *BSND* gene or by simultaneous mutations in both the *CLCNKA* and *CLCNKB* genes. Bartter syndrome has also been reported in patients who turned out to have mutations in the calcium-sensing receptor (*CASR*) gene, in which loss of function mutations usually leads to hypocalciuria and secondary hyperparathyroidism, whereas gain of function mutations leads to hypercalciuria and hypocalcemia. In Bartter syndrome, presentation is usually prior to 5 years of age. The initial presentation may be with dehydration and hypovolemia. Some patients present with cramps or weakness in the muscles resulting from the hypokalemia. Some have had convulsions or tetany. Chvostek and Trousseau signs may be positive. There may be polyuria, nocturia, or enuresis. A craving for salt is common. Some patients display vomiting, and constipation is common.

Shortness of stature or failure to thrive is the rule. Mental retardation has been observed in about two-thirds of the children. Some have had abnormal electroencephalography. Ileus has been observed and attacks of hypoventilation, both concomitants of hypokalemic alkalosis. Rickets has been reported rarely. Nephrocalcinosis has been observed in many, especially in those with the antenatal or infantile forms of Bartter syndrome.

Hypokalemia, alkalosis, hyperaldosteronism, and hyperreninemia are regular laboratory findings, as is normal blood pressure. Bartter and colleagues noted unresponsiveness of the blood pressure to intravenous angiotensin II and hypothesized that increased production of renin was a compensatory response to maintain blood pressure and that aldosterone production was also stimulated. Volume expansion can reduce levels of renin and aldosterone. Histologically there is hyperplasia of the renal juxtaglomerular apparatus. There is increased renal prostaglandin production. Large urinary losses of sodium and potassium lead to contraction of volume. Hypokalemia is usually profound. The concentration is less than 2.5 mEq/L. Patients are unable to form a concentrated urine, and this isosthenuria does not respond to administration of antidiuretic hormone.

Less common laboratory findings include hypomagnesemia, hypocalcemia, and glucose intolerance without fasting hyperglycemia. Some patients have hyperuricemia, and clinical gout has been observed.

The differential diagnosis of Bartter syndrome includes Gitelman syndrome (see below). It also includes primary hyperaldosteronism, a renin-producing tumor, and renal artery stenosis, all three of which are differentiated by the presence of hypertension. Chronic diarrhea, especially laxative abuse, and bulimia may mimic Bartter syndrome, as of course may chronic or surreptitious diuretic use.

The differential diagnosis of Bartter syndrome also includes chronic chloride diarrhea. Whole exome capture and massive parallel DNA sequencing led to an unanticipated diagnosis in a patient whose DNA was sent to confirm a clinical diagnosis of Bartter syndrome. The gene mutated was SLC26A3, the chloride diarrhea locus. Mutations were also found in five other patients in whom a diagnosis of Bartter syndrome was suspected, but mutations had not been found.

Remember

Both Bartter syndrome and Gitelman syndrome are characterized by renal loss of salt with hypokalemic alkalosis and hyponatremia. Bartter syndrome is clinically more severe with hypercalciuria and often nephrocalcinosis, failure to thrive, growth retardation, and muscle weakness. Clinical manifestation of the more frequent Gitelman syndrome is mild and often nonspecific; electrolyte changes resemble thiazide therapy.

26.4.4 Gitelman Syndrome

Gitelman syndrome is a disorder in which depletion of magnesium and potassium leads to

hypokalemic alkalosis. It was once thought to be a variant of Bartter syndrome, but it is caused by mutations in the thiazide-sensitive NaCl cotransporter gene on chromosome 16q13, referred to as *SLC12A3*, which codes for a transporter that mediates sodium and chloride reabsorption in the distal convoluted tubule. This transporter is the target of the thiazide diuretics used to treat hypertension. At least 17 different nonconservative mutations have been found.

Patients with this disorder present later than those with Bartter syndrome, often in adulthood, and without hypovolemia, but there are patients with findings that overlap both the syndromes.

Episodic muscle weakness has been one presentation. Tetany has been another, often precipitated by intercurrent infectious illness. Recurrent periodic paralysis has been reported. There may be paresthesias or carpopedal spasm. Patients have been described with a chronic dermatosis, in which the skin was thickened and had a purple-red hue. Erythema of the skin has been observed in experimental magnesium depletion in rats. Prolongation of the QT electrocardiographic interval is common; ventricular fibrillation is a rare complication.

In Gitelman syndrome, hypokalemic metabolic alkalosis is accompanied by hypomagnesemia and hypocalciuria. Renal wasting of potassium and magnesium is characteristic. There is an increased excretion of sodium, chloride, and magnesium in response to intravenous furosemide, as well as abolition of the hypocalciuria, consistent with a defect in transport in the distal tubule as opposed to the loop of Henle.

26.5 Investigation for Renal Tubular Dysfunction

Initial clinical chemistry is undertaken to determine serum values for Na, K, Cl, HCO_3, and pH. This will establish the presence or absence of acidosis and whether or not it is hyperchloremic. Other useful blood chemistry data include Ca, PO_4, Mg, alkaline phosphatase, urea, and creatinine.

The urine is studied to assess the presence of a Fanconi syndrome by analysis of glucose, amino acids, Ca, PO_4, and creatinine. Proteinuria or increased amount of retinol-binding protein or *N*-acetylglucosaminidase indicates tubular damage and proximal tubular leak. Decreased urinary concentrating ability may also indicate generalized tubular dysfunction.

In the presence of acidosis, the pH of urine is measured. For this purpose it is important to analyze promptly after passage. $NaHCO_3$ supplements should have been discontinued. In a patient suspected of having RTA in whom acidosis is not present, an ammonium chloride load may be useful (Table 26.8).

Table 26.8 Investigations for the analysis of renal tubular dysfunction

Initial clinical chemistry
Plasma: Na, K, C1, HCO_3, urea, creatinine, urate, Ca, Mg, PO_4, ionized Ca, alkaline phosphatase, parathyroid hormone
Urine: reducing substances, amino acids, tubular reabsorption of phosphate, Ca/creatinine ratio, albumin/creatinine ratio, retinol-binding protein/creatinine ratio, *N*-acetyl glucosaminidase/creatinine ratio
Further investigation aimed at a specific cause
Blood lactate and pyruvate
Pyruvate carboxylase leukocytes or fibroblasts
Leukocyte cysteine
Renal (liver) ultrasound
In the presence of hepatic dysfunction
Plasma: 1.25-dihydroxyvitamin D
Erythrocyte: galactose 1-phosphate uridyltransferase
Urine: organic acids for succinylacetone
DNA: common mutations for hereditary fructose intolerance and glycogenoses Ia, Ib
Plasma: ceruloplasmin, urine; plasma, copper
Roentgenograms: joints (rickets, osteomalacia)
Plasma: renin, aldosterone
Blood or muscle mtDNA
Blood acylcarnitine profile
Urine: toxicology
Muscle biopsy: electron transport chain activity
Liver biopsy: aldolase, GSD I, Fanconi–Bickel
Skin biopsy: phosphatidylinositol-4, 5-bisphosphate 5-phosphatase (Lowe syndrome)

General Suggestions for Reading

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Neurological Disease

27

Angels García-Cazorla, Nicole I. Wolf, Fanny Mochel, and Georg F. Hoffmann

Key Facts

- The correct diagnosis of inherited metabolic diseases that affect primarily the nervous system is a major challenge because the same neurological symptoms and often even disease course may be caused by non-metabolic disorders.
- Neurometabolic diseases often start with common and nonspecific signs, such as isolated developmental delay/mental retardation, seizures, dystonia, or ataxia. They are especially to be suspected when the course of the disease is progressive or when additional neurological systems or other organs become involved. An important clue is the coexistence of different neurological features that cannot be explained by a "simple" neuroanatomic approach.
- Acute or recurrent attacks of neurological manifestations such as coma, ataxia, or abnormal behavior are major presenting features especially in the late-onset inborn errors of metabolism (See also Chap. 18).
- The initial diagnostic approach to these disorders is based on a few metabolic screening tests. It is important that the biologic fluids are collected at the time of the acute attack. And always consider treatable disorders first when choosing your plan of investigations.

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Table 27.1 Signs and symptoms suggestive of a neurometabolic disorder

Positive family history, consanguinity
Unusual age and/of sequence of neurological symptoms
Slowing/plateau of development, regression
Progressive loss of hearing and/or vision
Simultaneous affection of different neurological systems
Suggestive neuroimaging findings
Involvement of other organs than the CNS

Remember (Table 27.1)

Metabolic investigations are usually not indicated in children with moderate static developmental delay; isolated delay of speech development in early childhood; occasional seizures, e.g., during fever; or well-defined epileptic syndromes responding to standard treatment. Other genetic etiologies outside the metabolic field have also to be considered, especially as causes of mental retardation, ataxia, dystonia, and spastic paraplegia.

27.1 General Remarks

Neurological disease can be considered the most common and important consequence of inherited metabolic diseases for three reasons. In building and maintaining structure and function of the brain, many more genes are involved as compared to other organs. Also, the brain seems to possess restricted capabilities for repair. As a consequence even slowly, both acute repeated and chronic insults, corresponding to the two most important patterns in inborn errors of metabolism, will result in lasting, often progressive neurological and in the end devastating disease. Finally, in the development of modern human societies, many physical disabilities from reduced vision or hearing, up to the loss of a limb or even an organ, will still allow relatively independent and unimpaired activities and life. On the contrary, mental abilities and capacities are being required at an ever-increasing level, and even marginal or specific partial inabilities can have profound negative effects on the status and well-being of an individual.

27.2 History and Neurological Examination

Family history, including possible consanguinity, and age at onset of symptoms are important aspects in the suspicion of a neurometabolic disorder. Five other aspects should be kept in mind:

1. *Multisystem involvement*: In patients with neurological diseases of undefined origin, searching for multisystem involvement can be very helpful. This includes special neurological functions, such as vision and hearing, as well as overall growth and physical development. The eye, liver, spleen, heart, kidney, skin, muscle, and the skeletal system are the most prominently affected organs in inborn errors of metabolism besides the nervous system.
2. *Stability/progression of the disease*: Some neurometabolic disorders classically lead to progressive neurodegenerative disease courses, e.g., due to organelle dysfunction in lysosomal and peroxisomal disorders and mitochondriopathies. However, many of them can present for a long time as static or nonprogressive neurological dysfunctions. Metabolic disorders affecting small molecules often show a stable neurological course. Examples include creatine deficiency syndromes, some defects of purine metabolism, and some CDG. The lack of long-term outcome series makes it often difficult to determine the static versus progressive nature of a particular disease. A recent example is the realization that succinyl semialdehyde dehydrogenase deficiency (4-hydroxybutyric aciduria) is, when following patients until older age, a slowly progressive condition (Lapalme-Remis et al. 2015), whereas the disease was traditionally considered as "static."
3. *Multi-neurological involvement*: When isolated neurological symptoms persist over time, the diagnosis of an inborn error of metabolism is less likely. Neurometabolic disorders tend to present with a mixed neurological symptomatology. However, some exceptions have to be kept in mind, including progressive dystonia without additional

symptoms in Segawa disease, isolated cerebellar ataxia in some forms of coenzyme Q_{10} deficiency and other complex lipid synthesis and remodeling defects, isolated mental retardation in creatine transporter defect, and benign forms of adenylosuccinate lyase deficiency, as well as in some patients with Sanfilippo disease, in particular type A. Isolated neurological symptoms are also more commonly observed in late-onset forms of neurometabolic disorders, such as hereditary spastic paraplegia in adults caused by mutations in *ABCD1* (adrenomyeloneuropathy), *CYP2U1* (SPG5), or *ALDH18A1*.

4. *Asymmetry of the symptoms*: Contrary to perceived wisdom, asymmetrical involvement does not argue against a neurometabolic diseases. Although the evolution of these disorders tends to result in generalized clinical manifestations, it is not uncommon to detect unilateral signs. Examples include unilateral dystonia in Segawa disease, Leigh disease, some cases of GLUT1 deficiency, focal status epilepticus in MELAS or Alpers disease, and unilateral tremor in atypical forms of glutaric aciduria type I.
5. *Fluctuation of symptoms*: In inborn errors of intermediate metabolism, symptoms often fluctuate. In neurotransmitter disorders with dopaminergic dysfunction, dystonia and oculogyric crises may worsen over the day and with fatigue and improve with rest; in GLUT-1 deficiency, symptoms like epilepsy, ataxia, dysarthria, and dyskinesia may worsen with fasting and exercise and improve after eating or rest; in urea cycle disorders, disturbed consciousness, hyperkinesia, and other symptoms may develop with intercurrent illnesses or fluctuate depending on protein intake.

27.3 Neurological Regression/ Deterioration

Progressive neurologic symptomatology and mental deterioration is commonly seen in inborn errors of metabolism. Therefore, it is helpful to take into consideration the age at onset, the presence or absence of epilepsy, other neurological signs such as ataxia or other movement disorders, and the coexistence of non-neurological symptoms, especially hepatosplenomegaly and bone, heart, kidney, or retinal involvement (Table 27.2). Altered metabolism of complex molecules, defects of energy metabolism, urea cycle defects, organic acidurias, and storage disorders are the main pathophysiological groups involved in neurological deterioration.

It is important to analyze the speed and slope of progression. In storage diseases, development usually slows down before reaching a plateau which precedes frank regression. The loss of acquired skills follows at a variable speed. In slowly progressive disorders, it is often difficult to determine whether there is true regression or simply a residual syndrome. Old family photographs or home videos may be helpful in making that distinction. Furthermore, cerebral imaging may evolve only slowly or even be normal, especially at the onset of symptoms. Organic acidurias, mitochondrial disorders, and defects of the urea cycle can manifest with acute and dramatic deterioration, with a plateau (or even partial recovery) later on. This is especially true for glutaric aciduria type I. Mitochondrial disorders may display acute deteriorations with regression, followed by partial recoveries. Over time, the course of these disorders is relentlessly downhill.

There are several non-metabolic disorders presenting with a pseudo-degenerative disease course. This is especially true for disorders from the autistic spectrum, as well as Rett syndrome in girls whose early development is usually normal, and deterioration may be rapid. Continuous spike waves during sleep (CSWS) are another differential diagnosis which is amenable to treatment. Sleep EEG studies must therefore be performed in all children with regression in addition to metabolic investigations, regardless whether seizures are present or not. Moreover, in other epileptic encephalopathies, the untreated epilepsy itself, if severe enough, can lead to regression mimicking a neurodegenerative disorder such as in Dravet or Lennox-Gastaut syndromes. Additional causes of motor and mental deterioration are side effects of antiepileptic drugs, especially valproate, intoxication by heavy

Table 27.2 Main causes of regression with additional leading symptoms

Additional leading symptom	Disease
Epilepsy	Late-infantile neuronal ceroid lipofuscinosis, Alpers disease, MELAS, MERRF, and other progressive myoclonus epilepsies
Ataxia	Late-infantile neuronal ceroid lipofuscinosis, GM_2 gangliosidosis, mitochondrial disorders, cerebrotendinous xanthomatosis, Refsum disease, Friedreich's ataxia, vitamin E-responsive ataxias
Dystonia	Glutaric aciduria type I, pterin disorders, Wilson disease, biotin-responsive basal ganglia disease, PKAN
Spasticity	Metachromatic leukodystrophy, adrenoleukodystrophy, Krabbe disease, and other leukodystrophies
Neuropathy	Metachromatic leukodystrophy, Krabbe disease, Refsum disease, Friedreich's ataxia, vitamin E-responsive ataxias, cerebrotendinous xanthomatosis, mitochondrial disorders
Retinopathy	Infantile and late-infantile neuronal ceroid lipofuscinoses, juvenile neuronal ceroid lipofuscinosis, PKAN, Refsum disease, mucolipidosis type IV, mitochondrial disorders
Cataract	Cerebrotendinous xanthomatosis, beta-mannosidosis, Fabry disease, mitochondrial disorders
Hepatosplenomegaly	Sandhoff disease, Gaucher disease, Niemann–Pick diseases, infantile form of Salla disease
Dysostosis multiplex	Mucopolysaccharidoses, oligosaccharidoses, GM_1 gangliosidosis

Table 27.3 Inborn errors of metabolism presenting with predominant psychiatric symptoms

Acute porphyria
Adrenoleukodystrophy
Cerebrotendinous xanthomatosis
GM2 gangliosidosis
Homocystinuria
Homocystein remethylation defects
Mitochondriopathies
Metachromatic leukodystrophy
Niemann–Pick C
Wilson disease
Urea cycle disorders

metal contamination (lead, mercury), and the Münchausen-by-proxy syndrome.

In adulthood neurometabolic disorders often present primarily with pure or prevailing psychiatric symptomatology. Often time lag to diagnosis and treatment is especially long (Table 27.3).

Remember

In the differential diagnosis of neurodegenerative disorders, age of onset, additional (leading) symptoms, and the dynamics of regression are important clues (Table 27.2).

Disease Info: *Metachromatic Leukodystrophy (MLD)*

The classical late-infantile form of this autosomal recessive disorder usually starts between 10 and 30 months of age with hypotonia or gait disturbance. Severe spasticity then develops, while tendon reflexes are decreased or absent reflecting polyneuropathy. CSF protein is elevated. Cognition remains relatively intact until late in the disease course and seizures are uncommon. Arylsulfatase A activity is severely impaired and the excretion of sulfatides increased. The latter can differentiate between true arylsulfatase A deficiency and a pseudodeficiency which occurs in 7–15 % of the general population. The juvenile and adult form present either as spastic ataxia or as frontotemporal dementia depending on the genotype. If the diagnosis is done before neurological symptoms develop or early in the disease course, hematopoietic stem cell transplantation can be a successful treatment, at least for some patients. MRI

shows involvement of the central and periventricular white matter with sparing of the subcortical areas; the corpus callosum is also involved (see Fig. 27.5c, d, Chap. 45).

Disease Info: *X-Linked Adrenoleukodystrophy (X-ALD)*

This X-chromosomal disorder is clinically characterized by two main phenotypes: adrenomyeloneuropathy (AMN) and the cerebral demyelinating form of X-ALD (cerebral ALD). Cerebral ALD only affects males and presents usually with a rapidly progressive inflammatory demyelination within the brain. Cerebral ALD results in severe cognitive and motor disability, a vegetative state within 2–5 years of clinical symptom onset and death thereafter. This phenotype is most common during childhood and adolescence, but up to 20 % of adult males initially presenting with AMN can develop cerebral ALD later in life. Neuroimaging reveals characteristic parieto-occipital white matter changes; see Fig. 27.5a, b, Chap. 45). Adrenocortical involvement occurs in about half of affected individuals. Conversely, the pathology of AMN, the most frequent phenotype of X-ALD in adults, is characterized by a non-inflammatory distal axonopathy involving mostly the long tracts of the spinal cord. AMN results in a progressive spastic paraplegia with the first symptoms developing in men between the age of 20 and 30 years. About 65 % of heterozygous women also develop symptoms of AMN by the age of 60 years. About 10 % of patients have isolated Addison's disease. Plasma very long-chain fatty acids (VLCFA) are elevated with an increased ratio of C26:C22 but may be normal in heterozygous women. Hematopoietic stem cell transplantation and autologous hematopoietic stem cell gene therapy are effective in arresting and even reversing neuro-inflammatory cerebral ALD in males, provided that the procedure is performed at an early inflammatory stage – i.e., before the onset of neurological symptoms. Genetic counseling is crucial in X-ALD. Whether Lorenzo's oil may lower the risk of developing the severe childhood form is still under debate.

Disease Info: *GM_2-Gangliosidosis*

The most common form of this autosomal recessive disorder, also called Tay–Sachs disease, is due to absent hexosaminidase A activity and starts in infancy with regression, acoustic startle reactions, and hypotonia. Spasticity, blindness, macrocephaly, and seizures develop later. A cherry-red spot in the macular region is almost invariably present. Sandhoff disease shows the same neurological symptoms but can also lead to liver involvement; hexosaminidase A and B activities are absent. The juvenile form of GM_2-gangliosidosis can present with ataxia, action myoclonus, dystonia, and dementia. Late-onset GM_2 gangliosidosis usually presents with lower motor neuron disease, cerebellar ataxia, or dystonia. In addition, psychiatric signs are observed in 20–40 % of patients and may precede motor signs for years. Cerebellar atrophy is a frequent neuroradiological finding in this form.

Disease Info: ***Niemann–Pick Disease Type C (NPC)***

About 95 % of patients with this autosomal recessive disorder have mutations in the NPC1 gene and the remainder in the NPC2 gene. Both genes encode for proteins important for intracellular cholesterol trafficking. Half of the patients have a cholestatic neonatal icterus which disappears spontaneously within the first 3 months of life. Some infants go on to develop severe cholestatic liver disease with impaired liver function; they do not yet display neurologic symptoms. Mental retardation and regression or initial normal development followed by regression in childhood is common. Vertical supranuclear (especially downward) gaze palsy is a typical symptom but is usually absent in young children. Laughing spells and cataplexy are common, epilepsy as well. Hepatosplenomegaly is not always present. If the disease starts later in childhood or in adulthood, cerebellar ataxia, slowly progressing dementia, and psychiatric symptoms are the leading symptoms. There is great clinical heterogeneity. In cultured fibroblasts, cholesterol esterification is defective, but this test may be normal especially in late-onset forms of the disease. Chitotriosidase activity is moderately increased and sea-blue histiocytes can be found in bone marrow aspirates. The significant elevation of two plasma oxysterols (cholestane-3β,5α,6β-triol and 7-ketocholesterol) is likely to become the most accessible biomarker to identify NPC patients in daily practice.

Disease Info: ***Neuronal Ceroid Lipofuscinoses (NCL)***

These disorders, all with autosomal recessive inheritance, show storage of autofluorescent material. There are several forms with onset from neonatal age until adulthood; the retina is involved in almost all of them. Infantile NCL (Santavuori–Haltia disease) is frequent in Finland but can also be found in other ethnic groups. Affected infants show an initially normal development, and regression becomes apparent at the end of the first year of life with ataxia, loss of vision, and myoclonus. EEG shows progressive slowing and in the later stages of the disease isoelectric tracing. The disease is due to absent activity of palmitoyl protein thioesterase 1 (CLN1). Mutations in the same gene can give rise to the juvenile form of the disease. Late-infantile NCL (Jansky–Bielschowsky disease) is caused by mutations in the gene coding for tripeptidyl-peptidase 1 (CLN2). The disease starts between 2 and 5 years of age with epilepsy, ataxia, and regression followed by vision loss and spasticity. Juvenile NCL (Batten disease) starts with vision loss due to retinitis pigmentosa around the age of 6 years. Regression starts thereafter; parkinsonism and epilepsy are additional symptoms. There is a common 1.02 kb deletion in the CLN3 gene coding for a membrane protein with palmitoyl protein Δ-9 desaturase activity. A useful and simple screening test is looking for lymphocyte vacuolizations in a peripheral blood smear. This should be done in every child with retinitis pigmentosa. CLN8 shows similar symptoms as the late-infantile form due to CLN2 mutations; it is caused by mutations in CLN8 which also lead to progressive epilepsy with mental retardation (EPMR, Northern epilepsy). There is also an adult-onset form of NCL, Kufs disease, caused by biallelic mutations in CLN6 or, in the dominant form, by mutations in DNAJC5.

Disease Info: ***Krabbe Disease***

The deficiency of β-galactocerebrosidase gives rise to this autosomal recessive disorder. Its infantile form starts around the age of 6 months with irritability, progressive stiffness, and opisthotonus. Later, infants become hypotonic because of neuropathy. CSF protein is elevated. Seizures are relatively common. Late-onset forms are extremely rare and manifest in adolescence and adulthood with predominantly spastic paraparesis or tetraparesis. A peripheral neuropathy, mostly demyelinating, is also observed in 60 % of patients. Less common symptoms include cerebellar ataxia (30 %), optic neuropathy, and cognitive decline. Brain MRI is characterized almost invariably by abnormal signal of the corticospinal tracts and the optic radiations.

Disease Info: ***Sanfilippo Disease***

This autosomal recessive disorder (mucopolysaccharidosis type III) is caused by mutations in four different genes coding for heparan N-sulfatase (type A), α-N-acetylglucosaminidase (type B), acetyl CoA/α-glucosaminide acetyltransferase (type C), and N-acetylglucosamine 6-sulfatase (type D). Mental retardation and insidious regression become apparent between 2 and 6 years of age. Coarse features may be absent or not very prominent. Quantitative determination of mucopolysaccharides in urine by glycosaminoglycanes electrophoresis should therefore be done in all children with mental retardation and/or regression. Screening and spot tests are likely to give false-negative results.

Disease Info: ***Infantile Neuroaxonal Dystrophy (INAD)***

This is a very rare neurodegenerative disorder which starts at the end of the first or in the second year of life after an initially normal development. First symptoms are stagnation of development, muscle hypotonia, and truncal ataxia. Nystagmus and further deterioration follow. Children develop spasticity, optic atrophy, and additional evidence of chronic denervation. Skin biopsy shows spheroid inclusions in nerve endings. Cerebral MRI is remarkable for T2 hyperintense cerebellar cortex; signal of globus pallidus may be hypointense (see Fig. 27.4h, i, Chap. 45). EEG displays fast rhythms (Fig. 27.1). A large proportion of cases have mutations in PLA2G6, a gene coding for a phospholipase A2, which plays an important role in the remodeling of membrane phospholipids.

27.4 Global Developmental Delay/Mental Retardation

Mental retardation is a frequently occurring condition, affecting up to 3 % of the general population, with a major impact on the life of the affected individuals, their family, and society. Establishing a diagnosis is challenging as the spectrum of possible etiologies is vast and the range of diagnostic investigations burdensome and costly. Although the hope that a specific diagnosis will lead to a rational therapy is mostly futile, reaching a diagnosis is beneficial by ending the diagnostic odyssey and providing knowledge about the short- and long-term prognosis, the possible comorbidities, recurrence risks, treatment options, as well as contact with other families. Often it is a great step toward the acceptance of the disability.

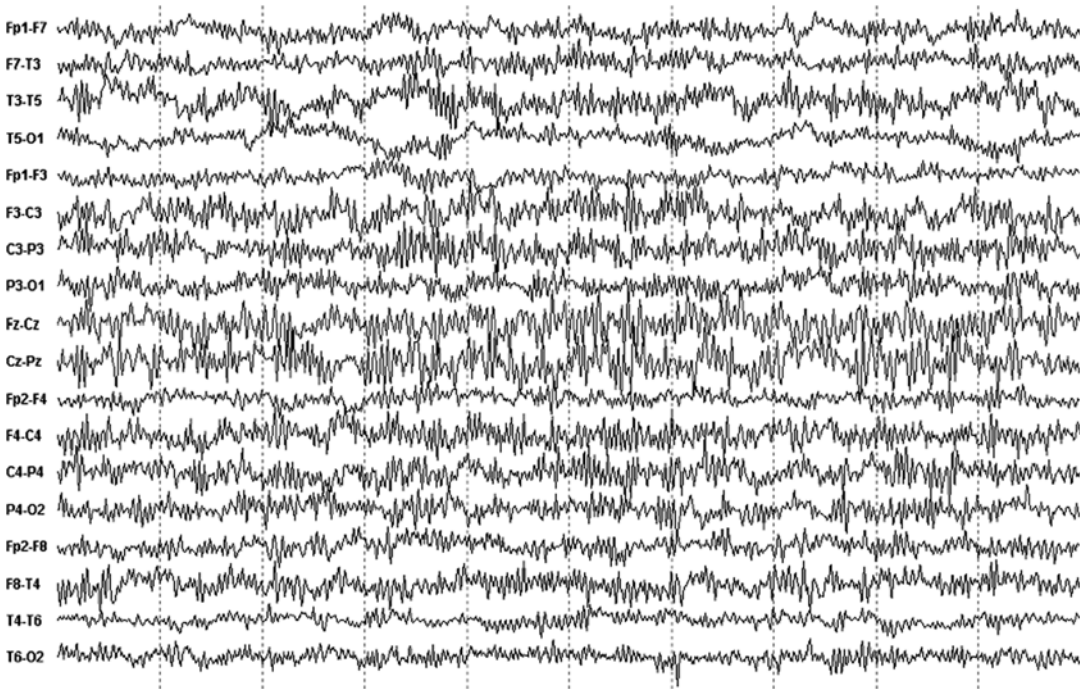

Fig. 27.1 Prominent general fast β-activity in this child with INAD during sleep stage II

Table 27.4 Inborn errors of metabolism that may present with primary mental retardation

Cobalamin disorders
Creatine deficiency syndromes
Defects of purine and pyrimidine metabolism
Fumaric aciduria
Homocystinuria
4-Hydroxybutyric aciduria
(Maternal) phenylketonuria
(Untreated) phenylketonuria
Mitochondriopathies
Mucopolysaccharidoses
Urea cycle disorders

There are as yet no guidelines available for the investigation of mental retardation established and tested in an evidence-based approach. Studies report the yield of different diagnostic approaches between 10 and 81 %. With regard to inborn errors of metabolism as a cause of mental retardation, there has been a heightened awareness in recent years as (i) there is an increasing number of disorders not included in even the most extended newborn screening programs, and (ii) most importantly, there is significant progress in successful therapies. The 2014 report of the American Academy of Pediatrics includes metabolic tests as a first-line investigation of mental retardation together with microarray and fragile X testing. Close to 100 metabolic disorders can result in mental retardation. Cumulatively they range between 1 and 5 % in studies delineating the etiology of mental retardation. Unfortunately, few studies are comparable with regard to parameters and disorders investigated. Especially, disorders possibly leading to nonspecific mental retardation (see Table 27.4) were mostly not investigated, even in the more recent studies. And there are new groups of metabolic disorders discovered with such a presentation, e.g., diverse GPI-anchor deficiencies (new category of CDG). Some of them are associated with hyperphosphatasia (Mabry syndrome) (Thompson et al. 2012).

What can be concluded is that inborn errors of metabolism are not a major but still important cause of isolated mental retardation. In inborn

errors additional neurological and/or somatic systems are often involved to guide the diagnostic process. They are elucidated in other sections of this chapter, e.g., neurodegeneration, movement disorders, etc. Nevertheless, inborn errors of metabolism remain responsible for ~2.5 % of nonspecific mental retardation even in countries offering extended newborn screening. Because of their high recurrence rate, the availability of prenatal diagnosis and, most importantly, specific treatment in a growing number of disorders – which in general can only prevent permanent handicap if initiated early – guidelines are needed regarding which metabolic tests to perform in patients with mental retardation.

The evaluation of a patient with mental retardation is typically multidisciplinary. Taking a good clinical history and performing a detailed clinical examination (including neurological and dysmorphological assessment) by a trained specialist remain the bases and must come before any laboratory testing. Auditory and visual capabilities must also be ascertained. Presently, chromosome microarray is replacing high-resolution chromosome testing as the first-line investigation in any child with mental retardation, even in the absence of dysmorphic features. Molecular testing for fragile X, *MECP2*, catch 22, and Angelman and/or Prader–Willi syndromes should be initiated using selection criteria, such as clinical checklists.

Neuroradiological studies display a high yield of relatively nonspecific brain abnormalities in patients with mental retardation, such as delayed myelination, but rarely allow establishing a specific diagnosis (see also Chap. 45). Therefore, it is debatable whether they should be routinely performed as they often involve invasive sedation. They have always to be initiated in the presence of specific symptoms such as abnormal head size, focal neurological findings, epilepsy, skin lesions, or neurodegeneration.

Basic metabolic studies should be included in the first "investigation round" since, in the absence of clues for other causes, the diagnostic yield is still sufficiently high (~2.5 % in an average population) to recommend testing (see Table 27.5). A special issue in testing for metabolic disorders is whether the disorder thought of is potentially treatable. Doing focused or sequential metabolic testing (i.e., based on results of basic tests) can increase the diagnostic yield up to 14 %. Next-generation sequencing such as whole exome sequencing is now entering into the diagnostic world. Metabolic investigations can and will nevertheless not be replaced in the foreseeable future. Indeed, the primary genomic information is still not exact, specific, and timely enough to allow skipping metabolic testing.

Certain relatively homogeneous populations can have a much higher yield of specific metabolic disorders, e.g., the Finnish or the Ashkenazi Jewish population. Parental consanguinity, loss of developmental milestones, and/or a previously affected sibling point to a monogenic disorder. In these circumstances, comprehensive metabolic evaluation should be initiated early together with neuroimaging, genetic, and ophthalmologic evaluation.

Remember

- Inborn errors of metabolism are rare but important causes of isolated nonspecific mental retardation.
- Treatable metabolic disorders should be included in the first round of diagnostic investigations.
- In metabolic disorders presenting with mental retardation, additional neurological and/or somatic systems can guide the diagnostic process.
- Doing a focused or sequential metabolic testing can increase the diagnostic yield up to 14 %.

27.5 Delayed Speech Development

Isolated delay in speech development is frequent in childhood. Per se, it does not pinpoint to a metabolic disorder and metabolic investigations are not warranted. If the speech delay is part of global developmental delay,

Table 27.5 Checklist of laboratory tests in mental retardation focusing on monogenic disorders

Basic laboratory tests in nonspecific mental retardation without dysmorphic features and additional neurological symptoms
Genetic analyses, e.g., chromosome microarray, consider fragile X syndrome
Basic laboratory tests (blood glucose, lactate, ammonia, acid–base status, blood counts, liver function tests, alkaline phosphatase, creatine kinase levels, uric acid)
Thyroid function including T3
Plasma/serum: quantitative amino acids, homocysteine, copper, ceruloplasmin, zinc
Biotinidase activity, if not included in newborn screening (dried blood spots)
Creatine metabolites (urine → creatine transporter deficiency)
Urine: simple tests, organic acids, oligosaccharides, sialic acid
Glycosaminoglycanes in urine (by electrophoresis → Sanfilippo disease)
Consider maternal phenylalanine
Consider purines and pyrimidines (urine)
Additional laboratory tests in mental retardation with neurological abnormalities
Consider additional genetic analyses, e.g., Rett syndrome, Angelman syndrome
Consider glycosylation disorders (CDG)
Consider vitamin-dependent diseases: thiamine deficiency
Consider 5-methyltetrahydrofolate (5-MTHF) in CSF (dihydrofolate reductase deficiency and cerebral folate transport deficiency)
Additional laboratory tests in mental retardation with dysmorphic features
Sterols, peroxisomal studies (very long-chain fatty acids, phytanic and pristanic acids, plasmalogens)
Transferrin isoelectric focusing for glycosylation studies (CDG)
Consider maternal phenylalanine
Psychomotor retardation and …
… progressive loss of skills or organomegaly: consider lysosomal disorders
… multisystem disorder: consider mitochondrial disorders peroxisomal disorders, glycosylation disorders (CDG)
… progressive myopia, dislocated eye lenses: measure total homocysteine
… abnormal hair: consider Menkes disease, argininosuccinic aciduria
… macrocephaly: check urinary organic acids (glutaric aciduria type I, Canavan disease), lysosomal disorders. MRI is recommended as hydrocephalus must be ruled out. Megalencephalic leukodystrophy with subcortical cysts can only be diagnosed by MRI
…microcephaly: defects of serine biosynthesis, defect of branched chain dehydrogenase kinase (low concentration of branched chain amino acids), defect of MFSD2A, (transporter required for omega-3 fatty acid transport in brain) (Guemez-Gamboa et al. 2015), defect of proline biosynthesis (PYCR2, pyrroline-E-carboxylate reductase 2 deficiency (Nakayama et al. 2015) that associates microcephaly and hypomyelination (see reference at the end of the article), asparaginase synthetase deficiency (Ruzzo et al. 2013)

Adapted from: Zschocke and Hoffmann (2011)

investigations should be performed as for mental retardation (see above). There are several disorders in which expressive speech is more profoundly affected than overall development. This is the case for creatine deficiency syndromes, 4-hydroxybutyric aciduria and adenylosuccinate lyase deficiency, but it may also be found in mitochondrial disorders, especially when associated with hearing impairment. It is important to evaluate cognitive abilities independently. Equally important is a thorough approach of therapists in order to enable assisted communication or to teach simple sign languages. Abnormal speech development may be seen in galactosemia despite treatment.

27.6 Deafness

Deafness can be caused by a great variety of genetic and environmental causes. Genetic factors account for at least half of all cases of

Table 27.6 Main causes syndromic deafness

Metabolic disorders and syndromic deafness	Other genetic syndromic deafness
Mitochondrial disorders Mutations in *MTTS1* and *MTRNR1*: non-syndromic deafness Mitochondrial DNA mutations (syndromic deafness): NARP, Pearson, Wolfram, MELAS, MERFF, Kearns–Sayre, MIDD, OPA1, DDP1 Vitamin disorders Biotinidase deficiency Riboflavin transporter 2 and 3 deficiency Lysosomal disorders Fabry disease Mucopolysaccharidoses Oligosaccharidoses Sphingolipidoses Peroxisomal disorders Peroxisome biogenesis disorder Refsum disease Purine disorders Phosphoribosylpyrophosphate deficiency	Pendred syndrome Sensorineural deafness, malformations of the inner ear and goiter. It is the most common form of syndromic deafness. Autosomal recessive. Mutations SLC26A4 gene Branchio-oto-renal syndrome Sensorineural or mixed. Branchial cleft fistulas, prehelical pits, deformities of the inner ear, dysplastic or polycystic kidneys Autosomal dominant. Mutations EYE 1 gene Waardenburg syndrome Sensorineural hearing loss, patches of cutaneous dyspigmentation, blue eyes, heterochromia, synophrys, lateral displacement of the inner canthi of the eyes. Genetically heterogeneous, at least eight loci involved Usher syndromes Sensorineural deafness with retinitis pigmentosa. Phenotypically and genetically heterogeneous. Autosomal dominant and recessive forms Jervell and Lange–Nielsen syndromes Sensorineural deafness and prolongation of the QT interval. Autosomal recessive mutations KVLQT1 potassium channel gene Alport syndrome Sensorineural deafness, progressive nephritis, ocular abnormalities. Mutations involving tissue-specific polypeptide subunits of collagen encoded by the COL4A genes. Autosomal recessive or X-linked

congenital deafness. In fact, about 400 independent genes have been identified as causes of hearing loss. Among them, 77–88 % are transmitted as autosomal recessive traits, 10–20 % as dominant, and 1–2 % as X-linked. In 20–30 % of cases, other associated clinical manifestations enable the diagnosis of a specific form of syndromic deafness. Metabolic disorders that exhibit deafness in variable forms (from profound deafness to variable degrees of hypacusis) belong commonly to this syndromic group (Table 27.6). Hearing loss in metabolic disorders is mostly sensorineural, progressive, symmetrical, and with a predominant involvement of high frequencies although, in advanced stages, all frequencies can be affected. In general, there is no specific medical treatment except in the early stage of biotinidase deficiency. Another treatable cause of deafness is Brown–Vialetto–van Laere syndrome, caused by a defect of the riboflavin transporter 2 and 3 encoded by *SLC52A2* and *SLC52A3*, respectively.

Mitochondrial dysfunction is the most important metabolic cause of deafness. Outer hair cells have a high ATP demand, do not divide, and receive poor, indirect metabolic support from Deiter cells, especially those at the basal coil, which are the most metabolically active. It is postulated that a drop in the level of ATP results in progressive ionic imbalances in both the outer hair cells and the stria vascularis, leading to cell injury and cell death. The great majority of the mitochondrial causes of deafness are represented by more than 50 nuclear genes involved in non-syndromic hearing loss. However, they are not included in our conventional classification of metabolic disorders. Several rare mutations in the mitochondrial *MTTS1* and *MTRNR1* genes can also cause non-syndromic hearing loss. By contrast, well-known mutations in the mitochondrial DNA, such as those associated with NARP, Pearson, MELAS, MERFF, or Kearns–Sayre syndromes, have multisystem involvement – varying degrees of myopathy, cardiomyopathy, and encephalopathy – in addition to hearing loss. In MIDD (maternally inherited diabetes and deafness), caused by mitochondrial DNA rearrangements,

neurosensory hearing loss is a very prominent component of the clinical picture. Diabetes is non-insulin dependent at onset but progresses to insulin dependency with age. Retinopathy, cardiomyopathy, myopathy, encephalopathy, and kidney disease are present in many patients resulting in a phenotypic overlap with MELAS syndrome. Cochlear implants have been reported to be effective in MELAS (mitochondrial encephalopathy, lactic acidosis, and stroke-like episodes), MIDD (maternally inherited diabetes and deafness), Kearns–Sayre, and CPEO (chronic progressive external ophthalmoplegia) syndromes but make MRI follow-up nearly impossible. Mutations in the *OPA1* gene, which encodes a dynamin-related GTPase involved in mitochondrial fusion, cristae organization, and control of apoptosis, have been linked to non-syndromic optic neuropathy transmitted as an autosomal-dominant trait. However, it can also be responsible for a syndromic association of sensorineural deafness, ataxia, axonal sensory-motor polyneuropathy, chronic progressive external ophthalmoplegia, and mitochondrial myopathy characterized by cytochrome c oxidase negative and ragged red fibers. Finally, DDP1 (deafness/dystonia peptide 1) is the product of a gene on Xq22, mutations which lead to the Mohr–Tranebjaerg syndrome characterized by early childhood-onset hearing loss followed by late-onset progressive dystonia or ataxia, visual impairment, and dementia.

Disease Info: ***Fabry Disease (See Also* Chap. 32*: Physical Abnormalities in Metabolic Diseases)***

Both progressive hearing impairment and sudden deafness have been reported in this disorder. Hearing loss on high-tone frequencies has also been found on audiograms in Fabry patients with clinically "normal" audition. Furthermore, the incidence of hearing loss appeared significantly increased in Fabry patients with kidney failure or cerebrovascular lesions.

The origin and mechanisms of deafness probably involve the inner ear. Neuropathologic studies have disclosed evidence of glycosphingolipids accumulation in the ear in vascular endothelial cells and in various ganglion cells. Sudden deafness could also be caused by vascular mechanisms due to the accumulation of glycosphingolipids within lysosomes of endothelial and smooth muscle cells leading to progressive narrowing, ischemia, and frank occlusion in the vessels feeding the cochlea. The diagnosis is made by enzymatic studies (α-galactosidase) in plasma, serum, leukocytes, or fibroblasts. Enzyme replacement therapy is available.

Besides Fabry disease, many lysosomal disorders can exhibit hypacusis or deafness. In mucopolysaccharidoses, especially type I (MPS I) and Morquio syndrome (MPS IV), otitis media with mixed hearing loss are common. Although audition can be preserved at initial stages of the disease, hearing loss tends to progress over time from mild/moderate to deafness. In a mouse model of MPS I, cells with lysosomal storage vacuoles were observed in spiral ligament, spiral prominence, spiral limbus, basilar membrane, epithelial and mesothelial cells of Reissner's membrane, endothelial cells of vessels, and some ganglion cells. The organ of Corti also disappeared over time arguing for early therapeutic intervention. Furthermore, sphingolipidoses, such as Krabbe disease, and oligosaccharidoses, such as mannosidoses, have a high incidence of profound neurosensorial deafness.

Perceptive deafness, either congenital or as progressive hearing loss, has been reported as a common symptom of peroxisome biogenesis disorders such as Zellweger syndrome and variants, as well as Refsum disease. In adult-onset forms of peroxisomal disorders, hypoacousia may precede motor dysfunction for years. Accordingly, the metabolic exploration of deafness should always include VLCFA, phytanic, and pristanic acids.

Phosphoribosylpyrophosphate deficiency is a purine disorder characterized in childhood by a variety of neurological deficits, including inability to walk or talk, abnormal facies, and sometimes inherited nerve deafness. In early adulthood it gives rise to gout or stones. PRPS deficiency may also manifest in the carrier female and should be suspected in any seemingly X-linked defect where the mother presents with gout or hyperuricemia and may be deaf. Both complete and "partial" defects can present as acute renal failure in infancy.

Remember

- Deafness in metabolic disorders is commonly neurosensorial, symmetrical, progressive, and syndromic (associated to other clinical signs).
- Mitochondrial disorders are the most frequent cause of deafness in metabolic diseases.
- Transmission or mixed hypacusis is observed in muco- or oligosaccharidoses.
- Consider always the possibility of biotinidase deficiency and the Brown–Vialetto–van Laere syndrome because of the therapeutic possibilities.

27.7 Epilepsy

Epilepsy is an important sign of many metabolic disorders, although metabolic disorders are rarely the underlying cause of epilepsy. Convulsions are considered to reflect gray matter involvement in neurodegenerative disorders but may also be prominent in acute metabolic decompensation. For the most part, the semiology of seizures or EEG patterns does not depend on the underlying disorder but is rather determined by age at presentation. However, seizures in metabolic disorders are more often focal than generalized. In most metabolic disorders, the treatment of seizures is guided by their semiology and the age of onset and requires conventional antiepileptic drugs. Specific therapy – mostly cofactors as in biotinidase deficiency but also dietary treatment as in phenylketonuria – only exists for few metabolic disorders. In such diseases, treatment must be started early in order to avoid chronic neurologic damage. Therefore, it is of utmost importance to think about metabolic causes of epilepsy early in the course of presentation, especially in neonates and infants, in order to provide adequate treatment.

In otherwise healthy children with febrile seizures or clear-cut epileptic syndromes responding promptly to adequate antiepileptic treatment, metabolic investigations are not warranted. In children with cryptogenic, difficult-to-treat epilepsy, metabolic investigations should be initiated. However, it is important to correctly classify the epileptic syndromes and to consider other underlying disorders, such as focal cortical dysplasia, which might be difficult to find even with advanced MRI technology or other genetic disorders such as channelopathies or mutations in genes like *ARX* or *CDKL5* instead of repeating or expanding metabolic testing, especially as a correct diagnosis of some channelopathies has consequences for the choice of antiepileptic treatment.

One of the most important aspects in the differential diagnosis of epilepsy caused by metabolic disorders is the age of onset of seizures. Diagnoses to consider in a neonate are completely different from possible diagnoses in a school-aged child. Exceptions are mitochondrial disorders in which epilepsy can start at any age. It is also important to look for other symptoms, such as movement disorders or cognitive impairment. EEG features are sometimes of help but usually nonspecific (see below).

27.7.1 Neonatal Period

The great majority of vitamin-responsive epilepsies start in the neonatal period or early infancy (Table 27.7). Although rare, they are treatable disorders. Seizures consist of a mixture of partial, massive myoclonus, erratic myoclonus of the face and extremities, or sometimes tonic seizures. If myoclonic seizures dominate the clinical pattern, the epilepsy syndrome

Table 27.7 Epileptic encephalopathies presenting in the neonatal period

	Treatment	Diagnostic test (in addition to molecular studies)
Treatable disorders		
Pyridoxine dependency	Pyridoxine (or pyridoxal-5-phosphate) 30 mg/kg (usually 100 mg) as starting dose, then 30 mg/kg/day	Response to pyridoxine; elevated pipecolic acid (CSF, plasma, urine) and α-aminoadipic semialdehyde (urine)
Folinic acid dependency	Folinic acid 2–3 mg/kg/day in 3 doses	Response to folinic acid; elevated pipecolic acid (CSF, plasma, urine) and α-aminoadipic semialdehyde (urine); unknown peak in CSF HPLC
PNPO deficiency	Pyridoxal-5-phosphate 30–40 mg/kg/day in 3–4 single doses	Response to pyridoxal 5-phosphate; elevated glycine, threonine, 3-orthomethyldopa, lactate
Diverse GPI-anchor deficiencies (new category of CDG)[a]	May respond to pyridoxine	Hyperphosphatasia in some (MABRY syndrome)
Phosphoserine aminotransferase deficiency	Serine supplementation	Low CSF and plasma serine and glycine
Urea cycle defects	Appropriate treatment	Hyperammonemia, plasma amino acids, urine orotic acid
Organic acidurias	Appropriate treatment	Urinary organic acids, acylcarnitines
Holocarboxylase synthetase deficiency	High-dose biotin	Organic acids
Maple syrup urine disease	Appropriate treatment	Plasma amino acids
Not treatable disorders		
Nonketotic hyperglycinemia		Elevated CSF: plasma glycine ratio
Zellweger syndrome		Elevated VLCFA, phytanic and pristanic acids
Neonatal adrenoleukodystrophy		Elevated VLCFA, phytanic and pristanic acids
Molybdenum cofactor disease		Sulfite test in fresh urine, fibroblast studies, uric acid
Sulfite oxidase deficiency		Sulfite test in fresh urine, fibroblast studies
GABA transaminase deficiency		GABA in CSF
Adenylosuccinate lyase deficiency		Modified Bratton–Marshall test, purines in urine
Respiratory chain disorders and PDHc deficiency		Lactate elevation in CSF, plasma and urine, activity of respiratory chain enzymes and PDHc in muscle and fibroblasts
Glutamate transporter deficiency		Glutamate oxidation in fibroblasts, genetic studies (sequencing of *SLC25A22*)
Congenital glutamine deficiency		Extremely low levels of plasma, urine, and CSF glutamine
Neonatal form of neuronal ceroid lipofuscinosis		Cathepsin D activity
Asparagine synthetase deficiency[a]		No biomarker. ASNS gene
Hyperprolinemia due to *SLC25A22* mutations		High plasma proline, low glutamate in the CSF, accumulation of lipids in fibroblasts

is called "early myoclonic encephalopathy" (EME). EEG often shows a burst-suppression pattern. Myoclonic jerks may be without EEG equivalent. Among the non-treatable disorders, nonketotic hyperglycinemia is the most frequent. In many cases, the etiology remains unclear despite adequate investigations. In Table 27.7, treatable and non-treatable epileptic encephalopathies starting in the neonatal period are summarized.

Disease Info: *Pyridoxine-Dependent Seizures (PDS)*

First described in 1954, the genetic basis of pyridoxine (B_6)-dependent seizures was discovered in 2006. Autosomal recessive mutations in *ALDH7A1*-encoding antiquitin, which has α-aminoadipic semialdehyde dehydrogenase activity, lead to accumulation of α-aminoadipic semialdehyde and Δ1-piperideine-6-carboxylate. The latter metabolite inactivates pyridoxal-5′-phosphate which is involved as an essential cofactor in many enzymatic reactions including neurotransmitter metabolism. The classical form of PDS starts within the neonatal period with therapy-resistant seizures, especially myoclonic, but also tonic seizures. Administration of pyridoxine usually has an immediate positive effect; children may become apneic. Atypical forms of PDS start later in infancy or show partial effect of conventional antiepileptic drugs. In some children the response to pyridoxine may initially not occur, and some respond to folinic acid instead. There is no universal protocol for a pyridoxine trial (see below). The disorder can now be unequivocally diagnosed by measuring α-aminoadipic semialdehyde and pipecolic acid, which are elevated in urine and plasma even under treatment. Psychomotor development is better the earlier pyridoxine treatment is started, but even with adequate and immediate treatment outcome is mostly not entirely normal.

Disease Info: *Pyridoxal-Phosphate-Dependent Seizures*

Autosomal recessive mutations in the *PNPO* gene-encoding pyridox(am)ine 5-phosphate oxidase lead to decreased levels of pyridoxal phosphate (PLP), the active form of vitamin B_6, in the CNS. The activity of different enzymes dependent of PLP is thus reduced, leading to disturbed metabolism of neurotransmitters and amino acids among others. The measurement of these metabolites often, but not invariably, gives abnormal results, with typically increased 3-methoxytyrosine, glycine, threonine, as well as lactate and decreased homovanillic and 5-hydroxyindolacetic acids in CSF. The latter constellation mimics aromatic L-amino acid decarboxylase deficiency. Affected neonates are often born prematurely with signs of fetal or neonatal distress. Seizures start early within the first days of life and are resistant to therapy. They respond promptly to administration of PLP (30–60 mg pyridoxal 5′-phosphate/kg b.w./d) which needs to be given at least three times per day in order to prevent the recurrence of seizures before the next dose. Some children with PNPO deficiency respond to pyridoxine instead and may even deteriorate on PLP. If the treatment is not initiated, the disorder leads to severe neurological deficits and is lethal. If the treatment is started in the neonatal period, children may survive even without neurological symptoms.

Disease Info: *Nonketotic Hyperglycinemia (NKH)*

NKH is caused by impaired function of the glycine cleavage system, a multienzyme complex, with the P protein subunit being the most frequently affected. Most patients suffer from the classical form starting in the first days of life with seizures including

singultus, lethargy progressing to coma, and apnea requiring artificial ventilation. Glycine concentrations in CSF are highly elevated especially in relation to its concentration in plasma (ratio >0.08, variants 0.02–0.08). EEG shows a burst-suppression pattern. If children are ventilated, they may survive, but prognosis regarding the neurologic outcome is extremely poor. Development is virtually absent, and refractory epilepsy often persists even under treatment with sodium benzoate which lowers glycine levels in plasma but also in CSF and can be combined with dietary restriction of glycine. Finally, it is important that treatment with sodium benzoate lowers glycine levels into the normal range, best ≤250 μmol/l, which often requires high doses.

27.7.2 Treatment Protocol in Neonates

Pyridoxal phosphate can be used as a first-line treatment as it stops both pyridoxine- and pyridoxal phosphate-dependent seizures, although recent descriptions of patients with PNPO deficiency responding to pyridoxine and not to pyridoxal phosphate argue for a reconsideration of pyridoxine as first-line treatment. The recommended dose is 30–60 mg/kg/day in at least three doses. To evaluate efficacy, therapy should be maintained for 5 days. CSF (and urine) studies should be ideally performed before treatment in order to have a biochemical marker, but should not delay treatment. If therapy is successful, the appropriate genetic studies should be performed as well.

If pyridoxal phosphate is not available, pyridoxine (B_6) should be used first: 100 mg in a neonate (or 30 mg/kg) either intravenously or orally in a single dose, preferably with EEG monitoring. High doses may be necessary to control seizures, at least initially. In classical cases, we suggest a starting dose of 100 mg intravenously. If there is no response within 24 h, the same dose should be repeated (and possibly increased up to 500 mg total) before excluding pyridoxine responsiveness. If there is uncertainty about a partial response, pyridoxine should be continued at 30 mg/kg/day for 7 days before final conclusions are drawn. The use of vitamins does not preclude the introduction of other drugs during this period of time if seizures do not stop. The possibility of late-onset pyridoxine-dependent seizures in children up to 3–4 years of age should be considered and pyridoxine be tried in this constellation of symptoms. Folinic acid is effective for the extremely rare folinic acid-dependent seizures at a dose of 2–3 mg/kg/day, which appeared to be due also to mutations in *ALDH7A1*-encoding antiquitin. It is recommended to maintain the treatment for at least 1 month to test efficacy. If biotinidase cannot be ruled out quickly, e.g., with newborn screening, biotin 10–100 mg/day can be administered as well. Urinary organic acids should be analyzed before starting treatment. Plasma biotinidase activity will remain diagnostic despite therapy.

Remember

In neonatal-onset epileptic encephalopathy, results of pending metabolic investigations must not delay treatment initiation. Every neonate with seizures should have a trial with pyridoxine, pyridoxal phosphate, and folinic acid if no other cause of seizures is evident.

27.7.3 Infancy

In infancy, several disorders present with epileptic encephalopathy, among them are untreated PKU – which has virtually disappeared in many countries with newborn screening – biotinidase deficiency, GLUT1 deficiency, infantile neuronal ceroid lipofuscinosis (CLN1), GAMT deficiency as well as creatine transporter deficiency, and Menkes disease. Late-onset forms of pyridoxine dependency may start during infancy and early childhood. In these disorders, seizures are (partially) resistant to treatment with conventional antiepileptic drugs. Many disorders that cause neonatal seizures can also start beyond the neonatal period and during early childhood.

Disease Info: *GLUT1 Deficiency*

Glucose enters the brain by crossing the blood–brain barrier. Its uptake into the brain is mediated by the glucose transporter type 1 (GLUT1). GLUT1 deficiency is characterized by persistent hypoglycorrhachia (a low CSF glucose <2.7 mmol/l and/or glucose CSF/serum quotient <0.45) in the absence of hypoglycemia and with normal or low lactate and alanine in CSF. The absolute CSF glucose level is more sensitive than the CSF to plasma ratio and below the tenth percentile for age in all patients. Mutation analysis of *SLC2A1*-encoding GLUT1 or measurement of erythrocyte uptake of 3-O-methyl-D-glucose confirms the diagnosis. Mutations are usually de novo, but familial cases have been described. Epilepsy is a core feature of the disease, affecting 80–90 % of the patients and usually starting during the first year of life. About 70 % of the patients have mixed types of seizures, generalized tonic–clonic seizures and absence seizures being the most common. In the classical form of the disease, other manifestations include acquired microcephaly, developmental delay, pyramidal signs, and a complex movement disorder with dystonia, ataxia, and spasticity. Milder forms of the disease include a broad phenotypic spectrum with or without mild mental retardation. *SCL2A1* mutations have been found in patients with paroxysmal exercise-induced dyskinesia, paroxysmal non-kinesigenic dyskinesia, and "idiopathic" generalized epilepsy. Overall, it seems that the epileptic manifestations improve with age whether paroxysmal dyskinesia worsen. GLUT1 deficiency can be treated with ketogenic diets, which are especially efficient on seizures control.

Disease Info: *Creatine Deficiency Syndromes*

Creatine is crucial for energy metabolism and is synthesized by a two-step process: the first is mediated by AGAT (arginine-glycine amidinotransferase) and the second by GAMT (guanidinoacetate methyltransferase). Furthermore, the creatine transporter (CRTR) encoded by an X-chromosomal gene is required for creatine uptake into the brain and muscle. Disorders of creatine synthesis and transport lead to psychomotor delay and epilepsy, which is frequently refractory to conventional antiepileptic drugs. In all these disorders, brain MRS (spectroscopy) displays a severe decrease or absence of the creatine peak in vivo. Disorders of creatine metabolism can be reliably diagnosed by the analysis of guanidino compounds and creatine in urine, plasma, or CSF. In GAMT deficiency, guanidinoacetate and other guanidino compounds are elevated, while they are rather decreased in AGAT deficiency. In CRTR deficiency, the creatine/creatinine ratio is elevated in urine. Refractory epilepsy is especially prominent in GAMT deficiency and may resemble West syndrome. The disease can be treated by creatine supplementation and a diet enriched in ornithine but restricted in arginine in order to lower the guanidino compounds that seem toxic for the CNS. The diet is efficient for seizure control even when started late in the course of the disease. In AGAT deficiency, creatine must be supplemented. There is no specific treatment for CRTR deficiency.

Disease Info: *Biotinidase Deficiency*

Epilepsy often starts at 3 or 4 months of life in this autosomal recessive disease. West syndrome is the most frequent epileptic

syndrome, and conventional antiepileptic drugs are only partially effective. Alopecia and (perioral) dermatitis are useful clinical clues. Psychomotor development is severely delayed, and, if untreated, optic atrophy and deafness develop. Lactate is mostly elevated as well as 3-methylcrotonylglycine, 3-hydroxyisovaleric, 3-hydroxypropionic, and 2-methylcitric acid in urine and other body fluids. Biotin (5–10 mg/day) stops the seizures and reverses cutaneous symptoms. The diagnosis is easily made by measuring biotinidase activity, which can be performed on dried blood spots.

Disease Info: ***Menkes Disease***

This is an X-linked condition which starts in early infancy. The neonatal period is usually normal. The first symptom is often epilepsy with refractory focal seizures, up to focal status epilepticus or West syndrome. Development is severely delayed with profound muscle hypotonia. Spasticity develops later. Hairs are abnormally brittle and steely with a microscopic aspect of pili torti. Plasma copper and ceruloplasmin are low. The genetic defect involves a copper-transporting ATPase (*ATP7A*). Copper deficiency also impairs other structures, especially

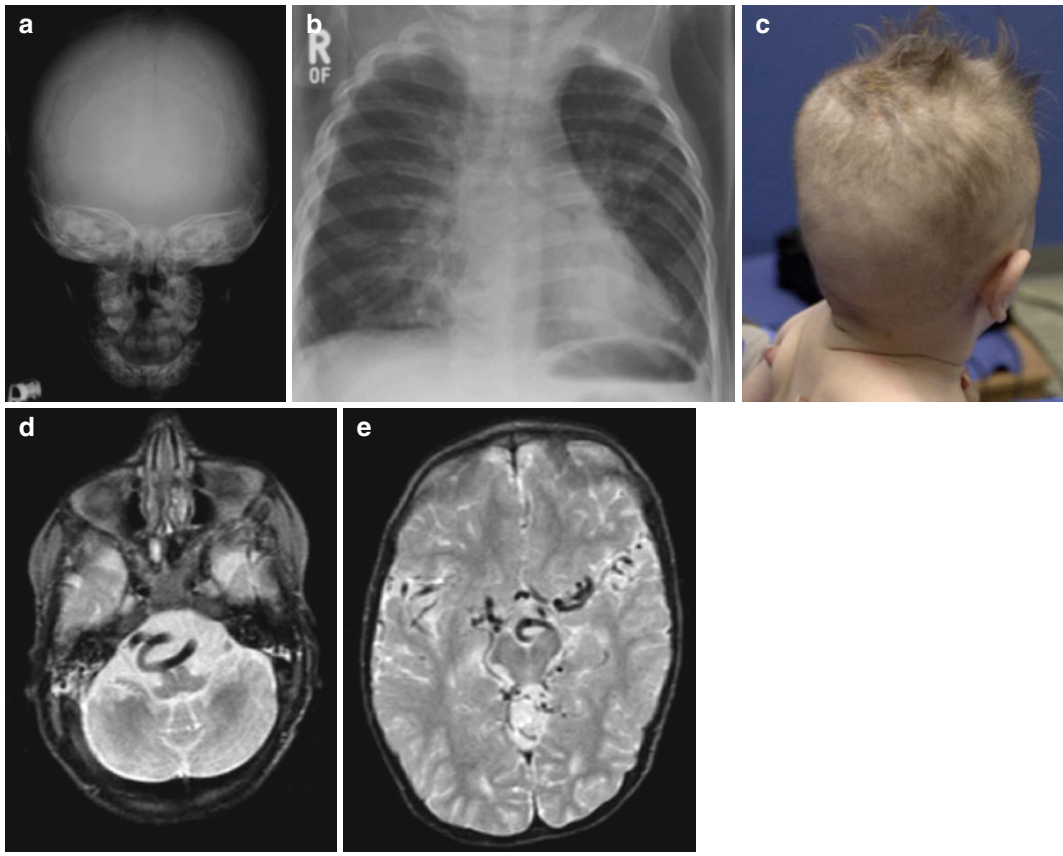

Fig. 27.2 Menkes disease. In this infant with Menkes disease, Wormian bones in the lambda and sagittal sutures are seen (**a**). (**b**) Depicts a serial rib fracture of ribs 4–8 on the right, another frequent finding. Hairs are steely and fragile and were never cut (**c**). Brain MRI at the age of 4 years shows tortuous and enlarged vessels on axial T2w images (**d**, **e**)

bones and connective tissue. Bone fractures and subdural hematomas sometimes lead to the diagnosis of non-accidental injuries. On cranial MRI, cerebral vessels show increased tortuosity (Fig. 27.2). Treatment consists of subcutaneous copper–histidine, but its effects are only marginal if started at the symptomatic phase.

27.7.4 Childhood and Adolescence

In this age group, late-infantile (CLN2) and juvenile (CLN3) forms of neuronal ceroid lipofuscinoses (CLN) as well as mitochondrial disorders are the most common neurometabolic conditions with prominent seizures. If brain MRI shows stroke-like lesions in the occipital or temporal regions, a mitochondrial disease is the most likely

Disease Info: ***Late Infantile Neuronal Ceroid Lipofuscinosis (CLN2, Jansky–Bielschowsky)***

This autosomal recessive disorder is caused by mutations in the gene coding for the lysosomal enzyme tripeptidyl-peptidase 1 (TPP1). The disease starts between the age of 2 and 5 years, usually with progressive ataxia and epilepsy. Generalized tonic–clonic seizures are frequent in the beginning; later on myoclonic and focal clonic seizures are seen. Epilepsy is difficult to treat, especially myoclonus. Dementia proceeds quickly. The retina is also involved with a pigmentary retinopathy leading to vision loss. EEG shows background changes and spikes with slow photic stimulation, which can trigger seizures (Fig. 27.3). Brain MRI displays cerebellar and to a lesser extent supratentorial atrophy and mild white matter changes.

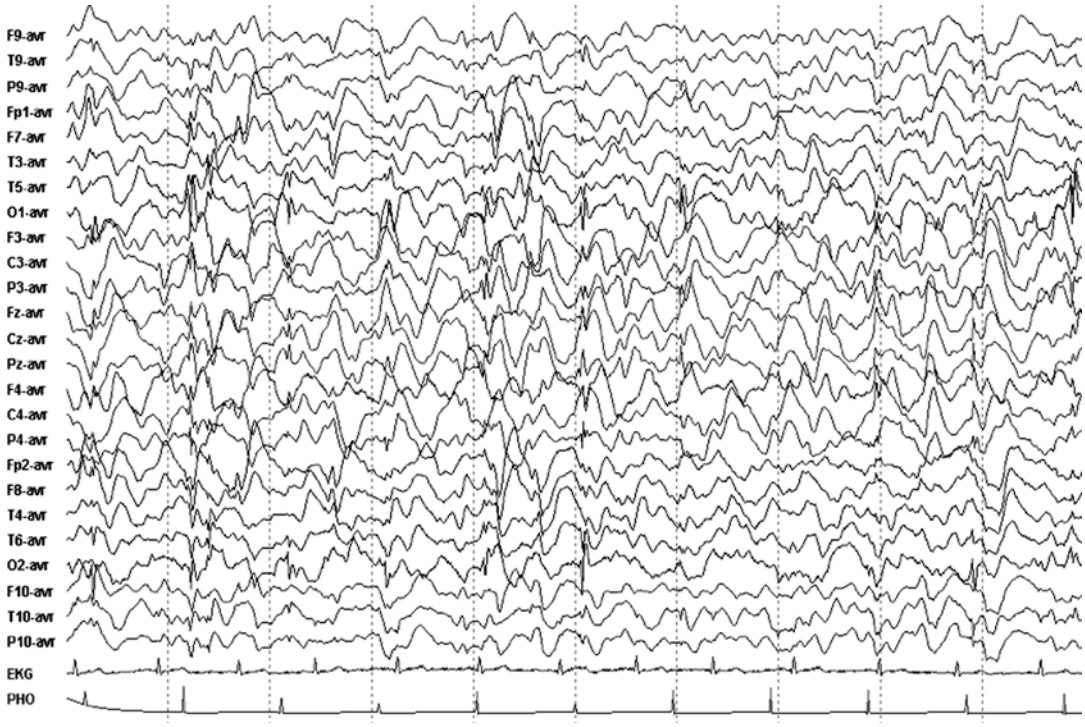

Fig. 27.3 Slow photic stimulation triggers posterior spikes in a child with late-infantile neuronal ceroid lipofuscinosis

Disease Info: *Alpers Disease*

This autosomal recessive disorder can start from infancy to young adulthood, but most frequently in childhood. First symptom is often refractory status epilepticus in a previously healthy child with normal or almost normal development. The EEG shows rhythmic high-amplitude delta with superimposed (poly)spikes (RHADS) involving either left or right posterior region (see Fig. 27.4). Liver function is usually normal then. If children survive status epilepticus, most of them develop a relentlessly downhill course with refractory seizures – especially epilepsia partialis continua – optic atrophy, and dementia. Some children have a more stable course with an almost complete recovery after status epilepticus. Administration of valproic acid almost invariably triggers liver failure, but liver failure can also develop without valproic acid. Neurological outcome is extremely poor and survival short, even when a liver transplantation is performed. The most frequent cause of Alpers syndrome are recessive mutations in polymerase γ 1 (*POLG1*), an enzyme involved in mtDNA maintenance. Accordingly, mtDNA is often depleted in the liver. In some patients, lactate and protein are elevated in CSF; in others, all investigations – including the activity of respiratory chain enzymes in the muscle – remain repeatedly normal.

diagnosis. Alpers disease and MELAS may manifest with refractory status epilepticus in a previously normal child. Antiepileptic drugs should be chosen according to the epileptic syndrome with the exception of valproic acid that should be avoided in mitochondrial disease, especially in Alpers disease due to a high risk of fatal liver failure.

Progressive myoclonic epilepsy is a group of genetic disorders in which myoclonic and generalized seizures appear in combination with

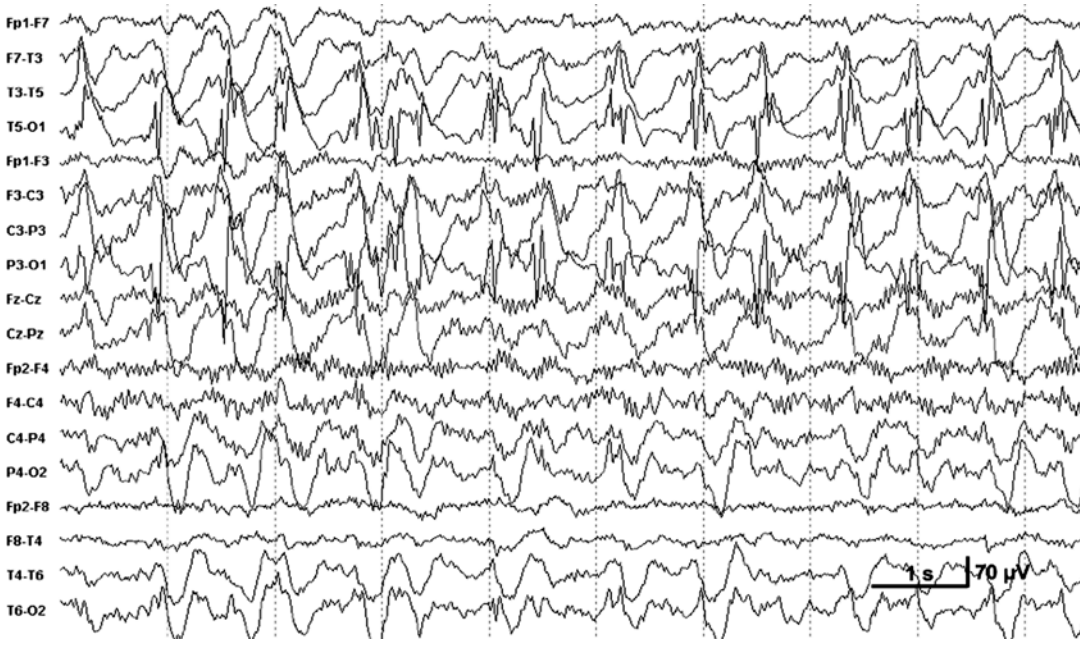

Fig. 27.4 RHADS over the left posterior region in a child with Alpers disease with compound heterozygous mutations in *POLG1*

cognitive decline and usually ataxia. Myoclonus is often exacerbated by external stimuli, such as light, sound, or touch, and they are very difficult to treat. This group of disorders comprises Unverricht–Lundborg disease (mutations in cystatin B, *CSTB*, formerly known as EPM1), Lafora disease (80 % of patients carry mutations in *EPM2A*, laforin; another gene involved: *EPM2B* or *NHLRC1*), myoclonic epilepsy with ragged red fibers (MERRF; mutations in *MTTK* which is located in the mtDNA), some forms of neuronal ceroid lipofuscinoses (the most frequent being late-infantile NCL), sialidosis (due to neuraminidase deficiency, commonly associated with a cherry-red spot), gangliosidosis, Gaucher disease, dentatorubral–pallidoluysian atrophy (DRPLA), and the recently described clinical picture that associates progressive myoclonic epilepsy and spinal muscle atrophy due to ceramide accumulation linked to *ASAH1* mutations (previously described as Farber disease) (Zhou et al. 2012).

Remember

Only a few metabolic epilepsies presenting beyond the neonatal period are amenable to metabolic interventions. In the majority of patients, conventional antiepileptic drugs must be used according to the seizure type. Valproic acid should be avoided in children with Alpers disease and other mitochondrial disorders as it can trigger fatal liver failure (Table 27.8).

27.8 Muscle Weakness

Several metabolic disorders can cause isolated or predominant muscle weakness. Important treatable disorders are Pompe disease (due to *a*-glucosidase deficiency), fatty acids oxidation defects (primary carnitine deficiency and multiple acyl-CoA dehydrogenase deficiency), and genetic defects of riboflavin transport (including Brown–Vialetto–van Laere syndrome). The latter can present in infancy with deteriorating head control and diaphragmatic paralysis, mimicking infantile myopathies. Ventilation may become necessary, but usually infants respond well to treatment with riboflavin. Mitochondrial disorders may also present with pure muscular involvement. An important differential diagnosis is the genetic myasthenic syndromes, as they are again amenable to specific therapies (see also Chap. 28).

27.9 Movement Disorders

Our understanding of movement disorders is rapidly evolving stimulated by progress in the discovery of monogenic disorders and the development of structured diagnostic and therapeutic approaches in the light of age-dependent pathophysiology. Next-generation sequencing studies and the recent description of a new category of inborn errors of metabolism, i.e., defects in the synthesis and remodeling of complex lipids, are challenging traditional classification systems and clinical phenotyping in movement disorders. Two specific phenotypes, spastic paraparesis and neurodegeneration with brain iron accumulation (NBIA), have importantly grown in the last few years. A series of investigations should be performed with the primary aim of an early diagnosis of treatable conditions.

27.9.1 Ataxia

Ataxia is defined as an inability to maintain normal posture and smoothness of movement, while force and sensation are intact. There are many variations in the clinical phenotype, ranging from findings of pure cerebellar dysfunction to mixed patterns of CNS (extrapyramidal pathways, brainstem, and/or cerebral cortical participation) or peripheral nervous system involvement. A wide range of molecular defects have been identified, among them are classical metabolic disorders, but also other neurogenetic defects. Autosomal recessive cerebellar ataxia with an age of onset between childhood to young adulthood is the most common type of ataxia. It is important to first consider treatable

Table 27.8 Checklist of laboratory tests in epilepsy focusing on monogenic disorders

Laboratory tests in epilepsy with neonatal onset
Basic laboratory tests (blood glucose, lactate, ammonia, acid–base status, calcium, magnesium, alkaline phosphatase)
Pipecolic acid and 5-aminoadipic semialdehyde (pyridoxine-dependent seizures)
CFS and plasma amino acids (nonketotic hyperglycinemia, serine biosynthesis defects, congenital glutamine deficiency, hyperprolinemia)
Homocysteine (MTHFR deficiency)
Organic acids (organic acidurias)
Neurotransmitters (PNPO deficiency)
CSF GABA (GABA transaminase deficiency)
Sulfite test (molybdenum cofactor deficiency and sulfite oxidase deficiency)
VLCFA (peroxisomal disorders)
Laboratory tests in epilepsy with infantile onset (<1 year)
Basic laboratory tests (blood glucose, lactate, ammonia, acid–base status, calcium, magnesium, alkaline phosphatase)
Copper and ceruloplasmin (Menkes disease)
Amino acids (PKU)
Homocysteine (MTHFR deficiency, cobalamin disorders)
Organic acids (organic acidurias, especially 2-methyl-3-hydroxybutyryl-CoA dehydrogenase (MHBD) deficiency)
Biotinidase activity
Creatine, creatinine and guanidinoacetate in urine (creatine synthesis defects and creatine transporter defect)
VLCFA (peroxisomal disorders)
Purines and pyrimidines in urine
Pipecolic acid and 5-AASA (pyridoxine-dependent seizures)
CSF and plasma glucose (GLUT1 deficiency)
CSF lactate (mitochondrial disorders), amino acids, and neurotransmitters
5-Methyltetrahydrofolate (5-MTHF) in CSF (dihydrofolate reductase deficiency and cerebral folate transport deficiency)
Consider lysosomal studies (e.g., CLN1, Tay–Sachs disease)
Laboratory tests in epilepsy with onset in late infancy, childhood, and adulthood (with usually additional signs and symptoms as ataxia or regression)
Basic laboratory tests (lactate, ammonia, acid–base status)
Copper and ceruloplasmin (Wilson disease)
Creatine, creatinine and guanidinoacetate in urine (creatine synthesis defects and creatine transporter defect)
Organic acids (organic acidurias, especially MHBD deficiency)
Purines and pyrimidines in urine
Homocysteine (Homocystinuria, MTHFR deficiency, cobalamine disorders)
Biotinidase activity
Cholestanol (cerebrotendinous xanthomatosis)
VLCFA (adrenoleukodystrophy)
Pipecolic acid and 5-AASA (pyridoxine-dependent seizures) for atypical late-onset cases, also consider therapeutic trial
CSF lactate (mitochondrial disorders)
CSF and plasma glucose (GLUT1 deficiency)
5-Methyltetrahydrofolate (5-MTHF) in CSF (MTHFR deficiency, dihydrofolate reductase deficiency and cerebral folate transport deficiency)
Lysosomal studies (e.g., CLN2, Gaucher disease type III)

VLCFA very long-chain fatty acids, *CLN* ceroid lipofuscinosis, neuronal

disorders – most of them partially treatable – for which ataxia is a prominent sign, including vitamin E deficiency, biotinidase deficiency, abetalipoproteinemia, thiamine-responsive pyruvate dehydrogenase deficiency, cerebrotendinous xanthomatosis, Refsum disease, GLUT-1 deficiency, Niemann–Pick type C, Hartnup disease, and coenzyme Q_{10} deficiency (Table 27.9). Ataxia can be classified according to their evolution mode: intermittent or episodic and stable or progressive.

27.9.1.1 Intermittent/Episodic Ataxias

These are mainly defects of intermediary metabolism. Ataxia can be a sign of acute or subacute decompensation of amino acid (especially MSUD) and organic acid disorders. Defects of energy metabolism presenting with intermittent ataxia may involve exclusively the nervous system and are often more difficult to diagnose. Lactate may be elevated only intermittently together with clinical symptoms. Intermittent ataxia can also be seen in Hartnup disease; additional symptoms are pellagra-like skin changes, photic dermatitis, and psychiatric symptoms. Carbohydrate-sensitive ataxia is a feature of mild pyruvate dehydrogenase deficiency and occurs only in boys. GLUT1 deficiency may present with isolated intermittent ataxia (or gait dyspraxia) mimicking other episodic ataxias. In such patients, ataxia is usually worse before meals. Some children improve greatly with frequent carbohydrate-rich snacks and do not need a ketogenic diet. Mild forms of biotinidase activity may also give rise to intermittent ataxia. The so-called episodic ataxias, which belong to the group of channelopathies, may be mistaken for metabolic disorders. Their correct diagnosis is important as effective treatment exists for these disorders too. Overall, since metabolic parameters may be normal between bouts of ataxia, testing must be performed when patients are symptomatic and involves CSF investigations. Important nonmetabolic differential diagnoses of intermittent ataxia are intoxications, especially with benzodiazepines and centrally acting drugs.

Table 27.9 Checklist of laboratory investigations in ataxia

Basal laboratory investigations (including blood gases, lactate, ammonia)
Plasma (and urinary) amino acids
Urinary organic acids
CSF and plasma glucose
CSF lactate (and pyruvate)
Vitamin E
Cholesterol, lipoprotein electrophoresis
Coenzyme Q_{10}
Oxysterols (cholestane-3β,5α,6β-triol and 7-ketocholesterol)
Cholestanol
Phytanic acid
Transferrin electrophoresis
Alpha-fetoprotein, albumin
Consider lysosomal studies if regression
Consider genetic panel testing: genetic panels of inherited ataxias and other next-generation sequencing techniques
In the future, consider studies of lipidome (plasma/CSF)

27.9.1.2 Nonprogressive Ataxia

Nonprogressive ataxias are mostly secondary to cerebellar malformations, e.g., in Joubert syndrome. True metabolic causes of nonprogressive ataxias include PMM2-CDG type and other types of congenital glycosylation defects, Marinescu–Sjögren syndrome, 4-hydroxybutyric aciduria deficiency, and L-2-hydroxyglutaric aciduria. In PMM2-CDG, brain MRI displays cerebellar atrophy or hypoplasia, usually accompanied by brainstem hypoplasia. Mitochondrial disorders rarely present as nonprogressive ataxia.

27.9.1.3 Progressive Ataxia

Many neurometabolic and neurodegenerative disorders involve the cerebellum and present with a more or less prominent progressive ataxia. Ataxia is a prominent symptom in Refsum disease, cerebrotendinous xanthomatosis, aceruloplasminemia, mevalonic aciduria, and vitamin

E-responsive ataxias. Albeit not a classical metabolic disorder, Friedreich's ataxia is also part of this group. Other disorders frequently associated with progressive ataxia are mitochondrial disorders such as *POLG1* mutations (in particular 4647A < T mutation), MERRF syndrome (8344A < G mtDNA mutation), and other mitochondrial syndromes such as MELAS, NARP, MILS, and KSS. Ataxia and gait impairment may be present as an associated sign in metabolic disorders that affect complex lipid synthesis and remodeling – see section on NBIA (neurodegeneration with brain iron accumulation) and HSP (hereditary spastic paraparesis). Disorders of coenzyme Q_{10} deficiency and Niemann–Pick type C can also present with ataxia as a prominent clinical sign. Of note, among the group of non-metabolic disorders causing progressive ataxia, DNA-repair disorders can be detected using simple biomarkers: α-fetoprotein for ataxia–telangiectasia (AT) and ataxia with oculomotor apraxia type 2 (AOA2) and albumin for ataxia with oculomotor apraxia type 1 (AOA1).

27.9.1.4 Vitamin E-Responsive Ataxias (AVED, Ataxias with Isolated Vitamin E Deficiency)

Vitamin E deficiency results from recessive mutations in the gene for alpha-tocopherol transfer protein (*TTP1*). Laboratory findings include low-to-absent serum vitamin E and high serum cholesterol, triglycerides, and beta-lipoprotein. High doses of vitamin E (400–1,200 IU/d) improve neurological function. This disorder can start from childhood to adulthood. MRI is usually normal. Retinitis pigmentosa, peripheral neuropathy, and pyramidal signs may be present, but cardiomyopathy is rare. *Abetalipoproteinemia* (Bassen–Kornzweig disease) is a rare disorder resulting from a dysfunction in the microsomal triglyceride transfer protein (*MTP*) gene. Acanthocytosis in peripheral blood smears is a constant finding. Decreased serum LDL and VLDL and increased HDL cholesterol levels together with low triglyceride levels are also present.

Disease Info: *Friedreich's Ataxia*

Friedreich's ataxia (FRDA) is the most common spinocerebellar degeneration with an age of onset usually before the age of 16 years. It is an autosomal recessive disorder, and virtually all affected individuals carry expanded GAA repeats in the gene coding for frataxin. Frataxin is involved in mitochondrial iron homeostasis and iron sulfur cluster regulation. The main clinical signs associated with ataxia are pes cavus, sensory neuronopathy, pyramidal signs, and cardiomyopathy. Brain MRI is usually normal, but spine MRI may show atrophy later in the disease course. Therapeutic metabolic attempts (idebenone, deferiprone, riboflavin) have been unsuccessful so far.

Disease Info: *Refsum Disease*

The accumulation of phytanic acid is the hallmark of this disorder, which is due to mutations in the genes coding for phytanoyl-CoA-hydroxylase (*PAHX* or *PHYH*) or peroxin 7 (*PEX7*). Affected patients usually present with peripheral neuropathy and retinitis pigmentosa, sometimes cardiac involvement and impaired hearing. Ichthyosis and multiple epiphyseal dysplasia are possible associated symptoms. The disease usually starts during adolescence but can start in adulthood. During acute decompensation, plasmapheresis (lipid apheresis) may be required to effectively lower phytanic acid levels. A diet restricted in phytanic acid and chlorophyll is otherwise recommended but hard to comply to.

Disease Info: ***Cerebrotendinous Xanthomatosis (CTX)***

This autosomal recessive disorder is caused by mutations in *CYP27A1* coding for sterol 27-hydroxylase. Almost all patients present with chronic diarrhea, which starts early in infancy, but this symptom is overlooked as there is usually no impact on growth curves. Few patients present with a transient liver disease in infancy. Mild to moderate cognitive delay is common. The disease is then usually characterized by the occurrence of psychiatric and motor symptoms (cerebellar ataxia, pyramidal signs, peripheral neuropathy) during late childhood to young adulthood. Characteristic features are bilateral cataracts. Xanthomata are not common and may appear only late in the disease course. Increased levels of plasma cholestanol are a hallmark of the disease. Treatment with chenodeoxycholic acid is efficient to lower cholestanol levels, digestive and psychiatric symptoms, as well as stabilizing motor dysfunction.

Disease Info: ***Coenzyme Q_{10}-Responsive Ataxias and Defects of Coenzyme Q_{10} Biosynthesis***

Mutations in *ADCK3* (also called *CABC1*), a gene involved in CoQ_{10} biosynthesis, have been identified in patients with autosomal recessive ataxia and epilepsy. Muscle CoQ_{10} deficiency may also result from genetic defects in aprataxin (AOA1).

Disease Info: ***Autosomal Recessive Cerebellar Ataxia with Hypogonadotropic Hypogonadism and Chorioretinal Dystrophy***

This clinical entity has been associated with metabolic defects involved in the synthesis and remodeling of complex lipids: Boucher–Neuhäuser (ataxia, hypogonadism, and chorioretinitis) and Gordon Holmes syndromes (ataxia, hypogonadism, and brisk reflexes) are due to mutations in *PNPLA6*-encoding NTE (neuropathy target esterase).

Remember

Consider treatable causes of ataxia first: vitamin E deficiency, biotinidase deficiency, abetalipoproteinemia, thiamine-responsive pyruvate dehydrogenase deficiency, cerebrotendinous xanthomatosis, Refsum disease, GLUT-1 deficiency, Niemann–Pick type C, Hartnup disease, and coenzyme Q_{10} deficiency. The most important test is brain MRI to rule out structural anomalies and brain tumors. Intermittent ataxias are often due to metabolic disorders. Important differential diagnoses for the latter are channelopathies and intoxications.

27.9.2 Dystonia, Parkinsonism, and Chorea

Extrapyramidal symptoms observed in inborn errors of metabolism include dystonia, parkinsonism, chorea, tremor, myoclonus, and tics. Different movement disorders may coexist in the same patient. However, dystonia is predominant in patients with inborn errors of metabolism. Usually, diseases causing parkinsonism in adults may present with dystonia in children. When movement disorders occur during intercurrent illnesses, their onset is usually abrupt and generalized – e.g., in glutaric aciduria type I. Otherwise, movement disorders tend to develop late in the disease course, with focal onset and progressive generalization, thereby causing major disability and wheelchair dependency – e.g., in propionic and methylmalonic acidemias, homocystinuria, Niemann–Pick type C, and GAMT deficiency. Although usually associated with other neurological symptoms, some inborn errors of metabolism

can initially present as an isolated dystonia – e.g., Segawa disease, PKAN, Leigh syndrome, Lesch–Nyhan disease, pyruvate dehydrogenase deficiency, and juvenile forms of metachromatic leukodystrophy.

27.9.3 Dystonia

Dystonia is defined by a sustained, abnormal muscular contraction causing fluctuating tone and abnormal posturing. Currently, next-generation sequencing techniques are unraveling a wide spectrum of dystonias of genetic origin. Based on their inheritance pattern, they can be divided into (1) autosomal-dominant forms such as DYT1-*TOR1A*, DYT5a-*GCH1*, DYT6-*THAP1*, DYT11-*SGCE*, DYT12-*ATP1A*, paroxysmal non-kinesigenic dyskinesias (DYT8-*MR1*, DYT10-*PRRT2*, DYT18-*SLC2A1*), and Huntington disease; (2) autosomal recessive forms such as DYT5a-*GCH1*, DYT5b-*TH*, Wilson's disease, and other neurometabolic disorders; (3) X-linked recessive forms such as Lesch–Nyhan disease; and (4) mitochondrial forms such as Leigh syndrome due to DNA mitochondrial mutations. Recently, a new classification approach renames the formerly called "primary dystonias" as genetic dystonias (DYTs), which can be either isolated (pure) or combined (with associated symptoms). Genetic dystonias can be due to various neurodegenerative or neurometabolic diseases (Table 27.10).

In many 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, and neurotransmitter defects are among the most relevant. In particular, neurodegeneration with brain iron accumulation (NBIA) is a growing group of disorders primarily characterized by progressive dystonia and parkinsonism. There are some clinical clues that can help in identifying dystonia as a presenting sign of a neurometabolic disease. If dystonia is focal, has progressed slowly, or remained isolated over time, it is unlikely to be due to an inborn error of metabolism. By contrast, if dystonia appears abruptly, settles rapidly, is generalized, and is postural from the very first stages of the disease, a metabolic cause should be strongly considered. As always, it is important to consider first inborn errors of metabolism for whom a therapeutic intervention is possible, such as dopa-responsive dystonia syndromes (Segawa disease and other neurotransmitter deficiencies), creatine deficiency syndromes, cerebral folate deficiency syndrome, Wilson disease, homocystinuria, and biotinidase, thiamine, vitamin E, and GLUT1 deficiencies. Of note, GLUT1 deficiency can cause paroxysmal exercise-induced dyskinesia and other paroxysmal complex movement disorders.

Inherited Disorders of Biogenic Amines

Disorders affecting dopamine and serotonin synthesis comprise aromatic L-amino acid decarboxylase deficiency, tyrosine hydroxylase deficiency, disorders of tetrahydrobiopterin (BH_4) synthesis, and "transportopathies" such as dopamine transporter deficiency and vesicular monoamine transporter type 2 deficiency. Unlike classical forms of BH_4 deficiency, Segawa disease (GTP cyclohydrolase I deficiency) and sepiapterin reductase deficiency present without hyperphenylalaninemia and are not detected by newborn screening. Childhood-onset dystonia or parkinsonism–dystonia is the hallmark of dopamine deficiency and the most suggestive clinical symptom of pediatric neurotransmitter diseases. In very young infants, the symptoms are less specific. Patients often present with truncal hypotonia, restlessness, feeding difficulties, or motor delay. Hypokinesia, increased limb tone, oculogyric crises, ptosis, and faulty temperature regulation are common signs in these diseases. Segawa disease, also called dopa-responsive dystonia, is an

Table 27.10 Genetic dystonias (DYTs)

Pure dystonia		
DYT1, DYT6, DYT25, DYT4		
Dystonia "Plus" (with associated signs)		
Myoclonus: DYT11		
Parkinsonism: DYT3, DYT5a (GTPCH dominant rarely recessive or Segawa disease), DYT5b (tyrosine hydroxylase deficiency), DYT-SPR (sepiapterin reductase deficiency), DYT12 (rapid-onset dystonia–parkinsonism)		
Plus other dyskinesias: DYT8 (paroxysmal non-kinesigenic), DYT10 (paroxysmal kinesigenic), DYT18 (paroxysmal exercise induced)		
Dystonia secondary to metabolic diseases		
Pterin disorders with hyperphenylalaninemias		
	Mode of inheritance	Gene defect
GTP cyclohydrolase I (GTPCH)	AR	*GCH*
6-Pyruvoly-tetrahydropterin synthase (PTPS)	AR	*PTS*
Dihydropteridine reductase (DHPR)	AR	*DHPR*
Copper metabolism disorders		
Wilson's disease	AR	*ATP7B*
Manganese metabolism disorders		
Dystonia, parkinsonism, hepatopathy, hypermanganesemia	AR	SLC30A10
Neurodegeneration with Brain Iron Accumulation (*NBIA*)		
Pantothenate kinase-associated neurodegeneration (PKAN)	AR	*PANK2*
PLA2G6-associated neurodegeneration (PLAN)	AR	*PLA2G6*
Mitochondrial protein-associated neurodegeneration (MPAN)	AR	*C19orf12*
Fatty acid hydroxylase-associated neurodegeneration	AR	*FA2H*
Beta-propeller protein-associated neurodegeneration (BPAN)	XL	*WDR45*
Neuroferritinopathy	AD	*FTL*
Aceruloplasminemia	AR	*CP*
Woodhouse–Sakati syndrome	AR	*DCAF17*
Coenzyme A synthase protein-associated neurodegeneration (CoPAN)		*COASY*
Kufor–Rakeb syndrome		*ATP13A2*
Lysosomal disorders		
Niemann–Pick C	AR	*NPC1*, *NPC2*
Neuronal ceroid lipofuscinoses (NCL)	AR, AD, or AR	*PPT1*, *TPP1*, *CLN3*, *DNAJC5*, *CLN5*, *CLN6*, *MFSD8*, *CLN8*, *CTSD*, *GRN*, *ATP13A2*, *CTSF*, *KCTD7*
Fucosidoses	AR	*FUCA1*
Arylsulfatase deficiency	AR	*ARSA*
GM1 gangliosidosis	AR	*GLB1*
GM2 gangliosidosis, AB variant	AR	*GM2A*
Krabbe disease	AR	*GALC*
Pelizaeus–Merzbacher disease	XL	*PLP1*
Purine metabolism disorders		
Lesch–Nyhan disease	XL	*HPRT1*
Mitochondrial diseases		

(continued)

Table 27.10 (continued)

Leigh syndrome, MEGDEL (3-methylglutaconic aciduria), LHON, MELAS, POLG-related diseases, MERFF, Mohr–Tranebjaerg syndrome	Mitochondrial, AR, AD, XL	Diverse genes
Organic acidurias		
Glutaric aciduria type 1	AR	*GCDH*
D-2-hydroxyglutaric aciduria	AR	*D2HGDH*
Methylmalonic aciduria	AR	*MUT*, *MMAA*, *MMAB*, *MCEE*, *MMADHC*
Aminoacidopathies		
Homocystinuria	AR	*CBS*
Hartnup disease	AR	*SLC6A19*
Biotin metabolism disorders		
Biotinidase deficiency	AR	*BTD*
Thiamine disorders		
Thiamine transporter-2 deficiency (Leigh syndrome and biotin–thiamine responsive basal ganglia disease)	AR	*SLC19A3*
Interferon disorders		
Aicardi–Goutières syndrome	AD, AR	*TREX1*, *RNASEH2B*, *RNASEH2C*, *RNASEH2A*, *SAMHD1*, *ADAR*
Other defects of energy metabolism		
Brain creatine defects		*SLC6A8*, *GAMT*, *GATM*
Glut1 transporter defect		*SLC1A2*
Other genetic causes of dystonia		
Ataxia–oculomotor apraxia		
Ataxia–telangiectasia		
Benign familial chorea		
Dentato-pallidoluysian atrophy		
Familial dystonic–amyotrophic paraplegia		
Hereditary nonprogressive athetoid hemiplegia		
Huntington disease		
Intranuclear neuronal inclusion disease		
Machado–Joseph disease (striatonigral autosomal-dominant degeneration)		
Myoclonic hereditary dystonia with nasal malformations		
Olivocerebellar atrophy		
Pelizaeus–Merzbacher disease		
Progressive calcification of the basal ganglia		
Progressive pallidal degeneration		
Rett syndrome		

autosomal-dominant disease characterized by progressive dystonia that normally appears during the first decade of life, is not associated with cognitive impairment, and has a dramatic and lifelong responsiveness to levodopa. Tyrosine hydroxylase and sepiapterin deficiencies respond in different degrees to levodopa, whereas amino acid decarboxylase and dopamine transporter deficiency do not have a satisfactory treatment. The most important tool for diagnosis of these diseases is cere-

brospinal fluid with investigations for neurotransmitter metabolites and pterins (see Sect. 27.13). A timely diagnosis is especially important for those conditions with specific treatments.

Disease Info: *Glutaric Aciduria Type I*

Glutaric aciduria type I is an autosomal recessive disease caused by deficient activity of glutaryl-CoA dehydrogenase. Classically, patients have an abrupt presentation, usually between 6 and 18 months of age, with encephalitis-like symptoms following an acute illness. Abnormal movement disorders then persist with dystonic or choreoathetotic movements; focal, segmental, or generalized dystonia; and twisting and torsion postures of hands and feet in a child otherwise alert, together with profound hypotonia of the neck and trunk and stiff arms and legs. Without diagnosis and treatment, psychomotor regression may develop later. Typically T_2-weighted brain MRI shows frontotemporal atrophy and hyperintense striatum. Macrocephaly is present in about 70 % of the cases. In a minority of cases, the onset is insidious with psychomotor delay, hypotonia, and dystonic postures. Urine organic acid analysis reveals increased glutaric and 3-hydroxyglutaric acids. Isolated and minor increases of 3-hydroxyglutaric aciduria have been described. Increased ratios of acylcarnitines to free carnitine in plasma and urine as well as increased glutaryl-carnitine in body fluids can also be detected. Treatment consists of a protein-restricted diet combined with oral supplementation of L-carnitine and an intensified emergency treatment during acute episodes of intercurrent illnesses. This strategy has significantly reduced the frequency of acute encephalopathic crises in early-diagnosed patients especially successful patients diagnosed by newborn screening. Anticholinergic drugs and botulinum toxin type A have proven to be beneficial as symptomatic treatments for severe movement disorders.

Disease Info: Lesch–Nyhan Disease

Lesch–Nyhan disease is an X-linked recessive disease caused by deficiency of the purine salvage enzyme hypoxanthine-guanine phosphoribosyltransferase (HPRT). Affected patients exhibit overproduction of uric acid and a characteristic neurobehavioral syndrome that includes mental retardation, recurrent self-injurious behavior, and a complex spectrum of motor disturbances. The pathogenesis of the neurological and behavioral features remains incompletely understood but has been related to dopaminergic function in the basal ganglia. All patients exhibit profound motor disabilities. Extrapyramidal and pyramidal signs typically develop between 6 and 18 months of age. The most prominent feature of the motor syndrome in all patients is dystonia affecting all parts of the body. Less than half of the patients display chorea, less severe than dystonia, and it typically emerges only with stress or excitement. Other movement disorders comprise tremor, opisthotonus, and extensor spasms of the trunk. A small number of patients with variants of HPRT do not display abnormal behavior. Therefore, the enzymatic assay is indicated in any patient with the motor symptoms detailed above. Allopurinol is indicated for renal manifestations but does not improve neurological symptoms. Levodopa, dopaminergic agonists, and dopamine-depleting agents have been used to treat movement disorders

without convincing results. Pimozide, haloperidol, fluphenazine, and risperidone have been used to control self-injurious behavior with poor response. Recent studies suggest a dramatic response to S-adenosylmethionine in only a small proportion of patients. Baclofen and benzodiazepines may be useful to control spasticity.

Disease Info: Wilson Disease

This autosomal recessive disorder is due to a defect in the copper adenosine triphosphatase transporter (ATP7B) resulting in abnormal deposition of copper in liver, brain, and other tissues. Hepatic abnormalities are the first manifestations of the disease in 50% of the patients. Neurological manifestations usually appear after the age of 10 years, although neurological symptoms have been reported in patients as young as 4 years old. In children, neurological symptoms may begin insidiously. Dysarthria, disturbed coordination of voluntary movements, tremor, dystonia, and a rigid–akinetic syndrome are common symptoms. Choreiform movements may also be observed. Psychiatric manifestations and behavior disorders are also common. Ocular abnormalities are usually silent but have a great diagnostic value. The Kayser–Fleischer ring (see Fig. 24.2 Chap. 24) is pathognomonic for the disease and precedes the appearance of neurological abnormalities. Brain MRI usually shows T1-hypointensity and T2-hyperintensity of the lenticular nuclei, thalami, brainstem, claustrum, and white matter (Fig. 27.5). The contrast between the normal low intensity of the red nuclei and substantia nigra and the abnormal high-intensity signal of the midbrain tegmentum results in a pattern called the "face of the giant panda." Plasma ceruloplasmin and copper levels are decreased, whereas cupruria is increased. Because of its frequent side effects and the initial neurological deterioration with penicillamine therapy, less toxic drugs such as trientine or zinc have progressively become first-line treatments. Initial zinc therapy for presymptomatic individuals and maintenance zinc therapy in patients after long-term chelation seem to be safe and effective.

Disease Info: *Neurodegeneration with Brain Iron Accumulation (NBIA)*

NBIA syndromes are a group of degenerative monogenic disorders with accumulation of iron in the brain, usually in the basal ganglia. In general, they cause progressive dystonia, bulbar symptoms, and pyramidal signs in young children, whereas parkinsonism usually appears later. Cognitive decline occurs in some subtypes of NBIA, but more often cognition is relatively spared. Cerebellar atrophy is a frequent finding. So far, ten different genetic forms have been described. Biochemical pathways involving the synthesis and remodeling of complex lipids seem to play an important mechanistic role in NBIA disorders. At least four genes, *PANK2*, *PLA2G6*, *COASY*, and *FA2H*, encode proteins involved in complex lipids metabolism.

Disease Info: *Pantothenate Kinase-Associated Neurodegeneration (PKAN)*

PKAN is one of the most frequent NBIA. This autosomal recessive disease is caused by mutations in *PANK2* that encodes pantothenate kinase 2. PKAN is characterized by a progressive movement disorder (dystonia or parkinsonism)

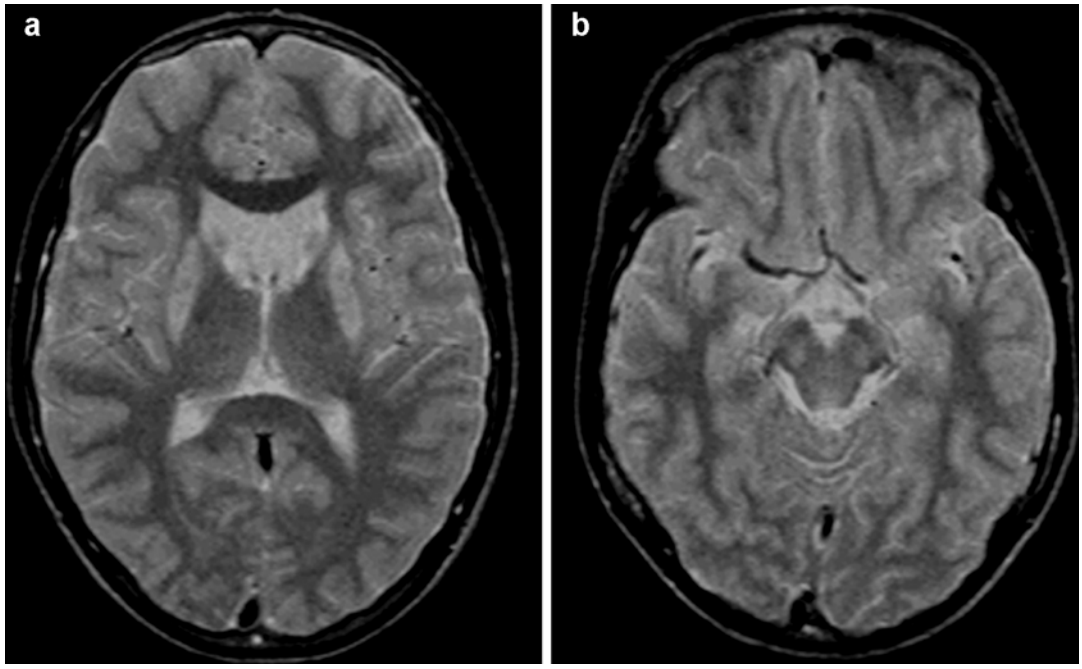

Fig. 27.5 Wilson disease axial T2w images of an adolescent demonstrating (**a**) symmetric hyperintense signal changes and atrophy of caudate nucleus and putamen, (**b**) hyperintense signal changes in the midbrain. The "sign of the giant panda" is marginally visible in this patient

usually associated with dementia and pyramidal tract signs. Neuropathologic findings include dysmyelination and deposition of iron-staining pigments in the pallidum and the pars reticulata of the substantia nigra, axonal swelling in the cerebral cortex and basal ganglia, increased lipofuscin deposition, and, in a few cases, Lewy bodies. In the early-onset form, after an initial silent period, gait becomes unsteady, and regression begins within 5–10 years. Dystonia (often in the upper limbs, trunk, and oromandibular muscles) may be an early manifestation but may develop 1 year after the onset of regression. Retinopathy and acanthocytosis are common. Death usually occurs between 11 and 15 years of age. Dystonia in the lower limbs and oromandibular muscles is a prominent sign in juvenile onset cases and is followed by intellectual deterioration, seizures, and ataxia. Parkinsonism is the predominant manifestation in late-onset (adult) cases. Brain MRI shows bilateral T2 hyperintensity in the medial part of the pallidum surrounded by a larger zone of markedly low signal, the so-called "eye-of-the-tiger" image (see Fig. 27.3f, g in Chap. 45). In some cases, only a markedly decreased signal of the pallidum without central hyperintensity is found. If the MRI is done early in the disease course, the hypointense signal may not be present yet. Of note, the pallidum may be hyperintense instead, mimicking a mitochondrial disorder.

Thiamine Transporter-2 Deficiency

Pathogenic mutations in the *SLC19A3* gene decrease the activity of a transmembrane protein, the second thiamine transporter, resulting in insufficient B_1 vitamin uptake.

Clinical presentations comprise neonatal lactic acidosis, infantile spasms with progressive brain atrophy, and basal ganglia-related diseases such as Leigh disease, Wernicke-like encephalopathy, and biotin–thiamine-responsive basal ganglia disease. Brain MRI findings in acute crises show a T2-hyperintensity with swelling of the basal ganglia (caudate nucleus and putamen) and the medial thalami and a diffuse involvement of cortical and subcortical white matter as well as of the infratentorial brain. Later on, atrophy and necrosis of the basal ganglia and thalami develop. Brainstem and cerebellum are also affected in 30 % of the cases (Fig. 27.6). Recommendations on the long-term doses of vitamins that should be administered in SLC19A3-deficient patients are still debated., Documented doses of thiamine and biotin supplementation vary from 100 to 900 mg per day and from 2 to 12 mg/kg per day, respectively.

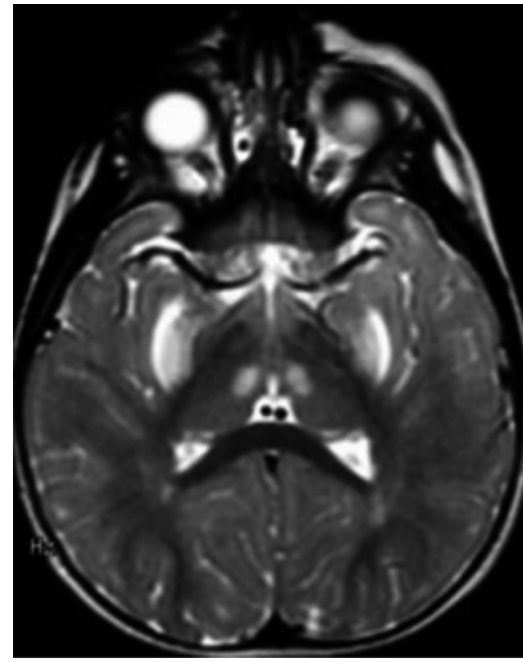

Fig. 27.6 Thiamine transporter-2 deficiency. This axial T2w image displays bilateral and symmetrical involvement of the putamen and medial thalamic nuclei

Remember

- Dystonia is a major symptom of many neurometabolic diseases and is usually associated with other neurological signs.
- If dystonia is associated with intercurrent illnesses, appears abruptly, and is suddenly generalized or if it is focal but has a progressive generalization, a metabolic disorder is likely.
- CSF studies are necessary when first-line investigations have failed.
- L-dopa therapy should be tried, especially when CSF analysis reveals low values of dopamine-related metabolites.
- Consider also a therapeutic trial with thiamine and biotin in dystonia of unknown origin (Table 27.11).

27.9.4 Parkinsonism or Rigid Akinetic Syndrome

Parkinsonism is a frequent neurological syndrome in adulthood but very rare in childhood. Early forms of parkinsonism have distinctive features as compared to parkinsonism in adults, and in general, the concepts "hypokinetic–rigid syndrome," "dystonia–parkinsonism," "parkinsonism plus," or "parkinsonism-like" are more accurate in children. Inborn errors of metabolism represent an important group among the genetic causes of parkinsonism at any age: (1) metal-storage diseases such as Wilson's disease, manganese transporter deficiency, and NBIA syndromes; (2) neurotransmitter defects, which cause early-onset dystonia and dystonia–parkinsonism; (3) lysosomal and complex molecule disorders, especially ceroid lipofuscinosis, GM_1, Niemann–Pick C, and cerebrotendinous xanthomatosis; and (4) energy metabolism defects such as POLG mutations and other mitochondrial diseases. These metabolic entities can respond to low L-dopa + carbidopa doses, especially in adolescents and adults.

27.9.5 Chorea

The term chorea is a derived from the Greek word "choreia" for dancing and refers to involuntary rapid spasmodic movements of the face, neck, and proximal limb muscles. It may extend to the oropharyngeal muscles generating swal-

Table 27.11 Checklist of laboratory tests in dystonia

1. Treatable causes of dystonia
Biogenic amines, pterins, glucose, folate, and thiamine in CSF
CSF and plasma glucose
Plasma and urinary amino acids
Plasma total homocysteine
Creatine and guanidinoacetate in urine
Copper (plasma and urine), ceruloplasmin (plasma)
Manganese (plasma), polycythemia
Plasma biotinidase activity
Plasma oxysterols (cholestane-3β,5α,6β-triol and 7-ketocholesterol)
2. Pure dystonia:
Biogenic amines in CSF
DYT genes
3. Dystonia associated with additional neurologic signs
Parkinsonism: biogenic amines and pterins in the CSF, copper and ceruloplasmin, lactate, pyruvate, plasma amino acids (consider respiratory chain activity and mitochondrial DNA study) – consider *PANK2* and other NBIA genes, juvenile parkinsonism genes
Myoclonus: urine organic acids, DYT5 gene – consider mitochondrial and lysosomal investigations
Developmental delay and spasticity: uric acid (plasma and urine), purines (urine), lactate, pyruvate, plasma amino acids, urine organic acids, manganese – consider mitochondrial and lysosomal investigations
Cerebellar ataxia: urine organic acids, plasma and urine amino acids, plasma vitamin E with cholesterol and triglycerides – consider mitochondrial investigations, aprataxin gene (if oculomotor apraxia) and immunological studies (ataxia–telangiectasia), Niemann–Pick C, and NBIA syndromes
4. Abrupt generalized dystonia (especially in a catabolic state)
Urine organic acids (including glutaric and 3-hydroxyglutaric acids), lactate, pyruvate, plasma amino acids (consider other mitochondrial and PDH studies), thiamine in the CSF (usually low in thiamine transporter deficiency) – consider DYT12 (*ATP1A3*)
5. Dystonia associated with other signs
Visceral signs: lysosomal investigations, urine organic acids
Ocular signs: optic atrophy (LHON gene), oculomotor apraxia (aprataxin gene), ocular telangiectasia (immunologic studies and ataxia–telangiectasia gene), retinitis pigmentosa (plasma vitamin E, mitochondrial, lysosomal investigations)
Deafness: plasma biotinidase activity, glycosaminoglycans, and oligosaccharides in urine – consider mitochondrial investigations and dystonia/deafness genes
6. If negative after first-line investigations, next-generation sequencing = dystonia panels and/or whole exome sequencing

lowing difficulties. Choreic movements can be observed in glutaric aciduria type I (in the context of severe acute dyskinetic syndrome), Lesch–Nyhan disease, PKAN and other NBIA syndromes, homocystinuria, and Niemann–Pick C, among others (Table 27.12).

27.9.6 Spasticity

Spasticity is a very common clinical situation in pediatric and adult neurology. It refers to a dysfunction of the motor system in which certain muscles are continuously contracted. This contraction gives rise to muscle stiffness interfering with voluntary movements. Control of voluntary movements is achieved through the upper motor neurons in the brain motor cortex, which send their axons via the corticospinal tract to connect to lower motor neurons in the spinal cord. Spasticity 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

Table 27.12 Genetic diseases that can present with chorea

Alternating hemiplegia (*ATP1A3*)
Ataxia with oculomotor apraxia type 1 and 2
Benign hereditary chorea (*NKX2.1*)
Dentatorubropallidoluysian atrophy
Huntington disease
Neuroacanthocytosis
Pontocerebellar hypoplasia type 2
Spinocerebellar ataxias (SCA2, SCA3, SCA17)
Inborn errors of metabolism
Glutaric aciduria I
Other organic acidurias such as propionic aciduria and methylmalonic aciduria
Galactosemia
Homocystinuria
Nonketotic hyperglycinemia
Pterin defects
Guanidinoacetate methyltransferase deficiency
Sulfite oxidase/molybdenum cofactor deficiency
Lesch–Nyhan disease
Infantile neuronal ceroid lipofuscinosis
Niemann–Pick C
PI4K2A (phosphatidylinositol 4-kinase type II-alpha) mutations (associated with cutis laxa)
PKAN and other NBIA syndromes
Wilson disease
Cerebral folate deficiency due to *FOLR* mutations
GLUT-1 deficiency

affected anatomical region, spasticity may be more evident or restricted to the lower extremities (spastic diplegia or paraplegia) and to one side of the body (spastic hemiplegia) or affecting all four limbs (spastic quadriplegia or tetraparesis).

In children, most events related to motor damage occur during late pregnancy and delivery such as prematurity, neonatal asphyxia, birth trauma, or infections. The resulting clinical manifestations are usually grouped under the general term cerebral palsy. In addition, traumatic injury, infections of the CNS that take place later on, as well as genetic conditions (including inborn errors of metabolism) may result in spasticity. Since some of these metabolic disorders are treatable and allow family genetic counseling, it is important to include them in the diagnostic approach of spasticity.

Spasticity in metabolic disorders is in general associated with additional neurological or organ dysfunctions (Table 27.13). Spasticity may develop acutely together with signs of metabolic intoxication such as loss of consciousness or vomiting. Some disorders can start with isolated spastic paraparesis such as X-linked adrenoleukodystrophy, remethylation defects of homocysteine metabolism, HHH syndrome (hyperammonemia, hyperornithinemia, homocitrullinuria), arginase deficiency, dominant mutations of *ALDH18A1*, and Segawa disease.

The main components of the motor system (motor neurons and myelin) are extremely vulnerable to inborn errors of metabolism. This is the reason why disorders of both intermediary metabolism and complex molecules may exhibit spasticity. Disorders that interfere with myelin metabolism, synthesis, and remodeling of complex lipids, defects in energy production, or small toxic molecules often cause pyramidal tract lesions. In fact, almost all progressive neurometabolic diseases end up manifesting with different degrees of spasticity. This section addresses inborn errors of metabolism in which spasticity is the dominant or one of the most prominent signs.

Remember

- The possibility of an inborn error of metabolism should be considered in every child with the diagnosis of cerebral palsy but without a history of perinatal or postnatal brain injury (prematurity, hypoxia, infections, traumatic brain injury).
- In inborn errors of metabolism, spasticity tends to be syndromic or complicated (other neurological signs and/or other organs are involved). However, in some cases spasticity may remain isolated for a long time.
- Spasticity is probably the most common neurological sign in neurometabolic disorders. It is therefore important to search carefully for other associated clinical signs.

Table 27.13 Main causes of spasticity

Clinical signs associated to spasticity	Disorders
Additional neurological signs	
Peripheral neuropathy	CTX, mitochondrial disorders (axonal neuropathy), biotinidase deficiency, beta-mannosidosis, sialidosis type I, Krabbe disease, MLD, homocysteine remethylation defects, vitamin E deficiency, PLA2G6, arylsulfatase I, complex lipids (CYP2U1, GBA2, B4GALNT1)
Leukoencephalopathy	Krabbe disease, MLD, Canavan disease, L-2-hydroxyglutaric aciduria, X-ALD, homocysteine remethylation defects, mitochondrial disorders, multiple sulfatase deficiency, Schindler disease
Ataxia	CTX, cerebral folate deficiency, biotinidase deficiency, HHH syndrome, some mitochondrial disorders, L-2-hydroxyglutaric aciduria, complex lipids (PNPLA6, FA2H, GBA2)
Movement disorders	Dopamine synthesis and transport defects, cerebral folate deficiencies, mitochondrial disorders, brain iron disorders
Epilepsy	Homocysteine remethylation defects, cerebral folate deficiencies, mitochondrial disorders, sialidosis, Schindler disease, multiple sulfatase deficiency, Canavan disease, L-2-hydroxyglutaric aciduria, fatty acid elongase ELOVL4
Microcephaly	Cerebral folate deficiencies, homocysteine remethylation defects, arginase deficiency, mitochondrial disorders
Macrocephaly	Cerebral organic acidurias, MPS
Developmental delay/mental retardation	In all of them except GTPCH I (dominant) and sialidosis type I
Additional non-neurological signs	
Visceral	Urea cycle disorders (liver involvement, cyclic vomiting), CTX (diarrhea), X-linked adrenoleukodystrophy (adrenal insufficiency), most lysosomal disorders (organomegaly)
Cutaneous signs	CTX (xanthomas), multiple sulfatase deficiency, Sjögren–Larsson and ELOVL4 (ichthyosis), biotinidase deficiency (alopecia, dermatitis), X-linked ALD (melanoderma), sialidosis II (angiokeratoma)
Ocular signs	Homocysteine remethylation defects (retinitis pigmentosa, optic nerve atrophy), CTX, ELOVL4 and GBA2 (cataracts), biotinidase deficiency, optic neuropathy, Sjögren–Larsson (retinopathy), cerebral folate deficiencies (optic atrophy), different lysosomal disorders (cherry-red spot), brain iron diseases, mitochondrial disorders, vitamin E deficiency (retinitis pigmentosa)

Abbreviations: *CTX* Cerebrotendinous xanthomatosis, *MLD* metachromatic leukodystrophy, *X-ALD* X-linked adrenoleukodystrophy

27.9.6.1 Treatable Inborn Errors of Metabolism with Prominent Di/Tetraparesis

Urea cycle disorders such as arginase deficiency, can result in progressive spasticity, seizures, and mental retardation with relatively mild hyperammonemia. HHH syndrome (hyperammonemia, hyperornithinemia, homocitrullinuria) is a disorder of ornithine transport between cytoplasm and mitochondria that causes progressive spastic diplegia in addition to other signs of neurologic dysfunction. *ALDH18A1* encodes delta-1-pyrroline-5-carboxylate synthase (P5CS), an enzyme that catalyzes the first and common step of proline and ornithine biosynthesis from glutamate. Monoallelic *ALDH18A1* mutations have been recently identified in adult patients with dominant forms of hereditary spastic paraparesis (HSP) who presented with low levels of plasma ornithine, citrulline, arginine, and proline. Accordingly, routine investigation of spasticity should include plasma ammonia and amino acids (in plasma and urine). Therapeutic strategies include diet, ammonia-lowering agents, or citrulline/arginine supplementation.

Biotinidase deficiency may also manifest with progressive spastic paraparesis. It may improve with biotin (5–10 mg/day). Measurement of plasma biotinidase activity reveals the diagnosis.

Homocysteine remethylation defects can lead to demyelination of the pyramidal tracts and produce subacute combined degeneration of the spinal cord. It is important to measure plasma amino acids, total homocysteine, vitamin B_{12}, and folate concentrations in every patient with isolated spasticity, as it may be the only clinical sign over a long period. The combination of betaine (up to 10 g/day in three doses), folic acid (5–10 mg/day), and hydroxocobalamin (1 mg i.m. monthly up to weekly in adults) is not as effective as it is in resolving other neurological or psychiatric symptoms.

Cerebrotendinous xanthomatosis is a sterol disorder that may present with progressive spasticity from the second decade of life. Increased plasma cholestanol and bile acid precursors (in plasma or urine) are diagnostic. Treatment with chenodeoxycholic acid (750 mg/day) improves the neuropsychiatric disability.

Dopamine synthesis defects , especially GTPCH I deficiency, may exhibit pyramidal signs, and, in some cases, lower limb dystonia can mimic spastic paraparesis. Therefore, CSF study and a trial of levodopa are advisable in all patients with unexplained spastic paraparesis or dystonic cerebral palsy.

Vitamin E deficiency (see also Sect. 27.9.1 "Ataxia") Spasticity may appear together with peripheral neuropathy and retinitis pigmentosa. Laboratory findings include low-to-absent serum vitamin E and high serum cholesterol, triglycerides, and beta-lipoprotein. High doses of vitamin E (400–1,200 IU/d) improve neurological functions.

Spastic paraplegia type 5 (SPG5) is due to mutations in *CYP7B1*, which encodes oxysterol 7α-hydroxylase – an enzyme involved in the synthesis of bile acids from cholesterol. *CYP7B1* mutations are responsible for rare forms of liver failure in infancy and HSP in adults. SPG5 is mostly characterized by a rather pure form of spastic paraplegia starting during adolescence or adulthood. Increased levels of 25 and 27-hydroxycholesterol in plasma are diagnostic. The dramatic therapeutic response of a child with liver failure due to *CYP7B1* mutations using chenodeoxycholic acid opens promising therapeutic perspectives for SPG5 patients, possibly as in cerebrotendinous xanthomatosis.

Remember

- Measure ammonia, amino acids, biotinidase activity, total homocysteine, vitamin B_{12}, folate, cholestanol, oxysterols (25-hydroxycholesterol and 27-hydroxycholesterol), and vitamin E in all patients with spasticity of unknown origin.
- Consider a trial of L-dopa and CSF studies to measure neurotransmitters and folate.

27.9.6.2 Progressive Spasticity Associated with Multiple Neurologic Signs, Irritability, and Global Deterioration

This is a group of heterogeneous disorders that usually present with a complex neurological picture in which progressive spasticity is one of the main features. Peripheral neuropathy, visual, auditory, and visceral involvement are frequently present. Brain MRI may disclose specific white matter patterns that can be very helpful to the diagnostic approach.

In *lysosomal disorders*, some of the most representative diseases giving rise to spasticity are Krabbe disease, metachromatic leukodystrophy, MPS III, fucosidosis, and mannosidoses. *X-linked adrenomyeloneuropathy* can present with spasticity as an isolated sign for a long time, whereas in *adrenoleukodystrophy* of childhood, cognitive and behavioral problems may appear before motor disturbances.

In *mitochondrial disorders* involvement of the pyramidal tract is also frequent; however, other signs and symptoms often guide the diagnostic approach.

Neuroaxonal dystrophy due to mutated *PLA2G6*, encoding phospholipase A2, and other causes of NBIA syndromes present with progressive neurological regression and distended axons (spheroid bodies) in the nervous system as well as increased iron content in the basal ganglia. Progressive spastic paraparesis, extrapyramidal signs, dementia, retinitis pigmentosa, and optic atrophy are present.

Some cerebral organic acidurias such as *Canavan disease* (high urine and brain MRS N-acetylaspartate) or *L-2-hydroxyglutaric aciduria* are examples of childhood leukodystrophy exhibiting different degrees of progressive spasticity and well-defined brain MRI patterns.

27.9.6.3 Hereditary Spastic Paraparesis (HSP)

HSP is a heterogeneous group of genetic disorders in which the main feature is progressive spasticity in the lower limbs due to pyramidal tract dysfunction. There are more than 50 genetic types of HSP, many of them described in recent years. Most of them present as complex HSP syndromes, i.e., spasticity is associated with other neurological signs. Cerebellar involvement, axonal neuropathy, ichthyosis, and ocular abnormalities are among the most commonly associated symptoms. Brain MRI often displays a thin corpus callosum and mild white matter abnormalities. About 13 HSP gene-encoding proteins involved in complex lipids biosynthesis and remodeling have been described until now. They participate in different biological functions, especially phospholipids and membrane lipids remodeling (*PNPLA6*, *CYP2U1*, *DDHD1*, *DDHD2*) as well as sphingolipids biosynthesis (*FA2H*, *GBA2*, *B4GALNT1*). Lipidomic studies may allow identifying new biomarkers for these new entities such as plasma oxysterols for the diagnosis of SPG5.

Remember

- Check nerve conduction, visual and auditory function, skeleton examination (X-ray), and urine glycosaminoglycans/oligosaccharides in patients with multiple neurological signs and progressive spasticity.
- Some brain MRI-specific patterns can give the diagnostic clue.
- Consider the possibility of a brain iron disorder in a patient with progressive spasticity, especially if episodes of regression are triggered by infectious events, and even in the absence of specific brain MRI findings.
- Defects of complex lipid biosynthesis and remodeling are an emerging group of inborn errors of metabolism frequently causing HSP.

27.9.6.4 Spastic Tetraparesis Associated with Ichthyosis

This association is typical of *Sjögren–Larsson syndrome* (fatty alcohol NAD oxidoreductase deficiency). The skin alteration consists of yellowish-brown hyperkeratosis (ichthyosis). Glistening dots are present in the macular fundus. Mental retardation is also present. Cutaneous signs may remain isolated for a long time, and spastic tetraparesis can appear in adulthood. High leukotriene B_4 in urine, enzymatic activity in fibroblasts or leukocytes, and mutation analysis are the diagnostic tools. Zileuton improves the cutaneous symptoms and may ameliorate the neurological disease. *Elongase type 4 deficiency* (ELOVL4) is responsible for a Sjögren–Larsson-like syndrome with ichthyosis, seizures, mental retardation, and spasticity.

Multiple sulfatase deficiency combines features of mucopolysaccharidosis, metachromatic leukodystrophy, and ichthyosis, starts in infancy, and is usually fatal in early childhood. Glycosaminoglycans are increased in urine, and enzyme studies show deficiencies of many sulfatases (Table 27.14).

27.10 Neurophysiology

Neurophysiologic studies are important for diagnosis and follow-up of neurometabolic disorders, especially when epilepsy or a peripheral neuropathy are suspected or part of the clinical picture. There are few pathognomonic or typical findings. Of major interest for neurometabolic disorders are electroencephalography (EEG), electroretinography (ERG), measurement of nerve conduction velocities, and evoked

Table 27.14 Checklist of laboratory tests in spasticity

Treatable causes of spasticity
Plasma ammonia, plasma, and urine amino acids
Biotinidase activity (plasma)
Vitamin B_{12}, folate, and total homocysteine (blood)
Cholestanol and 27-hydroxycholesterol (plasma)
Vitamin E, triglycerides, cholesterol and fractions, erythrocyte morphology (plasma)
Folate and biogenic amine metabolites in CSF
Progressive spasticity with signs of neurologic deterioration
Glycosaminoglycans, oligosaccharides, sialic acid (urine)
Lysosomal enzymes (blood)
Very long-chain fatty acids (plasma)
Lactate, pyruvate (plasma)
Organic acids (urine)
Consider *PLA2G6* mutation and other NBIA syndromes depending on clinical and MRI findings
Consider different genes related to HSP and plasma/CSF lipidome
Spasticity with ichthyosis:
Glycosaminoglycans in urine
Enzymatic activity of fatty alcohol NAD oxidoreductase in fibroblasts
Enzymatic activity of fatty acid elongase ELOVL4 in fibroblasts
Other non-metabolic causes of spasticity
Hereditary diseases: HSP genes, SCAs
Infections: AIDS, HTLV-1
Immunologic disorders: multiple sclerosis
Malformations: Arnold–Chiari, cervical/lumbar spondylosis
Cerebral palsy (prematurity, hypoxia, infections)
Tumors

potentials. Electromyography (EMG) is done if a myopathy or a motor neuropathy is suspected. It can reveal myotonia-like discharges in juvenile type II glycogenosis.

The EEG may be of value in the diagnosis of several neurometabolic disorders. In infantile neuroaxonal dystrophy (INAD), it shows a pronounced, diffuse fast β-activity in the absence of medication, especially in stage I and II sleep which helps in the diagnosis of this rare disorder (Fig. 27.1). In late-infantile neuronal ceroid lipofuscinosis, slow photic stimulation leads to occipital spikes (Fig. 27.3) and may even trigger focal occipital seizures. In the infantile form, EEG shows early a slowing of the background, later in the course an isoelectric tracing. In the neonatal manifestation of maple syrup urine disease, EEG displays comb-like rhythms. A burst-suppression pattern is not specific and may be found in different epileptic encephalopathies with neonatal onset, the most frequent being nonketotic hyperglycinemia. In patients with homocystinuria, centrotemporal spikes are often present resembling the epileptiform potentials seen in benign epilepsy with centrotemporal spikes. If cortical abnormalities are present, EEG reflects their localization and quality – in cobblestone lissencephaly, it shows generalized β-activity, usually of high amplitude. In Alpers disease, 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 *s*uperimposed (poly)spikes (RHADS), usually over the posterior regions (Fig. 27.4).

Somatosensory evoked potentials (SSEP) are helpful in delineating posterior column involvement, e.g., in Friedreich's 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 detecting an early involvement of the optic nerve, e.g., in mitochondrial disorders, infantile neuroaxonal dystrophy, or Alpers disease. ERG is important in detecting retinal involvement which is of use in the differential diagnosis of neurodegenerative disorders. Retinal involvement is common not only in neuronal ceroid lipofuscinoses but also in pantothenate-associated neurodegeneration (PKAN), mitochondrial disorders, and many others (see also Chap. 30).

27.11 Diagnostic Lumbar Puncture

In encephalopathies of unknown origin, CSF investigations can be instrumental in identifying the underlying neurometabolic disorder. As there is still widespread uncertainty about when to perform specialized CSF investigations and what

to investigate, many patients remain undiagnosed, while they often have recognizable phenotypes. This results in important therapeutic delays. On the other hand, a lumbar puncture should only be performed when basic analyses have been carried out in blood and urine (see Table 27.15), and neuroimaging studies have been carefully evaluated (Chap. 45). Some tests may need to be repeated as specificity and sensitivity are rarely 100 %. For example, lactate values may be intermittently normal in mitochondrial disorders, and the urine sulfite test for molybdenum cofactor deficiency may give a false-negative result. Keeping this in mind, there is no place for a selective screening in CSF. Reliable results of specialized CSF investigations can only be obtained if the appropriate protocol is strictly applied. This should be discussed beforehand with the neurometabolic laboratory (see Chap. 37 Biochemical Studies).

The diagnosis of monogenic defects of neurotransmission is almost exclusively based on the quantitative determination of the neurotransmitters and/or their metabolites in CSF, i.e., the amino acids glutamate, glycine, and γ-aminobutyric acid (GABA); the acidic metabolites of the biogenic monoamines, dopamine, serotonin, epinephrine, and norepinephrine; and individual pterin species. It is important to always include the determination of folate in CSF as deficiencies of the cerebral folate transporter and dihydrofolate reductase cannot be revealed otherwise. In other neurometabolic disorders, results of CSF investigations are an important although not always exclusive part of the diagnostic work-up, e.g., GLUT1 deficiency, mitochondriopathies, and serine synthesis disorders.

Remember

Whenever CSF investigations are performed, the analysis should include quantitative determination of lactate, pyruvate, and amino acids, the latter by methods especially suited for CSF, in addition to cells, glucose, protein, immunoglobulin classes, specific immunoglobulins, and an evaluation of the blood–brain barrier (Table 27.16).

Preprandial plasma amino acids, serum glucose, and blood lactate must always be determined at the time of the lumbar puncture, as ratios are highly informative for a large number of disorders and almost indispensable for the diagnosis of some disorders, e.g., nonketotic hyperglycinemia (CSF glycine almost always >30 μM and glycine CSF/plasma ratio >0.04), glucose transport protein deficiency (CSF glucose <2.7 mmol/l, glucose CSF/

Table 27.15 Investigations for neurometabolic diseases in blood and urine

Blood/plasma/serum: Full blood count and reticulocytes, clinical chemistry profile including Ca, P, alkaline phosphate, creatinine, uric acid, copper, ceruloplasmin, manganese, T3, T4, TSH, thyroid-binding globulin, prolactin (3 determinations hourly apart)
Amino acids, total homocysteine, lactate and pyruvate, ammonia, biotinidase, very long-chain fatty acids, pristanic acid, pipecolic acid, transferrin isoelectric focusing, 25-hydroxycholesterol/27-hydroxycholesterol and cholestane-3β,5α,6β-triol/7-ketocholesterol
Plasma lipidome (in case of lipid biosynthesis defect suspicion)
Urine: organic acids, lactic acid, uric acid (24-h urine or uric acid/creatinine ratio), guanidino compounds, sulfite, purines and pyrimidines, bile acid intermediates, mucopolysaccaccharides, oligosaccharides, sialic acid, creatinine (preferably 24-h urine)
Blood or urine: Acyl-carnitine esters
Enzymes: white cell hexosaminidase, sphingomyelinase, palmitoyl protein thioesterase activity, aryl sulfatase, glucocerebrosidase, galactocerebrosidase, fucosidase, tripeptidyl-peptidase activities

Table 27.16 Investigations for neurometabolic diseases in CSF

Cells, protein, immunoglobulin classes, and glucose (plus plasma glucose[a] and evaluation of blood–brain barrier)
Lactate (plus preprandial blood lactate[a])
Amino acids (plus preprandial plasma amino acids[a])
Biogenic amine metabolites
Individual pterin species
5-Methyltetrahydrofolate

[a]To be determined at the time of the lumbar puncture

plasma ratio <0.45), and defects of serine synthesis (CSF serine <14 μM and serine CSF/plasma ratio <0.2). Unreported blood contamination of CSF is the most common cause for an erroneous diagnosis of nonketotic hyperglycinemia. If the CSF is blood stained, the sample must be centrifuged immediately and supernatant transferred to fresh collection tubes before freezing. Because amino acids and glucose change dramatically after a meal, timing of the lumbar puncture and blood taking should not be post- but preprandially, i.e., at least 4–6 h after a meal. Blood should be taken first as glucose may rise stress related during the lumbar puncture.

Figure 27.7 depicts an algorithm for the interpretation of pathological CSF/plasma results of glucose and lactate. Glucose and lactate must be looked at together. Pathologically altered ratios may indicate a disease intrinsic to the CNS. Increased CSF lactate together with decreased glucose results from inadequate aerobic energy production, e.g., in mitochondriopathies but more commonly in the course of CNS infections. Conversely, decreased CSF glucose in GLUT1 deficiency is accompanied by low normal or even reduced levels of lactate and alanine. Postprandial or stress-related elevated blood glucose is the most common cause of a reduced CSF/blood ratio resulting in an erroneous suspicion of GLUT1 deficiency. On the other hand, some genetically diagnosed cases with GLUT1 deficiency have shown only mildly reduced or even borderline CSF glucose values but not the diagnostic CSF/plasma ratio.

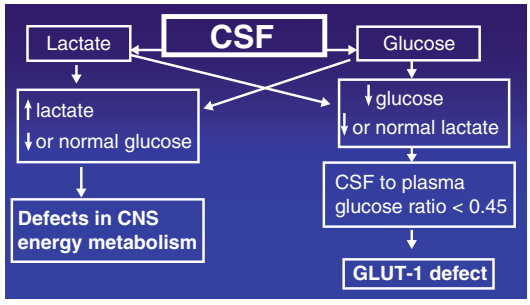

Fig. 27.7 Evaluation of CSF/blood results of glucose and lactate

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Metabolic Myopathies

28

Stephen G. Kahler

Key Facts

- The metabolic disorders which affect muscle can cause chronic weakness and hypotonia or episodic exercise intolerance cumulating in rhabdomyolysis or both. Rhabdomyolysis disorders can be conveniently separated according to tolerance of short, intense exercise compared to longer, milder efforts.
- Most metabolic disorders which affect skeletal muscle do so by altering energy metabolism. Muscle at rest uses fatty acids as the main energy source.
- During intense exercise, there will be anaerobic glycolysis and utilization of muscle glycogen. During sustained exercise, fatty acids become the source of fuel. Exercise, fasting, cold, infections, and medications may elicit symptoms.
- Important causes of metabolic myopathy include adenosine monophosphate (myoadenylate) deaminase deficiency and disorders of glycolysis, glycogenolysis, fatty acid oxidation, and oxidative phosphorylation. In many cases other organs are involved.
- Diagnosis requires careful attention to dietary and exercise history and appropriate laboratory investigations. Exercise testing, electromyogram, molecular testing, and muscle biopsy can provide essential information.
- Treatment depends on avoiding precipitating factors and optimizing muscle energetics.

28.1 General Remarks

Many metabolic disorders cause muscle dysfunction or damage (Table 28.1). The pathophysiological basis in most is impairment of energy production when stressed, particularly by exercise, cold, fasting, or infection (especially of viral origin). In some situations, the problem is confined to skeletal muscle, but in many there is cardiac involvement as well. Liver, brain, retina, and kidney, all of which have significant energy requirements, may also be involved. Even more extensive or patchy involvement, e.g., pancreas and bone marrow, is a characteristic of the mitochondrial disorders where heteroplasmy may occur (see also Chaps. 14 and 42).

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Table 28.1 Major presentations of metabolic myopathies

	Enzyme (position- in pathway – Fig. 28.1)	Rhabdo-myolysis	Hypotonia, weakness	Cramps	Worsened by fasting?	Worsened by exercise?	Worsened by cold?	Worsened by infection?	Increased baseline CK	Abnormal EMG	Muscle biopsy	Organic acids	Carnitine level	Acylcarnitine profile	Cardio-myopathy	Hepatic dysfunction[a]	Encephalo-pathy[a]	Diagnostics tissues	Comments
Rhabdomyolysis and myoglobinuria presentation																			
Intense exercise not tolerated; second wind phenomenon: disorders of glycogenolysis and glycolysis; AMP deficiency																			
GSD type V	Muscle phosphorylase (II)	++	+	++		++			+		+							M	Occ. severe infantile form. Proximal > distal
GSD type IX	Muscle phosphorylase kinase (II)	+	+	+		+			+		+				One form			M	Four syndromes, two involving skeletal muscle
GSD VII	Muscle phosphofructokinase (Oldfors and and DiMauro 2013) (II)	+	+	+		+	++		++	+	++				+			M and E	Hemolytic anemia, rare, hyperuricemia. Muscle cannot utilize glucose – worse after glucose, high-CHO meal. Severe infantile form with cardiomyopathy
PGK deficiency	Muscle phosphoglycerate kinase (III)	++		++		++			++		++	-					++	M	Hemolytic anemia. Neurologic abnormalities. X linked
PGAM deficiency	Phosphoglycerate mutase (III)	++		++		++			++	-	++	-						M	Very rare
β-Enolase deficiency	β-Enolase (III)	++		++		++			+	-	++	-						M	Very rare
LDH deficiency	Lactic dehydrogenase M subunit (Hogan and and Vladutiu 2009; Joshi et al. 2012)	++				++					-	-						M	Very rare. Lactate does not rise after ischemic exercise when pyruvate is elevated. Uterine stiffness

Myoadenylate deaminase deficiency	Muscle AMP deaminase	+	+	+		+			-	+	++							L	Impaired ammonia production with ischemic exercise
Short, intense exercise tolerated; prolonged exercise not tolerated: disorders of fatty acid oxidation and carnitine-assisted transport into mitochondria																			
Translocase deficiency	Carnitine-acylcarnitine translocase (Kishnani et al. 2010; Parikh et al. 2013a)	+	+	++	++	++	++	++	+			+	+	++	++	++	+	F	
CPT II deficiency	Carnitine palmitoyltransferase II (Magoulas and and El-Hattab 1993; Parikh et al. 2013b)	++			++	++	++	++			++	+	?	++	++	++	+	F, M, L, and W	Heterozygote may have symptoms, be vulnerable to malignant hyperthermia. Lactic acidosis. Sensorimotor neuropathy.
VLCAD deficiency	Very-long-chain acyl-CoA dehydrogenase (Roe and Brunengraber 2015)	++	+		++	+		+	+	++		++	++	++	++	++	++	F, W, and L	
LCHAD/ trifunctional enzyme deficiency	Long-chain 3-hydroxyacyl-CoA dehydrogenase (Niezgoda and and Morgan 2013; Roe et al. 2010))	++										++	++	++	++	++	++	F, M, L, and D	HELLP syndrome in pregnant heterozygote
Hypotonia and weakness. Carnitine deficiency or depletion; Pompe disease; glycogen brancher and debrancher deficiencies, mitochondrial myopathies																			
Carnitine transporter deficiency	Plasma membrane carnitine transporter (Lachmann and and Schoser 2013)		++		++				+	+	++	+	??		++	++	+	F, M, L, and K	

(continued)

Table 28.1 (continued)

	Enzyme (position- in pathway – Fig. 28.1)	Rhabdo-myolysis	Hypotonia, weakness	Cramps	Worsened by fasting?	Worsened by exercise?	Worsened by cold?	Worsened by infection?	Increased baseline CK	Abnormal EMG	Muscle biopsy	Organic acids	Carnitine level	Acylcarnitine profile	Cardio-myopathy	Hepatic dysfunction[a]	Encephalo-pathy[a]	Diagnostics tissues	Comments
Secondary carnitine depletion			++			+	+	+		+	+	+	??		+	++	+		Occurs in many settings
MCAD deficiency	Medium-chain acyl-CoA dehydrogenase (Roe and Brunengraber 2015)	–	(+)		++	+	-	++				+	++	++	+	++	++	F, L, W, and D	Myopathy is minimal; this is the commonest disorder of fatty acid oxidation. Most early cases called systemic carnitine deficiency
Mild multiple acyl-CoA dehydrogenase deficiency (MADD) deficiency	Electron-transfer flavoprotein (ETF) or ETF dehydrogenase ETF-QO		+							+	+	++	+	++	++	++	++	F, M, and L	Mild forms exist – may respond to riboflavin
Lysosomal glycogen storage disease (Pompe)	Lysosomal α-glucosidase		++						++	++	++				++			W, M, and F	Variable. Macroglossia, severe cardiomyopathy in infantile Pompe form
GSD type III	Glycogen debrancher (II)	+	++	+					++		++				Most, but sx rare	++		E, F, H, L, M, and W	Liver ± muscle. Distal > proximal.
GSD type IV	Glycogen brancher enzyme (I)		+								++				+	++		L, M, W, E, F, and D	Severe liver disease. Neuropathy, dementia in adult form

Neutral lipid storage disease	ATGL or comparative gene identification-58		+						+	+	++							M	Ichthyosiform nonbullous erythroderma
Mitochondrial Myopathies	Many disorders and mechanisms; see Chap. 14 and Fig. 14.2	+	++	+		+	+	+	+	+	++	+	+	+	++	+	++	M, W, L, H, and F	Several syndromes. See Table 42.3. Lactate/ pyruvate ratio usually increased
Coenzyme Q deficiency	Several known gene defects and mechanisms	+	++								+				+	+	+	M	Renal tubular dysfunction, ataxia. Myopathic forms may be due to MADD

This list is only a guide. Which tissue is required and which test is to be done depend on the laboratory performing the test and in some cases on which organs are affected. Molecular testing (DNA) has replaced many of the tissue-specific enzyme tests and protein stains (immunohistochemistry and other techniques)

D DNA (predominant or common mutation), *E* erythrocytes, *F* fibroblasts, *H* heart, *K* kidney, *L* liver, *M* muscle, *W* leukocytes

++ Often present or abnormal; + occasionally present or abnormal

[a]Hepatic dysfunction with encephalopathy is a "Reye-like syndrome"

28.1.1 Special Aspects of Skeletal Muscle Metabolism

Skeletal muscle relies on different fuel sources at different times and circumstances. Fatty acids are the primary fuel at rest. Glucose from the blood and derived from muscle glycogen is used during short-term intensive exercise. Fatty acids predominate again during prolonged exercise and during fasting. Impairment of muscle energy metabolism will lead to clinical symptoms. The history of events that elicit the symptoms is a guide to the likely area of the biochemical defect.

Triglycerides are stored in the cytoplasm as droplets close to the mitochondria. The mobilization of free fatty acids from the lipid droplets depends on hormone-sensitive lipases and involves the hydrolase ATGL and its activator protein comparative gene identification-58 (CGI-58). Lipid storage myopathies occur with deficiency of these proteins (Wu et al. 2015).

Resting muscle in the fed state uses fatty acids as the primary fuel; glucose is stored as muscle glycogen (in the cytoplasm). Preformed high-energy phosphate compounds and muscle glycogen, in addition to glucose and fatty acids in the blood stream, are a source of energy for short-term intense activity. Lactate is the end product of anaerobic glycolysis. Impaired ability to utilize muscle glycogen (e.g., glycogen storage diseases (GSD) III, debrancher deficiency, and V, muscle phosphorylase deficiency, and muscle phosphorylase kinase deficiency) results in significant limitation when the patient attempts short, intense exercise. There will be diminished production of pyruvate and hence lactate. The situation is magnified if the muscle being tested is deprived of oxygen and continuous fuel by a tourniquet. This is the principle of the ischemic exercise test.

Defects of glycolysis in muscle, e.g., deficiencies of muscle phosphofructokinase (PFK), phosphoglycerate kinase (PGK), phosphoglycerate mutase (PGAM), β-enolase, and lactate dehydrogenase (LDH), can cause symptoms similar to muscle phosphorylase deficiency.

When sustained muscle activity is initiated, there is an initial reliance on glucose and glycogen as a fuel source. Metabolic disorders affecting these pathways typically cause symptoms at this time. After a few minutes, glycogen stores will be depleted, and fatty acids become more important as a fuel. The carnitine cycle and the β-oxidation spiral of fatty acid oxidation are essential at this point, so defects in these pathways can result in easy fatigability and impaired tolerance of sustained exercise.

The pathways of glycogenolysis, glycolysis, and fatty acid oxidation are shown in Fig. 28.1. Not all the steps mentioned in the text are specifically indicated on the pathway figure.

28.1.2 Basic Patterns of Metabolic Myopathies

The two major distinct syndromes of muscle metabolic disorders are exercise intolerance and rhabdomyolysis (with or without myoglobinuria) and weakness (with or without hypotonia). Rhabdomyolysis can be further divided into syndromes where it occurs during strenuous exercise and those where it occurs afterward.

Remember

Major symptoms of myopathies are weakness, hypotonia, exercise intolerance, and rhabdomyolysis.

Rhabdomyolysis, the destruction of skeletal muscle cells, often results from failure of energy production, leading to an inability to maintain muscle membranes. The hallmark of rhabdomyolysis is elevation of muscle enzymes in the blood, particularly creatine kinase (CK or CPK). This elevation can persist for several days after an acute event. Chronic elevation of CK indicates continuous damage (Wu et al. 2015; Chan et al. 2015; Sharp and Haller 2014).

Rhabdomyolysis during short-term intensive exercise is a primary feature of the disorders of carbohydrate metabolism, especially muscle phosphorylase deficiency (McArdle disease). After a period of intense pain with or without cramping, however, there may be considerable relief and ability to continue exercise, called the "second wind" phenomenon, as the muscle

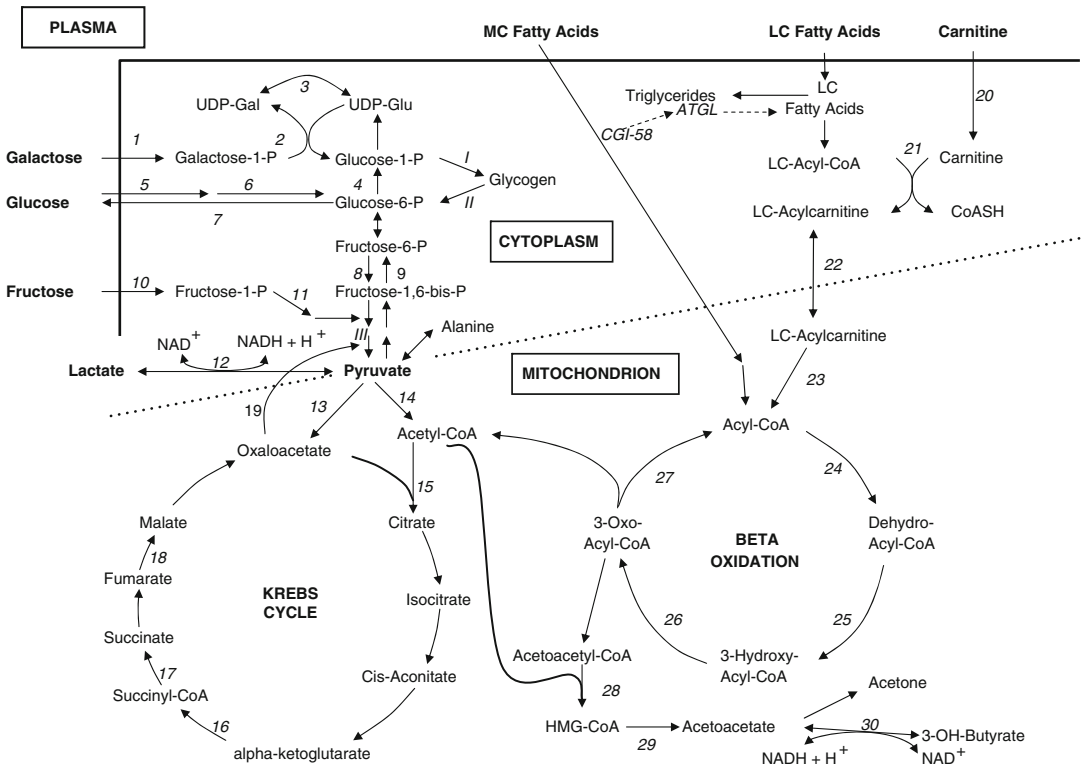

Fig. 28.1 Pathway of carbohydrate and fatty acid metabolism illustrating the relationships of glycogenolysis, glycolysis, and fatty acid oxidation. Major aspects of glucose and fatty acid metabolism. Single arrows represent single steps; sequential arrows (and Roman numerals) represent multiple steps. *I* Glycogen synthesis—glycogen synthase, brancher enzyme; *II* Glycogenolysis—phosphorylase kinase, phosphorylase, debrancher enzyme; *III* Glycolysis—phosphoglycerate kinase, phosphoglycerate mutase, etc. *1* Galactose kinase; *2* galactose-1-P uridyl transferase; *3* epimerase; *4* phosphoglucomutase; *5* glucose transporter; *6* hexokinase; *7* glucose-6-phosphatase; *8* phosphofructokinase; *9* fructose-1,6-bis-phosphatase; *10* fructokinase; *11* fructose aldolase; *12* lactate dehydrogenase; *13* pyruvate carboxylase; *14* pyruvate dehydrogenase complex; *15* citrate synthase; *16* alpha-ketoglutarate dehydrogenase complex; *17* succinyl-CoA synthetase; *18* fumarase; *19* phosphoenolpyruvate carboxykinase; *20* carnitine transporter; *21* carnitine palmitoyltransferase I (CPT I); *22* carnitine-acylcarnitine translocase; *23* carnitine palmitoyltransferase II (CPT II); *24* acyl-CoA dehydrogenases (very-long-chain (VLCAD), long-chain, medium-chain (MCAD), short-chain (SCAD)); *25* 2-enoyl-CoA hydratase (trifunctional enzyme, crotonase); *26* 3-hydroxyacyl-CoA dehydrogenases (long-chain (trifunctional enzyme/LCHAD), short-chain (SCHAD)); *27* 3-ketoacyl-CoA thiolase (long-chain (trifunctional enzyme), short-chain). (Acetoacetyl-CoA is the 4-carbon 3-ketoacyl-CoA.); *28* hydroxymethylglutaryl-CoA (HMG-CoA) synthase; *29* hydroxymethylglutaryl-CoA (HMG-CoA) lyase; *30* 3-OH-butyrate (beta-hydroxybutyrate) dehydrogenase

switches to increased use of fatty acids for fuel. Patients with McArdle disease will also benefit from glucose administration before exercise, because they are able to utilize glucose. In contrast, patients with a metabolic block in glycolysis, e.g., in PGK, cannot utilize glucose or glycogen. Glucose administration even diminishes the concentrations of the alternative fuels triglycerides and ketone bodies ("out of wind" phenomenon).

Postexercise cramps and rhabdomyolysis are the more common pattern in fatty acid disorders, especially deficiencies of carnitine palmitoyltransferase (CPT) II, very-long-chain acyl-CoA dehydrogenase (VLCAD), long-chain hydroxyacyl-CoA dehydrogenase (LCHAD) (see also Chap. 16), and mitochondrial oxidative phosphorylation disorders. Rhabdomyolysis may also be a chronic feature of the various muscular dystrophies, which are usually disorders of the

structural proteins of muscle (dystrophin, actin, tropomyosin, the dystroglycan complex, etc.).

Remember

Short, intense exercise stresses glycolysis and glycogen utilization.

Prolonged exercise requires adequate utilization of fatty acids.

Fasting, cold, infection, and medications can worsen many myopathies.

Myoglobinuria is an extreme result of rhabdomyolysis. When muscle cells lyse, myoglobin is released. Visible myoglobin in the urine indicates extensive damage. Typically, there is no myoglobin in the urine if the CK is <10,000 IU, so the absence of myoglobinuria provides no reassurance regarding absence of rhabdomyolysis.

Myoglobinuria is an emergency situation, as the pigment may precipitate in the renal tubules, leading to renal failure, which may become irreversible. Severe rhabdomyolysis can also raise the serum potassium level dangerously high, leading to cardiac rhythm disturbances. Accordingly, dark urine in a patient suffering from muscle symptoms (pain, weakness, cramping, etc.) must be tested for myoglobin using a specific test (to distinguish the pigment from hemoglobin). Hemoglobinuria most often accompanies hematuria, readily detectable by finding erythrocytes on microscopic analysis of the urine. Intravascular hemolysis will occasionally result in hemoglobinuria, without hematuria.

Myoglobinuria is treated with diuresis and careful monitoring of electrolyte, fluid status, and urine output, until the myoglobinuria resolves. Investigation of the underlying cause of myoglobinuria begins at the same time as its treatment.

Chronic weakness and hypotonia are typical features of disorders of endogenous triglyceride catabolism, glycogen breakdown, carnitine availability, fatty acid oxidation, and oxidative phosphorylation. Important causes include lysosomal GSD (acid maltase deficiency – Pompe disease), glycogen debrancher deficiency, carnitine transporter defect and secondary carnitine deficiencies, VLCAD, LCHAD, and mitochondrial myopathies. Chronic weakness may certainly result from rhabdomyolysis and consequent muscle destruction from any cause, especially if recurrent.

28.2 Approach to Metabolic Myopathies

The most urgent issues in the assessment of myopathy are to determine if there is weakness so severe to impair respiration, if there is sufficient damage to lead to myoglobinuria, and if there is cardiac involvement. Hepatic involvement, often manifest as hypoglycemia and fasting intolerance, occurs in many metabolic disorders, particularly those involving glycogen or fatty acid metabolism. A toxic encephalopathy, including cerebral edema, may also develop.

The history of muscle dysfunction may be easy to elicit from an adult or a child (or the parents), but may be difficult with infants. Hypotonia and weakness may first become evident as developmental delay. Careful assessment may then reveal that social, fine motor, and language skills are appropriate for age, and the only area of delay is in gross motor skills.

As a young child grows older, problems with exercise intolerance and easy fatigability become easier to detect, particularly if there is an unaffected older sibling to serve as a reference point for the parents. Occasionally, a child with a muscle disorder is thought to be "seeking attention" or malingering, but careful history and observation can usually eliminate this possibility quickly. Laboratory tests that convincingly demonstrate ongoing muscle injury (e.g., elevated CK) are most persuasive.

Disorders made worse by fasting may not be evident in infancy, as most infants are fed frequently. An inability to tolerate intense or prolonged exercise will not be evident in infancy and perhaps not until adulthood. Rhabdomyolysis in response to cold also may not become evident until adolescence or adulthood. Rhabdomyolysis triggered by infection (usually viral), or fasting, may present in infancy as sudden weakness, accompanied by dark urine. Rhabdomyolysis is often quite painful, but may be painless.

Some conditions that are not yet completely characterized can cause severe and potentially fatal rhabdomyolysis in children, particularly in the setting of viral infection. Children with such conditions, like children with named disorders of fatty acid oxidation or mitochondrial dysfunction, need to be monitored carefully during infections.

The history of exercise can provide preliminary guidance in determining the most likely causes of a myopathy. An inability to perform sudden intense exercise suggests a problem with glycogenolysis or glycolysis, while inability to perform at a sustained level suggests a problem with fatty acid oxidation.

Many mitochondrial disorders of oxidative phosphorylation first become apparent because of skeletal muscle weakness. Even isolated myopathies can occur. Rhabdomyolysis is uncommon. Mitochondrial disorders may have prominent muscle involvement. Mitochondrial disorders can involve any organ at any age. Other organs commonly affected include the brain, retina, extraocular muscles, cochlea, heart, liver, kidney, pancreas, gut, and bone marrow. Systemic growth may be impaired. Mild hypertrichosis often accompanies systemic lactic acidosis. Despite the diversity of mitochondrial dysfunction, there are several common syndromes in which many patients can conveniently be grouped. They include MERRF, MELAS, and infantile myopathy (Chap. 42).

Two very rare neutral lipid storage diseases have been identified. Neutral lipid storage disease type I or Chanarin–Dorfman syndrome has ichthyosiform nonbullous erythroderma with a slowly progressive proximal myopathy that spares the axial musculature. It is due to mutations in ABHD5. Neutral lipid storage disease type II is due to mutations in the activator gene PNPLA2, again leading to a lipid myopathy associated with cardiac dysfunction and hepatomegaly.

28.2.1 Genetics

Most metabolic myopathies, like most other metabolic disorders, are inherited in an autosomal recessive manner. All disorders of fatty acid oxidation and most disorders of glycogen and glucose metabolism are inherited this way. However, other mechanisms including X-linked (PGK deficiency and one form of phosphorylase b kinase deficiency), autosomal dominant (heterozygous CPT II deficiency), mitochondrial maternal transmission, and sporadic mitochondrial disorders occur. Specifics of inheritance are mentioned when appropriate in the discussion of the various disorders.

Because of the highly variable nature of most metabolic disorders of muscle, all siblings of patients should be checked for the condition which is in the family. If the disorder may be dominant, X linked, or mitochondrially inherited, other at-risk relatives should also be examined carefully.

28.2.2 Physical Examination

The general physical examination of a patient suspected of myopathy includes assessment of growth and development and particular attention to other organs. The muscles should be examined for bulk and regional (proximal and distal) or local evidence of wasting, texture and consistency, and tenderness. Deep tendon reflexes, which are generally preserved in myopathies, but lost in peripheral neuropathies, should be tested carefully. Attention should be especially directed to extraocular movements and the retina, hearing, the tongue, the heart, and the size and characteristics of the liver.

28.2.3 Laboratory Investigations

Laboratory investigation of suspected myopathies should be undertaken during the acute episode if possible and later repeated as indicated. Routine serum electrolytes and measurement of glucose, urea and creatinine, and "muscle enzymes" including CK, LDH including isoforms, aldolase, SGOT (ALT), SGPT (AST), total and free carnitine, plasma or blood spot acylcarnitines profile, plasma lactate and pyruvate, phosphate, calcium, thyroid hormone,

plasma and urine amino acids, and urine organic acids may all provide useful information.

Following the assessment of the first-order laboratory tests, further tests may be warranted. Functional testing using ischemic exercise (for suspected glycogen storage and glycolytic disorders and adenosine monophosphate deaminase deficiency) can be most helpful. Graded exercise or bicycle ergometry may help pinpoint the metabolic error, or define the general area of impairment, if history and blood tests have not done so. A "diagnostic fast" to evoke abnormal metabolites or provoke symptoms should only be done if information cannot be obtained by another method – challenges are better put to fibroblasts or tissue samples. However, a fast under controlled circumstances can provide valuable information regarding how long it is safe for a particular child to fast when healthy (see Chap. 41).

Third-order tests include electromyogram (often coupled with nerve conduction studies), chest X-ray, electrocardiogram, echocardiogram, and muscle biopsy (perhaps together with nerve biopsy). Light and electron microscopic examination and special stains for glycogen, lipid, and various enzymes may all be essential. "Classic" lipid storage is found in four conditions: primary carnitine deficiency due to a deficiency of the carnitine transporter, mild multiple acyl-CoA dehydrogenase deficiency (MADD) with secondary coenzyme Q_{10} deficiency, and the neutral lipid storage diseases types I and II.

Many enzymes can be studied in fibroblasts or lymphocytes, and DNA can be obtained from blood or a buccal brush instead of a tissue biopsy. Mitochondria can be prepared for functional and molecular studies from muscle (the preferred source) but also liver, leukocytes, and other samples. Coenzyme Q is best measured in muscle. Details of these tests are given in (Chap. 43). If an open muscle biopsy is done, a skin biopsy for fibroblast culture and DNA analysis can be taken from the edge of the incision. Some pathologists and mitochondrial laboratories are able to analyze muscle tissue obtained by needle biopsy. Table 28.1 provides a guide to the principal features of the major metabolic myopathies and the usefulness of the various diagnostic materials. Molecular diagnosis using panels of relevant genes is rapidly replacing biochemical and immunohistochemical testing done on biopsy samples and testing of individual genes. The new testing methods are also revealing that some patients have more than one condition contributing to their symptoms.

28.3 Specific Disorders of Muscle Metabolism

28.3.1 Exercise Intolerance/ Rhabdomyolysis and Myoglobinuria Presentation

28.3.1.1 Intense Exercise Not Tolerated. Mild, Prolonged Exercise Tolerated. Fasting Tolerated. Dietary Modifications Helpful

Muscle Glycogen Phosphorylase (Myophosphorylase) Deficiency: McArdle Disease (GSD Type V)

This dramatic disorder of muscle glycogen metabolism is a relatively common cause of rhabdomyolysis and myoglobinuria. Although it is inherited in an autosomal recessive manner, most symptomatic patients are men. Symptoms usually begin between late childhood and late middle age. Strenuous exercise leads rapidly to cramping and fatigue, but, after a period of rest (adaptation), exercise is tolerated. Some patients with McArdle disease have chronic progressive weakness and wasting, without pain or cramping.

Exceptional cases include a rapidly fatal form in infants or young children with hypotonia and generalized weakness, a late-onset form with chronic weakness, and a late-onset form with severe symptoms (pain, cramping, weakness, and muscle swelling), after decades of normal activity. Diagnosis after inadvertent discovery of elevated CK in a child without symptoms has been reported.

Diagnosis is suspected from the symptoms and response to ischemic exercise. The enzyme is expressed mainly in muscle. Muscle biopsy may show myopathic changes and increased glycogen content. There are two major mutations in the gene PYGM (p.Arg50Ter and p.Gly205Ser) which account for a majority of the mutations in most American and European populations. Other pathogenic variants may be relatively common in specific populations (Martin et al. 1993). Abnormalities in other enzymes may contribute to severity of symptoms (Gonzalez-Freire et al. 2009; Martinuzzi et al. 2003).

Specific treatment is generally not needed, as avoiding strenuous exercise prevents symptoms in most patients. A high-protein, low-carbohydrate diet has been suggested to improve endurance. Others have found increased carbohydrate intake (glucose and fructose) immediately before exercise to be helpful (Orngreen et al. 2009).

Muscle Glycogen Phosphorylase Kinase Deficiency (Formerly Phosphorylase b Kinase Deficiency, GSD IX)

There have been a few men with deficiency of muscle phosphorylase kinase. Weakness without cramps and cramps without weakness have both been reported. Increased muscle glycogen content, elevation of CK, and rhabdomyolysis have been reported. Enzyme deficiency was demonstrated in muscle. The gene encoding the alpha subunit of phosphorylase (PHKA1) in muscle is on the X chromosome. It is distinct from the liver isoform (also on X). There are also three autosomal components of the glycogen phosphorylase kinase system. Autosomal recessive defects have been found in the beta and gamma peptides. No defects in the three delta subunit isoforms (which are calmodulins) have been reported to date.

Muscle Glycolytic Disorders

Deficiencies of five glycolytic enzymes in muscle are rare causes of myopathy similar to muscle phosphorylase deficiency. They are PFK, PGK, PGAM, β-enolase, and triosephosphate isomerase. Hemolytic anemia can occur in all. PGK deficiency is X linked. Patients may have neurologic problems (mental retardation, behavioral abnormalities, seizures, and strokes). Other disorders are autosomal recessive (Oldfors and DiMauro 2013).

Lactate Dehydrogenase (LDH) Deficiency

Lactate dehydrogenase catalyzes the conversion of pyruvate and NADH to lactate and NAD^+. The enzyme is a tetramer of H and M peptides, produced from LDHB and LDHA genes. Homozygous deficiency of the M protein results in impaired muscle LDH activity. The result is impaired regeneration of NAD^+ for anaerobic glycolysis and impaired production of lactate (and resulting in high levels of pyruvate) with exercise, detectable by the ischemic exercise test. Cramps, weakness, and myoglobinuria can occur with strenuous exercise. Deficiency of LDH can result in a "false-negative" result if LDH is being measured to assess tissue damage in other situations. No syndrome is attributable to LDHB deficiency.

Adenosine Monophosphate (Myoadenylate) Deaminase Deficiency

This autosomal recessive disorder of purine metabolism is probably the commonest metabolic myopathy, with impaired exercise tolerance, postexercise cramps, and myalgias. Myoglobinuria is uncommon, but CK is often elevated after exercise. Onset of symptoms (usually pain after exercise) ranges from childhood to later adult life. In the US population perhaps, 2 % are homozygous for deficiency, but most have no symptoms. Because AMP deaminase deficiency is so common, it has sometimes been found coincidentally with a less common muscle disorder that by itself would account for the symptoms, e.g., muscle phosphorylase or PFK deficiency. As the enzyme deficiency will only be discovered by ischemic exercise testing, or specific assay or molecular test, there is a selection bias toward muscle problems.

Many patients discovered to have AMP deaminase deficiency have other symptoms as well, especially neuromuscular disease. Diminished synthesis of AMP deaminase occurs in a variety of situations. This is termed

acquired deficiency and does not seem to have a direct genetic basis, and the common mutation is not present at a frequency above the background rate.

AMP deaminase catalyzes the deamination of AMP to IMP (inosine monophosphate) in the purine nucleotide cycle (see Fig. 28.2). During exercise there will be increased production of

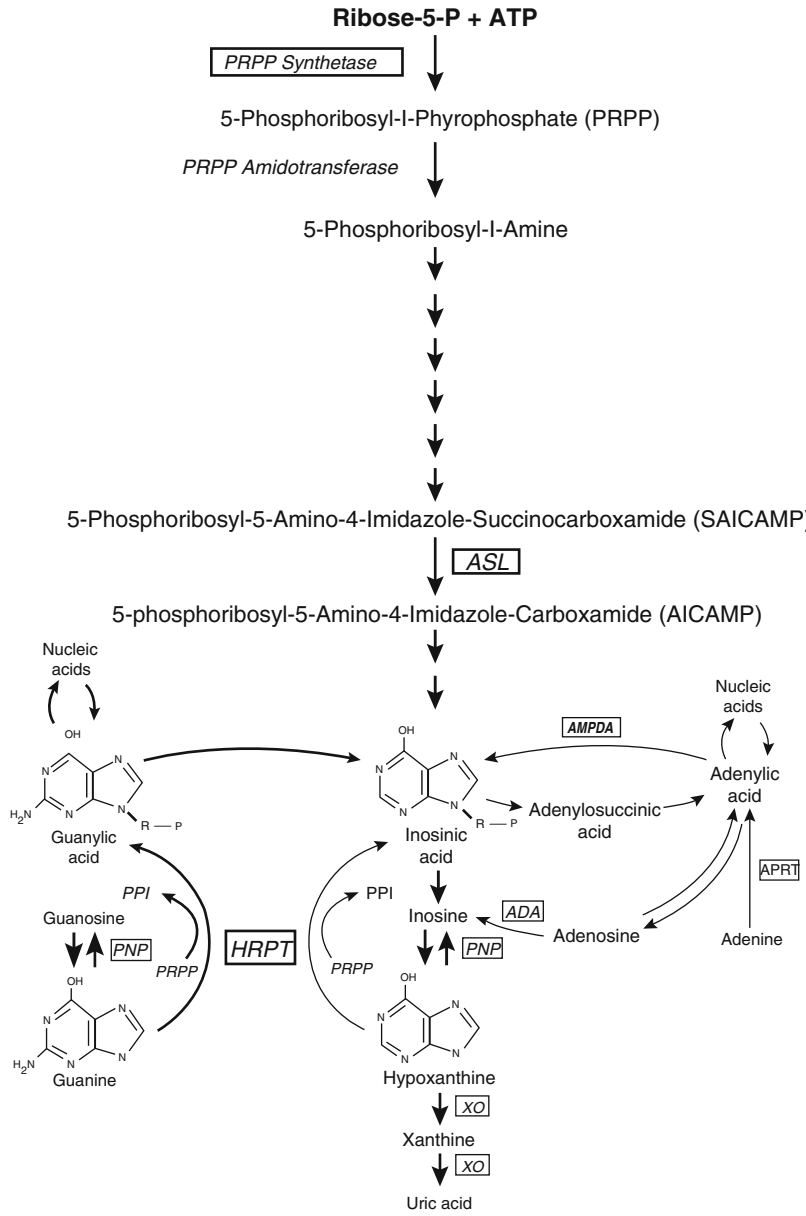

Fig. 28.2 Pathways of synthesis, salvage, and degradation of purines. The enzymatic steps in boxes indicate the sites of the commonly encountered disorders of purine metabolism *AMPDA* muscle adenosine monophosphate deaminase deficiency, *APRT* adenine phosphoribosyltransferase deficiency, *ASL* adenylosuccinate lyase (adenylosuccinase) deficiency, *HPRT* hypoxanthine–guanine phosphoribosyltransferase deficiency, *PNP* purine nucleoside phosphorylase deficiency, *SO* sulfite oxidase deficiency, *XO* xanthine oxidase (xanthine dehydrogenase) deficiency

IMP and ammonia and maintenance of the adenylate energy charge by preventing AMP accumulation. A decrease in ATP and increase in ammonia will also stimulate glycolysis by increasing the activity of PFK. The increase in IMP may also enhance glycogen phosphorylase. Finally, during intense exercise, AMP deaminase moves from the cytosol and becomes bound to myosin, which suggests that it is important in muscle metabolism during such times.

The diagnosis of AMP deaminase deficiency is approached by ischemic exercise which ordinarily provokes a rise in blood ammonia level. If AMP is deficient there will be diminished ammonia production. Muscle biopsy may be normal or show some myopathic changes. Specific staining for AMP deaminase is a generally reliable diagnostic test. Enzyme activity in deficient muscle ranges up to 15 % of normal; some authorities regard activity >2 % as adequate to prevent symptoms. A common mutation in AMPD1 accounts for most cases of inherited AMP deaminase deficiency. This common mutation is actually a pair of mutations in linkage disequilibrium (p.Q12X) and p.Pro48Leu. The nonsense mutation in exon 2, p.Q12X, can result in a severely truncated protein. However, an alternative splicing mechanism allows for phenotypic rescue by production of a shortened but functional protein, with p.Pro48Leu in exon 3 retained. This may account for the great variability in symptoms in homozygotes for this mutation. The allele frequency was 0.13 (Caucasians) and 0.19 (African-Americans) in one study, which accounts for the observed homozygote frequency of about 0.02. Symptoms may be due to combined deficiencies of AMP deaminase and another enzyme involved in muscle metabolism (Bruno et al. 1998; Rubio et al. 1997). Polymorphisms of angiotensin I-converting enzyme may contribute to the variability of symptoms seen with AMP deaminase deficiency.

AMP deaminase deficiency has been found in patients with aldolase deficiency, lactate dehydrogenase A (LDHA) deficiency, and β-enolase deficiency.

28.3.1.2 Short, Intense Exercise Tolerated, Prolonged Exercise Not Tolerated, Fasting Detrimental, Diet Effects Less Pronounced. Symptoms May Be Triggered by Infection. Restriction of Long-Chain Fats, with Supplementation of Medium-Chain Lipids, May Be Helpful, Especially for Cardiomyopathy

Carnitine-Acylcarnitine Translocase Deficiency

An inability to import long-chain acylcarnitines into the mitochondrial matrix would be expected to cause serious difficulties, especially in cardiac and skeletal muscle and in the liver. The severe infantile form of translocase deficiency typically does this, starting a day or two after birth. Cardiac (cardiomyopathy, usually hypertrophic) and hepatic dysfunction (hypoglycemia, vomiting, and hyperammonemia) are more prominent than hypotonia and weakness. Urinary organic acids show dicarboxylic aciduria, and the plasma/blood spot acylcarnitine profile is dominated by long-chain species (C16:1, C18:1, and C18:2), which can be formed but not used, and dicarboxylic acylcarnitines.

CPT II Deficiency

CPT II deficiency is the commonest disorder of fatty acid oxidation to cause episodic rhabdomyolysis. CPT II is needed to synthesize long-chain acylcarnitines once they have been translocated into the mitochondria, so the clinical features are similar to translocase deficiency. Prolonged exercise, cold, infection, and emotional stress (which will increase catecholamines and fatty acid metabolism) may precipitate episodes, which usually do not occur in children. Cardiac involvement is uncommon in this form of CPT II deficiency.

Plasma or blood spot acylcarnitine analysis shows prominent long-chain species, especially saturated and unsaturated C16 and C18 forms, and there may be dicarboxylic aciduria, similar to translocase deficiency.

A severe form of infantile CPT II deficiency also exists. Hepatic encephalopathy with hypoketotic hypoglycemia, severe cardiac involvement, renal malformations, and low plasma and tissue carnitine levels may be present. This form is usually fatal, from cardiac complications. It is discussed in more detail in Chap. 23.

Treatment includes avoiding fasting and providing adequate fuel for the muscles. Medium-chain fatty acids do not need the carnitine system in order to enter the mitochondria, so they can be used in place of long-chain fats as a source of energy.

Although this is an autosomal recessive disorder, heterozygosity for a mutation may be associated with myopathy (Joshi et al. 2012) and risk of malignant hyperthermia in response to anesthetics or muscle relaxants (Hogan and Vladutiu 2009; Vladutiu et al. 2000).

There is a form of CPT I that is solely expressed in muscle. No clinical deficiency has been recognized so far.

VLCAD Deficiency

VLCAD is the first enzyme of the β-oxidation spiral. It is bound to the inner mitochondrial membrane. Deficiency of VLCAD is a common cause of metabolic myopathy and cardiomyopathy. For several years, the enzyme now known as VLCAD was called LCAD (long-chain acyl-CoA dehydrogenase); reports from before about 1993 regarding LCAD deficiency almost always involve what is now called VLCAD. VLCAD utilizes fatty acids of 14–20 carbons. A major source of confusion is that fatty acids called very-long-chain fatty acids, of chain length >20, are metabolized by a different system altogether, in the peroxisomes.

Impairment of VLCAD will lead to variable skeletal, cardiac, liver, and brain symptoms, including recurrent Reye syndrome with coma, and hypoketotic hypoglycemia. Muscle soreness and episodic rhabdomyolysis may be provoked by infection, cold, fasting, or emotional stress (perhaps mediated by catecholamines). There is usually dicarboxylic aciduria, although during severe metabolic derangement it may be overlooked because of excessive lactic aciduria indistinguishable from a primary defect of the respiratory chain. Carnitine depletion, with low plasma and tissue levels, can occur, and acylcarnitine analysis shows prominence of C14:1 (tetradecenoyl) species, derived from oleic acid (C18:1). Hepatic dysfunction may result in hyperammonemia and lipid accumulation. Muscle biopsy may show lipid storage, and the EMG is often myopathic.

VLCAD deficiency, like other disorders of fatty acid oxidation, must be promptly treated during the acute episode with glucose sufficient to maintain the blood glucose level at 6–8 mM or even higher (see also Chap. 19). The use of carnitine supplementation has been controversial on theoretical and experimental grounds, particularly because of fear that long-chain acylcarnitines would accumulate and provoke arrhythmias. However, there are few convincing reports of this actually happening. Long-term management emphasizes adequate calories from carbohydrate, restricting long-chain dietary fats, avoiding fasting and other stressors, and supplementing with medium-chain triglycerides, which will provide a source of fuel that can be metabolized without requiring VLCAD. Triheptanoin, a novel treatment to provide a constant source of ketone bodies and propionyl groups for patients with defects of long-chain fatty acid oxidation, is showing great promise in preliminary studies (Roe and Brunengraber 2015).

The enzyme now known as LCAD is in the mitochondrial matrix. Its major substrates are unsaturated long-chain fatty acids, 12–18 carbons in length. Deficiency has not been convincingly demonstrated in humans.

LCHAD (Including Trifunctional Protein) Deficiency

LCHAD deficiency often results in chronic myopathy, with rhabdomyolysis, which may be extensive, particularly during viral infections. Like other disorders of long-chain fatty acids, there is often cardiomyopathy and significant liver dysfunction, both of which may be fulminant. The extent of chronic liver dysfunction can be greater than in other disorders, and fibrosis often occurs. There may be Reye-like episodes of hepatic

encephalopathy. In addition there may be peripheral neuropathy and retinopathy. The basis for these complications is not yet completely known.

The enzyme activity called LCHAD is found in an octameric protein (α4β4) called the trifunctional protein, for its ability to catalyze the 2-enoyl-CoA hydration, 3-hydroxyacyl-CoA dehydrogenation, and 3-oxoacyl-CoA thiolysis of long-chain acyl-CoAs. The first two activities reside in the α-subunit and thiolase activity in the β-subunit. The two subunits depend on each other for stability. The trifunctional protein is in the mitochondrial inner membrane.

There is some relationship between mutation and symptoms. The most common mutation (87 % in one study), c.1528 G > C (p.E510Q) in the α-subunit, usually causes liver dysfunction with hypoketotic hypoglycemia in infancy.

LCHAD deficiency is an autosomal recessive disorder, and carriers are generally symptom-free. A particular complication of heterozygous (carrier) status for LCHAD deficiency, especially the p.E510Q mutation, is serious liver disease during pregnancy when carrying an affected infant. The mother may suffer from acute fatty liver of pregnancy (AFLP, with nausea and anorexia, vomiting, and jaundice) or the HELLP (hemolysis, elevated liver enzymes, and low platelets) syndrome of hypertension (Ibdah et al. 2000; Jebbink et al. 2012). It may be that the production of abnormal fatty acid metabolites by the fetus overloads the mother's ability to deal with them, on top of the increased fatty acid mobilization that occurs during pregnancy. Prospective studies of women with AFLP or HELLP syndrome, however, have not always found an increased number of carriers of LCHAD deficiency, indicating there are other causes for these conditions. Women heterozygous for hepatic CPT I deficiency, which may cause a Reye-like syndrome in homozygotes, may also suffer from AFLP when carrying an affected fetus (Innes et al. 2000).

In untreated patients, the urine organic acids reveal increased saturated and unsaturated dicarboxylic and hydroxy species. The plasma acylcarnitine profile typically shows elevation of hydroxy-C18:1 species, which, in combination with an elevation of two of the three long-chain species C14, C14:1, and hydroxy-C16, identifies over 85 % of patients with high specificity (<0.1 % false-positive rate). Blood spot acylcarnitine analysis is not quite as sensitive, because of higher levels of long-chain species in blood samples. Dietary treatment (restriction of long-chain fats and supplementation with medium-chain triglycerides) will lower the long-chain acylcarnitine species, often to normal. Plasma carnitine levels are usually low, especially during acute illness. Carnitine is sometimes given at such times. Its usefulness as a treatment for this myopathy (and whether it might provoke arrhythmias in certain situations – see Chap. 23) is a subject of continuing investigation. A major complication not seen in most organic acidurias is a progressive retinopathy.

28.3.2 Weakness and Hypotonia Presentation

28.3.2.1 Carnitine Transporter Deficiency

The carnitine transport defect is the result of deficient activity of the high-affinity carnitine transporter (OCTN2, encoded by the gene SLC22A5), active in kidney, muscle, heart, fibroblasts, and lymphocytes. Renal fractional excretion of carnitine, calculated in relation to creatinine clearance, approaches 100 % (normal <5 %). The severe carnitine depletion that results (plasma levels can be <5 μmol/L, normal ≈ 45 μmol/L) will lead to tissue carnitine depletion as well. Hepatic carnitine depletion results in hypoketotic hypoglycemia. Onset of myopathy and cardiomyopathy can be in the first few months, or not for several years. Urine organic acids are typically normal. Muscle biopsy reveals lipid accumulation, and the muscle carnitine level is extremely low. Response to carnitine supplementation is dramatic, but because of the ongoing renal leak of carnitine, it is extremely difficult to maintain normal plasma carnitine levels or tissue levels, and exercise tolerance may be limited. Oral carnitine supplementation to an amount just short of provoking a fish odor (trimethylamine) by exceeding the oxidizing capacity is the usual approach. Inheritance is autosomal recessive.

28.3.2.2 Secondary Carnitine Depletion

Adults are able to synthesize all the carnitine they need. The major dietary source of carnitine is meat. The carnitine content of human breast milk is similar to that of plasma. Carnitine deficiency has occurred in several infants on prolonged parenteral nutrition (without added carnitine), resulting in myopathy and cardiomyopathy, impaired ketogenesis, and hepatic steatosis. Carnitine supplementation (intravenous or oral) was rapidly beneficial. This experience suggests that carnitine may be an essential nutrient in the very young and that routine carnitine supplementation of TPN solutions for infants should be considered.

Severe carnitine depletion can result from a generalized Fanconi syndrome, characteristic of cystinosis, Lowe syndrome, mitochondrial disorders (especially cytochrome C oxidase deficiency), etc. (see also Chap. 26). Recognition of the carnitine depletion usually occurs after the discovery of Fanconi syndrome. Plasma carnitine measurement and determination of the fractional excretion should be part of the investigation of all patients with Fanconi syndrome. Restoration of tissue carnitine levels may take a very long time even after correction of the renal leak, as after renal transplantation for cystinosis.

Some medications, such as valproic acid and the pivalic acid component of pivampicillin (and a few other medications), are organic acids and may be excreted as carnitine esters causing significant urinary losses of carnitine as valproylcarnitine or pivaloylcarnitine and consequently secondary systemic carnitine deficiency. Use of pivampicillin has been linked to life-threatening crisis, especially in patients with an underlying metabolic disorder [e.g., medium-chain acyl-CoA dehydrogenase (MCAD) deficiency].

MCAD deficiency is unusual among fatty acid oxidation disorders for having only minimal skeletal muscle symptoms. Hepatic and cerebral symptoms (Reye-like syndrome, hypoketotic hypoglycemia, and sudden unexplained death) are the usual features. However, carnitine depletion can occur. MCAD deficiency accounts for nearly all the patients described with "systemic carnitine deficiency," before the enzyme deficiency was discovered. This may be the mechanism for chronic weakness and impaired exercise tolerance in some older patients with MCAD deficiency. Long-term carnitine supplementation, whose use in MCAD deficiency is not universally accepted, might be expected to ameliorate this situation.

28.3.2.3 2,4-Dienoyl-CoA Reductase Deficiency

This extremely rare disorder has been described in at least two infants with hypotonia, normal deep tendon reflexes, poor feeding, and failure to thrive. There was plasma carnitine deficiency, and a unique unsaturated acylcarnitine species (C10:2) shown to be derived from long-chain unsaturated fatty acids, in plasma. Mutations in NADK2 were found in one patient.

28.3.2.4 MADD–ETF OR ETF-Dehydrogenase Deficiency (Synonymously Glutaric Aciduria Type II)

The severe form of this disorder causes overwhelming acidosis shortly after birth. Impairment of the ETF system blocks many different dehydrogenation systems for fatty acid and amino acid degradation. Milder deficiency of the same system can cause a lipid storage myopathy that may not become apparent for years or decades. Gradual onset of weakness and easy fatigability may be overlooked initially, and the history may first suggest an inflammatory myopathy. There may be liver dysfunction. Urine organic acids can show dicarboxylic aciduria, including ethylmalonic, adipic, and glutaric acids. Plasma acylcarnitine analysis shows elevation of short- and medium-chain species. A secondary deficiency of coenzyme Q_{10} (CoQ) may occur. Mitochondrial studies may show deficient activity complex (I and II). Response to supplemental riboflavin (50–100 mg/day), the precursor of the cofactor flavin adenine dinucleotide, is often dramatic in the milder forms. A disorder of riboflavin transport is the cause of a similar disorder, Brown–Vialetto–Van Laere syndrome and its allelic

variant Fazio Londe syndrome. Both conditions are motor neuron syndromes which respond well to high-dose (10 mg/kg per day) riboflavin supplementation.

28.3.2.5 Lysosomal GSD Type II (Pompe Disease)

Pompe disease is the severe infantile form of lysosomal α-glucosidase (GAA, or acid maltase) deficiency. It is one of the most common storage diseases presenting in infancy and the first one to be identified. The infant typically presents in the first few months with weakness and profound hypotonia. Feeding and respiratory difficulties are common. There is macroglossia, but minimal hepatomegaly. At least 80 % have significant cardiac involvement. Progressive weakness and cardiomyopathy usually lead to death within a year. Despite the hypotonia, the muscles feel firm or even woody (Howell et al. 2006).

There is a spectrum of deficiency of α-glucosidase, with onset of symptoms reported as late as the eighth decade (Winkel et al. 2005). Various terms, including juvenile and adult onset, are used to describe late-onset patients. The age of onset bears little relation to the rapidity of the course (Lachmann and Schoser 2013). The older the patient, the more likely that symptomatic muscle involvement will be patchy clinically and morphologically. However, enzyme activity will be deficient, regardless of the appearance of the cells. Late-onset Pompe disease may present with weakness of the diaphragm, or as a limb-girdle myopathy. The function of lysosomal glycogen is not known. Mutation analysis of GAA is readily performed on DNA from leukocytes or other sources. α-Glucosidase activity can be measured in leukocytes, as well as in muscle or in liver biopsy, or in cultured skin fibroblasts. Muscle biopsy, which is not necessary for diagnosis if enzyme assay can be performed, shows enlarged lysosomes engorged with glycogen, altering the ultrastructure of the cell.

There was no satisfactory treatment of Pompe disease until the development of enzyme replacement therapy. Recombinant α-glucosidase can arrest and even reverse the disease if begun early enough, so early diagnosis is now an urgent matter (Kishnani et al. 2013). Newborn screening for Pompe disease is now being implemented in the USA.

28.3.2.6 Glycogen Debrancher Deficiency: GSD Type III (Cori or Forbes Disease)

This relatively common disorder of glycogen metabolism results from varying deficiencies of amylo-1,6-glucosidase, 4-α-glucoanotransferase, the debrancher enzyme, a remarkable peptide which has two separate catalytic activities (transferase and glucosidase). There is always liver involvement, but muscle involvement is variable and does not occur at all in about 15 % (GSD IIIb). In infancy and childhood, the liver symptoms dominate, with hepatomegaly, hyperlipidemia, and fasting hypoglycemia similar to GSD I. Muscle weakness may not be apparent. After puberty the liver symptoms subside, but there is an ongoing risk for cirrhosis. The myopathy may persist as weakness and may worsen with time. CK may be elevated, but may be normal even if there is muscle involvement. There can be distal wasting, myopathic EMG, and peripheral neuropathy. There is usually cardiac involvement (mild) as well.

Diagnosis is suspected from the history. Enzyme assay and gene analysis can be done using fibroblasts, lymphocytes, muscle, or liver. Liver and muscle biopsies are often done to obtain direct information about these organs. Analysis of glycogen structure in muscle or liver can demonstrate abnormalities due to the lack of normal glycogen breakdown. The differences in tissue expression are traceable to alternate splicing of exon 1 of the gene. Differences in phenotype correlate with different mutations. Two mutations in exon 3 account for 90 % of patients with GSD IIIb (i.e., no muscle involvement). Molecular testing, to determine if muscle disease will occur, is replacing muscle biopsy. (If at least one of the mutations involves exon 3, the patient will not have muscle disease.) Support of glucose availability is achieved with cornstarch, similar to GSD I (Kishnani et al. 2010). Treatment of the myopathy using high-protein meals and

high-protein enteral feeds overnight has been attempted, but it does not seem to improve the long-term outlook.

28.3.2.7 Glycogen Brancher Deficiency: GSD Type IV (Anderson Disease)

Type IV GSD, glycogen brancher deficiency, can cause hypotonia and weakness, but the clinical picture is dominated by hepatic fibrosis and dysfunction. Cardiomyopathy may be significant in the severe infantile form (Magoulas and El-Hattab 1993). Partial deficiency of this enzyme is a cause of adult polyglucosan body disease (APBD), a storage myopathy. This condition may be ameliorated by triheptanoin, a compound being studied for disorders of fatty acid oxidation and other conditions with deficient intracellular energy (Roe et al. 2010).

28.3.2.8 Glycogen Synthase Deficiency: GSD Type 0

This disorder results in insufficient glycogen reserves in the liver, so that fasting hypoglycemia occurs relatively soon after a meal and excessive lactate is produced from dietary glucose. A few families have been reported with a muscle form of the disease (called GSD0B), which caused muscle weakness, reduced exercise capacity, and hypertrophic cardiomyopathy in the first decade.

28.3.2.9 Mitochondrial Myopathies and Coenzyme Q Deficiency

The mitochondrial myopathies are an extremely heterogeneous group of disorders. Symptoms may be confined to muscle, or may involve other organs, particularly the brain, heart, liver, and kidneys. Symptoms may already be present at birth or not appear for decades. Myopathy is particularly evident in the syndromes of chronic progressive external ophthalmoplegia (CPEO) including the Kearns–Sayre syndrome (KSS) or ophthalmoplegia-plus; mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes (MELAS); mitochondrial encephalomyopathy with ragged-red fibers (MERFF); fatal infantile mitochondrial myopathy; depletion of the mitochondrial DNA; and autosomal dominant and recessive mitochondrial myopathies. Molecular defects can be in the mitochondrially encoded tRNAs, mitochondrial- and nuclear-encoded subunits of the oxidative phosphorylation complexes, and many other proteins (Delonlay et al. 2013). Some of the most relevant disorders and etiologies are shown in Tables 42.2 and 42.3.

Muscle symptoms are generally those of chronic weakness and impaired exercise tolerance. Cramps are unusual. Rhabdomyolysis can occur, particularly, in the setting of sustained exercise or febrile illness. Malignant hyperthermia may occur with anesthesia or muscle relaxants (Niezgoda and Morgan 2013).

Systemic lactic acidosis may be present at rest or elicited with exercise. Some infants will have acidosis from the work of breathing, but be chemically normal with ventilator support. CK may be elevated. Plasma amino acids may show increased alanine. Urinary organic acids may show increased of lactate, citric acid cycle intermediates, and dicarboxylic fatty acids. The plasma carnitine level may be normal or low. Plasma acylcarnitine analysis may show a generalized increase in short- and medium-chain species, especially acetylcarnitine, or be unrevealing.

EMG may be normal or suggest myopathy; nerve conduction studies may reveal a peripheral neuropathy, usually axonal.

Muscle tissue can be analyzed for carnitine content and acylcarnitine species and coenzyme Q level. Muscle biopsy may show dense clusters of abnormal mitochondria, especially near the surface of the cell membrane ("ragged-red fibers"), as well as increased lipid, but may be normal (see also Chap. 43). Cells may stain strongly for succinate dehydrogenase (complex II) yet not stain for cytochrome oxidase (COX), particularly in CPEO, KSS, and MERFF. Maternally inherited Leigh syndrome patients may have deficient COX staining, but no ragged-red fibers. Electron microscopy may show abnormal mitochondrial morphology, including paracrystalline inclusions.

Studies of oxidative phosphorylation are best carried out in fresh muscle biopsy tissue. Some

laboratories will work with frozen muscle tissue or freshly isolated platelets. Mitochondrial DNA studies are optimally performed from muscle biopsy as well. If there is a heteroplasmic disorder in the mtDNA, other tissues (leukocytes and fibroblasts) can sometimes give a misleading normal result. Mitochondrial myopathies are a subset of the overall group of mitochondrial cytopathies, which are discussed in Chap. 42.

Mitochondrial disorders of oxidative phosphorylation are generally treated with a high-fat, low-carbohydrate diet and supplemental vitamins and antioxidants, especially coenzyme Q (ubiquinone) and riboflavin (which may be quite helpful for complex I deficiency myopathy) (Parikh et al. 2013a, b). Vitamin C, thiamin, vitamin E, vitamin K3 (as an artificial electron acceptor-donor), dichloroacetate, carnitine, and succinate have been used in various situations. Responses are generally subtle, but occasionally a patient responds dramatically to CoQ or other therapies.

The synthesis of coenzyme Q involves nine steps; recessive defects have been discovered in the genes SPSS1, SPSS2, CABC1, COQ2, COQ4, COQ6, ADCK3, and COQ9. Besides myopathy, there may be ataxia, deafness, encephalopathy, liver disease, renal tubular dysfunction, and cardiac valvulopathy, depending on the defect (Desbats et al. 2015; Garcia-Cazorla et al. 2015). In many patients suffering from the myopathic form of CoQ deficiency, MAD (multiple acyl-CoA dehydrogenase) deficiency is the primary defect. Secondary CoQ deficiency is also found in the ataxia-oculomotor apraxia disorders with hypoalbuminemia.

28.3.3 Myopathies with Major Cardiac Involvement

The cardiac manifestations of several disorders discussed in this chapter, including GSD types II and IV; fatty acid oxidation disorders including carnitine transport defect; deficiencies of carnitine-acylcarnitine translocase; CPT II, VLCAD, and LCHAD/trifunctional enzyme; and mitochondrial disorders, are discussed in Chap. 23.

28.3.4 Malignant Hyperthermia

Malignant hyperthermia (MH) in response to anesthetics occurs in many different situations and myopathies. MH is most commonly due to the failure of regulation of calcium concentration in the sarcoplasmic reticulum. Excess calcium permits continuous muscle contraction, leading to heat generation and a rise in body temperature. Severe myoglobinuria, irreversible kidney damage, and death from hyperthermia or arrhythmia due to hyperkalemia may occur. For these reasons, all patients with a myopathy must be especially carefully monitored during surgery or any other procedure where anesthesia is used, and the most risky anesthetics (e.g., halothane) and muscle relaxants (e.g., suxamethonium) should be avoided. Premedication with dantrolene can lessen the risk of untoward reactions. Several genes are now known for MH syndromes. MHS1 is due to mutations in the ryanodine receptor RYR1 (with or without central core disease) (Robinson et al. 2006). MHS2 is due to mutations in the alpha subunit of the gated sodium channel IV, SCN4A, also altered in hypokalemic periodic paralysis, paramyotonia congenita, and related disorders. MHS3 may be due to mutations in the calcium channel CACNL2A. MHS5 is due to changes in another calcium channel, CACNA1S. All of these conditions can be dominantly transmitted. Patients with muscular dystrophies or the recessive Native American myopathy with cleft palate and congenital contractures are also vulnerable (Stamm et al. 2008; Stewart et al. 1988). Even for these high-risk conditions, MH does not occur with each exposure to a triggering agent. Of the disorders of intermediary metabolism, which are the primary topic of this book, the greatest risk is to patients with CPT II deficiency (perhaps even in the heterozygous state) and with mitochondrial disorders, but all patients with metabolic myopathy should be regarded as at potential risk for MH.

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Psychiatric Disease

29

Ertan Mayatepek

Key Facts

- Psychiatric manifestations may be the only symptom of inherited metabolic diseases before additional neurological or other clinical signs are recognized.
- Inherited metabolic diseases can manifest acutely as attacks of delirium, hallucinations, mental confusion, hysteria, schizophrenia, or psychosis, e.g., in urea cycle disorders, organic acidurias, maple syrup urine disease, porphyrias, methylene tetrahydrofolate reductase deficiency, cobalamin metabolism defects, Morbus Fabry, and metachromatic leukodystrophy.
- In infancy, autistic features may be a leading clinical feature of metabolic diseases, e.g., in urea cycle disorders, inborn errors of biopterin, or purine metabolism.
- Psychiatric manifestations are mostly combined with regression of cognitive functions, e.g., in X-linked adrenoleukodystrophy or mucopolysaccharidosis type III (Sanfilippo).
- Patients with late-onset lysosomal storage disorders may initially present with psychiatric diagnoses such as dementia, psychosis, or emotional illness.
- In childhood psychotic behavior, depression and mania are typically found in GM2 gangliosidosis.
- Psychiatric manifestations can become most important in the long-term management of many patients with metabolic disorders.

29.1 General Remarks

Inherited metabolic diseases can manifest as acute attacks of delirium or psychosis as well as intellectual disintegration, mental regression, or chronic psychosis. Being aware of these manifestations allows to initiate appropriate diagnostic investigations and, if available, to institute rationale effective therapy (Tables 29.1 and 29.2).

In the course of many neurometabolic diseases, psychiatric manifestations often become of critical importance, and it is most relevant to inform families and to evaluate and treat them appropriately. Typical constellations in early childhood range from severe disturbances of mood and vegetative symptoms in patients with neurotransmitter defects and Smith-Lemli-Opitz syndrome to hyperactivity in patients with Sanfilippo disease and pterin defects to (auto-) aggressive behavior in Lesch-Nyhan disease. These aspects are beyond the scope of this book, except to stress the necessity of close collaboration with colleagues from child psychiatry in these instances.

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Table 29.1 Psychiatric symptoms suggestive of inherited metabolic diseases

Association with organic symptoms (organomegaly, neurological features, etc.)
Progressive cognitive decline
Confusion
Visual hallucinations
Catatonia
Fluctuating schizophrenia core symptoms
Treatment resistance or aggravation with treatment

Table 29.2 Investigations for the differential diagnosis of psychiatric manifestations of inherited metabolic diseases

Plasma/serum
Ammonia
Lactate
Amino acids
Homocysteine
Very long-chain fatty acids
Ceruloplasmin
Copper
Sterols
Urine
Amino acids
Organic acids
Mucopolysaccharides
Porphyrins (especially porphobilinogen)
Purines and pyrimidines
Oligosaccharides
N-Acetylneuraminic acid
Copper (24-h collection)
Histology including electron microscopy (skin biopsy, bone marrow, lymphocytes)
Biogenic amines and pterins in cerebrospinal fluid
Enzyme studies (lysosomal enzymes, respiratory chain enzymes)
Mitochondrial DNA
Brain MR spectroscopy

29.2 Acute Psychiatric Manifestations

Acute psychiatric attacks may be the first clinical correlate of an underlying metabolic defect. Presentations include mental confusion, hysteria, delirium, dizziness, aggressiveness, anxiety, bizarre behavior, agitation, agony, hallucinations or schizophrenic-like behavior, frank psychosis, and finally coma. Especially dramatic are acute attacks of hyperammonemia, i.e., *urea cycle disorders* (e.g., *ornithine transcarbamylase* (*OTC*) *deficiency* and *organic acidurias* or *maple syrup urine disease*). All these are in principle treatable disorders, especially the late-onset variants. However, if the appropriate metabolic investigations are not initiated, disease courses will become chronic leading to permanent handicap and early death. Topping the list of investigations in any patient presenting with acute encephalopathy including psychiatric presentation has to be ammonia. All too often acute psychosis after pregnancy or drunkenness were fatal misdiagnoses in patients suffering from urea cycle disorders, especially hemizygous females with OTC deficiency.

Psychiatric manifestations are classically found in *acute intermittent porphyria* (AIP) or *hereditary coproporphyria*. In AIP, a disorder of heme biosynthesis leading to intermittent elevations in porphobilinogen and related porphyrins, psychiatric manifestations of acute attacks comprise anxiety, depression, psychosis, or altered mental status. AIP is often mistaken for schizophrenia or hysteria. Before an initial attack, there is also a high frequency of histrionic personality traits in many patients with AIP. An even higher incidence of anxiety disorder is observed in otherwise asymptomatic carriers. For patients with AIP, it is essential to get the correct diagnosis early because many psychotropic drugs may induce or exacerbate an acute attack of porphyria. The metabolic crisis may induce or aggravate psychiatric symptoms, leading to a long-term psychiatric career because of misinterpretation as treatment resistance. Diagnosis of porphyria requires the demonstration of pathological metabolites in urine (e.g., porphobilinogen).

Patients suffering from *methylene tetrahydrofolate reductase deficiency* are sometimes initially (mis-)diagnosed as suffering from schizophrenia or psychosis. In these patients, further symptoms often include strokes, peripheral neuropathy, and a progressive myelopathy. Similar psychiatric signs are also found in cobalamin metabolism defects (Cbl C and Cbl G).

Besides symptoms like paresthesias stroke events, patients with *Fabry disease* are at higher risk of depression and even suicide.

29.3 Chronic Psychiatric Manifestations

Psychiatric symptoms are predominantly recognized in older children, adolescents, or adults suffering from neurometabolic diseases. The decisive manifestations in these disorders are mental deterioration and/or progressive neurological manifestations (see Chap. 16). However, these may be less obvious than psychiatric symptoms for some time. Psychiatric features can be the only presenting symptom before any significant neurological or extraneurological signs are recognized. These manifestations include behavior disturbances, changes of personality and character, mental regression, dementia, psychosis, depression, or schizophrenia. Such presentations are especially common in *X-linked adrenoleukodystrophy* (*cerebral form*); *Hallervorden-Spatz disease*; *Huntington chorea*; *urea cycle disorders*, especially *hemizygous OTC deficiency*; and *Wilson disease*.

It is helpful to consider the relationship of psychiatric manifestations to the age of the patient.

29.3.1 Infancy (1–12 Months)

In infancy, autistic traits may be a leading feature of metabolic disease. They have been observed in untreated phenylketonuria (PKU) and inborn errors of biopterin metabolism. Autistic features may also be present in infants affected with late-onset subacute forms of disorders associated with hyperammonemia, especially in *urea cycle disorders*, as well as in infants with succinic *semialdehyde dehydrogenase deficiency*, *Smith-Lemli-Opitz syndrome*, *adenylosuccinase deficiency*, *dihydropyrimidine dehydrogenase deficiency*, *homocystinuria*, *nonketotic hyperglycinemia or sulfite oxidase deficiency*. Children with the treatable pyrimidine nucleotide depletion disease due to *cytosolic 5′-nucleotidase superactivity* develop a pervasive developmental disorder. Patients with *Smith-Lemli-Opitz syndrome* often present severe sleeping problems as well as excessive screaming in early childhood.

29.3.2 Early Childhood (1–5 Years)

Episodes of psychotic behavior, depression, and mania are typical psychiatric signs of the late infantile form of GM_2 *gangliosidosis* (*Tay-Sachs and Sandhoff*). The disease is characterized mainly by developmental regression, spinocerebellar degeneration, ataxia, and spastic fright reaction. In this age group, it is important to be aware of other diseases with arrest or regression of cognitive function, most importantly *Rett syndrome*. In Rett syndrome girls are affected, and they present with characteristic behavior, regression of developmental achievements, and stereotyped movements of fingers and hands.

In *mucopolysaccharidosis type III* (*Sanfilippo*), major clinical manifestations include regression of high-level achievements as well as loss of speech. Affected children often show agitation, autism, and disintegrative behavior. Mental retardation with episodes of psychosis, hyperactivity, or confusion may be seen in *α- or β-mannosidosis*.

29.3.3 Childhood and Early Adolescence (5–15 Years)

Progressive neurological and mental deterioration along with psychiatric manifestations can be found in a number of neurometabolic diseases such as *juvenile neuronal ceroid lipofuscinosis* (*Spielmeyer-Vogt*). Intellectual deterioration and depression develop in addition to loss of sight and retinitis. In patients with this disease, alteration in behavior may be the presenting complaint and may be seen years before other manifestations are evident.

Dementia and deterioration are found alongside with psychiatric manifestations in metabolic diseases with predominant cerebellar ataxia such as mitochondrial disorders, e.g., *MELAS*, and in

cerebrotendinous xanthomatosis, *GM_1 gangliosidosis*, *Gaucher disease*, *Niemann-Pick disease type C*, the juvenile form of *Krabbe disease*, *Lafora disease*, *and metachromatic leukodystrophy* (*MLD*). Psychiatric manifestations are most often present in the late juvenile and adult age group and are characterized by psychosis with disorganized thoughts, delusions, and auditory hallucinations. The subsequent rapid intellectual deterioration to dementia should be the clue to the suspicion of an underlying metabolic disease.

Remember

- In childhood psychotic behavior, depression and mania are typically found in GM_2 gangliosidosis.

Severe behavioral problems in combination with mental retardation may be seen in creatine deficiency syndromes, in monoamine oxidase A deficiency, as well as in maternal PKU.

29.3.4 Late Adolescence and Adulthood (>15 Years)

In late adolescence and adulthood, progressive neurologic and mental deterioration along with preponderant psychosis and dementia may be found in a variety of metabolic diseases discussed in the preceding section including *urea cycle disorders*, *MLD*, *Niemann-Pick disease type C*, *ceroid lipofuscinosis*, *cerebrotendinous xanthomatosis*, *Huntington chorea*, *and Lafora disease*. In adult-onset MLD, psychotic changes may be those of schizophrenia. This is also true of Niemann-Pick type C disease. Paralysis of upward gaze is the characteristic giveaway sign, but it has been a missed in psychiatric evaluations.

In Wilson disease, toxic tissue levels of copper cause damage primarily to the liver and basal ganglia (see also Chap. 24). Psychiatric manifestations may vary and include personality changes, depressive episodes, cognitive dysfunction, and psychosis. The overall prevalence of psychiatric symptoms in Wilson disease is greater than 20 %. In total, 10 % of the patients present with psychiatric symptoms alone. Effective chelation treatment does improve the majority of psychiatric symptoms. However, initiation of chelation treatment in Wilson disease may precipitate an acute psychiatric crisis.

Psychiatric abnormalities have been reported in patients with homocystinuria due to *cystathionine β-synthase* (*CBS*) *deficiency*. Major diagnostic categories in patients with CBS deficiency include in order of decreasing incidence personality disorders, chronic disorders of behavior, episodic depression, and chronic obsessive-compulsive disorder. Schizophrenia or psychotic episodes are uncommon in CBS-deficient patients.

PKU in late-treated or untreated patients results not only in mental retardation but varying degrees of psychiatric pathology. Some adult patients with early-treated PKU have exhibited an atypical pattern of psychopathology with a variety of symptoms of anxiety and depression. PKU patients no longer observing dietary restriction have an increased risk of psychosocial difficulties; agoraphobia has been recognized as a common symptom. It is also possible that psychiatric disease, being common, simply coexists in some of these patients. Interestingly, psychiatric symptoms observed in PKU are not clearly related to phenylalanine levels.

Take Home Messages

- Many neurometabolic diseases manifest symptoms compatible with various psychiatric diagnoses. Psychiatric features may be a leading clinical correlate of certain metabolic diseases as well as an important sequel in long-term care. With improvements in treatment and prognosis, manifestations that affect long-term quality of life such as psychiatric problems become increasingly important in an older patient population.
- Recognition of metabolic diseases in the differential diagnosis of psychiatric manifestations is important since some

metabolic diseases may be mistaken for diagnoses such as schizophrenia. In AIP, psychiatric symptoms may be at the forefront of clinical manifestations. Careful examination of mental and neurological status, psychiatric history, and recognition of psychotropic drugs or treatment regimens potentially exacerbating metabolic crises, e.g., in porphyrias or in Wilson disease, are mandatory.

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Eye Disorders

30

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Key Facts

- Eye involvement plays a key role as a possible sign or symptom in several inherited metabolic diseases, and it is frequently helpful in the diagnostic workup.
- Ophthalmological impairment generally occurs early in life with symmetrical involvement. Monitoring the progression of eye disease is thus helpful in many inherited metabolic diseases (as it is for cataract in galactosemia).
- The expanding field of tailored therapies, as, for example, enzyme-replacement therapy in lysosomal disorders, makes ophthalmological signs and symptoms an easy-to-follow way to monitor treatment effectiveness and a practical way to test new treatment strategies.

30.1 General Remarks

In his Croonian lecture of 1908, Sir Archibald Garrod presented alkaptonuria as an iconic example to demonstrate his theory of inborn errors of metabolism in humans. The homogentisate 1,2 dioxygenase (HGD) enzyme deficiency, which is responsible for alkaptonuria disease impairing tyrosine breakdown, results in homogentisic acid accumulation in body fluids and tissues, thus leading to ochronosis.

The darkening of fluids and deposition in connective tissues, such as dark spots in the sclera and the cornea, leads to a multisystemic disease. Since then, the eye involvement has been described in many other inborn errors of metabolism, where it plays a key role representing a sign or a symptom which guides the diagnostic workup.

The eye is in fact a highly specialized sensor organ whose development involves mesenchymal, ectodermal and neural crest cells. His physiological links to the central nervous system account for eye involvement in those diseases that affect the brain. At the same time, the fact that the eye is accessible for investigations makes it especially suited for clinical monitoring. The eye is in medical practice the fourth most affected system by genetic-metabolic diseases, and, as for other complex organs, one or more of its structural or functional components may be affected by a single disease, resulting in a wide pattern of semiological signs or clinical symptoms. For most metabolic disorders, symmetrical bilateral involvement is the rule.

Even though the exact mechanisms by which a systemic metabolic disease might affect the eye are often not yet fully understood, generally speaking ocular involvement in metabolic disorders can be due to toxic compounds, errors of synthetic pathways and product accumulation and deficient energy metabolism.

From the clinical point of view, two situations may arise: either the patient presents with a known metabolic disorder and the ocular defect

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appears as a manifestation of the disease or the patient presents primarily with eye abnormalities and a metabolic disorder is then suspected.

Severe visual impairment is often not recognized until 2 months of age, when normal-sighted children develop eye contact. On the contrary, severe poor vision should be detected within the first weeks of life.

While in some conditions, abnormalities of the eye can be more easily detected, such as cataracts in galactosemia, in others, such as in some peroxisomal diseases, fundoscopic examination may still be normal in the neonatal period, whereas recordings of electroretinogram and visual-evoked responses are already abnormal.

In the present chapter, an overview of systemic genetic and metabolic disorders involving the eye will be introduced, focusing on those diseases which affect the eye, per sec, without discussing disorders which impair oculomotor functions (Poll-The et al. 2003; Saudubray et al. 2012).

In this sense, inborn errors of metabolism can develop different eye pathologies, which can be sorted into an involvement of the anterior chamber (comprising the sclera and cornea, iris, ciliary body and lens) or posterior segment (including the choroid, retina and optic nerve) of the eye.

Anterior chamber
- Corneal clouding
- Lens defects and dislocations

Posterior segment
- Retinal and choroid degeneration
- Optic neuropathy and atrophy

This chapter, as an overview on eye disorders, will first discuss ophthalmological assessment, and then it will focus on main ophthalmological disorders, sorted on the basis of different ocular structure impairments (Nyhan et al. 2012; Rajappa et al. 2010).

30.2 Ophthalmological Assessment

An ophthalmological assessment should include a detailed developmental and clinical history, including the family history, with a focus on the onset of eye symptoms. Ophthalmological visit (including ophthalmoscope, slit lamp and fundoscopic examination) and neurophysiological investigations can add further data, and regular follow-up is needed because most inborn errors of metabolism are chronic diseases with a variable clinical course.

30.3 Corneal Clouding

The three components of corneal tissue are epithelium, stroma and Descemet's membrane. Corneal transparency depends, in part, on stromal constituents, i.e. collagen fibrils and proteoglycans. The composition of proteoglycans in the cornea is involved in the organization of the collagen fibrils including fibrillar ultrastructure, fibril packing, organization, stability of the corneal lamellae, fibril size as well as corneal hydration. About 80% of the stromal dry weight is collagen, primarily type I, together with type V and VI collagens. The collagen fibrils are arranged in lamellae with adjacent lamellae arranged at right angles, forming an orthogonal grid. Corneal clarity is maintained through a crystalline array of stromal fibres and multiple translucent endothelial layers. The three components of corneal tissue (epithelium, stroma and Descemet's membrane) may be involved separately or simultaneously, depending on the disease.

Remember

The cornea is optically sensitive to abnormal storage products, which may accumulate as a result of a systemic disorder.

If the abnormal substrate is produced by the corneal tissue, it may be found throughout the cornea. If it is found in elevated amounts in the blood, it is more commonly accumulated in the corneal periphery. Lesions are relatively easy to detect by using simple instruments such as a hand light or the ophthalmoscope. More subtle changes can be seen using slit-lamp examination. Photophobia is frequently due to corneal involvement and can represent the warning symptom, often evolving in corneal scars, as it is for cystinosis or type II tyrosinemia.

Table 30.1 Corneal clouding

Lysosomal disorders
Mucopolysaccharidoses: types I, II, IV, VI, VII (severe form)
α-Mannosidosis
Sialidosis (severe infantile)
Galactosialidosis
Fabry's disease
Mucolipidosis I, II, IV
Fucosidosis type III
Multiple sulphatase deficiency
Farber's disease
Cystinosis
Lipid disorders
Homozygous familial hypercholesterolemia
Lecithin-cholesterol acyltransferase deficiency (Tangier disease)
Fish-eye disease
Amino acid disorders
Tyrosinemia type II
Alkaptonuria
Metal disorder
Wilson's disease

Remember

Corneal clouding is relatively easy to detect using instruments such as a hand light or the ophthalmoscope. Photophobia is frequently the main warning symptom.

Inherited metabolic diseases that affect the cornea are numerous and severe (Table 30.1).

Remember

The most frequent inherited metabolic diseases which present corneal clouding are lysosomal disorders (*including Anderson-Fabry disease*), *lipid disorders and tyrosinemia type II.*

Lysosomal Disorders

Lysosomal disorders constitute a wide spectrum of multisystemic storage diseases which encompasses over 50 different defects in which an ocular involvement often occurs. Eye signs and symptoms usually do not present in the neonatal period, with few exceptions (as it is for sialidosis and galactosialidosis). Later findings are strikingly represented by macular cherry-red spots. Often several different ophthalmological features coexist.

Disease Info: *Mucopolysaccharidoses*

In the mucopolysaccharidoses (MPS), some clinical manifestations such as coarse facial shape, thickened skin, organomegaly and eye lesions are the result of specific lysosomal enzyme dysfunction involved in the breakdown of glycosaminoglycans (GAG).

Ocular pathology is quite common in all types of MPS resulting in vision impairment. The ophthalmologic complications comprise corneal clouding, lens opacification, retinopathy, optic atrophy and raised intraocular pressure or glaucoma. Ocular hypertension is often difficult to monitor in patients with MPS because of corneal opacification and thickening.

Progressive corneal opacification (ground glass opacification) affects all patients with MPS types I, IV and VI. Corneal opacification is mild in MPS IS (Scheie disease) and MPS II (Hunter syndrome) and rarely requires corneal transplantation, while it is not a prominent feature of MPS III (Sanfilippo syndrome). In MPS II, in particular, corneal opacification has been reported as well as glaucoma, pigmentary retinopathy, optic atrophy and rhegmatogenous retinal detachment. Progressive corneal opacification is also seen in MPS VII (Sly syndrome). It is due to dermatan sulphate deposition in the cornea, which is not present in normal cornea, while in MPS IV (Morquio disease), it is due to keratin sulphate deposition.

Glycosaminoglycan deposition in the corneal stroma has been suggested by some authors to cause a progressive increase in corneal thickness.

In animals with systemic mucopolysaccharidoses, corneal clouding results from storage of glycosaminoglycans (GAG) in stromal keratocytes. The corneal epithelium is normal or minimally affected (as in MPS VI and VII

or in MPS I), and stromal oedema is not a feature even though the corneal endothelium demonstrated variable pathology. Corneal clouding is the result of a storage process in stromal keratocytes rather than corneal oedema from endothelial dysfunction.

Other ocular manifestations are also common in MPS. Glaucoma (with a variable prevalence between 2 and 12 % of patients), cataracts, optic nerve swelling and subsequently optic atrophy and pigmentary retinopathy have been reported in patients, often masked by corneal opacity which hampers optic disc evaluation and visualization of the drainage angle.

In the past, the ocular management of many patients with MPS has been conservative due to their short lifespan and to intellectual impairment. Now new treatments such as bone marrow transplantation and enzyme-replacement therapy (ERT) are leading to a longer surveillance and better quality of life for many MPS patients, and these treatments appear to be beneficial in stabilizing, but not preventing, the ocular involvement. Recently, several systemic gene therapy protocols, using viral and non-viral vectors methods, have been developed in animal models with promising benefits for the ocular features of the disease.

Disease Info: Anderson-Fabry Disease

Anderson-Fabry disease, also known as Fabry's disease, is an X-linked lysosomal disease that results from a deficient activity of the hydrolase α-galactosidase A. It is associated with severe multiorgan dysfunction, with cardiomyopathy, nephropathy and cerebrovascular events representing the most important striking complications.

Three main ocular signs have been linked to the disease: corneal changes, conjunctival and/or retinal vessel abnormalities and cataract (see Fig. 30.1).

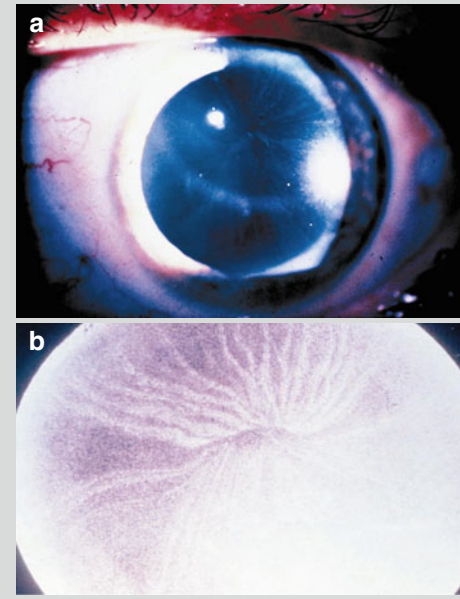

Fig. 30.1 Eye abnormalities associated with Fabry's disease: (**a**) conjunctival vessels tortuosity; (**b**) cornea verticillata (vortex opacities located in the superficial corneal layers)

Frequently, the subtle eye manifestations of Fabry's disease are visually not noticed by the patient. However, ophthalmologic signs prove to be useful in the diagnosis of the disease, in understanding its natural history and in assessing the response to enzyme-replacement therapy. These signs are the result of a progressive deposition of glycosphingolipids in the ocular structures.

A vortex keratopathy (known as "cornea verticillata") is the most common ocular sign reported in Fabry's disease. It can somehow be the only ocular sign and it is similar in different age groups. The reported prevalence for the cornea verticillata varies between 44 and 94 % among Fabry patients, irrespective of gender. Cornea verticillata is recognized only by slit-lamp examination. The earliest lesion is a diffuse haziness in

the sub-epithelial layer that later appears as fine, straight or curved lines radiating from the periphery towards the centre of the cornea in a characteristic pattern. Despite this finding, the vision is generally not affected. Indistinguishable, drug-induced phenocopies of Fabry cornea verticillata have been reported in patients on long-term chloroquine or amiodarone therapy.

A small number of patients show a characteristic lens opacity with a "spoke-like" pattern usually referred to as "Fabry cataract". It consists of two types, anterior and posterior subcapsular cataracts. The former, which only occurs in hemizygous males, appears as granular, radially arranged wedges. The latter has the appearance of nearly translucent spoke-like or dendritic projections; both can also occur in heterozygous female carriers. These signs can be detected by slit-lamp examination, a procedure that is non-invasive, inexpensive and not time-consuming.

Conjunctival and retinal vascular lesions are also common and can be part of the diffuse systemic vascular involvement. Conjunctival and retinal vessels are tortuous and may exhibit aneurysmal dilatations. Vessel tortuosity shows an association with both cornea verticillata and Fabry cataract; hence, the isolated presence of tortuous vessels (especially in the fundus), without any corneal or lens involvement, is not diagnostic for Fabry's disease. Vessel tortuosity has also been detected on the external superior lid of Fabry patients. Both Fabry cataract and tortuous vessels are more frequent in men than in women.

In terms of the relationship between ocular involvement and disease severity, in the past only the presence of vessel tortuosity appeared to be associated with a more rapid progression, and ophthalmological abnormalities were considered not informative in the accuracy of monitoring progression. In a recent retrospective analysis of the data collected through Fabry Outcome Survey (FOS), a large global Fabry database, the prevalence of ocular findings was regarded in correlation to disease severity and α-galactosidase A gene mutations. This study proved a correlation between the prevalence of ocular involvement and the severity of the disease, with null or missense mutations presenting a higher prevalence of eye findings as compared to patients with mild missense or p.N215S mutations (Pitz et al. 2015).

In a long-term study (over 10 years) of Fabry patients on ERT, a reduction in corneal deposits has been reported in 13 out of 32 patients treated with agalsidase beta (Fledelius et al. 2015).

Other lysosomal defects may lead to corneal manifestations as the earliest indication of a metabolic disease. In *mucolipidosis type IV*, corneal clouding with major visual impairment is a prominent feature. Corneal clouding is one of the earlier symptoms; later, retinal degeneration and blindness may develop. Cytoplasmic membranous bodies are found in several tissues, including the conjunctiva, fibroblasts, liver and spleen. Affected patients are usually mentally retarded. The late-onset form of *α-mannosidosis* also involves corneal clouding, in addition to cataracts, changes in bone and hearing loss. In *Farber's disease*, the severe form presents with eye changes including a cherry-red spot, a paint grey ring around the cornea, modular corneal opacity and a pingueculum-like conjunctival lesion.

In the *juvenile/adult type of galactosialidosis*, corneal clouding with loss of visual acuity develops in the second decade of life. Additional ophthalmologic abnormalities include bilateral cherry-red spots, punctate lens opacities and colour blindness. Other clinical symptoms include facial dysmorphism, growth retardation, cardiac involvement, inguinal hernias, angiokeratomata, joint stiffness, vertebral changes, hearing loss and a progressive neurologic course with mental retardation, seizures, myoclonus and ataxia. In type I sialidosis, early and late cataract

is a frequent finding together with macular cherry-red spots causing nystagmus and low visual acuity. In *steroid sulphatase deficiency*, corneal opacities are small punctate or filiform lesions located in the deep corneal stroma.

Disease Info: Cystinosis

The disease is a systemic metabolic disorder affecting the conjunctiva, cornea, iris, choroid and retinal pigment epithelium, as well as the kidney and other organs. It results from the accumulation of cystine within lysosomes. The disease is caused by mutations in the CTNS gene coding for cystinosin, a lysosomal carrier protein. In the cornea, the crystals are located in the anterior stroma; they are iridescent and polychromatic, presenting first in the periphery and extending centrally. The corneal changes and photophobia are due to the anterior location of the crystal deposition. Ophthalmological symptoms may be present before the nephropathy becomes severe, thus possibly representing the first sign of the disease. The anterior location of the crystals can predispose to recurrent erosions. Photophobia, watering and blepharospasm may become disabling; these symptoms are often related to the erosions of the corneal epithelium, leading eventually to keratopathy. Sight may be progressively reduced, due to cystine accumulation in the anterior and posterior chamber of the eye, with degenerative pigmentary retinopathy evolving in retinal blindness, in the most severe form of the disease. Cataract and pigmentary retinopathy have been also reported.

Cysteamine eye drops are effective in reducing the deposits of crystals in the cornea and in improving the extreme photophobia. Corneal transplantation may be indicated for visual rehabilitation, as well as for recurrent erosions (Shams et al. 2014).

Lipid Metabolism Disorders

Nearly 40 different genetic diseases are now reported to be involved in the biosynthesis and remodelling of phospholipids, sphingolipids and fatty acids. This represents an expanding issue of inborn metabolic diseases. Focusing on disorders presenting eye involvement, several high-density lipoproteins (HDLs), metabolism diseases, including Tangier disease, fish-eye disease, lecithin cholesterol acyltransferase deficiency (LCAT) and apoprotein A1 deficiency must be included in the differential diagnosis of corneal opacity. The central corneal changes are grey dots occupying the full thickness of the stroma, mainly centrally, with peripheral condensation to form the arcus-like changes. Occasionally, an arcus-like structure develops due to the deposition of a variety of phospholipids, low-density lipoproteins and triglycerides in the stroma of the peripheral cornea. Patients with these diseases have hypoalphalipoproteinemia with low HDL, low Apo A-I and elevated triglycerides. Those with Tangier disease have striking large yellow tonsils or pharyngeal plaques. They also have peripheral neuropathy leading to weakness, paresthesias, autonomic dysregulation and ptosis. In addition, premature coronary heart disease (CHD), abnormal rectal mucosa, anaemia, renal failure and hepatosplenomegaly may occur. In familial LCAT deficiency, marked corneal opacification is common together with splenomegaly, renal failure and anaemia, while corneal clouding is the only clinical manifestation in patients with fish-eye disease.

Amino Acid Disorders

Disease Info: Tyrosinemia Type II

Tyrosinemia type II (oculocutaneous tyrosinemia, Richner-Hanhart syndrome) is due to a defect in cytosolic tyrosine aminotransferase. Photophobia, redness, watering eyes and pain are often the most evocative symptoms of this disease. At slit-lamp examination, central pseudodendritic

corneal lesions are present that stain poorly with fluorescein, and, on in vivo confocal microscopy, hyperreflective linear deposits in the superficial epithelium can be seen. The lesions are bilateral in contrast with herpetic ulcers that are unilateral. If not early diagnosed and treated, ocular damages including corneal opacities, visual impairment, corneal plana, nystagmus, amblyopia and glaucoma are frequent complications.

Treatment consists of a phenylalanine- and tyrosine-restricted diet. There is no consensus as to the optimal blood level of tyrosine, but a level <500 uM is a reasonable goal. The eye symptoms resolve within a short period of treatment (few weeks). Treatment with systemic steroids should be avoided because it can worsen the disease.

Corneal deposits have also been described in few patients affected by type I tyrosinemia, mimicking type II disorder and probably representing the effect of nitisinone therapy with the need to treat the resulting hypertyrosinemia.

Alkaptonuria is a rare autosomal recessive condition caused by deficient homogentisic acid oxidase. Homogentisic acid is excreted in excess in the urine, but its oxidized pigment derivatives (alkapton) also bind collagen, leading to pigment accumulation in connective tissue of the nose, sclera and ear lobes. Affected patients develop a degenerative arthropathy. Ocular changes occur in 70 % of patients. Just inside the limbus, the cornea develops a black "oil-droplet" pigmentation that appears similar to spheroidal degeneration. Pigmentation gradually increases throughout adulthood.

Copper Disorders

Wilson's disease, also known as *hepatolenticular degeneration*, may be diagnosed by characteristic ocular findings. It is an autosomal recessive disorder that causes deposition of copper in the liver, corneas, kidneys and nervous system. The Kayser-Fleischer ring is the single most important diagnostic finding of the disease. When fully developed, it is seen with the naked eye, but subtle changes can be seen by slit-lamp examination (see Chap. 2). It consists of brownish-greenish deposit of copper in the Descemet's membrane just within the limbus of the cornea; it can be especially prominent at the upper pole; therefore, it requires lifting of the eyelid for recognition. It is present in 60 % of children at the stage of acute or subacute liver disease. It may be present in asymptomatic affected individual as well.

The ring is not exclusively pathognomonic for Wilson's disease, since other causes of liver failure such as carotenemia and multiple myeloma may lead to similar rings. In Wilson's disease, another rare but characteristic abnormality is the "sunflower" subcapsular cataract. The rings improve or disappear (in near half of the patients) with effective decoppering therapy or after liver transplantation, as does the cataract.

Remember

The cornea verticillata and the Kayser-Fleischer ring are important diagnostic ocular findings for Fabry's disease and Wilson's disease, respectively.

30.4 Lens Defects and Dislocations

30.4.1 Cataracts

Cataract is defined as any opacity within the lens. Cataracts can be graded through visual inspection with numerical assignment and severity scores. Several grading systems have been developed based on photographs of slit-lamp cross sections of the lens, mostly using a one to four grading system.

Cataracts and dislocation of the lens are the anomalies of the lens detectable in many inherited metabolic defects. At birth lens opacities, if not diagnosed or removed, are a major cause of blindness or amblyopia, while lens dislocation can present with nearsightedness and blurred or fluctuating vision.

Remember

Early cataract investigation: look for the orange or red retinal reflex with a dilute dilating eye drop at the first paediatric examination after birth with an ophthalmoscope.

Often, the retinal reflex can be detected with an ophthalmoscope alone. When cataracts are bilateral, they lead to irreversible nystagmus and amblyopia by 3 months of age. Thus, bilateral cataracts must be surgically removed within the first few days or weeks of life.

Remember

The etiologic classification of congenital cataract is challenging, and more than 90% of congenital cataracts remain unexplained. The most common aetiologies include infections, metabolic disorders and genetically transmitted syndromes.

A number of inherited metabolic diseases present with cataracts (Table 30.2). It is a constant and early finding in neonates with defects of carbohydrate metabolism (mainly galactose and polyol pathways), peroxisomal disorders (peroxisomal biogenesis) and Lowe's syndrome. Cataracts develop later in lysosomal disorders (sialidosis, galactosialidosis, α-mannosidosis, Fabry's disease and juvenile form of neuronal ceroid lipofuscinosis), Wilson's disease, Menkes disease, lipid disorders and some amino acid defects. In mitochondrial disorder, cataracts have been reported in Sengers disease, Sengers-like disease, β-methylglutaconic aciduria and mtDNA mutations linked to optic atrophy. Cataracts have been also reported in metachromatic leukodystrophy, hypobetalipoproteinemia, vitamin E or D deficiencies and lactose intolerance, and they are the characterizing feature of hypomyelination with congenital cataract (HCC): a newly recognized leukodystrophy due to hyccin protein dysfunction. Hypoglycaemic episodes of various origins during the perinatal period or in early infancy may result in lens opacities.

Remember

Cataracts are an early finding in defects of carbohydrate metabolism (galactose and polyol pathways), peroxisomal biogenesis, cholesterol biosynthesis and amino acid transport.

Table 30.2 Cataracts and the age of presentation

Newborn period
Galactosemia (all defects)
Sorbitol dehydrogenase deficiency
Zellweger syndrome
Rhizomelic chondrodysplasia punctata
Lowe's syndrome
Childhood
Carbohydrate disorders
Galactosemias
Sorbitol dehydrogenase deficiency
Aldose reductase deficiency
Lysosomal disorders
Oligosaccharidoses: a-mannosidosis; sialidosis; galactosialidosis
Fabry's disease; neuronal ceroid lipofuscinosis (juvenile form)
Amino acid disorders
Delta1-pyrroline-5-carboxylate synthase deficiency
Hyperornithinemia (ornithine aminotransferase deficiency)
Lysinuric protein intolerance
Lowe's syndrome
Lipid disorders
Sjögren-Larsson syndrome
Neutral lipid storage disorder
Cerebrotendinous xanthomatosis (cholestanollipidosis)
Mevalonate kinase deficiency (classic form)
Conradi-Hunermann syndrome
Smith-Lemli-Opitz syndrome
Peroxisomal disorders
Peroxisome biogenesis defects
Rhizomelic chondrodysplasia punctata
Mitochondrial oxidative phosphorylation disorders
Sengers disease
Sengers-like disease
β-Methylglutaconic aciduria
mtDNA mutations
Copper disorders
Wilson's disease
Menkes disease
Glucose-6-phosphate dehydrogenase deficiency
Congenital disorders of glycosylation
ALG8-CDG (CDG Ih), ALG2-CDG (Ii), ALG2-CDG (Ij)

Disease Info: Galactosemia

The lens is avascular by nature, receiving its nutrients from the aqueous humour. Most of

the glucose in the lens is handled by anaerobic glycolysis. The citric acid pathway produces about 20–30 % of the total ATP in the lens, even though only about 3 % of the glucose passes through the citric acid cycle. Three defects in galactose metabolism are known: galactose-1-phosphate uridyltransferase (GALT), galactose-1-phosphate epimerase and galactokinase. In the early stage, the "oil-droplet" cataracts are present, even though those are not true cataracts but the refracture changes in the lens nucleus (see Fig. 30.2). The lesion appears, in retroillumination, as a drop in the centre of the lens like an oil droplet floating in the water. The accumulation in the lens of galactitol, a metabolite of galactose, creates a shift of water into the lens, due to the lack of permeability of galactitol, with the ultimate disruption of the lenticular structure. In galactose-1-phosphate uridyltransferase deficiency, major manifestations include liver failure, jaundice and tubulopathy. Enzymatic (GALT) and molecular genetic testing both support the diagnosis (Viggiano et al. 2015). This can be achieved using the fluorescent spot test used in newborn screening (Beutler test) followed by quantitative tests for confirmation (enzyme activity in erythrocytes) and by mutation analysis. Patients with galactose epimerase deficiency may sometimes have a clinical picture resembling those present in GALT deficiency. In galactokinase deficiency, cataracts may not be recognized until late in the disease course because few or no other symptoms are usually present.

In all disorders, cataracts are usually reversible after the introduction of a galactose-free diet.

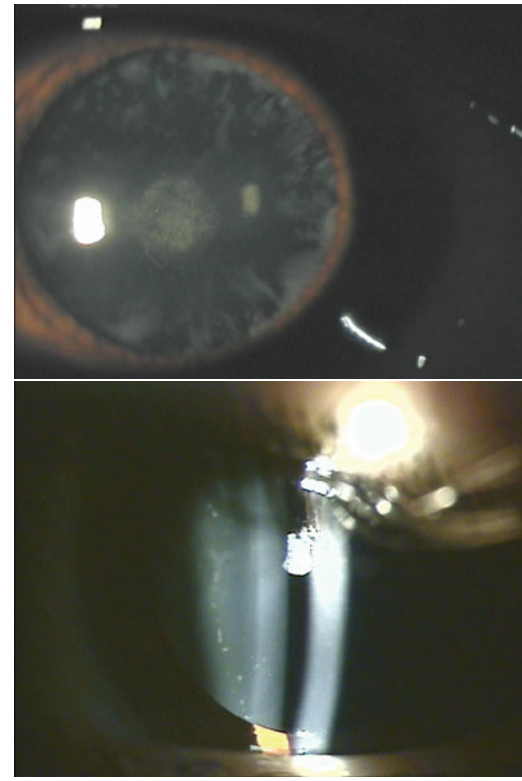

Fig. 30.2 Galactosemia: oil-droplet cataract in untreated patient visible by slit-lamp examination

Carbohydrate Metabolism

Remember

In all type of galactosemia, cataracts are usually reversible after the introduction of a galactose-free diet.

The polyol pathway consists of two enzymes: aldose reductase and sorbitol dehydrogenase. Aldose reductase reduces hexose sugars (such as glucose and galactose) to their respective polyols, sorbitol and galactitol. Polyol accumulation has been demonstrated to cause cataracts because of an increase in intracellular fluids resulting in lens swelling, augmented membrane permeability and electrolyte abnormalities. In sorbitol dehydrogenase deficiency, cataracts are present at birth as the only sign. Patients with glucose-6-phosphate dehydrogenase deficiency, who usually come to the clinician's attention because of haemolytic anaemia, may subsequently develop cataracts.

Lipid Metabolism Disorders

The lens membrane contains the highest cholesterol content as compared to any known membrane, indicating the importance of normal cholesterol metabolism in lens maintenance.

Disorders of cholesterol biosynthesis *(for instance*, *mevalonate kinase deficiency, Conradi-Hunermann syndrome and Smith-Lemli-Opitz syndrome)* present with a large and variable spectrum of morphogenic and congenital anomalies of the eyes. Cataracts are present early in the most severe forms, but, due to the clinical and biochemical continuum of these defects, they may not be present in milder forms. In *cerebrotendinous xanthomatosis*, bilateral, irregular, corticonuclear, anterior polar or posterior capsular cataracts can occur in the first decade. It may be associated with opacities of the crystalline lens. The lesion is associated with xanthomata and xanthelasma together with a neurological syndrome characterized by ataxia, pyramidal signs, cognitive impairment, epilepsy and peripheral neuropathy.

Patients with *Sjögren-Larsson syndrome* have cataracts, and they may be also manifest in another syndrome characterized by ichthyosis, the so-called neutral lipid storage disorder or Chanarin-Dorfman syndrome. This syndrome, a rare non-lysosomal inborn error of neutral lipid metabolism, is due to mutations in α- or β-hydrolase domain-containing protein 5 (ABHD5) and includes ataxia, myopathy and hepatomegaly as further clinical features. Vacuolated lymphocytes are a frequent finding in the peripheral blood.

Peroxisomal Disorders

Congenital cataracts in association with craniofacial dysmorphic features, hepatomegaly and renal cysts are frequently present in disorders of peroxisome biogenesis. To this group of disorders belongs to *Zellweger syndrome* and two related conditions: *neonatal adrenoleukodystrophy* and *infantile Refsum disease*. Other ocular abnormalities include pigmentary degeneration of the retina, corneal opacities and glaucoma. Differences between these syndromes are represented by the severity of the neurological abnormalities and by the variety of the clinical and pathological features. The measurement of plasma very-long-chain fatty acids (VLCFAs) allows the diagnosis. *Chondrodysplasia punctata* and rhizomelic dwarfism combined with congenital cataracts lead to the suggestion of rhizomelic chondrodysplasia punctata, which is associated with normal VLCFAs and low plasmalogen levels in tissues and red blood cells.

Recently, a new clinical entity has been described in 14 patients presenting progressive brain atrophy, intellectual disability, congenital neutropenia, movement disorders (ranging from stiffness to hypotonia and progressive tetraspasticity) and cataracts together with the biochemical marker of 3-methylglutaconic aciduria. Few patients presented also pigmentary retinopathy, cardiac involvement (dilated cardiomyopathy), facial dysmorphisms, endocrine abnormalities and behavioural and neuropsychological symptoms.

Amino Acid Disorders

In *Lowe's oculocerebrorenal syndrome*, cataracts are a constant and hallmark finding. The lesion is already present during the prenatal period as early as in the 24th week of gestation. Additional features are kidney impairment (Fanconi syndrome) and severe neurological signs and symptoms including muscular hypotonia, areflexia and mental retardation.

In other aminoacidopathies, including *ornithine aminotransferase deficiency* (which is characterized by gyrate atrophy of the choroid and retina), *delta1-pyrroline-5-synthase deficiency* and *lysinuric protein intolerance,* cataracts can also be an early sign.

Congenital Disorders of Glycosylation

Congenital disorders of glycosylation (CDG) are a group of 42 different enzymatic defects affecting the synthesis of N-linked oligosaccharides. Almost all CDGs develop during infancy with a broad clinical spectrum characterized by dysmorphic features, multiple organ involvement, severe developmental delay, seizures, ataxia and hypotonia, hypoglycaemia and protein-losing enteropathy. Brain involvement is frequently seen

with delayed myelination, cerebral and cerebellar atrophy and Dandy-Walker malformation that represent the most frequent features. Eye abnormalities have been reported in several CDG-affected patients with variable characteristics ranging from cataract (as in ALG8-CDG (formally CDG Ih), ALG2-CDG (Ii), ALG2-CDG (Ij)), eye malformations (ocular coloboma, optic nerve hypoplasia, visual loss) and eye movement disorders (such as in SRD5A3-CDG (formally CDG-Iq) and RFT1-CDG (In)).

30.4.2 Ectopia Lentis (Lens Dislocations)

Remember

An appropriate workup for patients with subluxation of the ocular lens should always include the measurement of total plasma homocysteine.

Dislocations of the ocular lens (ectopia lentis) are frequent, severe and characteristic complications, both in homocystinuria and in Marfan syndrome. Lenticular dislocation in Marfan syndrome is likely due to microfibril abnormalities of the lens capsule. The lens subluxation in homocystinuria most commonly occurs downwards and nasally, whereas in Marfan syndrome the lens usually subluxes upwards, although it can occur in any direction in both diseases. Marfan syndrome is most commonly due to alteration in microfibrils caused by mutations of the fibrillin-1 gene. The involvement of the ocular system includes a flat cornea, an increased axial length of the eye globe with hypoplastic iris or hypoplastic ciliary muscle causing decreased miosis.

Table 30.3 lists metabolic disorders in which ectopia lentis occurs, comprising also *molybdenum cofactor deficiency* and *sulphite oxidase deficiency* in which it may be one of the early presenting sign.

Table 30.3 Ectopia lentis (dislocation of the lens)

Homocystinuria
Marfan syndrome
Molybdenum cofactor deficiency
Sulphite oxidase deficiency
Weill-Marchesani syndrome

Disease Info: Homocystinuria

In patients with classic homocystinuria, subluxation of ocular lens is a very typical ocular sign, estimated to occur in over 90% of patients (see Fig. 30.3). The presence of the detached lens is often heralded by the clinical recognition of iridodonesis. It seldom occurs before 3 years of age and is usually present by the age of 10 years. Prior to this, patients usually develop rapidly worsening myopia, as well as astigmatism and glaucoma. Staphyloma may be the result of increased ocular pressure. Cataracts, which can be congenital, may also occur. All these features may be secondary to the ectopia lentis. The patient may also have retinal detachment and optic atrophy which may follow central retinal artery occlusion.

Although the body habitus of patients with homocystinuria resembles that of Marfan syndrome, the aetiology is completely different. Increased levels of plasma and urine homocysteine are found in all patients. Hypermethioninemia is, as well, an important finding. The diagnosis is confirmed firstly by assays of the enzyme cystathionine β-synthetase in cultured fibroblasts or lymphoblasts and, ultimately, by mutation analysis. Prevention of the lens dislocation appears to be possible in patients diagnosed through newborn screening and early treated by low methionine formulas or

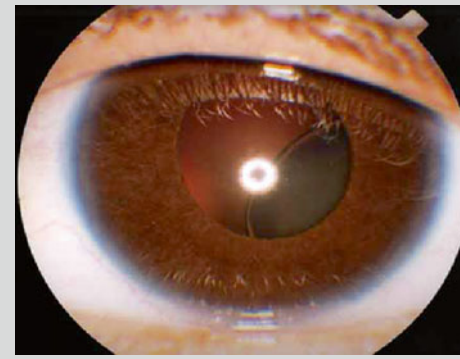

Fig. 30.3 The lens dislocation in homocystinuria is usually downward, while in Marfan syndrome it is upward

pyridoxine (vitamin B_6), in the case of B_6-responsive disease variants.

In homocystinuria due to 5,10-methylentetrahydrofolate reductase deficiency, ectopia lentis has never been reported, so far.

30.5 Degeneration of the Choroid and the Retina

To date 238 genes and 278 different genes and loci have been defined to be associated with retinal degeneration (http://www.sph.uth.tmc.edu/RetNet/).The number of inherited diseases increases up to 400, if retina, macula and choroid involvement are combined. The gyrate atrophy of choroid and retina is a progressive chorioretinic dystrophy leading to chorioretinal degeneration and atrophy presenting with nearsightedness, night blindness and loss of peripheral vision resulting in "tunnel vision".

Among the disorders that might be expected to affect the pigmented epithelium of the retina, there are diseases that are due to the storage of specific toxic compounds and others in which the normal ability to synthesize pigment is impaired.

This group of disorders includes those characterized by the presence of retinal pigmentation and a progressive red-corneal dystrophy as a prominent feature of retinal degeneration.

Disease Info: Ornithine Aminotransferase (OAT) Deficiency

Patients come to the attention of the ophthalmologists in late childhood or puberty for evaluation of increasing myopia or decreased night vision. At this age, sharply demarcated, circular areas of chorioretinal degeneration are present in mid-periphery of the ocular fundus (see Fig. 30.4). In few years, the retinal degeneration accelerates and the lesions enlarge, coalesce and extend towards the posterior pole of the fundus. Frequently, a posterior subcapsular cataract develops in the second decade. By the third decade, much of the fundus is involved, and increased pigmentation is common in the macula area, while the optic disc remains pink and does not become atrophic. The visual acuity decreases gradually and visual fields are progressively and concentrically reduced.

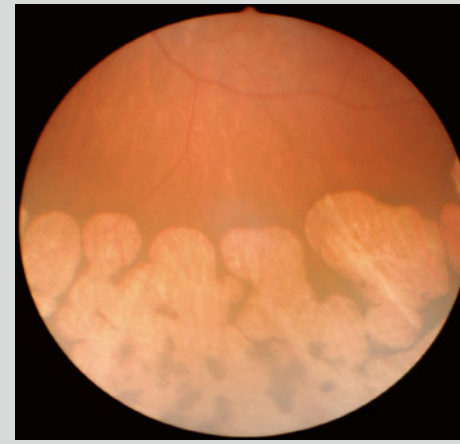

Fig. 30.4 Gyrate atrophy of choroid and retina in ornithine aminotransferase deficiency: the mid-periphery and far periphery show the typical atrophic areas in peripheral retina

OAT deficiency results in a 10- to 20-fold increase in ornithine in body fluids including aqueous humour leading to lesions in photoreceptors rather than retinal pigment epithelium. Therapeutic approaches include stimulation of residual OAT activity with pharmacological doses of pyridoxine, dietary reduction of ornithine through arginine restriction, increasing renal output by administration of pharmacological doses of lysine and/or administration of proline. Due to the slow disease progression, the evaluation of therapeutic approaches is difficult. Future treatment perspectives include gene or stem cell therapies.

In hyperornithinemia-hyperammonemia-homocitrullinuria (HHH) syndrome, the retina is not affected.

Gyrate Atrophy of the Choroid and Retina (GACR) and Retinitis Pigmentosa (RP)

The first group includes gyrate atrophy of the choroid and retina caused by ornithine aminotransferase (OAT) deficiency.

RP is a clinically and genetically heterogeneous group of hereditary disorders which belongs to the group of pigmentary retinopathies, presenting a progressive loss of photoreceptor and pigment epithelial function. Diagnostic criteria include bilateral involvement, loss of peripheral vision, rod dysfunction (with secondary degeneration of cones) and progressive loss of photoreceptor function. The anamnestic history should include information regarding the nature of the earliest symptoms, the age at onset and progression. The age at onset of RP varies but often begins in early childhood or infancy.

Remember

Retinitis pigmentosa is characterized by progressive loss of photoreceptor function causing visual problems such as defective adaptation to the dark, or night blindness, visual field decrease and reduction of visual activity.

In adults, careful questioning often elicits a history starting in childhood or adolescence when patients are asked to recall difficulties with outdoor activities at dusk or with indoor activities at night in minimal lighting. Patients rarely note a loss in peripheral vision as an early symptom, although they may be considered only clumsy until the restricted visual field becomes evident. Other symptoms include pendular eye movements and nystagmus. Patients who present with initial symptoms of photophobia, sensations of flashing lights, abnormal central vision, abnormal colour vision or marked asymmetry in ocular involvement may not have RP, but rather another retinal disease.

Remember

The diagnosis of RP is typically made by seeing a pigmentary retinopathy on fundus examination. An electroretinogram is an important confirmatory test.

The ocular examination should include measurements of the patient's best-corrected visual acuity, refraction, examination of the anterior segment and measurement of intraocular pressure. Attention should also be given to the lens, vitreous, optic disc, retinal vessels, macula and retinal periphery. The earliest ophthalmoscopic findings are a dull retinal reflex and a thread-like aspect of the retinal arteries. The electroretinogram (ERG) is abnormal before gross fundoscopic evidence of RP is found.

Remember

The most frequent RP associated with metabolic defects occurs in Sjögren-Larsson syndrome, neuronal ceroid lipofuscinoses, abetalipoproteinemia, 3-hydroxyacyl-CoA-dehydrogenase deficiency, Refsum disease, Kearns-Sayre syndrome and ornithine aminotransferase deficiency.

The pigmented retinopathies can be divided into two groups: primary RP, in which the disease process is confined to the eyes, and secondary RP, in which retinal degeneration is associated with involvement of additional organs, or otherwise non-syndromic and syndromic forms.

Secondary RP is most often associated with neurological disease, dysmorphic features, myopathy, nephropathy, deafness and skin abnormalities. Some of these conditions are well-defined inherited metabolic diseases (Table 30.4).

The *neuronal ceroid lipofuscinoses* (CLNs) are a group of progressive encephalopathies, which are characterized by neuronal and extraneuronal accumulation of ceroid and lipofuscin storage materials (see also Chap. 5). CLNs are among the most common inherited neurodegenerative disorders. Three main types that include RP have been distinguished based on clinical, neurophysiologic and genetic criteria: the infantile Santavuori-Haltia disease (CLN1), with onset between 6 and 18 months of age, showing psychomotor regression, myoclonic epilepsy, visual failure, microcephaly and "vanishing electroencephalogram (EEG)"; the late infantile Jansky-Bielschowsky disease (CLN2), with onset between 2 and 4 years of age, presenting with epilepsy, regression of mental skills, ataxia, myoclonic jerks, pathologic

Table 30.4 Secondary retinitis pigmentosa

Lysosomal disorders
Neuronal ceroid lipofuscinoses
Mucopolysaccharidoses: all except Morquio disease
Mucolipidosis IV, Krabbe disease (late onset)
Lipid disorders
Abetalipoproteinemia
Peroxisomal disorders: peroxisome biogenesis disorders; Refsum disease
β-Oxidation defects: long-chain hydroxyacyl-CoA dehydrogenase deficiency (LCHAD), mitochondrial trifunctional protein deficiency (MTP)
Sjögren-Larsson syndrome
Mitochondrial disorders
Kearns-Sayre syndrome
NARP (neuropathy, ataxia and retinitis pigmentosa)
2-Methyl-3-hydroxybutyryl-CoA dehydrogenase deficiency

Disease Info: Sjögren-Larsson Syndrome
Sjögren-Larsson syndrome, caused by deficiency of fatty aldehyde dehydrogenase (FALDH), is characterized by a triad of intellectual disability, spastic diplegia or tetraplegia and congenital ichthyosis with associated ocular features, which include pigmentary changes in the retina. Ocular features are characterized by a crystalline maculopathy and bilateral, glistening yellow-white dots involving the foveal and parafoveal areas from the age of 1–2 years. The number of dots may increase with age although the extent of macular involvement does not correlate with the severity of systemic features. Colour vision, electroretinography and electrooculography have been shown to be normal. Most patients exhibit photophobia, subnormal visual acuity, myopia and astigmatism. It is unknown if reduced visual acuity is due to retinal deposits or due to demyelination of the optic pathways, the latter having been demonstrated by MRI in affected patients.

EEG and impaired electroretinogram and visual-evoked potentials (VEPs); and the juvenile Spielmeyer-Vogt/Batten disease (CLN3), with onset of visual impairment between the ages of 4 and 10 years, showing psychiatric symptoms, motor dysfunction and epilepsy, retinal degeneration and vacuolated lymphocytes. The retinal disease starts with macular involvement, for instance, presenting with cherry-red spots.

Similar findings to that of neuronal ceroid lipofuscinosis have been linked to homozygous 15q13.3 microdeletions involving the CHRNA7 gene.

Besides cherry-red spot appearance, retinitis pigmentosa and optic atrophy have been recently reported together with yellowish flecks in the retinas and cortical lens opacities in hereditary spastic paraparesis due to SPG11 and SPG15. Granular macula and subsequent whole retina degeneration have also been described in spinocerebellar ataxia 7 (SCA7), an autosomal dominant trinucleotide repeat expansion disease. Macular dystrophy with a normal or subnormal ERG was reported in MFSD8 gene heterozygotes, a lysosomal transmembrane protein.

RP can be also found in different disorders of lipid metabolism. *Abetalipoproteinemia*, as an example, is caused by the absence of apoprotein B and the malabsorption of fat and fat-soluble vitamins, especially vitamin A and E. The most common clinical manifestations are diarrhoea and failure to thrive from early infancy. Other manifestations involve the nervous system, with signs of peripheral neuropathy, spinocerebellar ataxia and muscle weakness. Although the retinal dystrophy may occur at early age, it most commonly appears in late childhood. Fundus examination may be normal in the early stages; later there may be peripheral pigmentary retinopathy or a picture similar to retinitis punctata albescens with scattered white dots at the level of the retinal pigment epithelium. The ERG may be initially normal but later becomes abnormal with scotopic responses first to be lost. The retinal and neurologic complications may be prevented or stabilized by early supplementation with vitamin E. Disorder of fatty acid oxidation, mostly 3-hydroxyacyl-C-A-dehydrogenase deficiency, manifests with a generalized red-cone dystrophy.

Peroxisomal disorders are best diagnosed by determining plasma concentrations of very-long-chain fatty acids. Disorders with pigmentary retinopathy include the Zellweger syndrome, the neonatal adrenoleukodystrophy and other isolated enzyme defects, such as acyl-CoA oxidase deficiency and peroxisomal thiolase deficiency. Nystagmus is a common ocular symptom caused by retinal dystrophy, corneal clouding and cataract. Extensive photoreceptor degeneration and loss of ganglion cells with gliosis of nerve fibre layers have been also reported.

Patients with the *Refsum disease*, an inborn error caused by phytanic acid oxidase deficiency, present with RP (Fig. 30.5), peripheral polyneuropathy and elevated cerebrospinal fluid protein. Other symptoms include cerebellar ataxia, deafness, anaemia, ichthyosis and skeletal and cardiac manifestations. Night blindness may be the first clinical symptom at school age.

Adult-onset Refsum disease (20–50 years of age) have been reported. The patients show tapetoretinal degeneration as a precocious sign suggesting an isolated form of retinitis pigmentosa, therefore delaying the final diagnosis up to 11 years after the first visit (Rüether et al. 2010). The patients complain of night blindness during adolescence and later in life they present visual field restriction and impaired visual acuity. Miosis, attenuated papillary light response, iris atrophy and cataracts have been described. Fundoscopy reveals attenuated retinal vessels and pigment epithelium degeneration similar to retinitis pigmentosa. Besides ocular symptoms, patients briefly develop dysmorphic features (shortened metacarpals), ichthyosis, peripheral neuropathy, ataxia, anosmia, cardiac arrhythmias and sensorineural deafness.

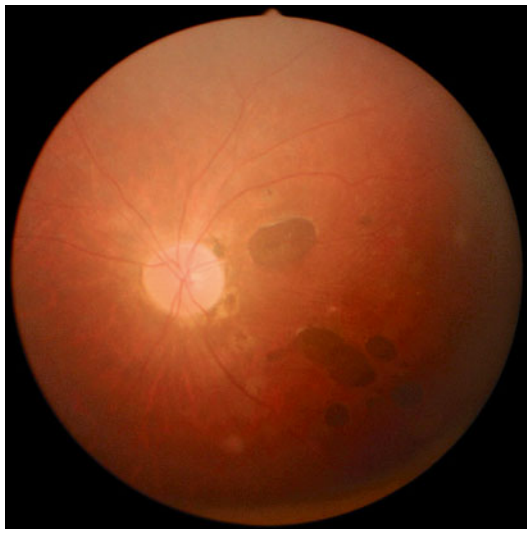

Fig. 30.5 Refsum disease showing retinal pigment abnormality

Defects in the mitochondrial electron transport chain cause a variety of ocular manifestations. Chronic external ophthalmoplegia and retinal dystrophy, similar to RP, are often found. ERG shows evidence of rod and cone dysfunction with the rods being more severely affected. Retinal degeneration has been consistently reported only in the *Kearns-Sayre syndrome*, a progressive multisystem disorder, with onset usually before the age of 20 years. Clinical features include chronic progressive external ophthalmoplegia, ptosis, retinopathy, cardiac conduction defects and deafness. It is caused by mutations, deletion ± duplications of mtDNA.

Finally, several well-known autosomal recessive disorders comprise RP. These disorders include *PKAN* (severe neurological regression, dystonia, acanthocytosis), *Laurence-Moon-Biedl* (obesity, polydactyly, mental retardation), *Usher type II* (deafness, severe mental retardation), *Joubert* (mental retardation, cerebellar vermis atrophy, attacks of hyperventilation) and *Cockayne* (dysmorphia, hypotonia, intracranial calcifications, deafness) syndromes and pyrimidine metabolism disorders such as phosphoribosyl pyrophosphate synthetase 1 deficiency or dihydropyrimidine dehydrogenase defect (ataxia, progressive peripheral neuropathy and hearing loss in the first and intellectual disability, seizures, autistic behaviour in the second).

30.5.1 Pigment Retinopathies

Pigment retinopathies due to the storage of specific compounds include many lysosomal storage diseases. The term cherry-red spot, as described by Warren Tay, refers to "…optic discs apparently

quite healthy, but in region of the yellow spot in each eye there was a conspicuous, tolerably well-defined, large white patch, more or less circular in outline, and showing at its center a brownish-red, nearly circular spot, contrasting strongly with the white patch surrounding it". The cherry-red spot is due to ganglioside accumulation in the retinal ganglion cells. The absence of ganglion cells at the fovea gives rise to the red spot surrounded by white cells filled with storage material. As the ganglion cells die, the cherry-red spot fades, and optic atrophy becomes apparent.

The differential diagnosis mainly includes, besides neuronal ceroid lipofuscinosis, lysosomal disorders (Table 30.5). The cherry-red spot is present in the early stages in most patients affected by *GM2 gangliosidosis* (GM2 types I, II and III) and *GM1 gangliosidosis* (type I); sialidosis types I and II; Niemann-Pick disease types A, B and C; mucopolysaccharidosis; Farber's disease; metachromatic leukodystrophy; Wolman disease; and dapsone toxicity. It is also a characterizing feature of cherry-red spot myoclonus syndrome, an old pathologic entity which presents with action myoclonus and can be due to sialidosis type I as well as several types of gangliosidosis and lysosomal disorders.

The ERG is consistently normal, but the visual-evoked potentials are abnormal from the early stages of the disease, and the cortical response is generally abolished from the first months of age.

The *Niemann-Pick disease type A* also leads to corneal opacification and dislocation of the anterior lens capsule.

In *sialidosis (mucolipidosis type I)*, the lesion presents in adulthood with a progressive myoclonus with normal intelligence. In *Farber's disease*, *metachromatic leukodystrophy*, *Gaucher disease type II*, a faint or irregular cherry-red spot can be present.

Table 30.5 Metabolic diseases with cherry-red spots

Ceroid lipofuscinosis
Sialidosis types I and II
Galactosialidosis (early infantile)
Tay-Sachs disease (GM2, variant B, infantile)
Sandhoff disease (GM2, variant O, infantile)
GM1-gangliosidosis (infantile)
Niemann-Pick disease type A
Gaucher disease type II
Farber's disease
Metachromatic leukodystrophy
Wolman disease

Retinal degeneration may be present in other defects of intermediary metabolism including defects of *intracellular cobalamin metabolism (Cbl C/D defects*) and *congenital disorders of glycosylation (CDG) syndromes* (especially PMM2-CDG, formally CDG-Ia).

A recent review on 100 patients with CblC defect describes the prominent ocular features of patients with early-onset phenotype (Weisfeld-Adams et al. 2015). A high prevalence of ocular involvement (affecting around 25 % of patients) with nystagmus and strabismus, presenting early after birth, has been reported. Pigmentary retinopathy with "bull's eye maculopathy" usually progresses into macular atrophy and pseudocolobomatous optic nerve. On the contrary, bilateral cataract and retinal haemorrhages have been reported only in few single cases.

Pigmentary changes develop early in the disease course and can progress to macular pseudocolobomas, similar to that seen in Leber's congenital amaurosis, which are quite distinctive of the disease.

Ophthalmologic disease progression is poorly or not clearly attenuated by current treatments.

Finally, *Leber's congenital amaurosis* is the most severe and the earliest form of inherited retinal dystrophies, accounting for over 5 % of the whole retinal dystrophies. To date, 18 genes have been identified to be responsible for the disease encoding for proteins involved in several retinal development and physiological retinal pathways, with wide clinical variability. Leber's congenital amaurosis is usually characterized by early visual loss, sluggish papillary responses and abnormal or silent ERG. The fundus appearance is variable, ranging from mild retinal impairment to macular coloboma or maculopathy. From a clinical point of view, photophobia progresses to refractory defects, nyctalopia and variable visual acuity impairment. Mental retardation, cerebellar involvement (Joubert like syndrome), stereotypic behaviour and olfactory dysfunction have been reported in association with the ocular findings. Recently, some gene therapy protocols

have been carried out bringing positive responses in the long-term (3 years follow-up) period.

30.6 Optic Atrophy

Optic atrophy results from loss of ganglion cell axons that form the optic nerve and/or a loss of the supporting surrounding microvascular tissue. Symptoms of optic atrophy include decreased visual acuity (ranging from no light perception to mild decreases in visual acuity), visual field defects and/or abnormalities in colour vision and contrast sensitivity (Huizing et al. 2005).

Remember

The hallmark clinical sign of optic atrophy is optic nerve pallor at fundoscopic examination.

Optic neuropathy is a more generic term for optic nerve dysfunction and includes the early phase of the disease, before clinical signs of optic atrophy may be present.

Currently, no effective treatment exists, although the identification and the correction of the underlying cause or the withdrawal of a toxic medication may halt progression.

Disease Info: Leber's Hereditary Optic Atrophy (LHON)

LHON is an inherited form of acute or subacute loss of central vision affecting predominantly young males. The disease is the paradigm of mitochondrial optic neuropathies where a primary role for mitochondrial dysfunction is caused by maternal inheritance, specifically by missense mutations in different mitochondrial DNA (mtDNA) genes, especially complex I subunits. The typical presentation is a rapid painless loss of central vision in one eye. Usually fading of colours (dyschromatopsia) in one eye is followed by a similar involvement of the other eye, within days, months or rarely years. Visual acuity stabilizes at or below 20/200 within a few months. The accompanying visual field defects usually involve the central vision in the form of a large centro-cecal absolute scotoma.

Fundus examination during the acute/subacute stages mostly reveals characteristic changes as it follows:

1. Circumpapillary telangiectatic microangiopathy
2. Swelling of the nerve fibre layer around the disc (pseudoedema)
3. Absence of leakage on fluorescein angiography (in contrast to true oedema).

Thus, the optic disc appears hyperemic, occasionally with peripapillar haemorrhages, and the axonal loss rapidly leads to temporal atrophy of the optic disc. With time, the optic disc turns pale. The microangiopathy may be present in a number of asymptomatic at-risk family members along the maternal line, in whom it may remain stable over the years.

Optic atrophy with permanent severe loss of central vision but with relative preservation of pupillary light responses is the usual endpoint of the disease. However, spontaneous recovery of visual acuity has occasionally been reported even years after disease onset. Visual function may improve progressively, sometimes suddenly, with contraction of the scotoma or reappearance of small islands of vision within it (fenestration). In long-lasting LHON, cupping of the optic disc has been frequently reported as a sign of the chronic stage of the pathologic process.

LHON is often due to homoplasmic mtDNA mutations, typically with wide variability in phenotypic penetrance. To date, LHON is the only human disease for which the influence of mtDNA background (haplogroups) has been solidly documented, particularly on the T14484C/ND6 and G11778A/ND4 LHON mutations. Recently, the association of LHON with a region of chromosome X suggests that one or more nuclear genes may act as modifiers, possibly explaining the male prevalence.

The recent identification of mutations in the nuclear gene OPA1 as the causative factor in dominant optic atrophy (DOA, Kjer's type) brought the unexpected finding that this gene encodes for a mitochondrial protein, suggesting that DOA and LHON may be linked by a similar pathogenesis.

Polymorphisms in this very same gene may be associated with normal tension glaucoma, which might be considered a genetically determined optic neuropathy that again shows similarities with both LHON and DOA.

Genetic defects are responsible for a part of optic atrophy. The lesion can be the only clinical feature (primary) or associated to various neurological and systemic symptoms (secondary).

30.6.1 Primary Optic Atrophy

Two examples of primary optic atrophy (OA) are Leber's hereditary optic atrophy (LHON) or Costeff optic atrophy syndrome (OPA 3).

Costeff optic atrophy syndrome, or type III 3-methylglutaconic aciduria (OPA 3), is a neuro-ophthalmologic disease that consists of early-onset bilateral optic atrophy and later-onset spasticity, extrapyramidal dysfunction and cognitive deficits. Urinary excretion of 3-methylglutaconic acid and of 3-methylglutaric acid is increased. The disorder is mapping chromosome 19q13.2–q13.3, and the causative gene has been identified in the FLJ22187-cDNA clone, which consists of two exons and encodes a peptide of 179 amino acid residues. Milder mutations in OPA3 should be sought in patients with optic atrophy with later onset, even in the absence of additional neurological abnormalities. *Behr syndrome* is clinically similar to Costeff syndrome, but it is distinguished by the absence of 3-methylglutaconic aciduria.

Optic atrophy with significant visual impairment as well as cataracts has also been reported in few patients presenting a recently defined mitochondrial disease due to FBXL4 protein impairment, involved in phosphorylation-directed ubiquitination pathway which was also associated with mitochondrial defective energy metabolism and mtDNA depletion.

30.6.2 Secondary Optic Atrophy

Secondary optic atrophy occurs often in inherited metabolic diseases and is due to accumulation or shortage of substrates or formation of harmful metabolites such as in mitochondrial defects, peroxisomal defects and lysosomal defects and occasionally in some other genetic-metabolic defects such as progressive encephalopathy with oedema, hypsarrhythmia and optic nerve atrophy (PEHO) syndrome (Table 30.6).

Mitochondrial Disorders

In addition to the relative selective involvement of the optic nerve in some disorders with mitochondrial dysfunction, optic atrophy may occur in mitochondrial encephalomyopathies due to mtDNA point mutations in *tRNA* genes, such as *myoclonic epilepsy with ragged-red fibres (MERRF)*, *lactic acidosis and stroke-like syndrome (MELAS*), *NARP (neuropathy, ataxia and pigmentary retinopathy)* and *Leigh syndrome (see also* Chaps. 4, 5, 6, and 7).

Although the visual impairment is not a primary feature, optic atrophy is frequently reported in *Leigh syndrome* (LS) in addition to the bilateral necrotic lesions affecting the periventricular white matter, basal ganglia and brainstem. Given the early onset of LS, it is difficult to document visual loss, but according to the few histopathological reports of the visual system, there is a typical loss of retinal ganglion cells and nerve fibre layer dropout in the papillomacular bundle there is a progression deterioration of brainstem functions, ataxia, seizures, peripheral neuropathy, intellectual deterioration, impaired hearing and poor vision are present. Visual loss may be secondary to optic atrophy or retinal degeneration. A variety of molecular defects in both nuclear DNA (nDNA) and mtDNA have been identified. LS may be inherited, depending on the particular defect,

Table 30.6 Secondary optic atrophy

Mitochondrial disorders
MERRF (mitochondrial encephalopathy with ragged-red fibres and stroke-like episodes)
MELAS (mitochondrial encephalomyopathy, lactic acidosis and stroke-like episodes)
Kearns-Sayre syndrome
NARP (neuropathy, ataxia and retinitis pigmentosa)
Leigh syndrome
Peroxisomal disorders
Zellweger spectrum disorders
Adrenoleukodystrophy
Primary hyperoxaluria type I
Lysosomal disorders
Mucopolysaccharidoses
Oligosaccharidoses
Niemann-Pick type C
Niemann-Pick type A or B
GM1 gangliosidosis
Sandhoff disease
Multiple sulphatase deficiency
Krabbe disease
Metachromatic leukodystrophy
Neuronal ceroid lipofucinoses
Cystinosis
Other metabolic disorders
2-Methyl-3-hydroxybutyryl- CoA dehydrogenase deficiency
Cobalamin C/D disorders
Propionic and methylmalonic acidemia
Homocystinuria
Smith-Lemli-Opitz syndrome
Mevalonic aciduria
Canavan disease
Alexander disease
Pelizaeus-Merzbacher disease
Menkes disease
PEHO syndrome

maternally (mtDNA defects), as an X-linked recessive trait (pyruvate dehydrogenase complex, PDHC defect) or as an autosomal recessive disease (defects in complexes I and II nuclear genes; defects in *SURF* gene for complex IV assembly).

Recently, optic neuropathy and atrophy have been described in association with a mitochondrial disease due to dysfunction in mitochondrial translation optimization 1 (MTO1) protein, a novel form of mitochondrial respiratory chain defect presenting with cardiac failure, cognitive disability, cortical atrophy, seizures, optic neuropathy and early death.

Optic atrophy has also been reported in mitochondrial diseases caused by defects in nuclear genes such as hereditary spastic paraplegia due to mutations in the paraplegin gene and in the deafness-dystonia-optic atrophy syndrome (Mohr-Tranebjaerg syndrome) due to mutations in the X-linked *DDP1* gene. In this latter disease, patients manifest an optic atrophy with severely constricted visual fields, a pattern opposite to LHON and the other optic neuropathies that affect primarily the central visual field due to predominant loss of the papillomacular bundle.

Remember

The most characteristic and primary manifestation of LHON is visual loss due to optic nerve dysfunction.

Peroxisomal Disorders

Remember

Ocular findings in peroxisomal biogenesis disorders include profound demyelination of the optic nerve.

Ocular findings in all peroxisomal biogenesis disorders, such as *Zellweger syndrome, neonatal adrenoleukodystrophy and infantile Refsum disease*, include profound demyelination of the optic nerve, reduced numbers of optic nerve fibres and inclusion-bearing macrophages surrounding the optic nerve retinal ganglion cells. Disorders in single peroxisomal enzyme defects that lead to optic atrophy are *X-linked adrenoleukodystrophy* and *primary hyperoxaluria type I*. Patients with X-linked adrenoleukodystrophy show loss of retinal ganglion cells and sporadic macrophages surrounding the optic nerve fibres. Patients with primary *hyperoxaluria type I* harbour oxalate crystals in various tissues, including the eye, due to a deficiency of the peroxisomal enzyme alanine-glyoxylate aminotransferase. Optic atrophy can occur secondary to increased intracranial pressure caused by impeded cerebrospinal fluid drainage due to oxalate crystals. Crystals within the retinal ganglion cells can also directly cause apoptosis.

Lysosomal Disorders

Lysosomal storage diseases can cause optic atrophy. In the *mucopolysaccharidoses*, pathology is due to the optic disc swelling, but secondary raised intracranial pressure must be excluded. In the absence of raised intracranial pressure, optic nerve swelling and consequently atrophy arise from compression of the nerve by a thickened dura and sclera, resulting in compression at the level of the lamina cribrosa. Moreover, accumulation of GAG within ganglion cells is thought to eventually lead to degeneration and optic atrophy.

Optic atrophy may also occur secondary to retinopathy or can be caused by glaucoma.

Several *oligosaccharidoses*, gangliosidoses (GM1 gangliosidosis, Tay-Sachs and Sandhoff disease) and, occasionally, Niemann-Pick A or B disease can manifest distinct ocular findings and optic neuropathies. Loss of myelinated nerve fibres or thickening of the pial septum of the optic nerve probably causes optic neuropathies in *sphingolipidoses*. Mostly ocular pathology in sphingolipidoses relates to the damage of the retinal ganglion cells that form the optic nerve. Abnormal accumulation of lipid material and loss of retinal ganglion cells results in optic atrophy.

Another lysosomal storage disease with optic atrophy is *Krabbe disease* (globoid cell leukodystrophy). In this disease the lesion is a consequence of the severe loss of myelin and oligodendroglia that is characteristic of this disorder. Although the optic atrophy occurs early, it is usually overshadowed by the neurological deterioration. Optic atrophy is also a frequent cause of severely impaired vision in *metachromatic leukodystrophy*.

Other Secondary Metabolic Optic Atrophies

In addition, some disorders of lysosomal membrane transport such as Niemann-Pick C disease and infantile sialic acid storage disease can present with marked ocular abnormalities and sporadic optic neuropathies. In cystinosis, benign intracranial hypertension has led, in some cases, to optic atrophy. In neuronal ceroid lipofuscinoses, ophthalmological abnormalities like optic atrophy, maculopathy and retinitis pigmentosa have been reported. It is likely that lysosomal storage of metabolites indirectly affects the optic nerve and its supporting system.

30.6.3 Other Secondary Metabolic Optic Atrophies

Optic atrophy is described in a variety of other inherited metabolic disorders. It should be emphasized that, while optic atrophy has been noted in these disorders, it is not an invariant finding. In some cases, it may be a secondary effect of a metabolic crisis. The first group includes disorders where critical metabolites are either deficient or accumulate within the retinal ganglion cells or the supporting cells. Disorders in this group include *biotinidase deficiency, Smith-Lemli-Opitz syndrome, Menkes disease* and a subset of disorders of amino acid metabolism such as *homocystinuria*, *cobalamin C disease* and methylmalonic and *propionic acidemia*.

A second group involves myelination defects of the optic nerve. Disorders in this group include some *hereditary ataxias* such as Friedreich ataxia, some forms of Charcot-Marie-Tooth disease, *Canavan disease*, *abetalipoproteinemia, Pelizaeus-Merzbacher disease*, *Alexander disease* and the spastic paraplegia form with peripheral polyneuropathy (SPOAN, spastic paraplegia, optic atrophy and neuropathy).

Take Home Message

- *Ocular manifestations* represent frequent and characteristic features of several inherited metabolic diseases. Examination of the eye by clinical means, with the ophthalmoscope and slit lamps, may detect the following pathognomonic abnormalities: corneal clouding, lens defects and dislocation, retinal degeneration and optic atrophy.

- *Ophthalmologic damage* is not always related to an impairment in terms of vision (cornea verticillata in Fabry's disease, Kayser-Fleischer ring in Wilson's disease). In other diseases, there are both signs and progressively evolving ocular symptoms (cataracts in galactosemia and ectopia lentis in classic homocystinuria).
- *Clinical symptoms:* While in several inherited metabolic diseases, the eye involvement is selective, in most of the cases there is an overall involvement of the different components of the eye. Photophobia characterizes the clinical onset of *corneal involvement*, amblyopia is associated with *cataract* and blurred vision is related to *lens dislocation*. Nystagmus is frequently a precociuos sign, followed by myopia, night blindness and progressive loss of peripheral vision and amblyopia in disorders affecting the *choroid and the retina*. Visual loss is generally acute or subacute and sometimes preceded by dyschromatopsia, visual field defects and eye pain in *optic neuropathies*.
- *Treatment strategy:* There are two different approaches that have been developed with encouraging results. Tailored systemic treatments have shown clinical improvement also for the eye involvement (as it is for ERT in Fabry's disease), while other diseases improve only with topical targeted organ therapy (cysteamine eye drops in cystinosis).

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Skin and Hair Disorders

31

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Key Facts

- A correct classification of skin and hair signs as well as the knowledge of characteristic cutaneous symptoms is important to understand and diagnose inherited metabolic diseases.
- The profile of cutaneous signs, the age of the patient when manifestations initially occurred, and the presence of associated symptoms are of particular importance.
- Dermatological evaluation can help identifying complications of the disease or side effects of treatments.
- Cutaneous signs of several types often occur in a single given disorder.
- The principal lesions can be grouped into the following main groups: vascular lesions, skin eruptions, ichthyosis, papular and nodular skin lesions, abnormal pigmentation, photosensitivity, hair disorders, and skin laxity. Rarest lesions include aplasia cutis congenital, recurrent skin infections, hypoplastic nails, and linear skin defects.

31.1 General Remarks

The skin is an important and easily accessible organ with pathology often early recognized. It is therefore of special importance in multisystem disorders such as inherited metabolic diseases. Cutaneous signs may represent a hallmark for a specific metabolic disorder, while in other instances a careful dermatological evaluation can help identifying complications of the disease or adverse side effects of (over-) treatment. Cutaneous signs of several types often occur in a single given disorder, and the principal lesions can be grouped in the following main categories: vascular lesions, skin eruptions, ichthyosis, papular and nodular skin lesions, abnormal pigmentation, photosensitivity, hair disorders, and skin laxity. We have summarized for simplicity these cutaneous signs in tables, containing the list of disorders that frequently cluster for each skin or hair lesion. For most disorders, skin and hair abnormalities have been obtained from the clinical synopsis of Online Mendelian Inheritance in Man (OMIM), available at http://www.ncbi.nlm.nih.gov/sites/entrez.

As shown in Table 31.1, vascular lesion can be subclassified in different forms.

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Table 31.1 Vascular skin lesions

Disease	MIM number	Type of vascular skin lesions	Additional dermatological manifestations	Hair
Fabry disease	301500	Angiokeratoma	Hypohidrosis	
Gangliosidosis GM1	230500	Angiokeratoma corporis diffusum	Dermal melanocytosis	Hypertrichosis
Fucosidosis	230000	Angiokeratoma	Thin, dry skin, anhidrosis	Heavy eyebrows
Aspartylglucosaminuria	208400	Angiokeratoma corporis diffusum	Acne	
Galactosialidosis	256540	Angiokeratoma Widespread hemangiomas		
Mannosidosis	248510	Angiokeratoma		
Schindler disease type II	609242	Angiokeratoma corporis diffusum	Hyperkeratosis	
			Dry skin	
		Telangiectasia on lips and oral mucosa	Maculopapular eruption	
Mucolipidosis II	252500	Cavernous hemangioma	Tight skin	
Ethylmalonic encephalopathy	602473	Petechiae		
		Orthostatic acrocyanosis		
Hyperoxaluria type I	259900	Livedo reticularis Acrocyanosis		
Neuraminidase deficiency	256540	Widespread hemangiomas		
Smith–Lemli–Opitz syndrome	270400	Facial capillary hemangioma	Severe photosensitivity	Blond hair
			Eczema	
Transaldolase deficiency	606003	Telangiectasia of the skin	Cutis laxa	Hypertrichosis
DPM1-CDG (CDG Ie)	608799	Telangiectasia	Dysplastic nails	
		Hemangiomas		
MGAT2-CDG (CDG IIa)	212066	Midfrontal capillary hemangioma		
SLC35A1-CDG (CDG IIf)	603585	Petechiae		
		Ecchymoses		
Prolidase deficiency	170100	Diffuse telangiectasia	Crusting erythematous dermatitis	
			Severe progressive ulceration of lower extremities	
Pyrroline-5-carboxylate synthetase deficiency	219150	Visible veins	Cutis laxa	
			Progeroid appearance	
Pyrroline-5-carboxylate reductase deficiency	612940	Visible veins	Cutis laxa	
	614438		Progeroid appearance	

Remember

Skin lesions can be grouped in the following main categories: vascular lesions, skin eruptions, ichthyosis, papular and nodular skin lesions, abnormal pigmentation, photosensitivity, hair disorders, and skin laxity.

31.2 Vascular Lesions

31.2.1 Angiokeratoma

The angiokeratomata is flat or raised vascular skin lesions, and they develop classically as clusters, punctuate, and reddish or blue-black angiectases. They do not blanch with pressure and the largest lesions may appear hyperkeratotic.

Remember

The main vascular abnormalities include angiokeratoma, hemangiomas, petechiae, acrocyanosis, and telangiectasia.

In Fabry disease, a metabolic disorder in which the skin lesions are a dominant feature of the clinical picture, angiokeratomata (also known as angiokeratomas corporis diffusum) may be the first manifestations of the disease, and in some heterozygous females, they may be the only sign of disease, but males with this X-linked disorder have usually a number of years of frequent excruciating and unexplained pains. Pain appears most commonly in the extremities, particularly in the lower extremities, but patients may present with unexplained pain in the abdomen or elsewhere. Affected boys may have been referred to a psychiatrist because it is so difficult to find why they are complaining so vehemently. The appearance of the skin lesions creates an entirely different scenario. They are dark red, and they do not blanch with pressure. They seek out pressure points such as buttocks or knees, but they are often profuse in distribution over the scrotum and penis. They increase in number with time. Older lesions also increase in size. They are usually flat at first but may become slightly palpable. Lesions may be seen on the oral mucosa or on the conjunctiva. Skin signs may also include hypohidrosis.

Remember

Angiokeratomas corporis may be the first manifestations of Fabry disease.

Angiokeratoma may also occur in some other lysosomal storage disorders. One or two angiokeratomata and dermal melanocytosis may be seen in GM1 gangliosidosis, but usually these patients will already have been diagnosed because of hepatosplenomegaly, developmental delay, coarse features, and dysostosis multiplex that occur very early in infancy, whereas the skin lesions are rare before the first year of age. Similarly, a patch of angiokeratomata with thin and dry skin may be seen in fucosidosis, but again not among the earliest features, although they may appear from 6 months to 4 years; organomegaly, bone changes, and developmental delay are evident earlier. Similar lesions may be seen in childhood in aspartylglucosaminuria but long after the patient is known to have coarse features, acne, cataracts, joint laxity, and neurodegeneration. They may be seen in the juvenile form of galactosialidosis together with corneal opacities, bone findings, neurological degeneration, and widespread hemangiomas that bring the patient to attention again usually before the skin lesions appear. Also in β-mannosidosis, angiokeratomata has been reported to occur along with mental retardation, coarse facial features, dysostosis multiplex, and hepatosplenomegaly. Schindler disease type II, also known as Kanzaki disease, is an adult-onset disorder characterized by angiokeratoma corporis diffusum, hyperkeratosis, dry skin, maculopapular eruption, telangiectasia on the lips and oral mucosa, and mild intellectual impairment.

31.2.2 Acrocyanosis, Angiomas, and Telangiectasia

Mitochondrial disorders have long been regarded as diseases of the neuromuscular system and internal organs. However, since almost every tissue, including the skin, is dependent on mitochondrial energy supply, it is not surprising to observe dermatological signs in this extremely heterogeneous group of disorders. Ten percent of patients with primary mitochondrial disorders

present skin manifestations that can be grouped into hair abnormalities, rashes, pigmentation abnormalities, and acrocyanosis: moreover, a growing number of skin disorders present with secondary mitochondrial pathology. Different molecular defects can cause dysfunctional mitochondria, including mutations in mitochondrial- and nuclear DNA-encoded subunits and assembly factors of oxidative phosphorylation (OXPHOS) complexes, mutations in intermediate filament proteins involved in mitochondria dynamics, disorders of mitochondrial DNA metabolism, fatty acid metabolism and heme synthesis, and disorders of mtDNA repair (Feichtinger et al. 2014).

We will describe these disorders separately in the different subchapters according to the primary skin manifestation.

Acrocyanosis appears as a bilateral mottled discoloration of the entire feet or hands. In relation to a vasospasm of small arterioles and venules with secondary dilatation of capillaries, the skin's color becomes bright red, with no trophic changes and pain. Orthostatic acrocyanosis is one of the principal signs of ethylmalonic encephalopathy, a devastating mitochondrial disorder affecting the brain, gastrointestinal tract, and peripheral vessels (Fig. 31.1).

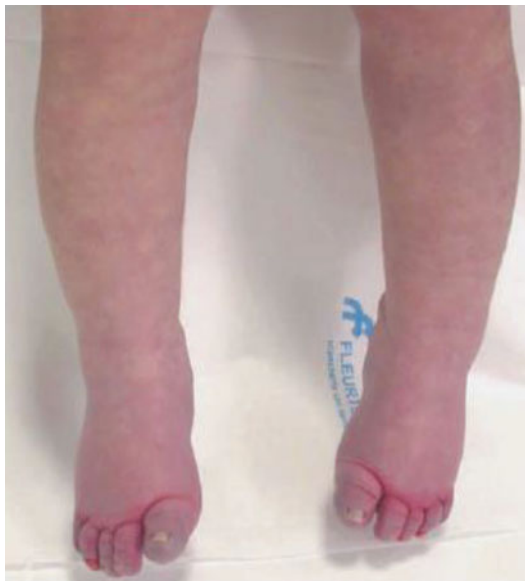

Fig. 31.1 Orthostatic acrocyanosis in ethylmalonic encephalopathy

Remember

Orthostatic acrocyanosis is a key sign of ethylmalonic encephalopathy.

Acrocyanosis may also be observed in other mitochondrial disorders with onset in early infancy and multisystem involvement (Bodemer et al. 1999). Acrocyanosis and livedo reticularis may be observed in hyperoxaluria type I along with urolithiasis, nephrocalcinosis, renal failure, peripheral vascular insufficiency, arterial occlusion, and Raynaud phenomenon.

Capillary malformations appearing as light pink to deep-red angiomas or as telangiectasia may occur in several metabolic diseases. Widespread hemangiomas, coarse facies, conjunctival telangiectasia, severe developmental delay, seizures, and skeletal abnormalities can be observed in neuraminidase deficiency. Facial capillary hemangioma, severe photosensitivity, eczema, and blond hair are typical features of the Smith–Lemli–Opitz syndrome, a disorder of cholesterol biosynthesis presenting with characteristic facial appearance, ambiguous genitalia, failure to thrive, syndactyly, microcephaly, and intellectual impairment (see also paragraph on photosensitivity). In transaldolase deficiency, a metabolic disease of pentose phosphate pathway reported in patients with liver failure and cirrhosis; dysmorphic facial features; renal, cardiac, and hematological involvement; and cutaneous signs include telangiectasia, cutis laxa, and hypertrichosis (Valayannopoulos et al. 2006). More rarely, vascular signs have been observed in patients with congenital disorder of glycosylation (CDG) along with neurologic, facial, and other multisystem abnormalities. These include DPM1-CDG (CDG type Ie), MGAT2-CDG (CDG type IIa), and SLC35A1-CDG (CDG type IIf) (Dyer et al. 2005).

Prolidase deficiency, which also presents with diffuse telangiectasia, will be discussed in the skin eruption paragraph.

Visible veins are frequently observed in two other disorders of proline metabolism, pyrroline-5-carboxylate synthetase and pyrroline-5-carboxylate reductase 1 deficiencies, which will be discussed in the skin laxity paragraph.

31.3 Skin Eruptions (Table 31.2)

The main lesions discussed in this paragraph include seborrheic dermatitis, eczema, skin rashes, psoriasiform lesions (erythematous squamous lesions), lupus-like lesions, hyperkeratosis, vesiculobullous lesions, and ulcers of the skin. *Seborrheic dermatitis* is associated with increased sebum production and involves the scalp, the skin folds, and the sebaceous follicle-rich areas of the face and trunk. The lesions appear pink to erythematous, with or without edema, covered with greasy yellow-brown scales. The scaling on the scalp can vary from fine to thick and could be mild to moderate on the folds. The facial involvement is most prominent on the forehead, the eyebrows, and around the nose. *Eczema* is an inflammatory dermatosis characterized by the appearance of erythema, edema, vesicles, scaling, and crusts. The lesions can be single, multiple, or confluent and pruritus is generally present and intense. *Psoriasiform lesions* consist of erythemato-squamous papules, sharply demarcated with clear-cut borders. *Hyperkeratosis* is a horny thickening that firmly adheres to the skin that may be epidermal or follicular. *Vesiculobullous lesions* are circumscribed, translucent elevated lesions containing fluid arising from cleavage at various level of the skin. *Ulcer* is the result of loss of the entire epidermis and at least the upper dermis (papillary), which heals with scarring.

Remember

Skin eruptions include seborrheic dermatitis, eczema, skin rashes, psoriasiform lesions (erythematous squamous lesions), lupus-like lesions, hyperkeratosis, vesiculobullous lesions, and ulcers.

In multiple carboxylase deficiency, either due to holocarboxylase synthetase or to biotinidase deficiency, skin and hair abnormalities are prominent findings. The usual initial presentation of holocarboxylase synthetase deficiency is the classic organic aciduria emergency with massive ketosis and acidosis progressing to coma. Usually the initial presentation in biotinidase deficiency is of later onset and more gradual; however, there is an overlap. Patients surviving the initial episode of metabolic decompensation often develop the dermatosis. Cutaneous signs consist in a bright red patchy or generalized body eruption, associated with alopecia. Lesions are often desquamative and typically periorificial. Complication by monilial infection is very common, especially around the mouth, the eyes, and in the diaper area. Skin lesions become vesicular when they are complicated by mucocutaneous fungal infection. The clinical manifestations in holocarboxylase synthetase deficiency are more severe than in biotinidase deficiency and some patients are unresponsive to biotine therapy. In biotinidase deficiency patients are less likely to develop life-threatening ketoacidosis. They may present with laryngeal stridor and convulsions and, if undiagnosed and untreated, all are hypotonic and most continue to become mentally retarded. In biotinidase deficiency, the effect of biotin treatment is spectacular with rapid and complete remission of skin, neurological, and metabolic abnormalities. Visual and auditory impairment are often irreversible despite biotin therapy. More rarely, patients with the isolated defect of 3-methylcrotonyl-CoA carboxylase may present with skin signs similar to multiple carboxylase deficiency. The differential diagnosis includes acrodermatitis enteropathica. In fact the first patients described with biotinidase deficiency carried the diagnosis of acrodermatitis enteropathica. Patients with acrodermatitis enteropathica have diarrhea, are zinc deficient, and respond to treatment with zinc, but the diagnosis may be a wastebasket term, and any infant with this diagnosis should at the least have organic acid analysis of the urine and biotinidase of the plasma, as well as a workup for immunodeficiency.

Epidermolysis bullosa simplex (EBS) is characterized by recurrent blistering of the skin following minor physical trauma. Mutations of the plectin 1 (Plec1) gene cause EBS with muscular dystrophy (EBS-MD) (Bauer et al. 2001). Plec1b localizes in the outer mitochondrial membrane and has a role in maintaining organelle shape and network formation by tethering mitochondria to intermediate filaments (Winter et al. 2008).

Table 31.2 Skin eruptions

Disease	MIM number	Type of skin eruptions	Additional dermatological manifestations	Hair
Biotinidase deficiency	253260	Skin rash	Cat odor	Alopecia, loss of eyebrows
Holocarboxylase synthetase deficiency	253270	Seborrheic dermatitis		Loss of eyelashes
3-Methylcrotonyl-CoA carboxylase deficiency	210210	Skin infections		Loss of eyebrows
		Periorificial dermatitis		
Epidermolysis bullosa simplex with muscular dystrophy (EBS-MD	226670	Recurrent blistering of the skin		
Methylmalonic aciduria	251000	Scalded skin, superficial desquamation		Fine hair
Propionic aciduria	606954	Periorificial dermatitis		Alopecia
		Psoriasis-like lesions		
		Skin rash	Edema and arthralgia during crisis	
Mevalonic aciduria	610377	Morbilliform rash, erythematous macules or papules	Edema	
Hyper-IgD syndrome	260920			
Smith–Lemli–Opitz syndrome	270400	See Table 31.1		
Acrodermatitis enteropathica	201100	Bullous, pustular dermatitis of extremities, oral, anal, and genital areas	Impaired would healing	Alopecia of scalp
			Paronychia	Alopecia of eyebrows
		Dermatitis, symmetric pattern		Alopecia of eyelashes
Hyperzynchemia with functional zinc depletion	601979	Skin rash		
Sulfite oxidase deficiency	272300	Mild eczema		Fine hair
Prolidase deficiency	170100	Severe progressive ulceration of lower extremities	Diffuse telangiectasia	
		Crusting erythematous dermatitis		
Lysinuric protein intolerance	222700	Lupus-like erythematous squamous lesions	Hyperelastic skin	Fine sparse hair
Hyperzincemia and hypercalprotectinemia	194470	Inflammatory skin lesions	Pyoderma gangrenosum	
Tyrosinemia type II	276600	Painful punctate keratoses of digits, palms, and soles		
Pustular psoriasis 15 (PSORS15)	616106	Sterile pustules	Dystrophic nails	
		Scaling		
Punctate palmoplantar keratoderma type IA (PPKP1A)	148600	Hyperkeratotic papules irregularly distributed on the palms and soles		
Non-epidermolytic palmoplantar keratoderma (NEPPK)	600962	Epidermolytic hyperkeratosis of the palms and sole		

Remember

Generalized body eruption with desquamative and periorificial skin lesions and alopecia are the characteristic signs of multiple carboxylase deficiency, either due to holocarboxylase synthetase or to biotinidase deficiency.

The typical skin lesions of methylmalonic aciduria and propionic aciduria usually occur in patients with the severe forms of these diseases, with no residual enzyme activity and subjected to a very severe natural protein-restricted diet (Fig. 31.2). Cutaneous manifestations could be divided into five categories: superficial scalded skin, superficial desquamation, bilateral and periorificial dermatitis, psoriasiform lesions, and alopecia (Bodemer et al. 1999). Different skin lesions can coexist in a given case and may be due to the enzyme deficiency itself or may be part of a multideficiency syndrome. Similar pictures, which strongly resemble to acrodermatitis enteropathica, may complicate any of the organic acidurias or disorders of amino acid metabolism that are overtreated with an excessive dietary restriction of natural protein and other essential nutrients. In patients with disorders related to branched-chain amino acid metabolism, skin lesions are often due to a selective deficiency of isoleucine and rapidly resolve when this essential amino acid is supplemented. As a presenting clinical manifestation of untreated disease, cheilitis and diffuse erythema with erosions and desquamation have been reported in two patients with methylmalonic aciduria with homocystinuria, cobalamin C type. In both cases, skin lesions were already present prior to diagnosis, in the absence of iatrogenic nutritional restrictions or deficiency (Howard et al. 1997).

Remember

Acrodermatitis enteropathica-like lesions may complicate any of the organic acidurias or disorders of amino acid metabolism that are overtreated with an excessive dietary restriction of natural protein and other essential nutrients.

Recurrent morbilliform rashes and erythematous macules and papules can be frequently observed in mevalonic aciduria, a disorder of

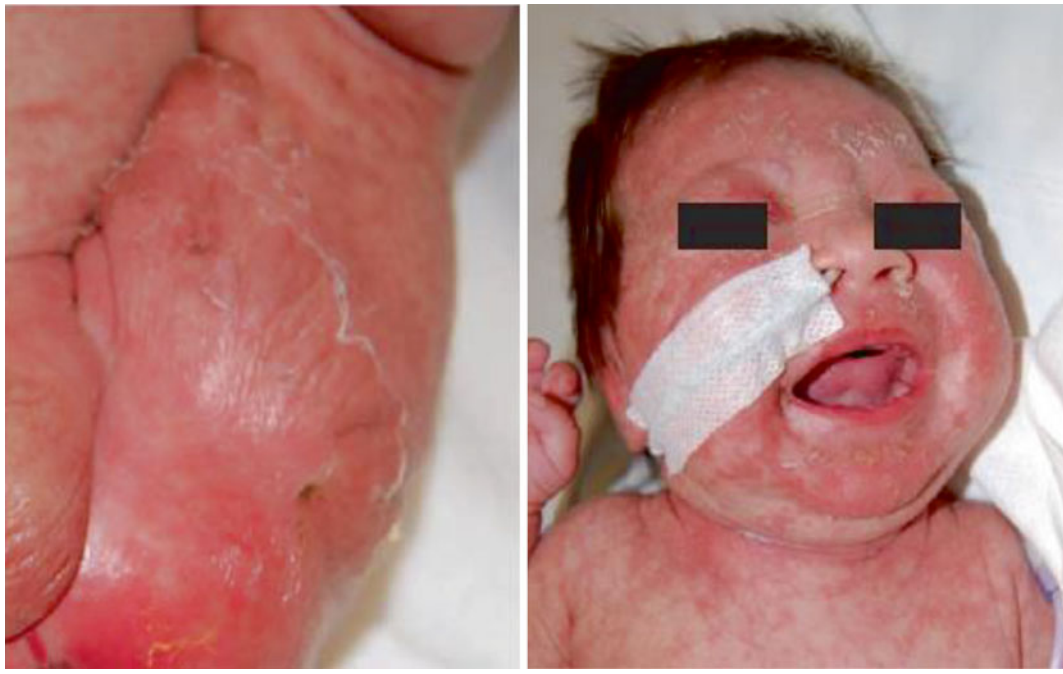

Fig. 31.2 Propionic aciduria with extensive skin lesions characterized by superficial scalded skin, desquamation, and periorificial dermatitis

cholesterol biosynthesis (Fig. 31.3). Patients have also facial dysmorphic features, global developmental delay, cataract, arthralgias with periarticular edema, recurrent febrile crises with lymphadenopathy, hepatosplenomegaly, vomiting, and diarrhea. Rash, edema, and arthralgia may occur during crisis. Similar skin signs can also be observed in hyper-IgD syndrome, the less severe allelic variant of mevalonic aciduria.

Cutaneous ulcers, mainly severe progressive ulceration of lower extremities, can represent the hallmark finding of prolidase deficiency. The ulcers may be further complicated by secondary infection. Skin changes also include telangiectasia, purpuric ecchymoses, rashes, or crusting erythematous dermatitis. In addition, patients may have mental retardation, ophthalmoplegia, splenomegaly, dysmorphic features, and susceptibility to infections. Severe immunological abnormalities, fulfilling the criteria for diagnosis of systemic lupus erythematous, have been reported in prolidase deficiency (Shrinath et al. 1997). Interestingly, skin lupus-like lesions (Fig. 31.4) and immunological abnormalities have also been observed in lysinuric protein intolerance, a transport defect of dibasic amino acid that usually presents with postprandial hyperammonemia, failure to thrive, and severe renal and pulmonary involvement (Dionisi-Vici et al. 1998).

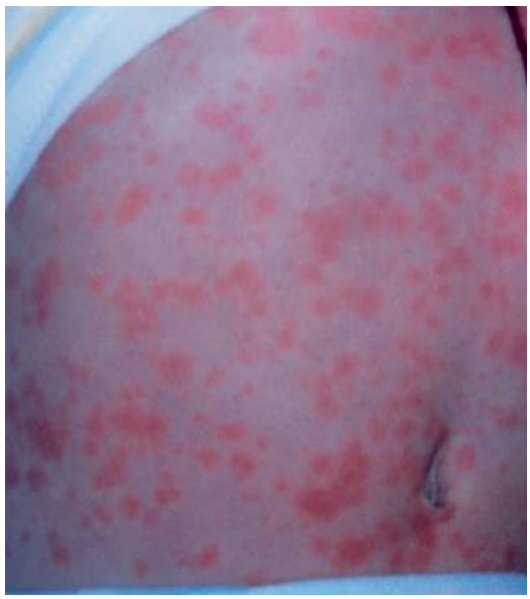

Fig. 31.3 Skin rash in mevalonic aciduria

Remember

Skin lesions and severe immunological abnormalities fulfilling the criteria for diagnosis of systemic lupus erythematous can be observed in prolidase deficiency and in lysinuric protein intolerance.

Ulcers in adolescence and adulthood may also develop in classical homocystinuria caused by cystathionine β-synthase deficiency; these ulcers result from the grounds of thromboembolic disease usually in the lower extremities. Inflammatory skin lesions of various degrees along with recurrent infections, growth failure, hepatosplenomegaly, arthritis, anemia, and persistently raised concentrations of C-reactive protein are the clinical features of hyperzincemia

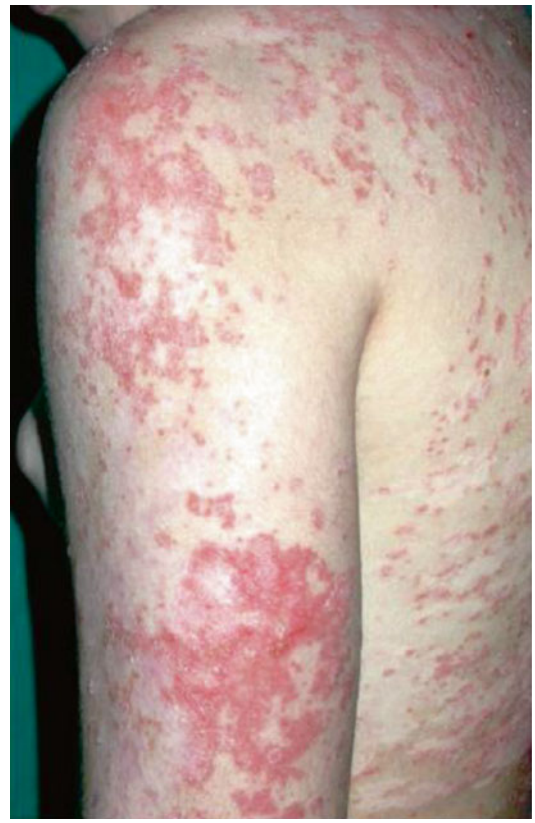

Fig. 31.4 Lupus-like skin lesions in lysinuric protein intolerance

and hypercalprotectinemia, probably caused by dysregulation of calprotectin metabolism.

Hyperkeratotic lesions are a characteristic sign of oculocutaneous tyrosinemia or tyrosinemia type II. The combination of ocular and these lesions has also been called the Richner–Hanhart syndrome (MIM 276600). The major manifestation of this disease is keratitis and corneal ulcers associated with skin lesions occurring on the palms and soles. The skin lesions that usually begin in early infancy are painful, nonpruritic, and frequently associated with hyperhidrosis. Bullous lesions may occur and progress rapidly to erosions that become crusted and hyperkeratotic. Rarely, lesions have a subungual localization.

Punctate palmoplantar keratoderma type I (PPKP1A; MIM 148600) is a rare autosomal dominant hereditary skin disease characterized by multiple hyperkeratotic centrally indented papules that develop in early adolescence or later and are irregularly distributed on the palms and soles. This disorder, which belongs to the adaptinopathy group (Martinelli and Dionisi-Vici 2014), is associated with mutations in the gene encoding AAGAB protein. Notably, the adaptinopathies include several disorders associated with defect in one of the subunits of adaptor protein (AP) complexes and are often associated with neurocutaneous abnormalities. AP complexes are cytosolic heterotetramers that direct the assembly and trafficking of small transport vesicles.

In patients with different forms of pustular psoriasis (PSORS15; MIM 616106), including generalized, palmoplantar, and acral, Setta-Kaffetzi et al. (2014) found heterozygous missense mutations in the gene, which encode for a subunit of the AP complex-1. Differential diagnosis for acral pustular psoriasis (or acrodermatitis continua of Hallopeau) includes defects of CARD14 (MIM 607211) and IL36RN (MIM 605507) genes.

31.4 Ichthyosis (Table 31.3)

Ichthyosis is a very striking scaling dermatosis that results from overactive proliferation of skin cells. Extra skin piles up in scales, reminiscent of the skin of fish, and falls off. Dependent on the rate of the process, the scales may appear old and black or there may be the pronounced erythroderma of new skin. Ichthyosis is characterized by abnormal differentiation (cornification) of epidermis. Several features are useful to distinguish different forms of ichthyosis. They generally may appear with fine or thick scales, covering the skin red or normally colored, sometimes sparing the folds, palms, and plantar areas. In some forms, it

Table 31.3 Ichthyosis

Disease	MIM number	Skin	Hair
Steroid sulfatase deficiency	308100	Hypertrophic ichthyosis	
Multiple sulfatase deficiency – Austin disease	272200	Dark adherent skin scales	
		Ichthyosis	
Refsum disease	266500	Ichthyosis	
Sjogren–Larsson syndrome	270200	Ichthyosis	
Chanarin–Dorfman syndrome/ neutral lipid storage disorder	275630	Nonbullous congenital ichthyosiform erythroderma	Diffuse alopecia
		Collodion baby	
Gaucher disease type II	608013	Erythematous skin ichthyosis, collodion skin	
		Desquamation of skin soon after birth	
		Petechiae	
		Purpura	

(continued)

Table 31.3 (continued)

Disease	MIM number	Skin	Hair
Conradi–Huenermann syndrome	302960	Congenital ichthyosiform erythroderma	Coarse, sparse hair
		Follicular atrophoderma	Patchy areas of alopecia
		"Orange peel" skin	
		Ichthyosis	Sparse eyebrows
			Sparse eyelashes
MEDNIK syndrome	609313	Ichthyosis	Trichorrhexis nodosa
		Hyperkeratosis	
		Erythroderma	
		Large scaling (variable)	
		Flexural involvement	
		Migratory erythema	
CEDNIK syndrome	609528	Ichthyosis	Patchy areas of alopecia
		Hyperkeratosis	
		Large scaling	
ARC syndrome	208085	Ichthyosis	
	613404	Jaundice	
CHILD syndrome	308050	Unilateral erythema and scaling	Unilateral alopecia
		Sharp midline demarcation	
		Hyperkeratosis	
		Onychorrhexis	
		Destruction of nails	
Rhizomelic chondrodysplasia punctata type I	215100	Ichthyosis	Alopecia
Chondrodysplasia punctata type 2	302960	Congenital ichthyosiform erythroderma	Coarse, sparse hair
		Follicular atrophoderma	Patchy areas of alopecia
		"Orange peel" skin	
		Ichthyosis	Sparse eyebrows
			Sparse eyelashes
MPDU1-CDG (CDG If)	609180	Ichthyosis	
DOLK-CDG (CDG Im)	610768	Ichthyosis	Sparse eyebrows
			Sparse eyelashes
			Minimal hair growth
SRD5A3-CDG (CDGIq)	612379	Ichthyosis	
		Hypertrichosis	
		Dark skin of the dorsum of hands and feet	
		Palmoplantar keratoderma	
PIGL-CDG		Migratory ichthyosis	Sparse and fine hair
		Erythroderma (variable)	
COG5-CDG (CDG IIi)	613612	Ichthyosis	
Serine deficiency		Ichthyosis	
Ichthyosis, split hairs, and aminoaciduria	242550	Lamellar ichthyosis	Split hair
		Collodion skin	

can be observed superficial fissuring or blistering especially on the folds. The scales could be generalized or localized. In lamellar ichthyosis, they are accentuated on the extremities, large plate-like brown covering most of the body. Collodion baby is the most severe presentation of congenital ichthyosis (Fig. 31.5). The child is born encased in a translucent membrane which is taut and may impair ectropion, eclabion, ears, and respiration and sucking. During the first 2 weeks of life, the membrane breaks up and peels off, often leaving fissures, with exposure to infection and water loss. This can lead to difficulties in thermal regulation and increased risk of infection and dehydration. On the basis of a clinical classification, two major group of ichthyosis can be identified: (1) primary ichthyoses limited to the skin and (2) syndromic ichthyoses, in which associated features are present (Schmuth et al. 2013).

Remember

Ichthyoses can be classified in primary ichthyoses and syndromic ichthyoses, in which associated features are present. Ichthyoses, in inborn error of metabolism, usually belong to the group of syndromic ichthyoses.

Most ichthyoses are not part of an inborn error of metabolism, but in many metabolic diseases, ichthyosis may be a very prominent presentation. As shown in Table 31.3, a large number of metabolic diseases – belonging to the group of syndromic ichthyoses – are listed. The purest form is X-linked ichthyosis resulting from defective activity of steroid sulfatase, which leads to deposition of cholesterol sulfate. These patients may have no other manifestations of the disease than dark, scaly skin. Mild corneal opacities, which do not interfere with vision, are found in about 25 % of patients. Mental retardation, hypogonadism, shortness of stature, and chondrodysplasia punctata have been observed. Patients with multiple sulfatase deficiency are most debilitated by manifestations of metachromatic leukodystrophy and those of mucopolysaccharidosis. A patient with features of both and who also has ichthyosis doubtless has multiple sulfatase deficiency, also known as Austin disease.

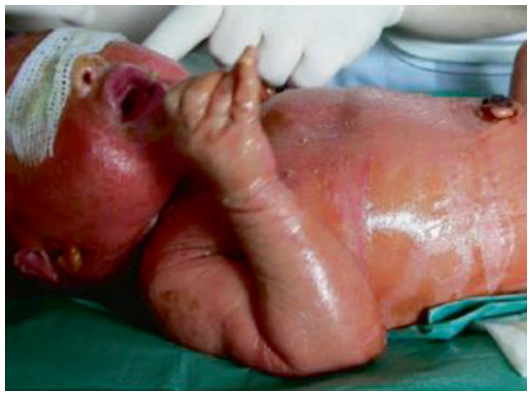

Fig. 31.5 Collodion skin in a neonate with Gaucher disease type II

In patients with Refsum disease, the skin may be the key to the diagnosis of this multisystem disease. Ataxia is an early manifestation. Deafness, peripheral neuropathy, and retinitis pigmentosa complete the syndrome.

Patients with Sjogren–Larsson syndrome in which the fatty alcohol oxidoreductase is defective have cataracts and ichthyosis (Fig. 31.6). In addition, they are mentally retarded and have spastic paraplegia. Retinitis pigmentosa may be evident on ophthalmoscopy; there may be glistening dots in the area of the macula.

Cataracts and ichthyosis are also seen in neutral lipid storage disorder, also known as Chanarin–Dorfman syndrome. There are vacuolated lymphocytes and, to distinguish it from Sjogren–Larrson disease, hepatomegaly. Patients also have muscle weakness ataxia and myopathy.

Ichthyosis is seen as an early, sometimes prenatal, congenital manifestation in neuronopathic Gaucher disease. There may even be a collodion baby appearance (Fig. 31.5). With time the skin may appear normal, but neurodegeneration is progressive. Ichthyotic lesions are also prominent in infants with CHILD syndrome, the acronym for "congenital hemidysplasia with ichthyosiform erythroderma and limb defects" (MIM 308050).

Erythroderma and ichthyosis. MEDNIK syndrome, acronym for mental retardation, enteropathy, deafness, peripheral neuropathy, ichthyosis, and keratodermia, is a severe neurocutaneous

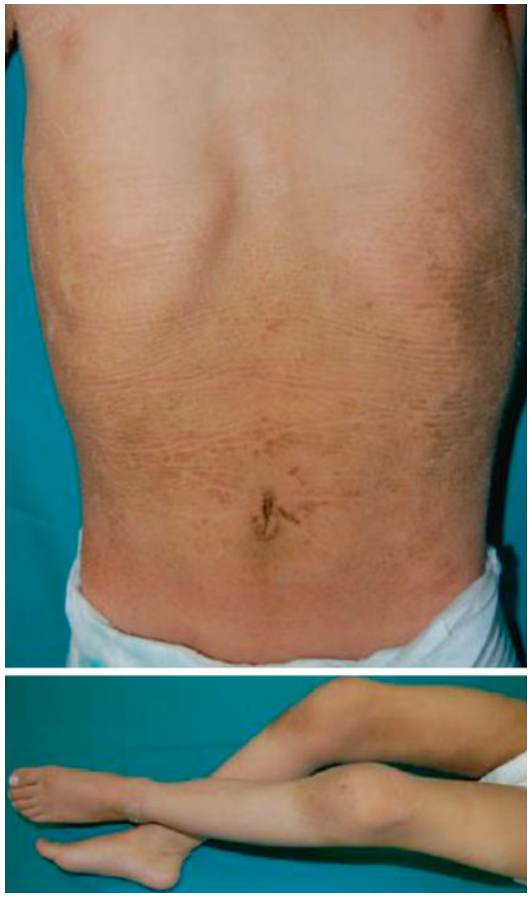

Fig. 31.6 Ichthyosis and spastic diplegia in a patient with Sjögren–Larsson syndrome

disorder with multisystem involvement (Saba et al. 2005; Montpetit et al. 2008; Martinelli et al. 2013) (Fig. 31.7). This condition, causing hyperkeratosis and red patches of variable sizes, shapes, and duration, was described in few families originating from the Kamouraska region in Quebec and originally designated as erythrokeratodermia variabilis-3 (Kamouraska type or EKV3) (Saba et al. 2005). In addition to neurocutaneous symptoms, patients also presented with congenital diarrhea and slight elevation of plasma VLCFA. Linkage analysis followed by candidate gene sequencing identified the same homozygous splice-site mutation in the *AP1S1* gene on chromosome 7q22.1, encoding σ1A, a subunit of AP-1 complex, which plays a crucial role in clathrin coat assembly and mediates trafficking between the trans-Golgi network, endosomes, and the plasma membrane (Montpetit et al. 2008). Afterward, severe perturbations of copper metabolism, combining the phenotype of both Menkes and Wilson disease (hypocupremia, hypoceruloplasminemia, liver copper accumulation, and intrahepatic cholestasis) were associated with MEDNIK phenotype; zinc acetate treatment ameliorated clinical conditions and reduced liver copper and bile acid overload (Martinelli et al. 2013). MEDNIK syndrome, which represents a more complex disorder of intracellular trafficking, falls in the group of adaptinopathies (Martinelli and Dionisi-Vici 2014). Interestingly, another syndromic ichthyosis, CEDNIK syndrome (acronym for cerebral dysgenesis, neuropathy, ichthyosis, and palmoplantar keratoderma), presents with a clinical phenotype resembling MEDNIK syndrome but can be easily distinguished by the lack of erythroderma (Fig. 31.8). Moreover, CEDNIK syndrome has not been associated so far either to abnormal copper metabolism or to a biochemical phenotype (Sprecher et al. 2005). The disease is inherited as an autosomal recessive condition, and it is due to mutations in the *SNAP29* gene (chr. 22q11.2) which encodes a SNARE protein involved in vesicle fusion. It is intriguing and still unsolved how these two different disorders of vesicle intracellular trafficking may show such a similar clinical phenotype.

Ichthyosis is also a characteristic feature of arthrogryposis–renal dysfunction–cholestasis syndrome (ARC; MIM 208085, 613404), a rare fatal autosomal recessive disorder caused by mutations in the *VPS33B* or *VIPAR*, two genes involved in intracellular trafficking. The classical presentation of ARC includes congenital joint contractures, renal tubular dysfunction, and cholestasis. Additional features include central nervous system malformation, platelet anomalies, and severe failure to thrive.

Erythroderma, ichthyosis, and limb defects. Unilateral hypomelia (digital hypoplasia to complete limb absence), elbow and knee webbing, joint contractures, and ipsilateral epiphyseal stippling complete the clinical findings. The disorder is characterized by unilateral ichthyotic skin

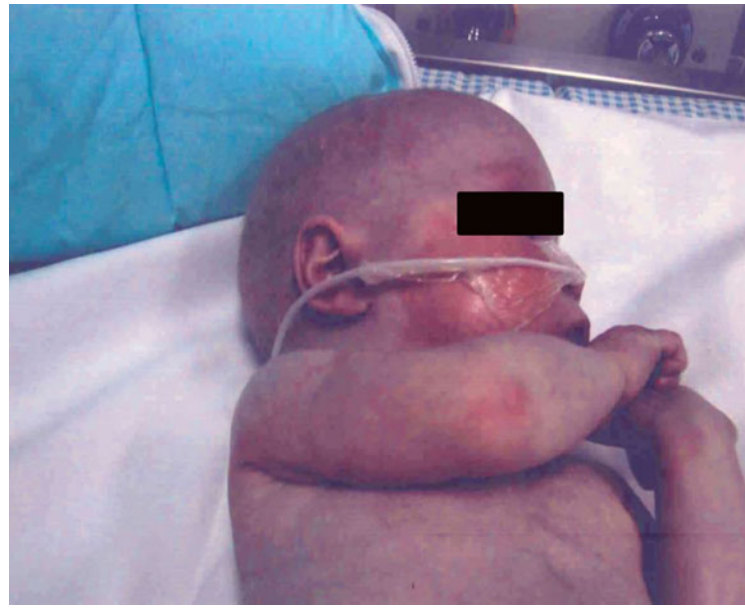

Fig. 31.7 Ichthyosis and erythrokeratoderma in a baby with MEDNIK syndrome

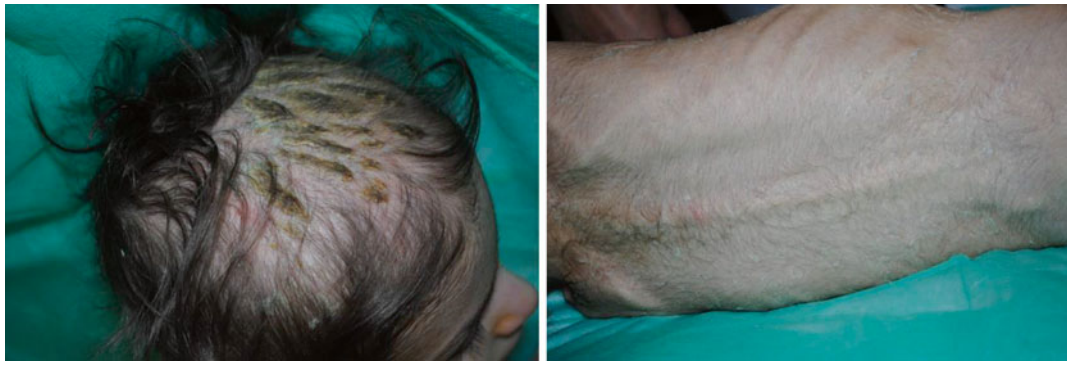

Fig. 31.8 Ichthyosis and patchy areas of alopecia in a patient with CEDNIK syndrome

lesions with a sharp demarcation at the midline of the trunk with the facial area typically spared, although the scalp may be involved. Studies of skin cell kinetics have indicated the lesions in this disease to be psoriasis. Punctate calcifications are present in the epiphyses and other cartilaginous structures of the affected side, which is usually the right side. The disease is inherited as an X-linked disorder and is thought to be lethal in males. Ichthyosis may also be seen in the Conradi–Huenermann or X-linked dominant chondrodysplasia punctata. Besides ichthyosis, Conradi–Huenermann syndrome is characterized by the typical calcifications and asymmetric rhizomelic limb shortness. Cataract and mental retardation have been found in a few affected females. The skin lesions may have a whorled pattern of hyperkeratotic, white adherent scales with underlying red skin and palmar and plantar hyperkeratosis and disappear by 3–6 months. Biochemically, Conradi–Huenermann syndrome patients have normal or decreased cholesterol levels and elevated concentrations of 8-dehydrocholesterol and 8(9)-cholestenol in plasma and tissues due to a defect of 3-β-hydroxysteroid-Δ8, Δ7-isomerase. The sterol-4-demethylase, the enzymatic step just prior to sterol-8-isomerase, is the underlying defect in CHILD syndrome.

Ichthyosis has recently been described in a growing group of congenital disorders of glycosylation (CDG). The different CDG associated with ichthyosis are due to defects within the dolichol pathway (DOLK; SRD5A3-CDG) or the glycosylphosphatidylinositol anchor biosynthesis (PIGL-CDG; MPDU1-CDG) (Rymen and Jaeken 2014). DOLK1 (or DK1)–CDG (formally CDG type Im), causing hypoketotic hypoglycemia and death in early infancy, can present with ichthyosis and hair abnormalities. SRD5A3-CDG (CDG type Iq) is characterized by ocular colobomas, ichthyosis, and endocrine abnormalities associated with midline brain malformations and mental retardation. PIGL-CDG has been associated with clinical phenotypes consistent with CHIME syndrome (coloboma, congenital heart disease, ichthyosiform dermatosis, mental retardation, and ear anomalies syndrome; MIM 280000) and Mabry syndrome (HPMRS, hyperphosphatasia mental retardation Syndrome; MIM 239300). MPDU1-CDG (CDG type 1f) presents with ichthyosis along with psychomotor retardation, night blindness, and dwarfism. Recently, mild ichthyosis has been observed in a patient with COG5-CDG (CDG type IIi) (Rymen et al. 2012). COG5-CDG patients show a wide range of clinical symptoms, from mild/moderate psychomotor retardation with language delay to a severe picture of cerebral and cerebellar atrophy, profound mental retardation, and multisystem involvement.

Ichthyosis has been observed in a girl with serine deficiency of unknown cause, presenting a progressive polyneuropathy, growth retardation, and delayed puberty. Despite no deficiency of any of the serine biosynthetic enzymes was detected, treatment with serine resulted in a clear improvement of neuromuscular and cutaneous signs (de Koning and Klomp 2004).

Differential diagnosis of syndromic ichthyosis includes other rare conditions such as KID syndrome (keratitis, hepatitis, ichthyosis, and deafness; MIM 148210), NISH syndrome (neonatal ichthyosis-sclerosing cholangitis syndrome; MIM 697626), Netherton syndrome (MIM 256500), Rud syndrome (MIM 308200) and other related disorders, and ichthyosis with hepatomegaly and cerebellar ataxia (MIM 242520).

31.5 Papular and Nodular Skin Lesions (Table 31.4)

Papules are small, elevated lesions that may have a variety of shapes. Papules can be differentiated according to the color, margin, number, surface, and distribution. A nodule is a circumscribed, palpable, solid, round, or ellipsoidal lesion up to 1 cm in size. Depth of involvement distinguishes papule from nodule. Nodules can be located in the deep dermal tissue, dermal–subdermal tissue, or subcutaneous fat.

The clinical expression of Farber disease, resulting from deficiency of ceramidase, is striking and the diagnosis can be easily suspected with a careful clinical evaluation. The characteristic features are nodular lesions in the subcutaneous tissues around joints and pressure point areas and painful swelling of joints appearing in early infancy. Lesions are located in the interphalangeal and metacarpal regions as well as in the ankle, wrist, knee, and elbow. The patient complains of pain and stiffness of the joints and may be carrying a diagnosis of arthritis. Hoarseness is another feature. Development may be severely and progressively delayed. Deep tendon reflexes may be diminished or absent. Interstitial pneumonia is an infiltrative component of the disease. Infantile systemic hyalinosis (MIM 228600) presents with similar features and should be considered in the differential diagnosis.

In Hunter disease, uniquely among the mucopolysaccharidoses, there are localized nodular accumulations of mucopolysaccharide in the skin of the scapular area.

Reviewing the literature of skin disorders associated with mitochondrial encephalomyopathies, the most recurring signs are lipomas (Birch-Machin 2000). Lipomas usually occur in the adult forms of mitochondrial disorders in patients bearing mutations of mitochondrial DNA and appear as multiple and symmetrical lesions. The mechanisms underlying the appearance of

Table 31.4 Papular and nodular skin lesions

Disease	MIM number	Papular and nodular skin lesions	Additional dermatological manifestations	Hair
Farber lipogranulomatosis	228000	Lipogranulomatosis periarticular subcutaneous nodules		
MPS type II	309900	Pebbly skin lesions on back, upper arms	Tight blue cutaneous pigmentation	Hypertrichosis
Infantile systemic hyalinosis	228600	Painful, fleshy papules or nodules (hands, scalp, ears, perinasal area) Subcutaneous tumors		
Mitochondrial diseases		Lipomas	Hyperpigmentation	See Table 31.7
			Hypopigmentation	
			Acrocyanosis	
			Skin rashes	
			Erythema	
			Keratoderma	
			Anhidrosis	
			Purpuric lesions	
			Photosensitivity	
			Linear defects	
Familial hypercholesterolemia	143890	Xanthomatosis		
Sitosterolemia	210250	Tendinous and tuberous xanthoma		
Lipoprotein lipase deficiency	238600	Xanthomatosis		
Apolipoprotein E deficiency	107741	Xanthomatosis (tuberous, tuberoeruptive, planar, and/or tendon)		
Cerebrotendinous xanthomatosis	213700	Tuberous xanthoma		
		Xanthelasma		
Glycogenosis type I a/b	232200	Xanthoma		
Niemann–Pick disease type A	257200	Xanthoma		
PMM2-CDG (CDG Ia)	212065	Abnormal subcutaneous fat tissue distribution (fat pads) “Orange peel” skin, inverted nipples		Sparse hair trichorrhexis nodosa pili torti
ALG8-CDG	608104	Abnormal subcutaneous fat tissue distribution (fat pads) “Orange peel” skin, wrinkled skin		
DPAGT1-CDG (CDG Ij)	608093	Fat pads		
SLC35C1-CDG (CDG IIc)	266265	Localized cellulitis		
Werner syndrome	277700	See Table 31.6		

lipomas have not yet been fully understood. A study demonstrated that lipomatosis in a patient with tRNA (Lys) mutations was associated with a pattern of altered expression of master regulators of adipogenesis and with a distorted pattern of brown vs. white adipocyte differentiation (Guallar et al. 2006).

In lipoprotein disorders, lipoproteins can enter the skin, subcutaneous tissues, and tendons producing xanthomata through lipid accumulation and infiltration. Different species of lipoproteins produce different types of xanthomata and characteristic phenotypes associated with specific metabolic defects. According to the morphological characteristics, different types of lipid infiltrates can be distinguished: eruptive-, tuberoeruptive-, tuberous-, tendinous-, planar-, and subcutaneous-xanthomata, xanthelasma, corneal arcus, and tonsillar infiltration (Goldsmith 2003). Diffuse cutaneous xanthomata of tuberous and subcutaneous types are seen along with tendinous xanthomata, xanthelasma, and corneal arcus in familial hypercholesterolemia homozygotes (Fig. 31.9). This disorder of low-density lipoprotein metabolism leads to early coronary artery disease and myocardial infarction; early adult myocardial infarction is seen in heterozygotes. In homozygotes the xanthomata are large, flat, and sometimes distinctly yellowish. A similar picture can occur in sitosterolemia, which, if untreated, also results in premature atherosclerosis and occasionally hemolysis. The xanthomata of patients with lipoprotein lipase deficiency and with other forms of chylomicronemia are very different. Severe elevation of circulating triglyceride concentrations is associated with eruptive skin xanthomata that appear as yellow papules on a slightly erythematous base. Typical locations are over the buttocks, shoulders, and extensor surfaces of the extremities. They occur when triglyceride levels are very high, and they disappear promptly on dietary reduction of triglycerides. These patients have chylomicronemia and do not develop vascular disease, whereas they are subject to recurrent attacks of pancreatitis. Diagnosis may be made by the appearance of the blood,

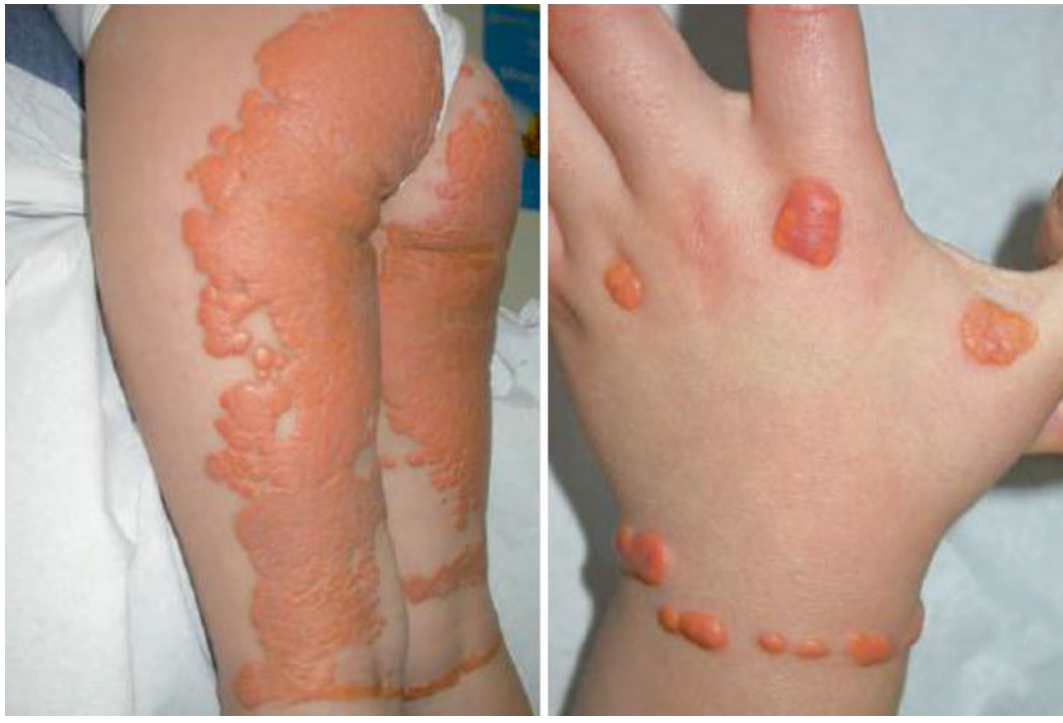

Fig. 31.9 Diffuse cutaneous xanthomata in homozygous hypercholesterolemia

which looks like milky tomato soup. In cerebrotendinous xanthomatosis, accumulation of abnormal sterol derivates is responsible for the development of xanthomata in the brain and tendons. Patients are at risk for myocardial infarction and also present with several neurologic signs and juvenile cataracts. Xanthomata may be observed as a late event in glycogen storage disease type 1 with poor metabolic control in relation to increased serum levels of triglycerides and cholesterol. In Niemann–Pick disease type A, xanthomata may also occur along with extreme hepatosplenomegaly, severe developmental delay, and dystonia.

The enlarged fat pads of the patient with phosphomannomutase 2 deficiency (PMM2-CDG; formally CDG type 1a), the most frequent CDG, are characteristic and almost diagnostic (Fig. 31.10a). The enlargement of fat pads is usually evident toward the end of the first year. They are typically located over the upper and outer areas of the buttocks or lower back, but they may be seen elsewhere including the lateral thighs and upper arms. The skin may feel thickened and later there may be lipoatrophy leaving streaks on the lower extremities. Hair changes, consisting of sparse appearance, slow growing, coarse texture, and trichorrhexis nodosa, have also been observed (Silengo et al. 2003). These infants present with predominant neurological signs, dysmorphic facial features, failure to thrive, and inverted nipples (Fig. 31.10b) associated with systemic manifestations affecting the heart, kidneys, and gastrointestinal system. Enlarged fat pads have been reported so far only in patients with ALG8-CDG (CDG type Ih), DPAGT1-CDG Carrera et al. 2012, and P5CS deficiency (see below).

Inverted nipples are typical of CDG but not specific and can be found in several CDG (especially PMM2- CDG) as well as in other genetic disorders (Turner, Robinow, Smith–Lemli–Opitz, Weaver) and also as a sporadic sign (Rymen and Jaeken 2014).

Hyperphosphatemic familial tumoral calcinosis (MIM 211900) is a rare autosomal recessive metabolic disorder characterized by the progressive deposition of basic calcium phosphate crystals in periarticular spaces, soft tissues, and sometimes bone. The biochemical hallmark of tumoral calcinosis is hyperphosphatemia caused by increased renal absorption of phosphate due to loss-of-function mutations in the FGF23 or *GALNT3* gene. *GALNT3* gene encodes an N-acetylgalactosaminyltransferase, involved in mucin-type O-glycosylation, and is now classified as GALNT3-CDG. Werner syndrome (MIM 277700) can present with subcutaneous calcification and ulceration. This disorder will be described in detail in the photosensitivity subchapter.

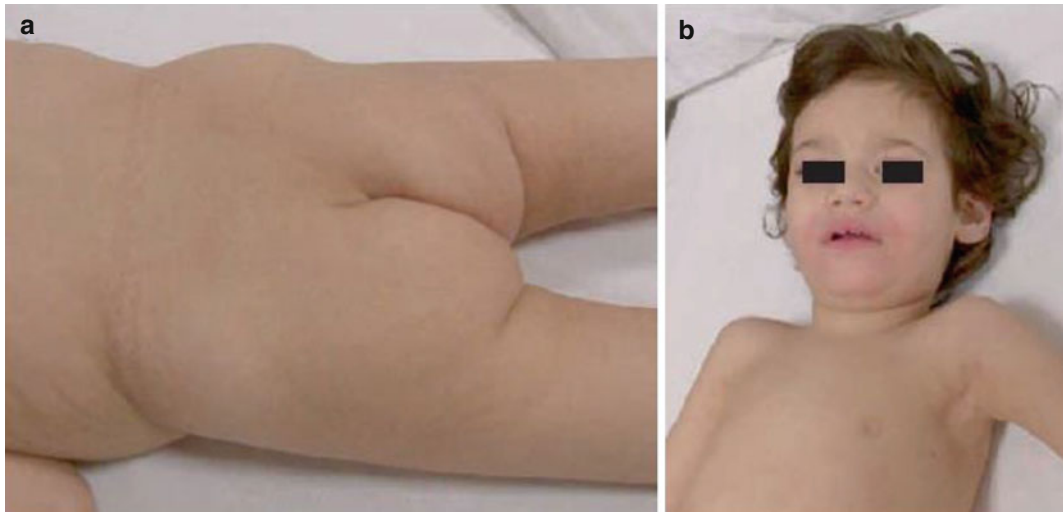

Fig. 31.10 Enlarged fat pads (**a**) and inverted nipples (**b**) in carbohydrate-deficient glycoprotein syndrome type Ia

Remember

The enlarged fat pads of the patient with PMM2-CDG (CDG type Ia) are characteristic and almost diagnostic; consider in differential diagnosis ALG8-CDG, DPAGT1-CDG, and P5CS deficiency.

31.6 Abnormal Pigmentation (Table 31.5)

31.6.1 Hypopigmentation

Skin hypopigmentation is in most cases caused by an enzyme deficiency involving the production, metabolism, or distribution of melanin. Oculocutaneous albinism, not discussed in this chapter, is a group of inherited disorders characterized by a generalized reduction in pigmentation of the hair, skin, and/or eyes. Besides oculocutaneous albinism, differential diagnosis of skin hypopigmentation includes several genetically determined disorders of intracellular trafficking often associated with immune dysfunctions as well as some metabolic diseases.

Remember

Differential diagnosis of skin hypopigmentation includes some metabolic diseases and several genetically determined disorders of intracellular trafficking often associated with immune dysfunctions.

Patients with phenylketonuria prior to the development of screening diagnosis and early treatment were fair of the hair and skin and blue eyed in the majority of cases. Early treatment has made it clear that it is not the gene but the abnormal chemical environment in the untreated state that interferes with normal pigmentation. Also patients with cystinosis and some with homocystinuria have thin hypopigmented skin and fine brittle hair. Moreover, skin hypopigmentation, along with early-onset severe developmental delay, coarse facial features, hepatosplenomegaly, failure to thrive, and dysostosis multiplex, is one of the characteristic signs of infantile sialic acid storage disease.

Hypopigmented skin is characteristic of Menkes syndrome, also known as kinky hair disease, an X-linked recessive neurodegenerative disorder caused by missense mutations in the *ATP7A* gene (copper transport gene on chromosome Xq21.1). Clinical symptoms include impaired intestinal copper absorption, reduced activity of copper-dependent enzymes, progressive neurodegeneration, seizures, and failure to thrive. Skin and hair abnormalities (including hypopigmentation of hair, twisted hairs or pili torti, and pale and lax skin) are due to decreased keratin fiber strength, impaired tyrosinase activity, and melanin synthesis.

Some CDG may present with pigmentary alterations. ST3GAL5-CDG, formerly known as the Amish infantile epilepsy syndrome (MIM 609056), can present with hyper- and hypopigmented macules. ST3GAL5-CDG is caused by a deficiency of GM3 synthase which normally converts lactosylceramide (LacCer) to GM3, a plasma membrane glycosphingolipid, and it has been suggested that a deficiency of GM3 synthase could lead to deficient melanogenesis.

POFUT1-CDG, a novel glycosylation disorder with a phenotype restricted to skin, shows a clinical picture characterized by hypopigmented macules and reticular hyperpigmentation of the skin. Skin biopsies of the hypopigmented macules documented small melanocytes lacking melanosomes, while the regions of hyperpigmentation displayed hyperkeratosis and altered basal pigment distribution.

Kearns–Sayre syndrome is a mitochondrial multisystem disorder, generally caused by a single large deletion of mtDNA in muscle. Skin involvement (e.g., hypomelanosis/hyperpigmentation) has also been shown in a few cases of Kearns–Sayre syndrome.

Several disorders of intracellular trafficking are associated with cutaneous hypopigmentation and in most cases are associated with immune and/or hematological dysfunction. Another adaptinopathy, Hermansky–Pudlak syndrome type 2 (HPS 2; MIM 608233), caused by mutations in *AP3B1* gene, encoding one of the two large subunit of AP3 complex, involved in intracellular vesicles transport, is characterized by oculocutaneous albinism, bleeding diathesis with absence of platelet

Table 31.5 Abnormal pigmentation

Disease	MIM number	Abnormal pigmentation	Additional dermatological manifestations	Hair
Phenylketonuria	261600	Pale pigmentation	Dry skin	Blond hair
			Eczema	
			Scleroderma	
			Mousy odor	
Cystinosis	219800	Light skin pigmentation	Recurrent corneal erosions	Light hair pigmentation
Homocystinuria	236200	Hypopigmentation	Livedo	Fine, brittle hair
			Ulceration	
Infantile sialic acid storage disease	269920	Hypopigmented skin		Fair hair
Methionine malabsorption syndrome	250900			White hair
Alkaptonuria	203500	Ochronosis		
Adrenoleukodystrophy/ adrenomyeloneuropathy	300100	Hyperpigmentation		Scarce and thin hair
				Alopecia
Menkes disease	309400	Hypopigmented and lax skin		Steely, kinky, sparse hair
ST3GAL5-CDG	609056	Hyper- and hypopigmented macules		
POFUT1-CDG	615327	Hypopigmented macules and reticular hyperpigmentation		
Hermansky–Pudlak syndrome type 2	608233	Fair skin		Fair hair
		Cutaneous albinism		
Hermansky–Pudlak syndrome type 9	614171	Hypopigmented skin		Blond, silvery hair
Chediak–Higashi syndrome	214500	Mild/severe skin hypopigmentation	Jaundice	Mild hair hypopigmentation
Griscelli syndrome type 1	214450	Skin hypopigmentation		Silver-gray hair
Griscelli syndrome type 2	607624	Skin hypopigmentation		Silver-gray hair
Griscelli syndrome type 3	609227			Silver-gray hair
				Silver-gray eyelashes
				Silver-gray eyebrows
Vici syndrome	242840	Skin hypopigmentation	Chronic mucocutaneous candidiasis	Hair hypopigmentation
		Cutaneous albinism		
Glycerol kinase deficiency with adrenal insufficiency	307030	Hyperpigmentation		
Kearns–Sayre syndrome	530000	Hypo/ hyperpigmentation		
Hemochromatosis	235200	Hyperpigmentation		Alopecia
		Telangiectasia		
Wilson disease	277900	Hyperpigmentation		
Gaucher disease type I	230800	Hyperpigmentation		

dense bodies, and abnormal depositions of ceroid lipofuscin in various organs. Hermansky–Pudlak syndrome type 9 (HPS 9; MIM 614171), caused by mutations in *PLDN* gene, presents with skin hypopigmentation, congenital nystagmus, and absent platelet delta granules.

The characteristic features of Chediak–Higashi syndrome (MIM 214500), caused by mutation in *LYST* gene, include decreased pigmentation of hair and eyes (partial albinism), photophobia, nystagmus, large eosinophilic, peroxidase-positive inclusion bodies in the myeloblasts and promyelocytes of the bone marrow, neutropenia, abnormal susceptibility to infection, and peculiar susceptibility to malignant lymphoma. Griscelli syndrome type 1 (MIM 214450), type 2 (607624), and type 3 (609227) are also associated with skin and hair hypopigmentation. In type 1, caused by mutation in *MYO5A* gene, additional features include developmental delay, hypotonia, and seizures but not immunologic abnormalities; in type 2, caused by mutation in the *RAB27A* gene, variable neurological deterioration is associated with severe immunological changes, including hemophagocytic syndrome (HS), deficiency of delayed skin hypersensitivity, humoral deficiency, and frequent pyogenic infections; in type 3, due to mutation in *MLPH* gene, hypopigmentation occurs without any immunological and neurological manifestations. In Vici syndrome (MIM 242840), a severe disorder due to mutation in *EPG5* gene encoding for a key regulator of autophagy, cutaneous (and ocular) hypopigmentation is associated with agenesis of the corpus callosum, cataracts, cardiomyopathy, and combined immunodeficiency.

31.6.2 Hyperpigmentation

In alkaptonuria, defective activity of homogentisic acid oxidase leads to the accumulation of homogentisic acid, which is then oxidized to form black insoluble pigment. Pigment deposition is greatest in cartilage, so it is prominent in the ears. It may also be seen early in the sclerae and in the nose, initially as salt and pepper spots, but later these may become confluent. In an older patient, it may be widely distributed, especially distally on the fingers. Deposition in joint cartilage leads to debilitating early osteoarthritis. The skin may serve as an alerting clue to the diagnosis.

Skin hyperpigmentation is characteristic of adrenal insufficiency, and this may be the first substantial clue in late childhood to the presence of adrenoleukodystrophy or, later in life, of adrenomyeloneuropathy, an X-linked recessive trait characterized by progressive demyelination of the nervous system, resulting in the accumulation of very long chain fatty acids in the nervous system, adrenal gland, and testes, which disrupts normal activity.

Remember

Skin hyperpigmentation is characteristic of adrenal insufficiency and may be the first substantial clue in late childhood to the presence of adrenoleukodystrophy.

Adrenal insufficiency is also seen in those patients with glucokinase deficiency who have a contiguous gene deletion syndrome. Dependent on the size of the deletion, some also have Duchenne muscular dystrophy and/or ornithine transcarbamylase deficiency. Likewise, adrenal insufficiency can cause skin hyperpigmentation in Kearns–Sayre syndrome.

In hereditary hemochromatosis, bluish-gray hyperpigmentations are seen on the light-exposed sebostatic scaling skin areas. Bronze diabetes with liver cirrhosis, hypogonadism, and loss of libido are found in more advanced cases. Hyperpigmentation of mucous membranes and conjunctival membranes occur in 15–20 % of patients. There can be a loss of axillary and pubic hair because of hepatotesticular insufficiency. Besides skin signs, fully developed clinical manifestations of hereditary hemochromatosis include a multiorgan involvement affecting the liver, heart, pancreas, endocrine organs, and joints. Patients with Wilson disease suffer from progressive severe dystonia, and skin manifestations are characterized by reticulated brownish hyperpigmentation of the lower legs, blue lunulae, and corneal pigmenta-

tion known as Kayser–Fleischer ring. There is additionally a premature graying of the hair and development of liver cirrhosis. Cutaneous hyperpigmentation can be observed in Gaucher type 1.

31.7 Photosensitivity (Table 31.6)

The phenotypic expression of diseases with increased photosensitivity mainly depends on UV or light exposure. Early recognition and

Table 31.6 Photosensitivity

Disease	MIM number	Photosensitivity	Additional dermatological manifestations	Hair
Erythropoietic protoporphyria	177000	Light-sensitive dermatitis	Edema Mild scarring	
		Itching		
		Burning		
		Erythema		
Congenital erythropoietic porphyria	263700	Photosensitivity	Conjunctivitis	Hypertrichosis
		Blistering	Corneal scarring	
		Scarring	Red stained teeth	Alopecia
			Mutilating skin deformity	Loss of eyelashes
			Pseudoscleroderma	Loss of eyebrows
			Hyperpigmentation	
			Hypopigmentation	
Porphyria cutanea tarda type I	176090	Photosensitivity	Mechanically fragile skin	Facial hypertrichosis
Porphyria cutanea tarda type II	176100	Blisters in sun-exposed areas		
			Pseudoscleroderma	
		Hyperpigmentation in sun-exposed areas	Fingernail onycholysis	Alopecia
Porphyria variegata	176200	Photosensitivity		
Coproporphyria	121300	Photosensitivity		
Ferrochelatase deficiency	177000	Light-sensitive dermatitis		
Hartnup disorder	234500	Light-sensitive dermatitis	Atrophic glossitis	
Tryptophanuria with dwarfism	276100	Cutaneous photosensitivity		
Cockayne syndrome type B	133540	Cutaneous photosensitivity	Progeoid features	
Rothmund–Thomson syndrome	268400	Skin atrophy, telangiectasia, hyper-/hypopigmentation	Premature aging	
Werner syndrome	277700	Scleroderma-like skin, especially of face and distal extremities Subcutaneous calcification Ulceration	Thin, sparse, gray Premature balding	
Ataxia-telangiectasia	208900	Cutaneous telangiectasia Cafe au lait spots Progeric skin changes Sclerodermatous skin changes	Progeric hair changes	
Smith–Lemli–Opitz syndrome	270400	See Table 31.1		

prompt diagnosis may prevent complications associated with prolonged unprotected exposure to sunlight allowing recognition of families at risk for rare heritable disorders associated with photosensitivity. Drugs and chemicals may interact with UV to induce photosensitivity, and recognition of the associated reaction patterns greatly assists clinical classification of these disorders, which include some metabolic diseases, the DNA repair-deficient disorders, and other genodermatoses.

Photosensitive skin lesions characterize many of the porphyries, a clinically and genetically heterogeneous group of diseases arising from enzymatic defects along the pathway of porphyrin–heme biosynthesis. In erythropoietic protoporphyria, photosensitivity begins early in life. Skin signs can occur acutely with burning, stinging, and pruritus in sun-exposed areas accompanied by pain in the extremities. These are followed by erythema, edema, erosions, and scarring. The vesicular lesions in response to sun tend to be smaller and patchier than in the other porphyrias of childhood. They leave tiny areas of flat depressed atrophy of the skin that may be very subtle but are telltale markers of the disease. Unusual late complications include biliary tract stones and hepatic failure. The most dramatic and earliest in onset is congenital erythropoietic porphyria, the disease in which there is pink urine and red teeth. These patients may also have hemolytic anemia and splenomegaly. The skin lesions are vesicular and bullous. The skin appears quite fragile, and ulcers or erosions appear. There may be residual scarring and alternating hyperpigmentation and depigmentation. Mutilation of fingers, nasal tips, and ears may eventually occur. Patients with porphyria cutanea tarda type I, the heterozygous, autosomal dominant form of uroporphyrinogen decarboxylase-III deficiency, present first symptoms in adulthood. The skin is fragile and minor trauma leads to erosions. Patients also develop hepatic siderosis. In patients with hepatoerythropoietic porphyria (also called porphyria cutanea tarda type II), the homozygous form of uroporphyrinogen decarboxylase-III deficiency, onset is neonatal or shortly thereafter. Patients may also have hemolytic anemia and splenomegaly. The urine may be black or pink. The skin is fragile and develops vesicles and blisters in response to sun exposure. Hypertrichosis about the face is common. Liver disease is a complication. Patients with variegate porphyria have abdominal and neurological crises. Onset is from puberty to adulthood. Late changes in the skin may be pseudosclerodermatous. Patients with hereditary coproporphyria have hemolytic anemia and abdominal and neurological crises. Onset is in adulthood. Abdominal or psychiatric symptoms may be precipitated by drugs that increase hepatic cytochrome P450. Heme synthesis and Fe–S cluster biogenesis are strictly related: in fact, heme, which is an important prosthetic group in different relevant proteins such as cytochrome c, is partly synthesized in the mitochondrion. Ferrochelatase deficiency (protoporphyria, erythropoietic, autosomal recessive; MIM 177000), the enzyme that catalyzes the final step in heme synthesis, is associated with a light-sensitive dermatitis (Murphy 1999). Microphthalmia syndromic 7 (MCOPS7) or linear skin defects with multiple congenital anomalies 1 (LSDMCA 1) is due to mutations in the X-linked gene encoding mitochondrial holocytochrome c synthase (*HCCS*); defect in HCCS protein is associated with lack of cytochrome c and secondary OXPHOS defects and increase of apoptosis (San Francisco et al. 2013). This disorder, which is not associated with photosensitivity, will be further discussed in the "Other lesions" subchapter.

Hartnup disease is characterized by a pellagra-like light-sensitive rash, cerebellar ataxia, emotional instability, and aminoaciduria. Patients have a transport disorder involving the intestine and the renal tubule, and failure to absorb tryptophan leads to the dermatological picture of pellagra. Cutaneous manifestations of this disease have been rare in the USA, presumably because most eat such a high-protein diet that deficiency is avoided. Tryptophanuria with dwarfism was described in three siblings with mental defect, cutaneous photosensitivity, and gait disturbance resembling cerebellar ataxia. The clinical features resembled Hartnup disease but the chemical findings were

different, and the defect was thought to concern the conversion of tryptophane to kynurenine. The differential diagnosis of photosensitive dermatoses includes the different syndromes of DNA repair defect, such as xeroderma pigmentosum and of Fanconi anemia, Cockayne syndrome type A (MIM 216400) and B (MIM 133540), Bloom syndrome (MIM 210900), and trichothiodystrophy (MIM 601675). All of these disorders of DNA repair are important to recognize because of their frequent complication by neoplasia. Interestingly, some of these syndromes have been associated with a defect in mtDNA repair (Fanconi syndrome, Cockayne syndrome type B, Rothmund–Thomson syndrome) or secondary mitochondrial dysfunction (Werner syndrome, ataxia-telangiectasia).

Rothmund–Thomson syndrome (MIM 268400), a disorder characterized by skin atrophy, telangiectasia, hyper- and hypopigmentation, congenital skeletal abnormalities, short stature, premature aging, and increased risk of malignant diseases, is associated with mutations in the helicase *RECQL4*, which can localize to mitochondria. Accumulation of mtDNA damage, decreased mtDNA integrity, and increased mtDNA copy number have all been reported.

Werner syndrome (MIM 277700), characterized by scleroderma-like skin changes, especially in the extremities, cataract, subcutaneous calcification, premature arteriosclerosis, diabetes mellitus, and a wizened and prematurely aged facies is caused by mutation of *RECQL2* gene.

Ataxia-telangiectasia (AT; MIM 208900), a disorder defined by cerebellar ataxia, telangiectasia, immune defects, and a predisposition to malignancy, is caused by mutations in the ataxia-telangiectasia-mutated (ATM) gene, a master mediator of the DNA damage response to double-strand breaks. ATM loss causes defects in mtDNA integrity, abnormal mitochondrial morphology, defect in MRC electron transport, and dysregulation of mitophagy (for a review see Feichtinger et al. 2014).

Remember

The differential diagnosis of photosensitive dermatoses includes the different forms of DNA repair disorders.

31.8 Hair Disorders

31.8.1 Hair Shaft Abnormalities

Hair shaft abnormalities are highly heterogeneous and range from changes in color, density, length, and structure of the hair to absence of the hair (alopecia). The hair of patients with hair shaft diseases that may occur as localized or generalized disorders feels dry and looks lusterless. Alopecia may be universal (loss of scalp hair, eyebrows, and eyelashes), total (loss of scalp hair), and partial.

Trichorrhexis nodosa is a characteristic sign in some patients with argininosuccinic aciduria. The patient is recognized for an appearance at a distance of alopecia, but on close examination it is clear that very short hairs are abundant. The hair shafts are fragile and break easily. A longer hair under the microscope displays the characteristic nodules. Abnormal appearance of the hair is also seen in Menkes disease. The typical appearance is that of pili torti, in which the hair shaft is twisted. These patients may also have trichorrhexis nodosa or monilethrix in which there is segmental narrowing of the hair shaft. These hairs tend to break readily too, but the patient never appears to have alopecia. As a consequence of reduced tyrosinase activity, hair and skin appear hypopigmented. In addition, patients show skin laxity. Menkes disease, also called kinky hair disease, is a devastating cerebral degenerative disease, with refractory seizures, bone lesions, skin and joint laxity, and tortuous cerebral arteries. In the occipital horn syndrome, the milder allelic variant of Menkes disease, the hair appears coarse (Fig. 31.11), and the skin is soft, mildly extensible, and redundant with easy bruisability.

In some cases of PMM2-CDG, hair aspects appear sparse and are unusual, slow growing, lacking luster, and coarse in texture. Enhanced fragility in the form of trichorrhexis nodosa, torsion of the shaft along the longitudinal axis, and pili torti has also been observed (Silengo et al. 2003).

Pili torti, in association with sensorineural hearing loss, are found in Bjornstad syndrome,

Fig. 31.11 Coarse, sparse, and fragile hair in Menkes disease

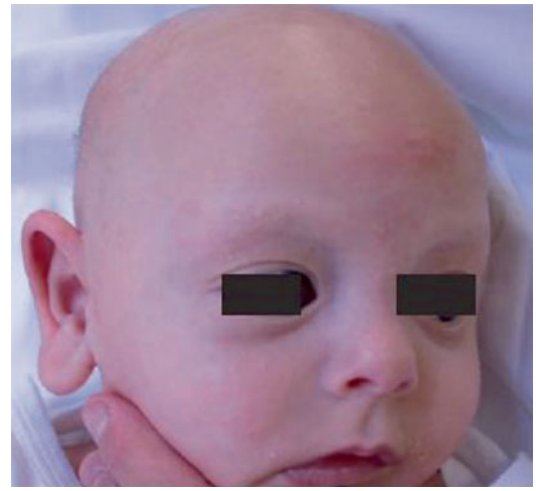

Fig. 31.12 Alopecia totalis with absence of eyebrows and eyelashes in a child with multiple carboxylase defect due to holocarboxylase synthetase deficiency

an autosomal recessive disorder due to mutations in the *BCS1L* gene. The protein encoded by this gene facilitates the insertion of Rieske iron–sulfur protein into complex III of mitochondrial respiratory chain during assembly. Mutations in the *BCS1L* gene had previously associated to GRACILE syndrome, a severe disorder of intrauterine growth retardation, aminoaciduria, cholestasis, iron overload, lactic acidosis, and early death. Mutations in the *RMRP* gene cause three different phenotypes, anauxetic dysplasia, cartilage-hair hypoplasia, and metaphyseal dysplasia without hypotrichosis. All these three allelic disorders are characterized a by fine, sparse, and light-colored hair, with an abnormal reduction in the diameter of the hair shaft on microscopic examination.

The classic presentation of sulfite oxidase deficiency involves neonatal seizures, progressive encephalopathy, dislocation of lenses, and death at an early age. Dermatological signs include mild eczema and fine hair. Alopecia totalis is the characteristic appearance of holocarboxylase synthetase (Fig. 31.12). Patients have no hair on the head, eyebrows, eyelashes, or lanugo hair. Patients with biotinidase deficiency have patchy alopecia in the pattern of the patient with acrodermatitis enteropathica.

Patients with organic acidurias, such as methylmalonic aciduria, propionic aciduria, or maple syrup urine disease, all of whom must be treated with very strict restriction of the intake of protein, may have fine hair or develop alopecia when the protein restriction is too stringent or when intercurrent infection increases demand (see Sect. C9.2).

As shown in Table 31.7, in many other metabolic diseases already discussed in previous paragraphs of this chapter, patients may have hair shaft abnormalities or alopecia.

31.8.2 Hypertrichosis (Table 31.8)

Hirsutism or hypertrichosis is a common finding in several lysosomal storage diseases. In most cases, patients have a characteristic coarse facial features, neurological abnormalities, intellectual delay, dysostosis multiplex, and hepatosplenomegaly.

Remember

Hirsutism, hypertrichosis, coarse facial features, neurological abnormalities, intellectual delay, dysostosis multiplex, and hepatosplenomegaly are common findings in several lysosomal storage diseases.

Table 31.7 Hair shaft abnormalities

Disease	MIM number	Skin	Hair
Argininosuccinic aciduria	207900		Trichorrhexis nodosa Dry brittle hair
Menkes disease	309400	Hypopigmentation, skin laxity	Steely, kinky, sparse hair
Occipital horn syndrome	304150	Soft, extensible, and redundant skin	Coarse hair
		Easy bruisability	
PMM2- CDG	212065	See Table 31.4	Sparse hair
			Trichorrhexis nodosa
			Pili torti
Sulfite oxidase deficiency	272300	Mild eczema	Fine hair
Mitochondrial diseases		See Table 31.4	Hypertrichosis
			Alopecia
			Thin, dry, brittle, sparse hair
			Trichothiodystrophy
			Trichorrhexis nodosa
Bjornstad syndrome	262000		Pili torti
Anauxetic dysplasia	607095		Fine, sparse, and light-colored hair
Cartilage-hair hypoplasia	250250		Fine, sparse, and light-colored hair
Metaphyseal dysplasia without hypotrichosis	250460		Fine, sparse, and light-colored hair
Biotinidase deficiency	253260	See Table 31.2	Alopecia
Holocarboxylase synthetase deficiency	253270		Loss of eyelashes
3-Methylcrotonyl-CoA carboxylase deficiency	210210		Loss of eyebrows
Congenital erythropoietic porphyria	263700	See Table 31.6	Hypertrichosis
			Alopecia
			Loss of eyelashes
			Loss of eyebrows
Porphyria cutanea tarda type I	176090	See Table 31.6	Facial hypertrichosis
Porphyria cutanea tarda type II	176100		Alopecia
Hemochromatosis	235200	See Table 31.5	Alopecia
Adrenoleukodystrophy/ adrenomyeloneuropathy	300100	See Table 31.5	Scarce and thin hair
			Alopecia
Chanarin–Dorfman syndrome/ neutral lipid storage disorder	275630	See Table 31.3	Diffuse alopecia
Rhizomelic chondrodysplasia punctata type I	215100	See Table 31.3	Alopecia
CHILD syndrome	308050	See Table 31.3	Unilateral alopecia
Conradi–Huenermann syndrome	302960	See Table 31.3	Coarse, sparse hair
			Patchy areas of alopecia
			Sparse eyebrows
			Sparse eyelashes
Acrodermatitis enteropathica	201100	See Table 31.2	Alopecia of scalp
			Alopecia of eyebrows
			Alopecia of eyelashes

Table 31.8 Hypertrichosis

Disease	MIM number	Skin	Hair
MPS type I	607014	Skin thickening	Hypertrichosis
MPS type II	309900	Coarse facies	Synophrys
MPS type IIIA	252900	Pebbly skin lesions on back, upper arms	Hirsutism
MPS type IIIB	252920		Coarse hair
MPS type IIIC	252930	Thigh blue cutaneous pigmentation	
MPS type IIID	252940		Hypertrichosis
MPS type VI	253200		Hypertrichosis
MPS type VII	253220		Hypertrichosis
MPS type VIII	253230		Hypertrichosis
Mucolipidosis type IIIA	252600		Hypertrichosis
Gangliosidosis GM1	230500		Hypertrichosis
Mannosidosis	248500		Hypertrichosis
Sialuria	269921		Hypertrichosis
Transaldolase deficiency	606003	See Table 31.1	Hypertrichosis
Leigh syndrome – *SURF1* mutations	256000		Hypertrichosis
Infantile lactic acidosis/*SUCLG1* mutations	611224		Hypertrichosis
Mitochondrial diseases		See Table 31.4	See Table 31.4

In the wide spectrum of clinical abnormalities of mitochondrial diseases, diffuse hypertrichosis is a typical sign of the Leigh syndrome encephalomyopathy with cytochrome c oxidase deficiency and mutations in the *SURF1* gene (Fig. 31.13).

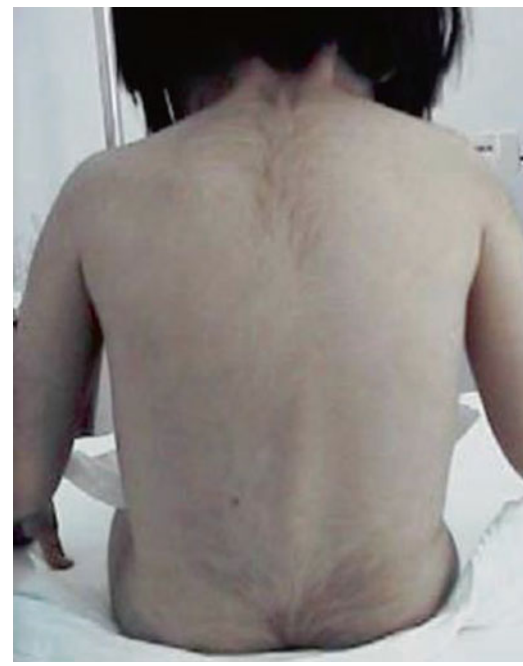

Fig. 31.13 Diffuse hypertrichosis in a child with Leigh syndrome, cytochrome c oxidase deficiency, and mutations in the *SURF1* gene

31.9 Skin Laxity (Table 31.9)

Cutis laxa Mohamed et al. 2014 is a group of disorders characterized by loose and/or wrinkled skin that determines a prematurely aged appearance. The skin lacks elastic recoil, in contrast to the hyperelasticity apparent in classical Ehlers–Danlos syndrome.

The clinical spectrum of autosomal recessive cutis laxa is highly heterogeneous with respect to organ involvement and severity. Type I autosomal recessive cutis laxa (ARCL1) is a specific, life-threatening disorder with organ involvement, lung atelectasis and emphysema, diverticula of the gastrointestinal and genitourinary systems, and vascular anomalies. Classification of autosomal recessive cutis laxa is further divided into

Table 31.9 Skin laxity

Disease	MIM number	Skin	Hair
Type II autosomal recessive cutis laxa	219200	Loose skin with redundant folds	
COG7-CDG (CDG IIe)	606978	Skin laxity	
MAN1B1-CDG	614202	Skin laxity	
Delta-1-pyrroline-5-carboxylate synthetase (*P5CS*) deficiency	138250	Skin hyperelasticity Visible veins	
Delta-1-pyrroline-5-carboxylate reductase (*PYCR1*) deficiency,	179035	Skin hyperelasticity Visible veins	
Hutchinson–Gilford progeria	176670	Absence of subcutaneous fat	Alopecia
ADCL	123700	Cutis laxa Loose redundant skin Inelastic skin Excessive skin folds Sparse, fragmented elastic fibers	
ARCL1	219100	Cutis laxa Loose redundant skin Excessive skin folds Increased vascularization, reduced collagen bundle size Underdeveloped elastic fibers in dermis	
MACS syndrome	613075	Soft, redundant skin Ichthyosis (rare) Hyperextensible skin Multiple pigmented moles Easy bruising	Receding anterior hairline Sparse hair Sparse androgenic hair
Menkes disease	309400	See Table 31.7	See Table 31.7
Occipital horn syndrome	340150	See Table 31.7	See Table 31.7
Transaldolase deficiency	606003	See Table 31.1	See Table 31.8
Lysinuric protein intolerance	222700	See Table 31.2	

type II (ARCL2), associated with bone dystrophy, joint laxity, and developmental delay, and type III (ARCL3), or De Barsy syndrome, which presents very severe symptoms, with ocular involvement and mental retardation.

ARCL syndromes includes geroderma osteodysplasticum (GO; MIM231070), wrinkly skin syndrome (WSS; MIM 278250), cutis laxa type 2A and 2B (ARCL2A; MIM 219200 and ARCL2B; MIM 612940), and De Barsy syndrome (DBS; ARCL3A, MIM 219150 and ARCL3B, MIM 614438) (Mohamed et al. 2011).

The continuous progress in the genetic field has led to the identification of a number of the responsible causative genes, helping in the reclassification of these disorders and clarifying the confusion caused by the highly overlapping phenotypes. In this section we will focus on those disorders characterized by a confirmed metabolic derangement. Type IIA autosomal recessive cutis laxa (ARCL2A), characterized by loose skin with redundant folds, slow return on stretching, and unaffected facial skin, is a defect of *N*-glycosylation at the level of Golgi apparatus (Fig. 31.14, kindly provided by E. Morava) (Mohamed et al. 2011). In addition to skin signs, patients present with widely persistent fontanelles, slight oxycephaly, dental caries, frontal bossing, downward slanted palpebral fissures, hip dislocation, scoliosis, and inguinal hernia. Isoelectrofocusing of serum transferring shows a type II CDG pattern, and the disorder is caused by loss-of-function mutations in the *ATP6V0A2* gene. The occurrence of mutations in the same gene in wrinkly skin syndrome indicates that autosomal recessive cutis laxa type II and some cases of wrinkly skin syndrome may represent variable manifestations of the same genetic defect. Other CDGs may present with

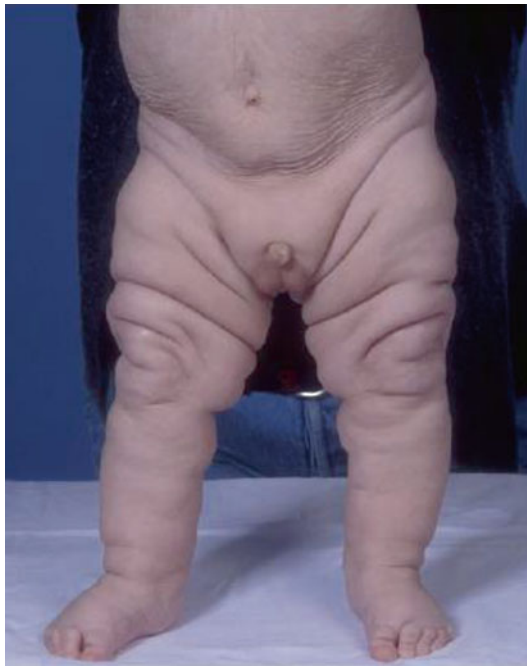

Fig. 31.14 Characteristic skin features in a child with type II autosomal recessive cutis laxa (kindly provided by E. Morava, Children's Hospital Leuven, University of Leuven, Belgium)

increased skin laxity, including COG7-CDG and MAN1B1-CDG. Both disorders fall in the group of CDG type II. COG7-CDG is a multisystem disorder dominated by cerebral atrophy, mental retardation, ventricular septal defect, and wrinkly skin. MAN1B1-CDG presents with slight dysmorphic features, cerebellar hypoplasia with vermian atrophy, moderate/severe psychomotor retardation and sometimes stereotypic behavior, skin and joint hyperlaxity, and obesity. Differential diagnosis of ARCL2A includes clinical cutis laxa syndromes of geroderma dysplasticum (GO) due to mutation in the *GORAB* gene (MIM 607983), The disorder is also known as "Walt Disney dwarfism" due to the typical facial features; mental retardation is exceptional. GORAB protein interacts with Rab6, involved in intracellular trafficking. Although Rab6 has been shown to interact with the COG complex, glycosylation defects have not been documented in GORAB deficiency (Rymen and Jaeken 2014).

Skin laxity, lax joints, microcephaly, bilateral subcapsular cataract, severe mental retardation, structural brain abnormalities, progressive neurodegeneration, peripheral neuropathy, and dystonia, along with fasting hyperammonemia, hypoprolinemia, hypocitrullinemia, and hypoornithinemia, are the characteristic findings of delta-1-pyrroline-5-carboxylate synthetase (P5CS) deficiency (MIM 138250) due to defect of *ALDH18A1* gene, a disorder of proline biosynthesis, responsible of autosomal recessive cutis laxa type IIIA (ARCL3A) or De Barsy syndrome type A. The main differential diagnosis is Δ-1-pyrroline-5-carboxylate reductase deficiency due to mutations in *PYCR1* gene. Affected patients fall in the category of autosomal recessive cutis laxa type IIB (ARCL2B) and autosomal recessive cutis laxa type IIIB (ARCL3B) or De Barsy syndrome type B. Reported patients showed a phenotype of intrauterine growth retardation, progeroid appearance, lax and wrinkly skin, connective tissue weakness, and mild to moderate mental retardation. Although these patients did not show abnormalities in proline metabolism in blood and urine, electron microscopy of skin biopsy documented rarefaction and fragmentation of elastic fibers.

Other differential diagnostic syndromes for P5CS and PYCR1 deficiency include: Hutchinson–Gilford progeria syndrome (MIM 176670) caused by mutations in the lamin A gene, autosomal dominant cutis laxa (ADCL; MIM 123700), and autosomal recessive cutis laxa type I (ARCL1; MIM 219100).

ADCL is a genetically heterogeneous connective tissue disorder characterized by wrinkled and inelastic skin associated with variable degree of internal organ involvement. The disorder is due to mutations in the elastin gene (*ELN*; MIM 130160) as well as in the fibulin-5 gene (*FBLN5*; MIM 604580).

ARCLI is a generalized connective tissue disorder with severe systemic manifestations, usually lethal for the presence of early-onset pulmonary emphysema, arterial aneurysms causing progressive heart failure, and genitourinary tract diverticula leading to severe infections. Causative mutations have been identified in the genes *ELN*, *FBLN5*, and *EFEMP2*, respectively. However, most patients are negative at the molecular investigation for these genes.

MACS syndrome, acronym for macrocephaly, alopecia, cutis laxa, and scoliosis, is due to

mutations in *RIN2* gene, which is involved in endosomal trafficking. In Menkes disease, the skin appears loose and redundant, particularly at the nape of the neck and on the trunk. In occipital horn syndrome, the less severe variant of Menkes disease, clinical findings include cutis laxa and joint hypermobility. Skin laxity can also be observed in transaldolase deficiency and in lysinuric protein intolerance.

Non-metabolic differential diagnosis of skin laxity includes the subtypes of Ehlers–Danlos syndrome, an umbrella term, which encompasses a heterogeneous group of connective tissue disorders with distinct inheritance patterns, genetic defects, and prognostic implications.

31.10 Other Lesions (Table 31.10)

Aplasia cutis congenital or Adams–Oliver syndrome (AOS; MIM 100300) is a genetically heterogeneous disorder defined by the combination of aplasia cutis congenita of the scalp vertex and terminal transverse limb defects. In 2013 autosomal recessive mutations in a glycosylation gene, *EOGT*, were associated with a subtype of AOS (AOS type 4; MIM 615297), but the link between this protein and aplasia cutis congenital has not been elucidated so far; it has been suggested that EOGT might act as a transcriptional regulator for the Notch pathway (Rymen and Jaeken 2014).

Recurrent skin infections may be present in SLC35C1-CDG (CDG type IIc). Clinical signs include unusual facial appearance, severe mental retardation, microcephaly, cortical atrophy, seizures, hypotonia, dwarfism, and recurrent infections with neutrophilia. The neutrophil defect has been described as "leukocyte adhesion deficiency type II" (LAD2).

Hypoplastic nails can be found in chondrodysplasia punctata type 2 (MIM 302960), in ALG12-CDG (CDG type Ig; MIM 607143) and in PIGV-CDG, which causes hyperphosphatasia with mental retardation syndrome type 1 (MIM 239300).

Mitochondrial disorders are also a rare case of syndromic linear skin defect syndrome, a disorder characterized by linear areas of erythematous skin hypoplasia involving the head and neck. For example, linear skin defects with multiple congenital abnormalities (LSDMCA) syndrome are an X-linked male-lethal disorder also known as MIDAS (microphthalmia, dermal aplasia, and sclerocornea). Additional clinical features include neurological and cardiac abnormalities and the syndrome is also called APLCC (aplasia cutis congenita, reticulolinear, with microcephaly, facial dysmorphism and other congenital anomalies). MLS syndrome is genetically heterogeneous and have been associated with mutations in *HCCS* (LSDMCA type 1), *COX7B* (LSDMCA type 2), or *NDUFB11* gene (LSDMCA type 3), which encodes one of 30 supernumerary subunits of complex I. These findings suggest a role of MRC dysfunction in causing an abnormal skin developmental phenotype (van Rahden et al. 2015).

Dupuytren's disease is a disorder of the soft tissues of the palm and fingers characterized by a progressive thickening and shortening of the fascial structures of the palm. An autosomal recessive or maternal mode of inheritance has been reported for only a very small number of patients. The mtDNA mutation C2839A, affecting the 16S rRNA, was detected in some patients with maternal inheritance (Anderson et al. 2012).

Table 31.10 Other lesions

Disease	MIM number	Type of lesion
Aplasia cutis congenital type 4	615297	Aplasia cutis congenital of the scalp vertex
SLC35C1-CDG (CDG type IIc)	266265	Recurrent skin infections
Chondrodysplasia punctate type 2	302960	Hypoplastic nails
ALG12-CDG (CDG type Ig)	607143	Hypoplastic nails
PIGV-CDG	239300	Hypoplastic nails
LSDMCA type 1 (microphthalmia syndromic type 7)	309801	Linear skin defect
LSDMCA type 2	300887	Linear skin defect
LSDMCA type 3	300952	Linear skin defect
mtDNA mutation (C2839A)		Dupuytren's disease

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The Bone in Genetic and Metabolic Diseases: A Practical Approach

32

Andrea Superti-Furga, Luisa Bonafé, and Sheila Unger

Key Facts

- Skeletal elements are composed of cartilage and bone tissues.
- Both tissues have complex, and different, metabolic activities that are related not only to cell metabolism and proliferation but also to the deposition and the homeostasis of the extracellular matrix.
- Bone and cartilage tissue participate in systemic metabolism, and, thus, many IEMs have an impact on bone homeostasis and skeletal growth.
- Among the >500 genetic disorders of bone, a significant proportion are caused by defects in enzymes and transporters and are as such "metabolic" disorders.
- Clinical telltale signs suggesting a possible skeletal disorder are short stature; disproportion between head, trunk, and limbs, recurrent or inappropriate fractures; and deformity. However, clinical signs may be present in many tissues and organs (e.g., the skin, hair, teeth, eye, heart, kidney, immune system, or brain).
- Bone elements are still best evaluated by standard radiography, but the interpretation of cartilage and bone changes in radiographs requires special expert training and is best done by expert centers.
- Detailed history and clinical examination are the basis for the correct formulation of a differential diagnosis and the interpretation of radiographic changes.
- Radiographic changes in the skeleton are a precious source of diagnostic information for intrinsic skeletal disorders as well as for IEMs.

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The skeleton as an organ is often regarded as bradytrophic; perhaps this idea is fostered by the observation that what remains of a human body years after death are often the bare bones. However, the bones have an active metabolism during life, and many "metabolic" disorders affect bone, cartilage, or both. Several of the genes responsible for genetic disorders of the skeleton code for enzymes, and a separation between inborn errors of metabolism

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and chondrodysplasias may be arbitrary; for example, deficiency of the enzyme carbohydrate sulfotransferase 3 results in recessive Larsen syndrome, while deficiency in N-acetylgalactosamine-sulfatase results in mucopolysaccharidosis type IV (Morquio disease); these two enzymes are responsible for the anabolic and catabolic side of the same reaction. Examples for genetic disorders of the skeleton that are caused by genes coding for membrane transporters and enzymes are given in Table 32.1. An unusual link between metabolism and bone was illustrated by the finding of somatic mutations in the isocitrate dehydrogenase genes (IDH1 and IDH2) that lead to the production of D-2-hydroxyglutaric acid and secondarily to dysregulation of chondrocyte proliferation at the metaphyses (metaphyseal chondromatosis and Ollier disease). These are only examples of the many connections between metabolism and the bone.

To add to the potential confusion, "metabolic bone disease" is an old but still widely used term for any condition associated with perturbed mineral homeostasis. It includes rickets in all its forms; renal tubular disorders including hypophosphatemia and acidosis, hyperparathyroidism, hypophosphatasia, and hyperphosphatasia; and more (Table 32.2). Conditions with marked osteoporosis are usually caused by defects in the intrinsic bone mass regulation pathways and should not be included in the group of metabolic bone diseases. Unfortunately, in spite of the term "metabolic," this group does not include the manifold skeletal manifestations of primary metabolic or lysosomal disorders. In growing children, metabolic bone disease will show best at the site of mineralization, i.e., at the metaphyses, taking the clinical and radiographic appearance of rickets (Fig. 32.1). Finally, chronic disease in children will often have repercussions on the skeletal system, such as delay of epiphyseal

Table 32.1 Examples of enzymes and transporters a deficiency of which results in a primary or significant skeletal phenotype

Acid (lysosomal) hydrolases:
Mucopolysaccharidoses (several types)
Oligosaccharidoses (several types)
GNPTAB, GNPTG (mucolipidosis 2 (I-cell disease), mucolipidosis 3)
GBA (Gaucher disease)
ASAH1 (Farber disease)
Proteoglycan synthesis:
Disorders of sugar-nucleotide metabolism: CANT1, SLC35D1
"Linkeropathies": B3GAT3, B3GALT6, B4GALT7, XYLT1, XYLT2; GALNT3
Proteoglycan chain extension disorders: CHSY1, EXT1, EXT2
Disorders of proteoglycan sulfation: DTDST(SLC26A2), PAPSS2, IMPAD1 (gPAPP), CHST3, CHST14
Bone reabsorption: tartrate-resistant acid phosphatase (ACP5), carboanhydrase 2 (CA2), cathepsin K (CTSK), chloride channel ClCN7, proton pump subunit TCIRG1
Cholesterol synthesis and lipid metabolism: ARSE, NSDHL, DHPAT, AGPS, DHCR24, INPPL1
Matrix proteinases: MATN3, MMP2, MMP9, MMP13, MMP14 (MT1-MMP), ADAMTS10, ADAMTS17, ADAMSTL2
Prostaglandin synthesis: thromboxane A synthase (TBXAS1), hydroxyprostaglandin dehydrogenase (HPGD)
Cyclic nucleotide metabolism: PDE3A, PDE4D, PRKAR1A, (GNAS), *and many others*

Table 32.2 Some causes of so-called "metabolic" bone disease in children

Vitamin D-deficient rickets
Calcium-deficient rickets
Phosphopenic rickets
Familial X-linked hypophosphatemia
Other genetic forms (recessive and dominant)
Proximal tubular insufficiency (De Toni–Debré–Fanconi syndrome)
Cystinosis
Lowe syndrome
Renal tubular acidosis
Hypophosphatasia (various forms)
Chronic hyperphosphatasia (juvenile Paget disease)
Hyperparathyroidism:
CASR mutations
Secondary to prenatal I-cell disease
Renal osteodystrophy

maturation, osteopenia, and in some cases mild metaphyseal dysplasia. This state is often accompanied by growth retardation. The differential diagnosis should be made according to the presence (or absence) of a known underlying disease, such as propionic academia or glycogen storage disease, or a chronic inflammatory bowel disease; good metabolic (or inflammation) control may result in improvement or disappearance of the skeletal changes.

In this chapter we will not review the large field of genetic skeletal disorders; the interested reader may consult review articles or monographs on this fascinating subject (Bonafe et al. 2015; Spranger et al. 2012). Rather, we will try to provide some practical considerations on how to approach the skeletal aspects when evaluating a newborn, child, or adolescent with a diagnostic suspicion of a genetic disorder. We will not limit ourselves to the differential diagnosis of metabolic diseases: firstly because of the intrinsic difficulty in defining what is "metabolic" and what is not, but also, and perhaps more importantly,

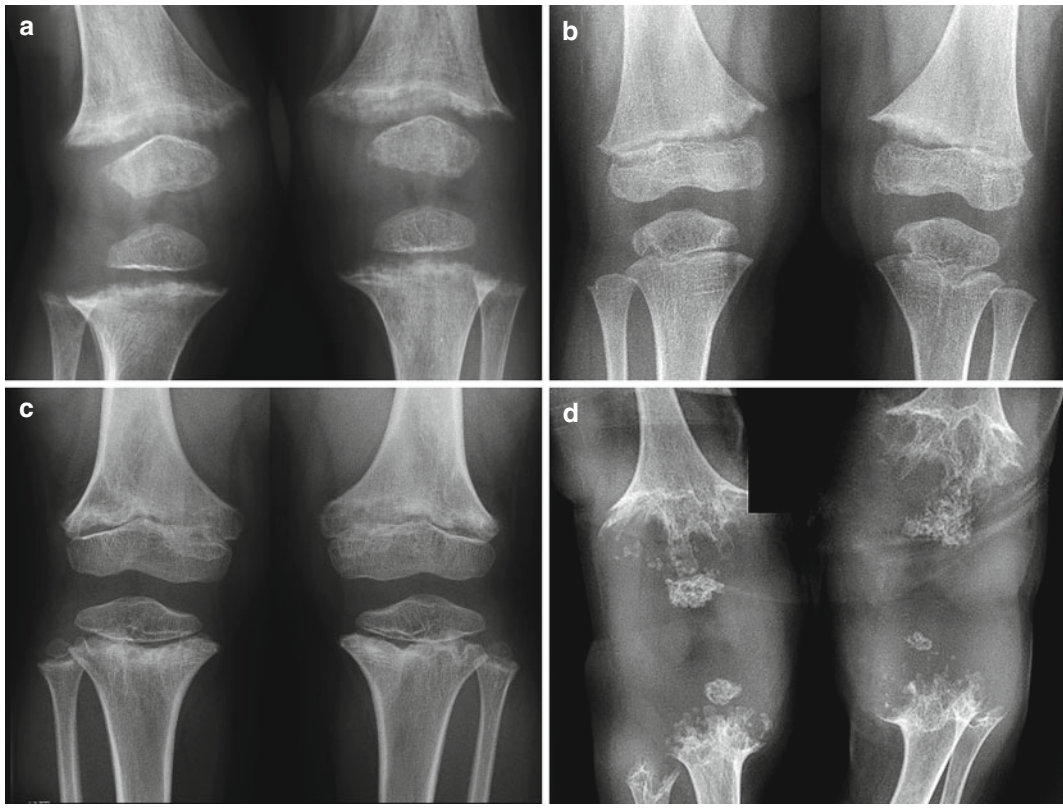

Fig. 32.1 Radiographs of the knee showing similar metaphyseal changes with different pathogenesis. (**a**) Renal tubular acidosis with rickets: low serum phosphate and acidosis impair the mineralization of the bone tissue that is newly deposited at the growth plate. There is also osteopenia of the adjacent bone structures. The image is typical for all forms of rickets. (**b**) Spahr metaphyseal dysplasia (MMP13 mutations): the metaphyseal fraying is probably caused by an impairment in the resorption of cartilage matrix. (**c**) Cartilage-hair hypoplasia (RMRP mutations): the metaphyseal irregularities may resemble those seen in (**b**); their pathogenesis is not determined but has possibly to do with impaired proliferation of chondrocytes at the growth plate. (**d**) Metaphyseal enchondromatosis (somatic IDH1 mutation): there are extensive radiolucent areas that reveal the presence of "enchondromas," i.e., areas of anarchic (but not malignant) proliferation of cartilage cells. The pathogenesis is believed to be the disequilibrium between alpha-ketoglutarate and D-2-hydroxyglutarate, leading to prolyl hydroxylase dysregulation and in activation of the anoxia sensing pathway (HIF1alpha and VEGF dependent)

because in the early diagnostic phase such a distinction is not possible and indeed, not meaningful. Our experience shows that young patients may transition from the metabolic clinic to the short stature consultation to the genetics clinic before a conclusive diagnosis is made. A multidisciplinary approach is helpful in this context, and it is important that physicians involved in the diagnosis and care of children with inborn conditions (may they be metabolic pediatricians, geneticists, endocrinologists, orthopedists, and pediatric radiologists) be able to address the differential diagnosis of conditions that affect children's bones.

32.1 "Skeletal" Findings – Accidental or Not

The diagnostic suspicion of a generalized skeletal disorder may be prompted by an accidental finding. Thus, a hand radiograph done to determine bone age may reveal brachydactyly, cone-shaped phalangeal epiphyses, or irregular carpals. Likewise, radiographs taken for a possible fracture may reveal generalized osteopenia suggestive of osteogenesis imperfecta or its opposite – hyperostotic bones suggestive of osteopetrosis (fractures are also more frequent in osteopetrosis). In a newborn or infant, chest radiographs taken for respiratory problems may reveal thin ribs, rib fractures, a vertebral segmentation defect, or irregularities of the proximal humeral metaphysis suggestive of a mineralization disorder. These "accidental" findings should prompt a skeletal survey to ascertain the distribution and nature of the changes and to allow for a differential diagnosis.

More frequently, the clinician may be alerted of the possibility of an underlying skeletal disorder by clinical signs such a short stature, disproportion between the trunk and the limbs, or the head and the rest of the body. Short stature alone is the least specific sign for skeletal dysplasia: the differential diagnosis is very large. While most cases are of genetic origin (from familial to syndromic), it is essential to obtain information on the growth curve: is the short stature of prenatal origin or did it develop in the first year of life or later? The differential diagnosis of a prenatal-onset short stature is quite different from that of a short stature that manifests in the first year of life or later. Overlooking these basic differential diagnostic considerations may lead to long diagnostic odysseys.

There may be clinical situations where a skeletal radiograph may reveal the presence of a specific sign and thereby support a diagnostic suspicion. These situations are rare but should be known. Examples are listed here:

- In a newborn, stippling in the patella may confirm a diagnosis of Zellweger syndrome (Fig. 32.2).
- Periosteal thickening and tarsal stippling may suggest the diagnosis of I-cell disease (Fig. 32.2).
- Acetabular dysplasia, proximally pointed metacarpals, and a vertebral "hook" may confirm the diagnosis of a mucopolysaccharidosis in a child with coarsening facial features (Fig. 32.4).
- The rare observation of D-2-hydroxyglutaric acid in the urine may alert to the presence of metaphyseal chondromatosis (Fig. 32.1).

In such rare situations, matching the clinical and laboratory findings with the skeletal finding may provide a useful and rapid diagnostic orientation. Some of the more common indications to obtain a skeletal survey for a suspected bone disorder in children are given in Table 32.3.

32.2 What Skeletal Radiographs Are Needed?

The *distal femur* (Fig. 32.1) is the site of most rapid bone growth in the child, and the distal femoral metaphysis will be the most sensitive indicator for disturbances of endochondral bone growth, including all forms of rickets and most metaphyseal dysplasias. An AP view of the

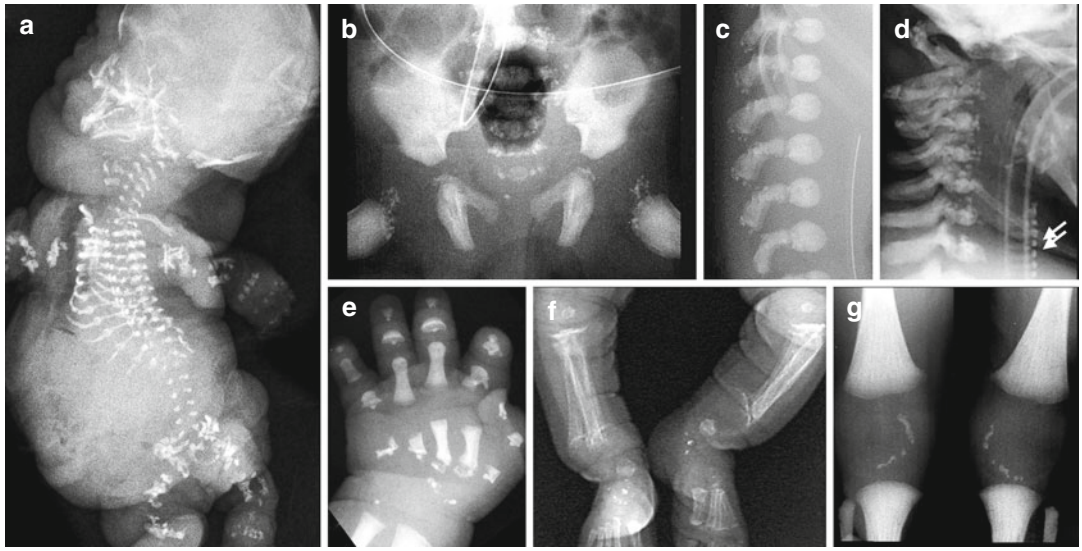

Fig. 32.2 Examples of "stipplings" (punctate calcifications) in different disorders. (**a**) Fetus with Greenberg dysplasia (recessive mutations in the lamin B receptor gene (*LBR*) that also has 3-beta-hydroxysterol delta(14)-reductase activity) with generalized punctate calcifications in cartilage elements leading to growth disruption and lethal chondrodysplasia; (**b–d**) radiographs of a newborn with X-linked brachytelephalangic chondrodysplasia punctate (arylsulfatase E deficiency (*ARSE*)) showing much more discrete stipplings at the proximal femurs, at the ilio-ischiadic junction at around the spine; however, note (in **d**) severe hypoplasia of cervical vertebrae (leading to instability) and calcification of the tracheal rings (*arrows*); these two elements are those with the most severe clinical implications. (**e**) Hand of a female newborn with so-called tibial-metacarpal type of chondrodysplasia punctate; its etiology is still unclear and some cases may be associated with maternal autoimmune disease and antibodies transferred through the placenta to the fetus. (**f**) Stippling in the distal tibia and tarsal area in a newborn with severe mucolipidosis 2 (I-cell disease) and neonatal hyperparathyroidism associated with fetal calcium deficiency; (**g**) bilateral soft tissue stipplings at the knee (seemingly delineating the patella but the patella is much smaller at that age) in a newborn with Zellweger disease

Table 32.3 Possible indications to obtain skeletal radiographs or a skeletal survey

Short stature
Disproportion between trunk and extremities (short extremities, short trunk)
Deformity (genua valga, genua vara, pectus carinatum, pectus excavatum, scoliosis, asymmetry, others)
Clinically apparent brachydactyly
Recurrent fractures
Coarse facial features
Chronic joint or bone pain

knee(s) is a prerequisite for the diagnosis of metabolic bone disease and epiphyseal and metaphyseal dysplasias in the growing skeleton.

The *hand* (Fig. 32.3) contains a large number of skeletal elements, and the hand radiograph is the single most informative radiographic view to recognize a generalized skeletal disorder. *Epiphyseal dysplasia* may be revealed by the shape and maturation of the radial, ulnar, metacarpal, and phalangeal epiphyses as well as by carpal maturation; *metaphyseal dysplasia* will be evident at the distal radius and ulna; *rickets* will be observed at the distal radius and ulna; *metabolic bone disease and hyperparathyroidism* will be seen in the metacarpals and phalanges (thin cortex, periosteal irregularities); the *dysostosis multiplex* typical of lysosomal disease will be seen at the metacarpals and phalanges; and, finally, *systemic osteolysis* is usually seen first at the carpal region.

A view of the *pelvis* with the *femoral neck and head* should always be obtained in the workup of a generalized skeletal disorder. The pelvis has a

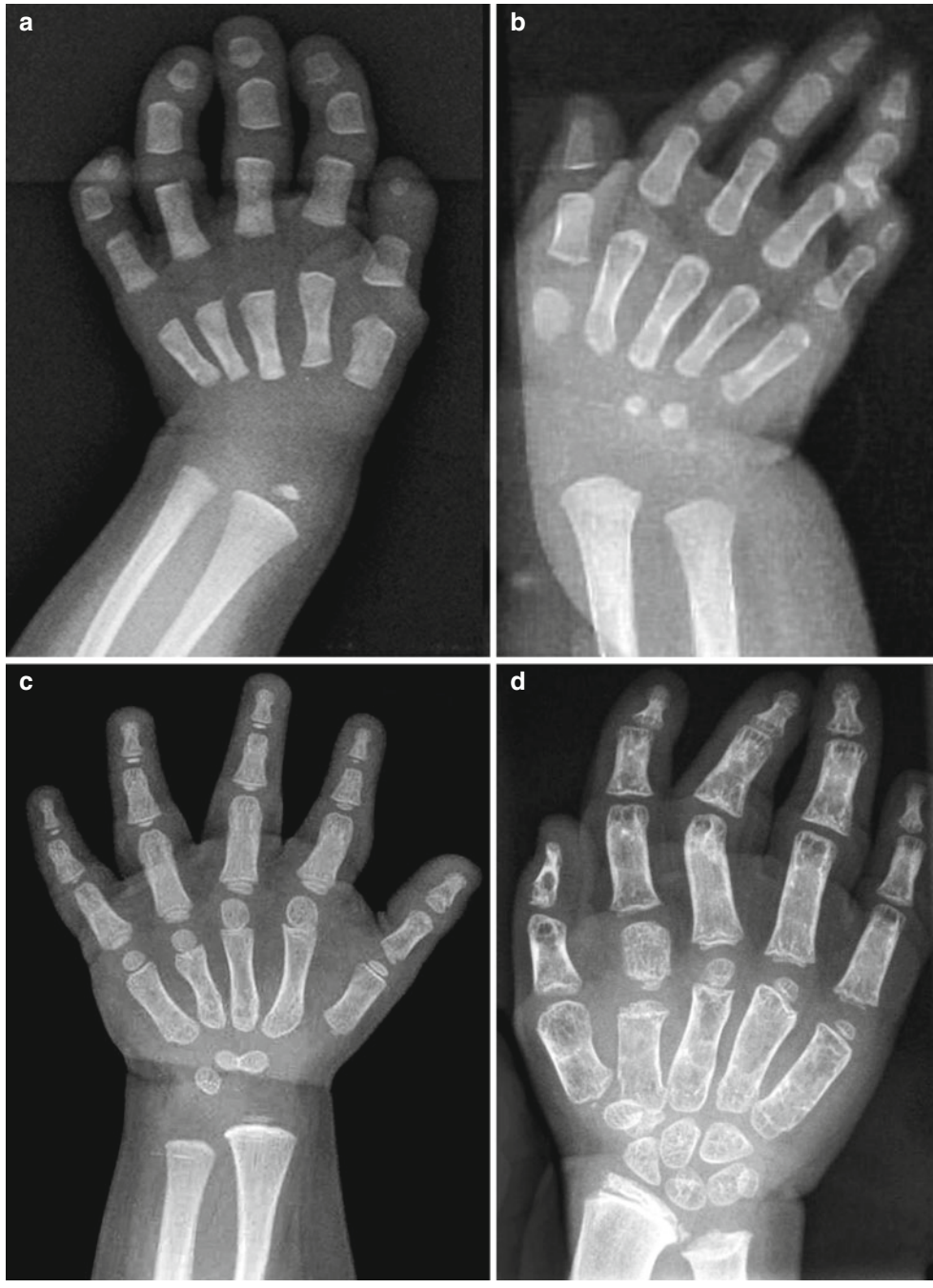

Fig. 32.3 Hand radiographs in (**a**) a newborn with Larsen syndrome (dominant filamin B mutation) showing precocious ossification of the distal epiphysis of the radius (normal ossification occurs around age 15–18 months) as well as broad phalanges ("tombstone"-like); (**b**) newborn with diastrophic dysplasia (recessive sulfate transporter (*DTDST*/*SLC26A2*) mutations) showing precocious ossification of carpal bones (normal ossification at age 3–6 months); (**c**) a 2-year-old girl with Golgi-resident phosphoadenosine phosphate phosphatase (*gPAPP*) deficiency (*IMPAD1* mutations) showing large metacarpal epiphyses, short metacarpals, and carpal bone fusion; (**d**) a 5-year-old girl with Desbuquois syndrome caused by calcium-activated nucleotidase 1 (*CANT1*) deficiency showing short metacarpals and hyperphalangy (second ray), markedly advanced ossification of carpal bones, and phalangeal subluxations

distinctive shape in several disorders; it offers a good impression of general mineralization. The proximal femur with its large epiphysis and metaphysis is a sensitive revelator of epiphyseal and metaphyseal changes. Most disorders of the growth plate, metabolic bone disorders, as well as lysosomal disorders will show at the pelvis and proximal femurs.

The *vertebrae* (Fig. 32.4) are frequently affected in bone density disorders, in metabolic bone disorders, and in a large number of dysplasias. They can also be affected by vertebral dysostoses, commonly disorders of vertebral segmentation. Thus, they can show reduced or increased density (osteogenesis imperfecta, osteopetrosis), an abnormal shape of the vertebral body (platyspondyly, dysostosis multiplex, single-hump, double-hump, and many other form variants), or abnormalities in their number or their pattern (coronal or sagittal clefts, vertebral fusions, hemivertebrae, and others). The lateral view of the spine is more useful than the AP view, as the lateral profile of the vertebrae can be revealing for a large number of conditions, but both AP and lateral views should be obtained whenever possible.

The *skull* (Fig. 32.5) is almost always involved in genetic bone disorders but the findings are in general subtle and of more difficult interpretation. Density disorders will show up either as hyperostosis (osteopetrosis and the craniotubular disorders) or as thinning, deformation, and Wormian bones (osteogenesis imperfecta). The characteristic finding of an *omega*- or *J*-shaped sella turcica is a useful telltale sign for the dysostoses multiplex typical of the lysosomal storage disorders. Clinically, the shape of the cranium and its circumference often give diagnostic indications.

In summary, the minimal skeletal survey for a suspected systemic bone disorder should include one or both hands AP, a lateral spine, and AP pelvis and one or both knees AP. Other views may be added later depending on a more restricted differential diagnosis. In the newborn and infant, a good-quality *babygram* is usually the most useful radiograph, as it allows for the appreciation of body proportions. In the newborn it is especially

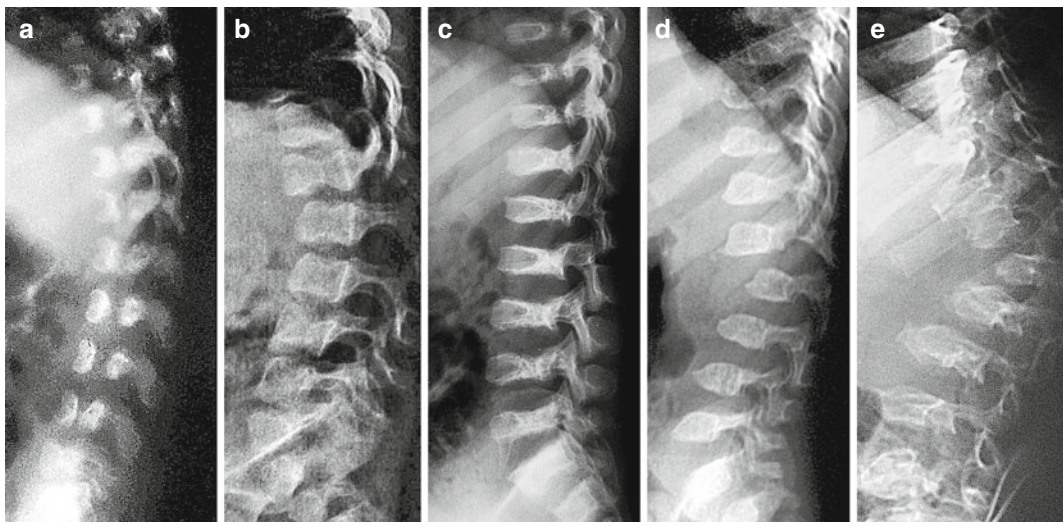

Fig. 32.4 Spine radiographs in (**a**) rhizomelic chondrodysplasia punctata (*rCDP*) showing marked coronal clefts; (**b**) carbohydrate sulfotransferase 3 deficiency (*CHST3*; recessive Larsen syndrome) showing irregular shape and size of vertebral bodies with flattening and residual clefting; (**c**) tartrate-resistant acid phosphatase deficiency (*ACP5*; spondyloenchondrodysplasia) with flattening of the vertebrae and posterior sclerotic enchondromatosis; (**d**) Morquio disease showing anterior pointing of vertebral bodies, anterosuperior notches ("hooks"), and a hypoplastic L1 already producing thoracolumbar kyphosis; and (**e**) a more progressed stage of Morquio disease with severe kyphosis from a small and posteriorly displaced L1 vertebral body

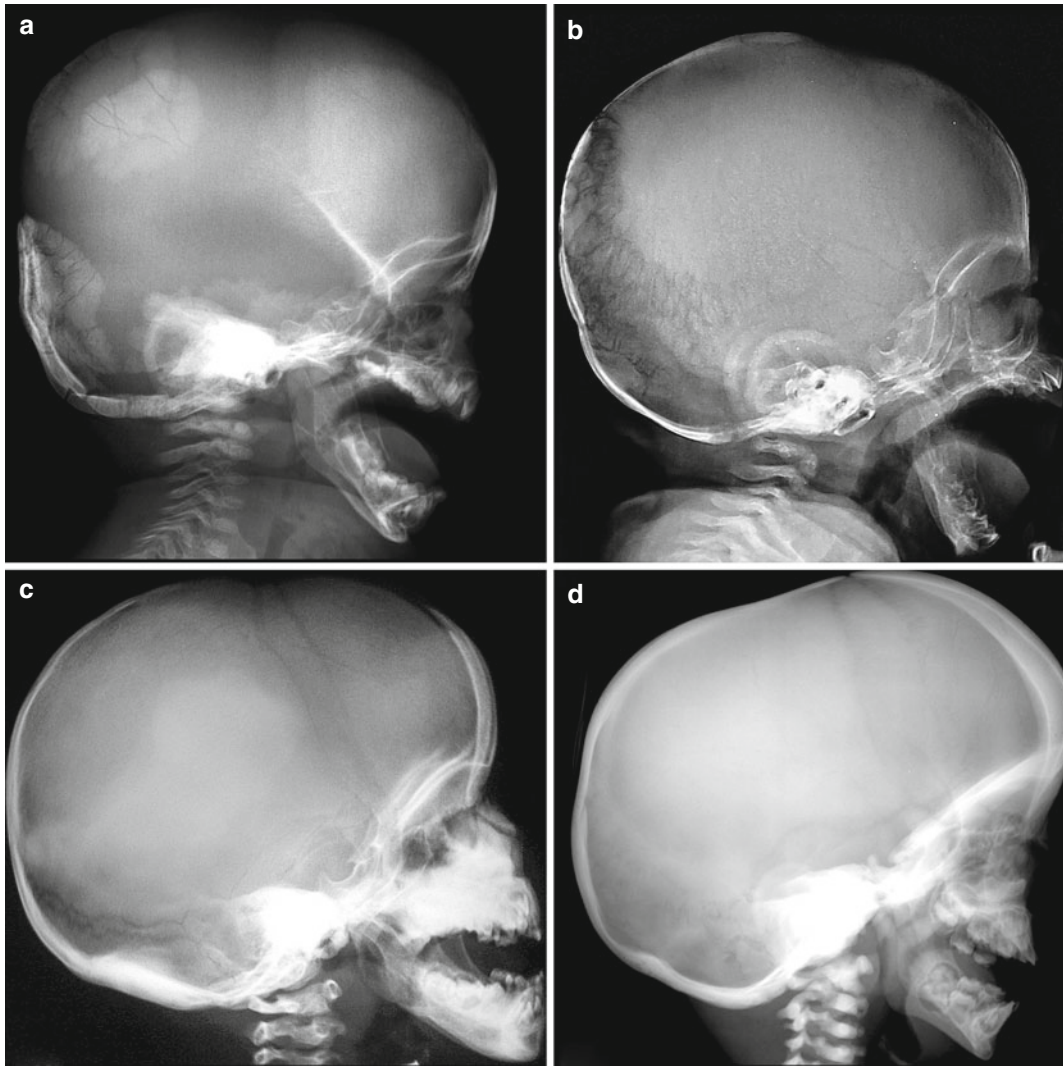

Fig. 32.5 Lateral skull radiographs. (**a**) Newborn with severe hypophosphatasia (recessive alkaline phosphatase (*TNSALP*) mutations): they are ossification "island" in the occipital, parietal, and frontal regions with large unossified lacunae in between. Wormian bones (mosaic-like) can be seen within the ossified regions. The patchy ossification is typical for severe hypophosphatasia and can be seen in the spine as well. (**b**) Newborn with osteogenesis imperfecta (dominant *COL1A1* mutation). There is general poor mineralization with numerous Wormian bones in the occipital region. (**c**) 3-year-old girl with dominant craniometaphyseal dysplasia (heterozygous mutation in the ANKH gene coding for a pyrophosphate transporter): there is sclerosis of the skull base and of the facial bones (the face showed paranasal "fullness"), while the cranial vault is relatively unaffected. In craniometaphyseal dysplasia, there is only mild involvement of the other skeletal elements (metaphyseal widening of long bones). (**d**) 4-year-old boy with severe (but not lethal) osteopetrosis (recessive mutations in the proton pump subunit *TCIRG1*): bone density is diffusely increased, in the cranial vault and in the vertebral bodies even more than in the facial bones (compare with (**c**)). The skull is elongated. All skeletal segments are affected in this condition; complications include blindness and hypoacusis and anemia with extramedullary hematopoiesis and hepatosplenomegaly

important to obtain good-quality films with the patient well positioned, and restrained, with the extremities well extended. As the hands tend to be overexposed (black) on babygrams, a case can be made to obtain separate hand films.

32.3 Quality Control in Skeletal Radiography

In the era of widespread quality control in medicine, it is surprising to see how often poor films are obtained (either technically over- or underexposed or with poor positioning of the patient) and how often poor reproductions are sent for expert review. As radiography does carry an intrinsic risk for exposed individuals, one should insist on critical quality management to extract the best possible information.

The interpretation of skeletal radiographs is also becoming a rare art in the era of multimodal imaging (ultrasound, MRI, and other modalities being more modern). It is important to have trained and – if possible – experienced pediatric radiographers to look at, and interpret, the radiographs. Also, one should not hesitate to consult experts using the e-mail or Internet. There are situations where a timely diagnosis may be crucial, as, for example, in the diagnosis of *thanatophoric dysplasia* in a newborn (a decision on intubation or not may be dependent on it), of *hypophosphatasia* in a critically ill infant (enzyme therapy being available), or of *osteopetrosis* (with a high risk of blindness because of optic nerve encroaching without surgery or stem cell transplantation). Some of the main categories of findings to be looked for in skeletal radiographs done for a suspected genetic disorder are listed in Table 32.4. Conversely, the correct interpretation of skeletal radiographs may end diagnostic errors with significant therapeutic odysseys, such as in *multiple epiphyseal dysplasias* and *pseudoachondroplasia* (often first seen by neuropediatricians because of muscle hypotonia and abnormal gait) or in *progressive pseudorheumatoid dysplasia*, where affected individuals are usually treated with anti-inflammatory or immunosuppressive therapies for long periods before the genetic and non-inflammatory nature of the disorder is recognized.

Table 32.4 "What to look for": cardinal findings on skeletal radiographs done for genetic disorders

Bone maturation: retarded or advanced for age
Increased bone density
Reduced bone density
Coarse trabeculation
Fractures and their callus
Hyperostosis (thick vortices, thick cranial base, thick cranial vault)
Shortening or elongation of tubular bones: rhizomelic, mesomelic or acromelic shortening; brachydactyly
Shape change: wide or narrow diaphyses, wide metaphyses, bowing, pointing
Dysplastic changes: epiphyseal dysplasia, metaphyseal dysplasia, vertebral dysplasia
Fractures
Phalangeal changes – "cone-shaped epiphyses" and other shape variants
Stippling (punctate calcifications)
Osteolysis (secondary reabsorption of bone)

32.4 Therapeutic Implications

Recognizing changes in skeletal radiology not only helps in making specific genetic-metabolic diagnosis, in reducing unnecessary investigations, or in stopping non-indicated therapies but may also pave the way to specific therapeutic approaches. Enzyme replacement therapy for several lysosomal storage disorders is well established, although its efficacy specifically for the skeletal manifestations of the disorders is less

than for the soft tissue manifestations. Conversely, enzyme replacement therapy for hypophosphatasia is presently the most effective among bone-directed metabolic therapies. Other molecular approaches are also proving helpful, such as the CNP analog therapy for achondroplasia, that works by counteracting the hyperactivation of the STAT and MAPK pathways induced by the FGFR3 mutations. Finally, biologicals acting on key players of bone homeostasis, such as monoclonal antibodies against RANKL or against sclerostin, are entering clinical practice for adult disorders but will find applications in pediatric disorders as well. Undoubtedly, more and more approaches will be developed, and skeletal disorders will become better amenable to treatment much as the "metabolic" diseases are today.

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Physical Abnormalities in Metabolic Diseases

33

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Key Facts

- Inherited metabolic disease with significant morphological anomalies is often caused by disturbances in cellular organelles and in the metabolism of complex molecules.
- Important disease groups include lysosomal storage disorders, peroxisomal disorders, congenital disorders of glycosylation (CDG), glycosylphosphatidylinositol (GPI) anchor protein disorders and cholesterol biosynthesis disorders.
- Identification of diseases in this group is based on careful clinical evaluation and may involve special biochemical investigations. Enzyme analyses are being replaced by molecular studies as primary tests for exact diagnoses.

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33.1 Introduction

The advances in massively parallel (next generation) sequencing technologies are changing our understanding of the link between inherited metabolic diseases and physical abnormalities. Previously there was only a limited range of metabolic diseases recognised through biochemical screening that are associated with multiple congenital anomalies and/or changes in physical appearance. Now there is a rapidly increasing number of dysmorphic syndromes that are caused by mutations affecting enzymes (and where the pathomechanism is linked to a metabolic disturbance) but are not associated with biochemical alterations that can be recognised through metabolic tests. Diagnostic algorithms thus are shifting from metabolic 'selective screening' to gene panels or exome sequencing. In this chapter we wish to highlight those conditions associated with physical abnormalities that can be diagnosed by the astute clinician through traditional biochemical investigations. We will discuss the typical clinical features and the underlying pathogenetic mechanisms, as well as the indications and limitations of the respective laboratory tests.

33.2 Definitions

For this chapter, physical abnormalities are confined to those detectable by physical examination or screening of internal organs and exclude both structural brain and eye anomalies. They comprise congenital malformations which are disor-

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ders of blasto- and organogenesis, and minor anomalies which result from disturbed phenogenesis (Epstein et al. 2008; Hennekam et al. 2010). Metabolic diseases sensu stricto are defined as inborn disturbances of biosynthesis or degradation of substances within metabolic pathways; classical examples are genetic enzymopathies. Metabolic diseases in this sense are essentially different from primary disturbances of structural proteins, membrane channels and transporters (unless their primary function is in a metabolic pathway), as well as signalling proteins and transcription factors; consequently, the respective disorders will not be considered. In addition, primary endocrinopathies or disorders of hormone syntheses are not brought into focus; they may be mediated by enzyme deficiencies but the main pathogenetic mechanism is alteration of other effector molecules often involved in intercellular signalling or cellular regulation. Although arbitrary to some extent, these definitions help to narrow down the vast fields of physical anomalies and of diseases with biochemical abnormalities. In consequence, six subgroups and mechanisms can be identified which are presented in this chapter illustrated by prototypic examples (Clayton and Thompson 1988).

33.3 Lysosomal Storage Disorders: Disturbed Degradation of Macromolecules in Lysosomes

Genetic defects of lysosomal enzymes cause the accumulation of incompletely degraded macromolecules in lysosomes resulting in progressive impairment of the function of affected cell systems such as connective tissue, cartilage, bone, solid organs and, above all, the nervous system. The storage of macromolecules typically causes organomegaly and other morphological features. There are no acute metabolic crises although some conditions, notably Fabry disease, may have acute presentations.

Remember

Cardinal clinical features of lysosomal disorders comprise:

- Hydrops fetalis
- Organomegaly and visceral disease
- Dysostosis multiplex and dysmorphism
- CNS disease – regression

In most cases, the typical clinical course is characterised by normal appearance at birth and normal development for a variable time span. The age of onset varies greatly in this group of disorders and for individual conditions often depends on the amount of residual enzyme activity; non-immunologic hydrops fetalis is the most severe form and of the earliest (prenatal) onset. Severely affected children may show facial dysmorphism or cardiomyopathy at birth. More frequently, patients present with muscular hypotonia and developmental delay. Coarse facial features, typical skeletal changes (radiologically classified as dysostosis multiplex) and thickening of the skin in addition to hepatosplenomegaly, cardiomegaly and hernias are important physical signs. Lysosomal storage disorders with predominant central nervous system involvement frequently present with ataxia, hyperexcitability and spasticity; ophthalmological examination may reveal corneal clouding or a characteristic cherry-red spot in the macula region in some disorders. A differential diagnosis of typical findings in lysosomal storage diseases is given in Table 33.1; however, clinical presentation is generally variable.

Diagnostic workup in children with suspected lysosomal storage disorders include:

- Physical examination
- Ultrasound of parenchymatous organs
- Neurological examination, hearing tests and cranial MRI scan to consider
- X-ray examinations for dysostosis multiplex
- Cardiological examination (ECG, echocardiography)
- Ophthalmological examinations (retina, macula, lens, cornea)

Table 33.1 Clinical findings and biochemical tests in lysosomal storage diseases

								Neuro				Eyes		Diagnosis				
	Coarse facial features	Dysostosis multiplex	Organomegaly	Macroglossia	Cardiac involvement	Hydrops fetalis	Angiokeratoma	Intellectual disability	Spasticity	Peripheral neuropathy	Myoclonic seizures	Corneal clouding	Cherry-red macular spot	Vacuolated lymphocytes	GAG (urine) elevated	Pathol. oligosaccharide s	Enzyme studies in:	OMIM
Mucopolysaccharidoses																		
Hurler (MPS I)	++	++	+	++	+			++				++			+		L/F	607014
Scheie (MPS I, atten.)	+	+	+	+				(+)				+			+		L/F	607016
Hunter (MPS II)	++	(+)	+	+	+			++							+		S/L/F	309900
Sanfilippo (MPS III)	(+)	(+)	(+)		(+)			++	+			+			(+)		L/F	252920
Morquio (MPS IV)	+	(+)	+			+						(+)			+		L/F	253000
Maroteaux-Lamy (MPS VI)	+	+	+									+			+		L/F	253200
Oligosaccharidoses																		
Fucosidosis	++	(+)	(+)		+		(+)	++	+		+			+		+	L/F	230000
α-Mannosidosis	++	+	+				(+)	++	(+)			++		+		+	L/F	248500
β-Mannosidosis	+						(+)	+	+	+						+	L/F	248510
Aspartylglucosaminuria	+	(+)	(+)		(+)		(+)	+				(+)		(+)		+	L/F	208400
Schindler								+	+		+					+	L/F	104170
Sialidosis type I						+			+	+	++		++	+		+	F	256550
Sialidosis type II	++	(+)	+		+	(+)	+	++			(+)		++	+		+	F	256550
Sphingolipidoses																		
GM_1-Gangliosidosis	++	+	+	+	(+)	+	+	++	(+)			+	++			+	L/F	230500
Tay-Sachs, Sandhoff			(+)					++	+		+		++			+	L/F	272750

(continued)

Table 33.1 (continued)

								Neuro				Eyes		Diagnosis				
	Coarse facial features	Dysostosis multiplex	Organomegaly	Macroglossia	Cardiac involvement	Hydrops fetalis	Angiokeratoma	Intellectual disability	Spasticity	Peripheral neuropathy	Myoclonic seizures	Corneal clouding	Cherry-red macular spot	Vacuolated lymphocytes	GAG (urine) elevated	Pathol. oligosaccharide s	Enzyme studies in:	OMIM
Metachromatic leukodystrophy								++	+	++			(+)				L/F	250200
Krabbe								++	+	++			(+)				L/F	245200
Niemann-Pick			++			+		+			(+)	(+)	(+)	+			F	257200
Gaucher Type I			++													+	L/F	230800
Gaucher Type II			++			+		++	+		+					+	L/F	230900
Fabry					+		+										S/L/F	301500

++ = prominent feature, + = often present, (+) = sometimes present
Angiokeratoma = red to dark-blue lesions (<1 mm, slightly hyperkeratotic, do not blanche on pressure) mostly on buttocks, genitalia, lower trunk, thighs)
Cherry-red spot – in the macula region
Cardiac involvement = cardiomyopathy, valve lesions, coronary artery disease
Vacuolated lymphocytes = typical vacuoles or evidence of storage in lymphocytes
F fibroblasts, *L* leukocytes, *S* serum, *M* muscle

Remember

Characteristic features of dysostosis multiplex are:

- Lateral skull – enlarged sella turcica; thickened diploic space
- Hands – proximally pointed, distally broadened metacarpals
- Lateral spine – hypoplastic L1

Metabolic tests: There are few general laboratory tests for lysosomal disorders. Several conditions are associated with increased concentrations of glycosaminoglycans (GAGs) or oligosaccharides in the urine. Microscopic examination of fresh leukocytes (bedside blood smear, not from an EDTA tube), bone marrow cells or biopsies may show vacuoles in some conditions. Chitotriosidase, a chitinolytic enzyme and marker of monocyte/macrophage activation, is highly elevated in several lysosomal storage disorders including Gaucher and Niemann-Pick C diseases. It may be used for screening as well as monitoring of treatment; however, results may be false negative due to a common null allele of the *CHIT1* gene, and chitotriosidase activity is also increased in a number of non-metabolic conditions including atherosclerosis, sarcoidosis, beta-thalassemia or malaria. Reliable confirmation or exclusion of lysosomal storage disorders requires molecular genetic analyses and/or the measurement of specific enzyme activities in leukocytes, plasma or fibroblasts (Winchester 2014).

Treatment: There has been considerable progress in the treatment of lysosomal storage disorders in the last years. Bone marrow transplantation has proven beneficial in (pre-)symptomatic patients in some disorders (e.g. MPS I, late-onset Krabbe, metachromatic leukodystrophy) but not in others (MPS III, MPS IV). Enzyme replacement therapy is available for Gaucher disease, Fabry disease, many types of MPS, Pompe disease and other disorders. For some disorders, treatment is still largely symptomatic (Hollak and Wijburg 2014).

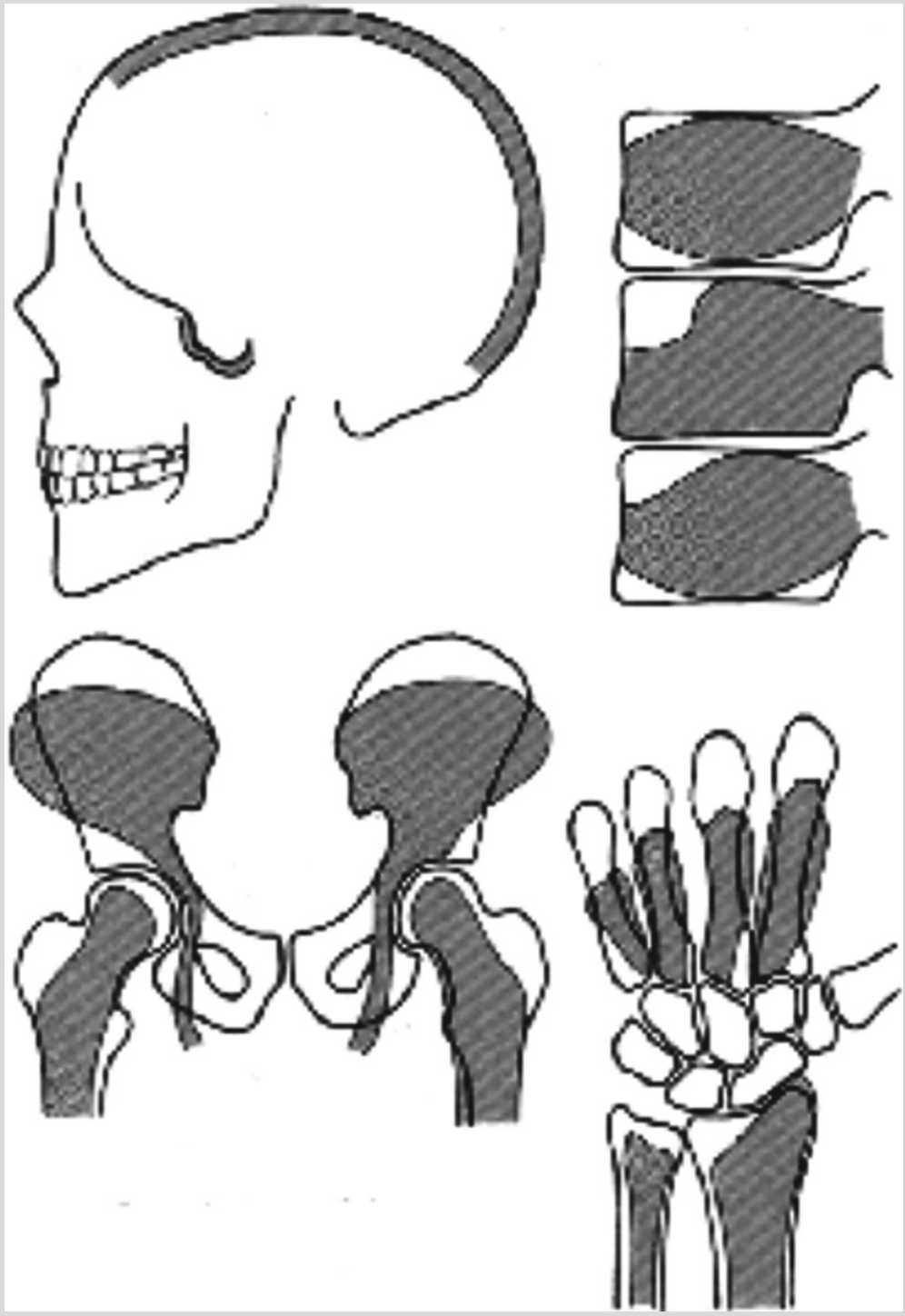
One of the most common lysosomal disorders, Pompe disease, a glycogen storage disease caused by the deficiency of acid maltase, usually shows no obvious morphological anomalies apart from sometimes an enlarged tongue. It is characterised by progressive muscle disease which in the severe infantile form presents in the neonatal period and leads to cardiac failure and death usually in the first year of life. There are also attenuated, juvenile and adult forms of the disease. The liver usually is not enlarged except in cardiac failure; CK is usually highly elevated. Enzyme replacement therapy is available.

Mucopolysaccharidoses (MPS) arise from the lack of specific lysosomal enzymes involved in the degradation of GAGs, long chains of sulphated or acetylated amino sugars attached to a protein skeleton. GAGs are major components of the viscous extracellular matrix. There are three major types of GAGs, and the clinical presentation of the different MPS types is linked to the tissue distribution of these subtypes. *Dermatan sulphate* is found in the connective tissue and skin as well as in the bone, cartilage and tendons, internal organs, blood vessels, cornea and other tissues. Disorders affecting dermatan sulphate breakdown show typical progressive morphological changes as seen, e.g. in Hurler's disease, but are not necessarily associated with mental decline (e.g. intelligence is usually normal in MPS type VI, Maroteaux-Lamy). In contrast disorders affecting *heparan sulphate* (a ubiquitous constituent of glycoproteins and the basal membrane) are usually associated with intellectual disability but, as in Sanfilippo's disease (MPS type III), do not always show characteristic morphological changes. Similarly, in MPS type IV (Morquio), the deficient breakdown of *keratan sulphate*, a constituent of bone, cartilage and the cornea, causes severe skeletal dysplasia but is usually not associated with intellectual disability. Most patients are recognised by GAG analysis in the urine but results may be false negative particularly in MPS types III and IV. Electrophoretic analysis should be performed from the beginning especially when there is strong clinical suspicion; the different MPS types may be distinguished through the electrophoretic separation of the different GAGs. All MPS except the X-linked type II (Hunter) are inherited as autosomal recessive traits.

Disease Info: Hurler Disease and Scheie Disease

In *Hurler disease* (MPS IH, OMIM 607 014), the degradation of mucopolysaccharides, in particular of dermatan sulphate, is disturbed due to a deficiency of α-L-iduronidase. It is the classical form of MPS. Substrate accumulation takes place in both lysosomes and the extracellular matrix, in particular those of chondrocytes (causing disturbance of enchondral ossification), hepatocytes, dermis and subcutis and synovia. Inflammation and apoptosis in cartilage and synovial tissue are caused by stimulation of lipopolysaccharide signalling pathways. Somatic features affecting the skeleton, facies and internal organs, as well as neurologic features, evolve within the first year of life. Death occurs by age 1–10 years, with a mean age at death of 6 years. The craniofacial phenotype is characterised by progressive coarsening apparent at 3–6 months, a large scaphocephalic head with bulging frontal bones, depressed nasal bridge, broad tip of the nose, full cheek and lips, a large tongue, hypertrophy of the gums and a mouth held open. The patients are generally hirsute and have thick and abundant hair. A protuberant abdomen; recurrent (umbilical) hernias; corneal clouding, optic nerve swelling and retinal degeneration; frequent ear, nose and throat infections; decreasing growth velocity by the age of 2 years resulting in short stature, short neck and short, broad hands and feet; joint stiffness; and cardiac disease are all frequent physical findings. Radiologically, a pattern of skeletal changes called 'dysostosis multiplex' may be seen. As in other lysosomal storage diseases, progressive dermal melanocytosis, e.g. extensive Mongolian spots, may be a clue to the diagnosis, particularly in infants with darker skin types. Dermal melanocytosis results from arrest of transdermal migration of melanocytes from the neural crest to the epidermis. Pathogenetically, abnormal increases in nerve growth factor (NGF) activity caused by the binding of accumulating metabolites to the tyrosine kinase-type receptors for NGF have been discussed as a primary cause. The attenuated form of α-L-iduronidase deficiency is denoted as *Scheie disease* (MPS IS, OMIM 607 016); it has a much later onset, a milder course and symptoms restricted to milder somatic features; stature, intelligence and lifespan are normal. *Hurler-Scheie syndrome* (MPS IH/S, OMIM 607 015) has an intermediate phenotypic expression. Bone marrow transplantation has proven benefit in presymptomatic patients with MPS I or early diagnosed patients with minimal CNS manifestation. Enzyme replacement therapy is licensed and available but has no beneficial effect on brain manifestation as the intravenously applied enzyme does not cross the blood-brain barrier.

Dysostosis Multiplex



Dysostosis multiplex is the typical skeletal abnormality found in MPS I and other lysosomal disorders. In severe MPS I, defective ossification may not be evident until the characteristic gibbus deformity of the lumbar spine is apparent at 6–14 months of age. As vertebrae become progressively flattened and beaked, spinal deformities, including kyphosis, scoliosis and kyphoscoliosis, may develop. Hips may be affected, resulting in dysplasia or subluxation. Long bone irregularities produce valgus and varus deformities, and genu valgum may occur. Phalangeal dysostosis and synovial thickening produce the characteristic claw deformity and trigger digits. Carpal tunnel syndrome and phalangeal involvement diminish hand function. By the time severely affected children are 2 years of age, joint stiffening and progressive arthropathy affect all joints. Radiographs obtained at birth can detect dysostosis in some patients with MPS I.

Patients with attenuated MPS I have progressive arthropathy, which ultimately leads to loss of joint range of motion. Patients present with mild to severe skeletal involvement and tend to have short stature. Kyphosis and/or scoliosis are frequent symptoms, with attendant hip and back pain. Patients may experience generalised pain and malaise, which may be attributable to osteopenia and microfractures. Patients with moderate/severe skeletal disease should be monitored by an orthopaedic surgeon, preferably one who is familiar with MPS disorders. Early detection of skeletal abnormalities, such as kyphoscoliosis, before irreversible changes occur, may provide more interventional options. Particularly upper spine deformities may require fusion; acetabular hip dysplasia can be addressed with osteotomy and genu valgum with epiphyseal stapling and/or eight-plates. Flexor tendon or carpal tunnel release can provide relief and the return of some hand function. Premature cessation of skeletal growth may occur in MPS I and should be taken into account when surgical procedures are being planned. Physical therapists can assess the degree of joint restriction and develop interventions to maintain joint function and muscle strength. In patients with attenuated MPS I, joint stiffness and pain may be lessened through passive and active range-of-motion exercises and hydrotherapy. In severe MPS I, these interventions may stabilise but not improve joint function and stiffness. The benefit of physical therapy in patients with severe orthopaedic compromise is controversial. Occupational therapists can improve quality of life and functional independence; the most effective occupational therapies are those that patients can perform on their own.

(*Bernhard Zabel*, *University Children's Hospital*, *Freiburg i. Brsg*., *Germany*)

Oligosaccharidoses are disorders in the breakdown of complex carbohydrate side chains of glycosylated proteins (glycoproteins), leading to increased concentration of oligosaccharides in the urine. They are less common than MPS which they resemble clinically, with variable coarsening of the face, skeletal deformities, hepatomegaly and sometimes corneal clouding. Psychomotor development is usually delayed; progressive neurological symptoms and seizures are common. Early presentation with hydrops fetalis or neonatal cardiomegaly is more frequent than in patients with MPS. Elevation of urinary oligosaccharides is also found in several sphingolipidoses such as GM_1 and GM_2 gangliosidoses and galactosialidosis.

Sphingolipidoses are disorders in the breakdown of membrane lipids that are found throughout the body but are of special importance in the nervous tissue. Some sphingolipids are essential components of myelin sheaths; others are prevalent particularly in the grey matter of the

brain. Sphingolipidoses thus usually present with primary disturbances of the central or peripheral nervous system; in addition, sphingolipids frequently accumulate in the reticuloendothelial system or other cells. Typical clinical features include progressive psychomotor retardation and neurological problems such as epilepsy, ataxia and/or spasticity. Hepatosplenomegaly is not uncommon. Progressive facial coarsening and dysostosis multiplex are found in GM_1 gangliosidosis; liver disease is a characteristic feature of Niemann-Pick disease; and a lysosomal leukodystrophy is found in Krabbe disease and metachromatic leukodystrophy. Several sphingolipidoses show a cherry-red macula spot; there may be foam cells in the bone marrow or vacuolated lymphocytes. Neurological and neuroradiological findings are not always specific. The diagnosis is made by enzyme or molecular analyses. All sphingolipidoses except Fabry disease are inherited as autosomal recessive traits.

Disease Info: Gaucher Disease and Fabry Disease

The most common form of *Gaucher disease* (OMIM 230800), the non-neuronopathic type I, is characterised by accumulation of sphingolipids in cells of the reticuloendothelial system leading to massive (hepato)splenomegaly and anaemia, thrombocytopenia and haematomas. Progressive bone changes may be associated with acute 'bone crises' (pain, fever) as well as with osteonecrosis, arthrosis and osteopenia. Long-term complications include growth retardation and lung fibrosis. The diagnosis of Gaucher disease is supported by highly elevated chitotriosidase activity in serum and the presence of characteristic Gaucher cells, e.g. in bone marrow, and is confirmed by enzyme analysis (deficient glucocerebrosidase) and molecular genetic testing of the *GBA* gene. Enzyme replacement therapy is available but has no effect on brain manifestation present in the neuronopathic types II and III Gaucher disease (10 % of cases). The International Collaborative *Gaucher* Group Registry (ICGG) and other groups have provided surveillance recommendations.

Fabry disease (OMIM 301500 (*see also* Chap. 27) typically presents in males with <1 % α-galactosidase (α-Gal A) enzyme activity in the first or second decade of life with episodes of acute painful acroparesthesias in arms or legs triggered, e.g. by physical exertion or temperature changes and lasting for hours or days. There may be hypohidrosis and episodic fever; 80 % of patients show typical angiokeratoma skin lesions. Corneal and lenticular opacities and proteinuria are common. In middle age, deterioration of renal function usually leads to renal failure and cardiovascular disease may occur. Intelligence is normal; there is no hepatosplenomegaly or facial dysmorphy. Fabry disease is inherited as an X-linked trait but carrier females frequently show complications in adulthood including progressive renal failure, hypertrophic cardiomyopathy and stroke. The diagnosis is confirmed by α-Gal A analysis or mutation testing of the *GLA* gene; confirmation of suspected female carriers should be based on molecular testing. Enzyme replacement therapy is available.

33.4 Peroxisomal Disorders: Disturbance of Biogenesis or of Modification of Substrates

Many oxygen-dependent reactions take place in the peroxisomes to protect the cell against oxygen radicals; the produced H_2O_2 is metabolised by a catalase. Important peroxisomal functions also include beta-oxidation of very long-chain fatty acids (VLCFA) and related substances, alpha-oxidation of 3-methyl fatty acids, degradation of phytanic acid, as well as biosynthesis of plasmalogens and other ether lipids and bile

acids. Peroxisomal disorders are classified into generalised defects of peroxisome biogenesis and single peroxisomal enzyme deficiencies, but clinical phenotypes are variable and overlapping. Generalised peroxisomal disorders are multisystem diseases that cause dysmorphic features and skeletal changes (specifically proximal shortening of the limbs) as well as neurological and ophthalmological abnormalities, hepatointestinal and renal dysfunction (see Disease Info Zellweger syndrome) (Faust et al. 2005; Wanders 2004). Morphological abnormalities such as rhizomelic chondrodysplasia punctata are generally related to a deficiency in biosynthetic functions and may be caused both by single enzyme deficiencies in this pathway and generalised peroxisomal disorders. Variant, milder phenotypes (e.g. neonatal adrenoleukodystrophy, infantile Refsum disease) are dominated by neurological manifestations. Patients follow a neurodegenerative course after a period of normal development. An important clue may be typical white matter abnormalities in the MRI. Peroxisomal disorders should therefore be included in the differential diagnosis of infants and young children with severe hypotonia and seizure disorders. Patients with rhizomelic chondrodysplasia punctate type 1 (RCDP1, OMIM 215100) caused by mutations in *PEX 7* show characteristic skeletal changes, facial dysmorphism reminiscent of Zellweger syndrome, hypotonia and psychomotor retardation. Cataracts and ichthyosis may also occur.

Children with peroxisomal disorders may show elevated transaminases and a tendency to hypoglycemia, as well as hypocholesterolemia and increased serum concentrations of iron and transferrin. Organic acid analysis may reveal dicarboxylic aciduria, in particular elevated 2-hydroxysebacic acid reflecting impaired peroxisomal β-oxidation.

Metabolic tests: The specific laboratory finding in peroxisomal disorders (with the exception of rhizomelic chondrodysplasia punctata) is an elevation of VLCFAs in serum or cultured fibroblasts, usually determined by GC-MS. VLCFAs are found consistently and reliably elevated in all disorders of peroxisome biogenesis as well as in single defects of VLCFA transport or β-oxidation. The ratio of C26/C22 is the most sensitive index. The analysis of plasmalogens in erythrocytes is indicated in suspected rhizomelic chondrodysplasia punctata. If levels are decreased, the analysis of phytanic acid in plasma will distinguish classical rhizomelic chondrodysplasia punctata, in which phytanic acid is increased (at least after the first months of life), from single enzyme defects that may produce the same clinical and radiologic features. If plasmalogens are normal, other disorders with punctate calcifications such as warfarin embryopathy (should be evident from the prenatal history) or X-linked chondrodysplasia punctata (due to a defect in sterol biosynthesis) must be considered.

Remember

The decisive marker for peroxisomal diseases, with the exception of rhizomelic chondrodysplasia punctata, is the elevation of very long-chain fatty acids (VLCFA). Additional biochemical investigations for peroxisomal disorders are not usually warranted.

Disease Info: Zellweger Syndrome

The prototypic disorder of disturbed peroxisomal biogenesis with multiple enzyme abnormalities is *Zellweger syndrome* (ZS, cerebrohepatorenal syndrome, OMIM 214100) inherited in an autosomal recessive manner. It is caused by defects in a number of *PEX* genes which encode peroxins, proteins necessary for peroxisomal biogenesis and for the import of proteins. Children with ZS typically present in the neonatal period with severe hypotonia, no or little psychomotor development, seizures and other neurologic disturbances. MRI of the brain often shows gross abnormalities reflecting defects of early brain development. The craniofacial features in ZS are characteristic with macrocephaly, large fontanels and wide sutures, a high forehead, flat and square face, shallow supraorbital ridges, telecanthus, epicanthic folds, broad and depressed nasal bridge,

anteverted nares, dysplastic ears and redundant skin in the neck. The limbs show variable contractures; calcified stippling of the patella and other bones is seen in about half of the patients. Hepatomegaly, hepatic fibrosis and renal cysts are additional characteristic findings. Eye anomalies include cataracts, nystagmus, optic atrophy and retinal changes. Most patients with severe ZS die within the first year of life; survivors often show severely impaired development, postnatal growth failure, blindness or severe visual handicap and significant sensorineural hearing impairment. Facial dysmorphy in these cases may be less marked with a high forehead and, typically, attached ear lobules.

Zellweger syndrome spectrum (ZSS) comprises neonatal adrenoleukodystrophy (NALD) and infantile Refsum disease (IRD), later the least severe phenotype. The clinical courses of NALD and IRD are often slowly progressive and very variable. Global development, hearing, vision and liver function may be impaired to a variable degree, and episodes of haemorrhage and intracranial bleeding can occur. Treatment in ZSS is symptomatic.

33.5 Disorders of Catabolism of Small Molecules: Substrate Accumulation Can Affect Macromolecular Functions

Disorders of biosynthesis and catabolism of small molecules, e.g. amino acids, organic acids, lipids, vitamins and cofactors, are usually not associated with physical abnormalities. There are, however, exceptions, in particular when accumulating substrates or their metabolites affect macromolecules. A well-known example is *homocystinuria* (OMIM 236 200). Classical homocystinuria is due to cystathionine β-synthase deficiency, leading to accumulation of homocystine which is the dimeric form of the amino acid homocysteine. The major clinical features of homocystinuria consist of intellectual disability, a characteristic marfanoid habitus but with joint mobility limitations and generalised osteoporosis, eye features such as ectopia lentis and myopia and vascular problems with increased risk for thromboembolic events (Skovby et al. 2010). Clinical variability is high. Homocystinuria may present as unspecific intellectual disability; however, about one third of patients have normal intelligence. Psychiatric problems are seen in about 50% of patients. Physical findings in homocystinuria are partly due to the effects of the accumulating homocystine on collagen, fibrillin and other elements of the connective tissue. Lens dislocation results from disruption of disulphide bonds of fibrous proteins. The *diagnosis* is best made through the identification of highly elevated concentrations of total homocysteine in plasma or even a pathological sulphite test in urine. About half of the patients respond well to substitution of the cofactor pyridoxine (vitamin B_6), sometimes in combination with folic acid and betaine. In all other patients including partial responders, a diet restricted in methionine and intake of betaine must be initiated.

33.6 Disorders of Cholesterol Biosynthesis: Disorders Affecting Developmental Signalling Pathways

Sterol synthesis defects present clinically as multisystem disorders with dysmorphic features and variable skeletal dysplasias; they should be considered in cases of otherwise unexplained recurrent abortions and foetal dysmorphy (Hennekam 2005; Herman 2003; McLarren et al. 2010; Waterham et al. 2001). The *biochemical diagnosis* is not always straightforward: Serum cholesterol is usually normal in all disorders but may be decreased in Smith-Lemli-Opitz syndrome (SLOS, OMIM 270400) and SC4MOL deficiency (OMIM 607545), and even specific sterol analysis may yield normal results. In these instances the diagnosis can only be reached by

mutation analysis or functional studies (fibroblasts cultured in sterol-free media).

The identification of SLOS as a disorder of cholesterol biosynthesis proved that enzymopathies within metabolic pathways can have a major impact on developmental signalling pathways and lead to syndromes with malformations and dysmorphism. Other disorders of post-squalene cholesterol biosynthesis include CHILD syndrome (OMIM 308050), X-linked chondrodysplasia punctata (CDPX2; OMIM 302960), SC4MOL deficiency (OMIM 607545), Greenberg dysplasia (OMIM 215140), Antley-Bixler syndrome (ABS 1, lanosterolosis, OMIM 201750), lathosterolosis (607330) and desmosterolosis (OMIM 602398). They are all associated with congenital malformations. A summary of clinical features in primary deficiencies of sterol biosynthetic enzymes is given in Table 33.2.

Mevalonic aciduria is caused by a deficiency of mevalonate kinase which catalyses an early (pre-squalene) step in sterol biosynthesis. Affected children show facial dysmorphism, congenital malformations and severe neurological abnormalities including progressive ataxia due to cerebellar atrophy, psychomotor retardation, cataracts and retinitis pigmentosa. In addition there may be dystrophy and recurrent crises with fever,

Table 33.2 Clinical features in disorders of distal (post-squalene) sterol biosynthesis

Disorder (inheritance)	Clinical features	Enzyme	OMIM
CHILD syndrome (X-linked)	*C*ong. *h*emidysplasia, ipsilateral *i*chthyotic skin lesions with a sharp demarcation at the midline of the trunk, *l*imb *d*efects, stippled epiphyses on the affected side; lethal in males	Sterol-4-demethylase	308050
CK syndrome (X-linked)	Cortical CNS malformations, seizures and microcephaly	Sterol-4-demethylase	308050
Chondrodysplasia punctata Conradi-Hünermann, CDPX2 (X-linked)	Psychomotor retardation, rhizomelic short stature with asymmetric shortening of limbs, stippled epiphyses, cataracts, ichthyosis; lethal in males	3β-Hydroxysterol Δ^8,Δ^7-isomerase	302960
Hemizygous male emopamil-binding protein (EBP) deficiency (X-linked)	Dysmorphic facies; developmental CNS malformations (Dandy-Walker, agenesis of corpus callosum); cardiac and GU malformations; occasional symmetric congenital ichthyosis and skeletal defects; high infant mortality	3β-Hydroxysterol Δ^8,Δ^7-isomerase	302960
Greenberg dysplasia (AR)	Non-immune hydrops fetalis, severe chondrodysplasia punctata, moth-eaten bone lesions, prenatally lethal	3β-Hydroxysterol Δ^{14}-reductase (lamin B receptor; *LBR* gene)	215140
Antley-Bixler syndrome, ABS1 (AR)	Craniosynostosis, midface hypoplasia, radiohumeral synostosis, contractures, choanal atresia/stenosis, genital anomalies	Cytochrome P450 oxidoreductase (*POR* gene)	201750
Lathosterolosis (AR)	Severe malformations, overlapping the spectrum of Smith-Lemli-Opitz syndrome, lipid storage	3β-Hydroxysterol Δ^5-desaturase	607330
SC4MOL deficiency (AR)	Postnatal onset of microcephaly and poor growth; psoriasiform dermatitis; mild intellectual disability	Sterol C4 methyl oxidase	607545
SLOS (AR)	Psychomotor retardation, growth retardation, microcephaly, facial dysmorphism, cleft palate, genital anomalies in males, postaxial polydactyly, syndactyly of toes 2/3, photosensitivity	7-Dehydrocholesterol reductase	270400
Desmosterolosis (AR)	Psychomotor retardation, facial dysmorphy, cleft palate, multiple malformations, ambiguous genitalia, short limbs, osteosclerosis	3β-Hydroxysterol Δ24-reductase	602398

skin rash, lymphadenopathy and hepato(spleno) megaly. The *diagnosis* is usually made through urinary organic acid (OA) analysis, but in patients with normal OA, diagnosis will rely on genetics. *Hyper-IgD syndrome* with recurrent febrile attacks represents an attenuated form of the disease, with residual enzyme function.

Disease Info: Smith-Lemli-Opitz Syndrome (SLOS)

SLOS is the most common defect of the cholesterol pathway (Porter 2008). It is caused by the deficiency of 7-dehydrocholesterol reductase, the last step of cholesterol synthesis, leading to elevated levels of 7- and 8-dehydrocholesterol and (often) reduced levels of cholesterol. As diagnostic markers, 7- and 8-dehydrocholesterol are measured by GC-MS in serum or tissues. SLOS is characterised clinically by recognisable craniofacial features in childhood with microcephaly and bitemporal narrowing, ptosis, broad nasal tip with anteverted nostrils, broad upper alveolar ridges (and later, broad upper secondary incisors) and micrognathia. In addition, children with SLOS show syndactyly of the second and third toes, genital anomalies in males, short stature, photosensitivity and mostly severe intellectual disability. The phenotypic spectrum, however, is very broad. Facial appearances may change with age and become less characteristic in adulthood. Also, genital anomalies may become less prominent. Structural brain anomalies occur in 37 %; not infrequently they belong to the holoprosencephaly spectrum, a failure of normal bilobar development of the forebrain. Malformations of the heart, lungs and the gastrointestinal system may be associated. Behavioural problems, sleep disturbances and feeding difficulties are common. Dietary cholesterol supplementation may have benefits especially on the behavioural problems in some SLOS patients. Trials combining high-cholesterol diets with statin treatment so far have given inconclusive results.

Given the multiple biological functions of cholesterol, the link between abnormal cholesterol metabolism and abnormal morphogenesis in SLOS is complex and only partly unravelled. Some of the malformations relate to impairment of sonic hedgehog (SHH) functioning, one of the major embryonic signalling pathways. Genetic disorders in this pathway cause holoprosencephaly, and mutations in *GLI3*, one of the downstream effectors of *SHH*, cause Pallister-Hall syndrome (PHS, OMIM 146 510) with clinical features overlapping those of SLOS. The different conditions may thus be grouped to one syndrome family that share a common pathway and part of their phenotypic characteristics. Other SLOS features may relate to deficient total sterols and alteration of cellular membrane properties and to possible toxic effects of cholesterol precursors.

33.7 Energy Defects: Mitochondrial Defects Potentially Affecting Morphogenesis

Mitochondrial disorders in a strict sense are disorders of enzymes or enzyme complexes directly involved in the generation of ATP by oxidative phosphorylation, discussed in detail in Chap. D7. They are often multisystem disorders affecting neuromuscular and other systems and may also affect morphogenesis. Whilst mitochondrial dysfunction caused by mtDNA mutations is not usually associated with physical abnormalities, mitochondrial energy deficiency resulting from mutations in nuclear-encoded genes may occasionally result in congenital malformations and (various) dysmorphism (Brown 2005).

An important disorder to consider is *multiple acyl-CoA dehydrogenase deficiency* (*MADD*),

also known as glutaric aciduria type II. This is a defect in the mitochondrial transfer of electrons from fatty acids to the electron transport chain, by genetic defects of the electron transfer flavoprotein (ETF) or ETF/ubiquinone oxidoreductase (ETFQO). Attenuated forms of the disease are characterised by predominant muscle and hepatic disease, but severe multiple acyl-CoA dehydrogenase deficiency results in the virtual absence of fatty acid oxidation with significant physical abnormalities, overwhelming metabolic decompensation hence poor prognosis. Malformations are predominantly found in the brain, kidneys, e.g. renal cysts, and genitals. Craniofacial dysmorphism may be reminiscent of Zellweger syndrome, as it is characterised by macrocephaly, large anterior fontanel, high forehead, flat nasal bridge, increased inner canthal distance and malformed ears. An unpleasant acrid body odour may be noted. Overwhelming acidosis, cardiomyopathy, liver dysfunction and epileptic encephalopathy all contribute to early fatal course. The *diagnosis* is made on identification of a wide variety of abnormal metabolites in urinary organic acid analysis and acylcarnitines.

33.8 Disorders of Post-translational Protein Modification: Congenital Disorders of Glycosylation and GPI Anchor Disorders

Glycosylation, i.e. the transfer of glycans to proteins or lipids, is the most complex post-translational modification of molecules in humans. Human disorders in glycosylation pathways are mainly known for N-linked (attached to the amide group of an asparagine of the protein) or O-linked glycans (to the hydroxyl group of serine or threonine). N-glycosylation requires assembly of glycans in the cytosol and ER, transfer to the protein and a subsequent processing pathway mainly located in the Golgi apparatus. Multiple enzymes, transporters and transferases are involved. Type I disorders of N-glycosylation affect the early assembly pathway; in type II disorders, the processing pathway is disturbed. O-glycosylation, mainly confined to the Golgi apparatus, is a much shorter – however more variable – pathway, consisting of assembly and transfer of the glycans to a protein. The diversity of O-glycosylation is due to variable sugars in the first position of the glycans, suchlike mannose in the O-mannosylation pathway. However, in view of the multitude of enzymes involved, it has recently been suggested to discontinue this system of classification and describe the individual disorders by their enzymatic deficiency.

The clinical features of *congenital disorders of glycosylation* (CDG) are as diverse as the numerous functions of glycoproteins which can be enzymes, transporter proteins, membrane components or hormones. CDGs may present as single organ diseases but are often multisystem disorders, comprising structural malformations and abnormal function, occurring in various combinations and covering a spectrum of severity (de Lonlay et al. 2001; Grunewald 2007; Hess et al. 2008; Jaeken and Matthijs 2001; Scott et al. 2014). Symptoms comprise psychomotor retardation and neurological abnormalities, multiple function deficits like bleeding diathesis, endocrine or gastrointestinal disturbances, organ manifestations (e.g. heart or kidney), skeletal manifestations (e.g. short stature, contractures and osteopenia), as well as other physical abnormalities. Many CDG patients already display symptoms at birth; this is also the case in the most common primary glycosylation disorder, phosphomannomutase deficiency (PMM2-CDG, formerly CDG-Ia).

Most N-glycosylation disorders are readily detected by isoelectric focussing (IEF) of serum transferrin although results may be false negative in some disorders and young infants, and abnormal results may be caused by a variety of other conditions. *Abnormal O-glycosylation* involving the so-called core 1 O-glycans with the first sugar being N-acetylgalactosamine (the most commonly observed O-glycosylation type) can be detected by IEF of apolipoprotein CIII in serum. For other O-glycosylation defects, suchlike O-mannosylation defects (Walker-Warburg-Syndrome or muscle-eye-brain disease), particular staining of the alpha-dystroglycan complex in a muscle biopsy will

show abnormal O-mannosylation. For the majority of CDG subtypes identified so far, mutational analysis can be performed to confirm the diagnosis.

Alongside disorders of N-glycosylation of proteins and defects in the O-glycosylation pathways, combined N- and O-glycosylation defects have been identified. As so far only very few patients have been identified for many of the CDGs, it is impossible to describe characteristic dysmorphic features. However, recognisable phenotypes, particularly dysmorphic features as reported in the literature, have been listed in Table 33.3.

Disease Info: Phosphomannomutase (PMM) Deficiency

By far the most common and prototypic CDG is PMM deficiency (OMIM 212065), previously denoted CDG 1a, caused by mutations in the *PMM2* gene. The phenotypic spectrum is very broad. Commonly, the central nervous system is involved and children show psychomotor retardation, hypotonia, hyporeflexia, ataxia and seizures. Dysmorphic features include a prominent forehead, large ears and a thin upper lip. Fat distribution is abnormal with fat pads at the buttocks or other sites and lipoatrophic changes. The nipples are typically inverted. Retinitis pigmentosa, strabismus and myopia are the most common ophthalmologic signs. Skeletal manifestations consist of growth retardation, osteopenia, contractures, spine anomalies, 'bone-in-bone' appearance and other changes. In the multivisceral form of PMM deficiency, multiple other manifestations may exist and include liver, cardiac, renal and gastrointestinal involvements.

A recently emerging group are *glycosylphosphadidylinositol* (*GPI*) *anchor-related diseases* (Ng and Freeze 2015). GPI-anchored proteins are attached to membranes undergoing several assembly steps in the ER and remodelling in the Golgi. Whilst undergoing the modifi-

Table 33.3 Human genetic disorders of glycosylation

Disorders of protein N-glycosylation			
Relevant gene	Deficient protein	OMIM	Clinical features
PMM2 (CDG-Ia)	Phosphomannomutase 2	601785	ID, cerebellar hypoplasia, abnormal fat pads, hypotonia, failure to thrive, multiorgan involvement, stroke-like episodes, seizures, cardiomyopathy, nephrotic syndrome, hyperinsulinaemic hypoglycemia, coagulopathy, hypergonadotropic hypogonadism
MPI (CDG-Ib)	Phosphomannose isomerase	602579	Protein-loosing enteropathy, hepatic fibrosis, coagulopathy, hyperinsulinaemic hypoglycemia
ALG6 (CDG-Ic)	Glucosyltransferase 1	603147	ID, hypotonia, epilepsy, coagulopathy, strabismus, skeletal malformations
NOT56L (CDG-Id)	Mannosyltransferase 6	601110	ID, optic atrophy, iris coloboma, secondary microcephaly, arthrogryposis multiplex, epilepsy
ALG 12 (CDG-Ig)	Mannosyltransferase 8	607143	ID, genital hypoplasia, microcephaly, undetectable IgF1 and IgFB3, cardiomegaly
ALG 8 (CDG-Ih)	Glucosyltransferase 2	608104	Protein-loosing enteropathy, renal failure, hepatic failure, cataracts, osteopenia, skeletal dysplasia, ID

(continued)

Table 33.3 (continued)

Disorders of protein N-glycosylation			
Relevant gene	Deficient protein	OMIM	Clinical features
ALG2 (CDG-Ii)	Mannosyltransferase 2	607906	Intractable seizures, iris coloboma, hepatomegaly, poor vision
DPAGT1 (CDG-Ij)	UDP-GlcNAc:Dol-P-GlcNAc-P transferase	608093	ID, seizures, esotropia, microcephaly
HMT1 (CDG-Ik)	Mannosyltransferase 1	608540	ID, secondary microcephaly, nephrotic syndrome, early death
DIBD1 (CDG-Il)	Mannosyltransferase 7-9	608776	Severe microcephaly, seizures, hepatomegaly, renal cysts, early death
RFT1 (CDG-In)	Flippase of Man5GlcNAc2-PP-Dol	611633	Intrauterine growth retardation, arthrogryposis, failure to thrive, impaired vision, drug resistant epilepsy, abnormal coagulation
MGAT2 (CDG-IIa)	N-acetylglucosaminyl-transferase 2	602616	ID, facial dysmorphism, stereotypic behaviour, seizures, dysmorphy, liver disease
GLS1 (CDG-IIb)	Glucosidase 1	606056	Seizures, liver disease, early death
TUSC3	Oligosaccharyltransfer-ase subunit	601385	Non dysmorphic, intellectual disability
SRD5A3 (CDG-Iq)	5-alpha-reductase	612379	Intellectual disability, cataracts, coloboma, kyphosis
PGM1 (CDG-It)	Phosphoglucomutase 1	614921	Cleft palate, uvula bifida, short stature, hypoglycemia, cardiomyopathy, normal intelligence
Disorders of protein O-glycosylation			
Defective gene	Defective protein	OMIM	Clinical features
**O-Xylosylglycan synthesis*			
EXT1/*EXT2* (multiple cartilaginous exostoses)	Glucuronyltransferase/*N*-acetylglucosaminyltrans-ferase	608177/608210	ID, multiple exostoses, hypertrichosis
B4GALT7 (progeroid type of Ehlers-Danlos syndrome)	β-1,4-Galactosyltransferase 7	604327	ID, short stature, macrocephaly, abnormal bones, loose skin
**O-N*-Acetylgalactosaminylglycan synthesis			
GALNT3 (familial tumoral calcinosis)	Polypeptide *N*-acetylgalac-tosaminyltransferase 3	601756	Massive calcium deposits in skin and tissue
**O*-Xylosyl/*N*-acetylgalactosaminylglycan synthesis			
SLC35D1 (Schneckenbecken dysplasia)	Solute carrier family 35	610804	Micromelic dwarfism, macrocephaly, abnormal bones, precocious ossified bones
**O-Mannosylglycan synthesis*			
POMT1/*POMT2*	Protein-*O*-mannosyltransferase 1 and 2	607423	Type II lissencephaly, cerebellar malformation, ventriculomegaly, anterior chamber malformations, severe muscular hypotonia, severe ID, death in infancy
POMGNT1	Protein-*O*-mannose β-1,2-*N*-acetylglucosaminyltrans-ferase	606822	Congenital muscular dystrophy, ocular abnormalities and lissencephaly
FKTN	Fukutin	607440	Fukuyama congenital muscular dystrophy

(continued)

Table 33.3 (continued)

Disorders of protein O-glycosylation			
Defective gene	Defective protein	OMIM	Clinical features
FKRP	Fukutin-related protein	606596	Limb-girdle muscular dystrophy
LARGE	*N*-Acetylglucosaminyl-transferase-like protein	603590	Congenital muscular dystrophy, brain abnormalities
**O-Fucosylglycan synthesis*			
SCDO3 (spondylocostal dysostosis type 3)	*O*-Fucose-specific β-1,3-*N*-acetylglucosaminyl-transferase	602576	Spondylocostal dysostosis
B3GALTL (Peters plus syndrome)	*O*-Fucose-specific β-1,3-glucosyltransferase	610308	ID, anterior eye chamber defects, disproportionate short stature, cleft palate

Disorders of glycosphingolipid and glycosylphosphatidylinositol anchor glycosylation			
Defective gene	Defective protein	OMIM	Clinical features
SIAT9 (Amish infantile epilepsy)	Lactosylceramide α-2,3 sialyltransferase (GM_3 synthase)	609056	Amish infantile epilepsy
PIGM (glycosylphosphatidylinositol deficiency)	Phosphatidylinositolglycan, class M	610273	Portal venous thrombosis, absence seizures
PIGA	GlcNAc-PI synthesis protein (X-linked)	311770	Dysmorphism, Pierre Robin sequence, seizures, early death
PIGL	GlcNAc-PI de-N-acetylase	280000	Colobomas, heart defects, ichthyosis
PIGN	GPI ethanolamine phosphate transferase	614080	Severe neurological impairment, multiple congenital abnormalities, early death
ST3GAL5	Sia2,3 Galbeta1,4 Glc-Cer synthase	604402	Severe delay, seizures, altered dermal pigmentation
PIGY	Part of GPI-acetylglucosaminyltransferase complex		Dysmorphic features, short neck, developmental delay, hip dysplasia, shortening of long bones
PIGW	Acetylation of GPI anchor	616025	Developmental delay, seizures, facial abnormalities
PIGV	Alpha-mannosyltransferase in GPI anchor synthesis	239300	Facial abnormalities, macrocephaly, brachytelephalangy, seizures, hyperphosphatasia
PIGO	GPI ethanolamine phosphate transferase	614749	Facial dysmorphism, nail hypoplasia, anal stenosis, mental retardation, hyperphosphatasia
PIGT	GPI transamidase defect	615398	Facial dysmorphism, severe developmental delay, structural brain abnormalities, abnormal bones, low alkaline phosphatase

Disorders of multiple glycosylation functions and other pathways			
Defective gene	Defective protein	OMIM	Clinical features
DPM1 (CDG-Ie)	GDP-Man/Dol-P-mannosyltransferase (Dol-P-Man synthase 1)	603503	ID, microcephaly, epilepsy, blindness
MPDU1 (CDG-If)	Lec35 (Man-P-Dol utilisation 1)	608799	ID, growth retardation, ichthyosis, ataxia, pigmentary retinopathy
B4GALT1 (CDG-IId)	β-1,4-galactosyltransferase 1	607091	ID, myopathy, Dandy-Walker malformation, macrocephaly
GNE (hereditary inclusion body myopathy)	UDP-GlcNAc epimerase/kinase	600737	Ascending muscle weakness, 'rimmed vacuoles' on biopsy

(continued)

Table 33.3 (continued)

Disorders of multiple glycosylation functions and other pathways			
Defective gene	Defective protein	OMIM	Clinical features
SLC35A1 (CDG-IIf) (CMP-sialic acid transporter deficiency)	CMP-sialic acid transporter	605634	Thrombocytopenia, haemorrhages, respiratory distress syndrome, opportunistic infections
SLC35A2 (CDG-IIm)	UDP-galactose transporter	300896	Severe ID, brain malformation, skeletal abnormalities
MAN1B1	1,2 alpha – mannosidase	614202	Mental retardation, dysmorphic features, obesity, facial dysmorphism
TMEM165 (CDG-IIk)	Abnormal trafficking	614727	Developmental delay, growth delay, skeletal dysplasia, brachydactyly, very short stature
SLC35C1 (CDG-IIc) (GDP-fucose transporter deficiency)	GDP-fucose transporter	605881	ID, growth retardation, microcephaly, persistent neutrophilia, recurrent fever
PGM3	Phosphoglucomutase 3	172100	Severe atopic dermatitis, immune dysfunction, vasculitis, connective tissue involvement
**Dolichol pathway*			
DK1 (CDG-Im)	Dolichol kinase	610768	Seizures, ichthyosis, dilated cardiomyopathy, early death
**COG complex*			
COG7 (CDG-IIe)	Component of conserved oligomeric Golgi complex 7	606978	ID, progressive microcephaly, dysmorphy, growth retardation, hypotonia, adducted thumbs, cardiac defects, wrinkled skin, hyperthermia, early death
COG1 (CDG-IIg)	Component of conserved oligomeric Golgi complex 1	606973	ID, growth retardation, progressive microcephaly, cerebrocostomandibular syndrome, hypotonia
COG8	Component of conserved oligomeric Golgi complex 8	606979	Severe psychomotor retardation, seizures, failure to thrive
**V-ATPase*			
ATP6VOA2 (cutis laxa type II)	V0 subunit A2 of vesicular H(+)-ATPase	611716	ID, wrinkled skin/cutis laxa, pachygyria, seizures, late closure of the fontanelle

cations, the lipids remain a functional asset to their protein. Disorders in this pathway have mainly been solved by homozygosity mapping and exome sequencing. The protein affected includes PIGA, PIGL, PIGM, PIGN, PIGO, PIGT, PIGV, PIGW, PIGQ, PIGY, PGAP1, PGAP2 and PGAP3. So far only few patients have been described in each entity. Common features are multisystem disorders, epilepsy and dysmorphic features. Skeletal abnormalities were seen in PIGA, PIGN, PIGO, PIGT, PIGV and PIGY. A helpful marker for some of these disorders is alkaline phosphatase (ALP), being elevated in several disorders (PIGO, PIGV, PIGW, PIGY, PGAP2 and PGAP3). In PIGT, ALP has been reported as low.

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Hematological Disorders

34

Ellen Crushell and Joe T.R. Clarke

Abbreviations

ACE	Angiotensin-converting enzyme
AcoD	Autosomal codominant
AD	Autosomal dominant
ALAS	5-Aminolevulinic acid synthase
AR	Autosomal recessive
ASAT	Anemia sideroblastic and spinocerebellar ataxia
CBC	Complete blood count
CDG	Congenital disorders of glycosylation
GSD	Glycogen storage disease
HDL	High-density lipoprotein
HPRT	Hypoxanthine guanine phosphoribosyltransferase
IRT	Immunoreactive trypsin
IVA	Isovaleric aciduria
LCAT	Lecithin-cholesterol acyltransferase
MCV	Mean corpuscular volume
ML	Mucolipidosis
MLASA	Mitochondrial myopathy and sideroblastic anemia
MMA	Methylmalonic aciduria
MPS	Mucopolysaccharides/oses
NCL	Neuronal ceroid-lipofuscinosis
NP	Niemann-Pick
OA	Organic acid analysis
PA	Propionic aciduria
RBC	Red blood cell
TG	Triglycerides
TPI	Triose phosphate isomerase
UMP	Uridine monophosphate
XLR	X-linked recessive

Key Facts

- CBC and blood film are indicated in all metabolic diagnostic workups.
- Hematological disorders may be the lead symptom or a secondary finding in many metabolic disorders.
- Hemolytic anemia may be the only sign of an inherited disorder of red cell energy metabolism.
- Macrocytic anemia is an important pointer to many metabolic disorders.
- Hematological abnormalities due to a metabolic disorder are rarely seen in isolation

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34.1 General Remarks

While many metabolic disorders are associated with secondary hematological findings (e.g. hypersplenism in Gaucher disease, neutropenia in propionic acidemia), sometimes the hematological disorder is the lead feature, as in Pearson marrow-pancreas syndrome or the macrocytic anemia of inherited disorders of cobalamin metabolism. In many instances the hematological abnormalities involve all the formed elements of the blood. In this chapter, these are discussed in the context of the most common or the most prominent abnormality. For example, type 1 Gaucher disease often presents as thrombocytopenia; however, neutropenia and anemia are almost always present, though rarely as prominent as the decrease in platelet counts.

34.2 Abnormal Cell Morphology

Abnormal cell morphology on blood film or bone marrow aspirate may be the first pointer toward a metabolic diagnosis. See Table 34.1 for various abnormal cell morphologies found in metabolic disease. It is important to be aware that various primary hematological disorders (including malignancies) may produce similar morphological findings. Also, a "normal" blood film does not rule out a metabolic diagnosis such as a mucopolysaccharidosis where vacuolated lymphocytes may be few and far between.

34.3 Hemolytic Anemia

Hemolytic anemia may be caused by factors within the red cell (intrinsic) or in the environment of the cell (extrinsic) (Table 34.2). Mature red cells contain no mitochondria and are totally dependent upon anaerobic glycolysis and the pentose phosphate pathway to meet their energy needs and requirements for NADPH. Deficiencies of any of the enzymes involved may be associated with hemolytic anemia as a result of a deficiency of ATP or of reducing equivalents.

By far the most common of the hereditary intrinsic defects causing hemolytic anemia is glucose-6-phosphate dehydrogenase (G6PD) deficiency. It is an X-linked recessive condition of variable severity which occurs with very high frequency in Mediterranean, African, and Asian populations. Acute hemolysis is usually associated with exposure to oxidizing chemicals or drugs or ingestion of certain foods, such as fava beans, hence the historical name for the disease: favism (Table 34.3). Some countries have introduced newborn screening for G6PD in an attempt to prevent the development of acute hemolysis, severe hyperbilirubinemia, and kernicterus.

Chronic, isolated hemolytic anemia, characterized by jaundice, gallstones, indirect hyperbilirubinemia, and splenomegaly, is also seen in patients with inherited deficiencies of pyruvate kinase, 2,3 diphosphoglyceromutase, glucose phosphate isomerase, or hexokinase. The diagnosis may be suspected from the red cell morphology; however, confirmation generally requires analysis of the specific red cell enzymes; pyruvate kinase deficiency is the most common of this group of non-spherocytic hemolytic anemias (Table 34.4).

Deficiencies of other enzymes involved in anaerobic glycolysis also lead to hemolytic anemia with significant non-hematological problems, such as mental retardation, myopathy (phosphofructokinase deficiency or GSDVII), ataxia, chronic metabolic acidosis, or stroke. The extrinsic hemolytic anemias may be caused by any inborn error that produces severe liver disease in infancy, such as galactosemia and neonatal hemochromatosis. Erythropoietic protoporphyria and Wilson disease also lead to hemolytic anemia (Table 34.2). Skin changes such as photosensitivity, ulcers, or abnormal pigmentation should initiate a porphyria screen in neonates and young children. Hemolytic anemia is often not the lead sign but

Table 34.1 Abnormal blood cell morphology

Cell type	Morphology	Disorder
Red cells	Target cells Spherocytes Spiculated red cells: Echinocytes ("burr") or acanthocytes ("spur")	Liver disease Lecithin: cholesterol acyltransferase deficiency Abetalipoproteinemia Hypersplenism G6PD Liver disease Renal failure Vitamin E deficiency Abetalipoproteinemia Wolman disease Cbl C defects Hallervorden-Spatz (pantothenate kinase) Pyruvate kinase deficiency
White cells	Basophilic stippling Vacuolated lymphocytes (Alder) Reilly bodies "Sea-blue" histiocytes	Lead poisoning Iron deficiency Hemolytic anemias Pyrimidine-5′ nucleotidase deficiency Aspartylglucosaminuria Multiple sulfatase deficiency Ceroid lipofuscinoses Mucolipidosis II GM_1 gangliosidosis Mucopolysaccharidoses Niemann-Pick A, C Pompe Sialidosis Wolman Mucopolysaccharidoses Niemann-Pick Ceroid lipofuscinoses Adult cholesterol ester storage disease GM_1 gangliosidosis
Bone marrow	"Foam cells"	Niemann-Pick A,B,C,D Gaucher disease types 1,2,3 Gangliosidosis – GM_1 and GM_2 Sialidosis I, II (late infantile) Mucolipidosis II,III,IV Fucosidosis Mannosidosis Ceroid lipofuscinoses Farber disease Wolman disease Cholesterol ester storage disease Cerebrotendinous xanthomatosis Chronic hyperlipidemia

Adapted from Lanzkowsky (2011)

an associated finding, along with poor growth, poor feeding, vomiting, and hypotonia in the organic acidurias MMA, PA, and IVA, abetalipoproteinemia, and Wolman disease in the first year of life.

Early-onset xanthomas and hypercholesterolemia (and elevated phytosterols) are seen in phytosterolemia. Lecithin-cholesterol acyltransferase deficiency (LCAT) is associated with corneal opacities and proteinuria (Table 34.2).

Table 34.2 Hemolytic anemias

	Disorder/enzyme deficiency	Associated abnormalities and diagnosis
Intrinsic defects		
Anaerobic glycolytic enzyme deficiencies		
Isolated hemolytic anemia	Pyruvate kinase	Non-spherocytic hemolytic anemia, red cell assay
	Glucose phosphate isomerase	Non-spherocytic hemolytic anemia, red cell assay
	Hexokinase	Non-spherocytic hemolytic anemia, red cell assay
	2,3-Diphosphoglyceromutase	Non-spherocytic hemolytic anemia, red cell assay
+ neurological abnormalities	Phosphofructokinase	Prominent myopathy, cardiomyopathy in infants with severe disease. Diagnosis by enzyme assay, or *PFKM* mutation analysis
	Triosephosphate isomerase	Autosomal dominant, early-onset, neurodegenerative disease. Diagnosis by enzyme assay, or *TPI* mutation analysis
	Phosphoglycerate kinase	X-linked recessive myopathy with chronic progressive encephalopathy. Diagnosis by enzyme assay, or *PGK1* mutation analysis
Pentose phosphate pathway defects		
	Glucose-6-phosphate dehydrogenase	Common X-linked recessive hemolytic anemia. Diagnosis by red cell enzyme assay. *G6PD* mutation analysis
	Glutathione synthetase	Autosomal recessive, metabolic acidosis, pyroglutamic aciduria (5-oxoprolinuria). Diagnosis enzyme analysis and *GS* mutation analysis
	Glutamylcysteine synthetase	Autosomal recessive, rare, mild chronic hemolytic anemia, spinocerebellar degeneration reported. Diagnosis by enzyme assay and mutation analysis
Abnormal erythrocyte nucleotide metabolism		
	Adenylate kinase	Enzyme assay, AK1 mutation analysis
	Pyrimidine 5-nucleotidase	Accumulation of pyrimidine nucleotides in red cells (basophilic stippling), most common disorder of nucleotide metabolism causing hemolytic anemia
	Adenosine deaminase superactivity	Elevated red cell ADA activity
Extrinsic causes of hemolysis		
Hypersplenism	Gaucher disease	Leukocyte or fibroblast β-glucosidase, *GBA* mutation analysis
	Niemann-Pick disease	Leukocyte or fibroblast enzyme assays, oxysterol analysis, mutation analysis
Defects of porphyrin metabolism	Congenital erythropoietic porphyria	Autosomal recessive photosensitivity of the skin with blistering and scarring, red coloring of teeth and urine. Diagnosis by urinary porphyrin analysis, measurement of enzyme (uroporphyrinogen III cosynthetase), *UROS* mutation analysis
Disorders of lipid metabolism	LCAT	Corneal opacities, "target cell" anemia, proteinuria, progressive renal impairment, low plasma esterified cholesterol. Diagnosis by plasma LCAT assay and by *LCAT* mutation analysis
	Abetalipoproteinemia	Steatorrhea, ataxia, "burr" cells (acanthocytes), hypocholesterolemia. Diagnosis by plasma apolipoprotein analysis, *MTP* mutation analysis
Defects of sterol metabolism	Phytosterolemia (sitosterolemia)	Premature coronary artery disease. Diagnosis by plasma sterol analysis. *ABCG5* and/or *ABCG8* mutation analysis
Intoxications	Wilson disease	Hepatocellular dysfunction, neurological abnormalities, Kayser-Fleischer rings. Diagnosis by plasma ceruloplasmin analysis, urinary copper excretion, *ATP7B* mutation analysis

Table 34.3 Partial list of agents inducing hemolytic anemia in G6PD deficiency

Antimalarials	*Antipyretics/analgesics*	*Others*
Primaquine	Acetylsalicylic acid	Chloramphenicol
Pamaquine	Acetanilide	Isoniazid
Quinine		Ciprofloxacin Menadione
Sulfonamides	*Infections*	*p*-Aminosalicylic acid
Sulfadiazine	Viral respiratory infections	Phenytoin
Sulfisoxazole	Viral hepatitis	Vitamin K (water soluble)
Nitrofurans	Typhoid	Naphthalene (moth balls)
Nitrofurantoin		Nalidixic acid

Table 34.4 Hemolytic anemia + neurological abnormalities

Disorder/enzyme deficiency	Gene	Inheritance	Tests to aid diagnosis
Triosephosphate isomerase	*TPI1*	AD	Red cell assay, *TPI1* mutation analysis
Phosphoglycerate kinase	*PGK1*	XLR	Red cell assay, *PGK* mutation analysis
Phosphofructokinase[a]	*PFKM*	AR	(GSD VII) CK, enzyme assay, muscle biopsy, *PFKM* mutation analysis
Abetalipoproteinemia	*MTP*	AR	Plasma apolipoprotein analysis, *MTP* mutation analysis
Hypobetalipoproteinemia	*APOB*	AcoD	Plasma apolipoprotein analysis, *APOB* mutation analysis
Glutathione synthetase	*GS*	AR	Red cell assay, urinary organic acids (pyroglutamic aciduria), *GS* mutation analysis
Wilson disease	*ATP7B*	AR	Plasma ceruloplasmin, urinary copper excretion, mutation analysis

[a]Myopathy

Table 34.5 Macrocytosis

	Disorder/enzyme deficiency	Associated abnormalities and diagnosis
Disorders of nucleotide metabolism		
Orotic aciduria	UMP synthase deficiency	Autosomal recessive megaloblastic anemia and marked orotic aciduria responsive to treatment with uridine. Confirmation of diagnosis by *UMPS* mutation analysis
Thiamine-responsive megaloblastic anemia	Thiamine transporter protein	Autosomal recessive anemia responsive to high-dose thiamine treatment. Diagnosis confirmed by *SLC19A2* mutation analysis
Lesch-Nyhan disease	HPRT deficiency	X-linked recessive spasticity, variable psychomotor retardation, self-mutilation, with high plasma and urinary urate. Diagnosis confirmed by enzyme assay and *HPRT* mutation analysis
Cobalamin disorders		
Malabsorption	Imerslund-Grasbeck syndrome (defective cobalamin transport by enterocytes)	Autosomal recessive megaloblastic anemia, proteinuria, with low plasma vitamin B_{12} levels. Schilling test, no correction. Normal intrinsic factor (IF) levels and absent IF antibodies. *GIF, CUBN*, *AMN* genes
	Intrinsic factor deficiency	Low plasma vitamin B_{12} analysis; Schilling test – corrected by intrinsic factor. *GIF* mutation analysis

(continued)

Table 34.5 (continued)

	Disorder/enzyme deficiency	Associated abnormalities and diagnosis
Transport	Transcobalamin II deficiency with normal serum cobalamin levels	Variable psychomotor retardation, hypotonia, MMA, hyperhomocysteinemia, low methionine, normal plasma vitamin B_{12} levels. Diagnosis confirmed by measurement of plasma TC II, Mutation analysis *TCN2*
Metabolism	Defective synthesis of methylcobalamin – *cbl*E, *cbl*G	Variable psychomotor retardation, associated with hyperhomocysteinemia with low methionine and without MMA, normal plasma folate and vitamin B_{12}. Diagnosis by complementation studies in fibroblasts and mutation analysis
	Defective synthesis of both adenosylcobalamin and methylcobalamin – *cbl*C, *cbl*D, *cbl*F	Psychomotor retardation, variable retinal dystrophy, associated with MMA and hyperhomocysteinemia with low methionine, normal plasma folate and vitamin B_{12}. Diagnosis by complementation studies in fibroblasts and mutation analysis
Folate disorders		
Malabsorption	Congenital folate malabsorption	Chronic encephalopathy, movement disorder, psychomotor retardation, pancytopenia, hypomethioninemia, low plasma folate. *SLC46A1* mutation analysis
Metabolism	Methylenetetrahydrofolate reductase (MTHFR) deficiency	Psychiatric disturbances, psychomotor retardation, epilepsy, hyperhomocysteinemia and low methionine. Low CSF folate. Diagnosis confirmed by enzyme assay or *MTHFR* mutation analysis
	Glutamate formiminotransferase deficiency	Clinical significance uncertain. Variable psychomotor retardation, marked increase in urinary FIGLU excretion
	Dihydrofolate reductase deficiency	Phenotype unclear, psychomotor retardation and seizures described. Low CSF folate. Megaloblastic anemia responds to treatment with folinic acid. Confirmation by *DHFR* mutation analysis
Others		
Mevalonic aciduria	Mevalonic acid kinase deficiency	Developmental delay, failure to thrive, hepatosplenomegaly, dysmorphic facies, recurrent fever with lymphadenopathy, with elevated urinary excretion of mevalonic acid. Diagnosis confirmed by enzyme assay or *MVK* mutation analysis

MCV >85 fL and megaloblasts in bone marrow

34.4 Macrocytic Anemia

Inherited disorders of absorption, transport, or metabolism of vitamin B_{12} (cobalamin) and folate may present with macrocytic anemia as the leading sign. Elevated homocysteine with low methionine points toward a remethylation defect of homocysteine to methionine.

Remember

- Over 95 % of patients with megaloblastic anemia in childhood are secondary to deficiency of folate or cobalamin.
- Macrocytosis may be masked by iron deficiency and thalassemia.
- High homocysteine and low methionine suggest inborn errors of cobalamin metabolism.
- Intermittent orotic aciduria may be seen in congenital folate deficiency.
- Absence of methylmalonic aciduria does not rule out a cobalamin defect.

Congenital (or acquired) disorders of nucleotide metabolism may also present with macrocytic anemia. In orotic aciduria caused by UMP synthase deficiency, pyrimidine biosynthesis is interrupted, while in Lesch-Nyhan (HPRT deficiency) disease, purine nucleotide regeneration is impaired (the resulting macrocytic anemia is usually a late feature).

Thiamine-responsive megaloblastic anemia is associated with diabetes and sensorineural deafness (in some, the full DIDMOAD spectrum – diabetes insipidus, diabetes mellitus, optic atrophy, and deafness – is present); only the anemia, however, will respond to high-dose thiamine treatment; the underlying defect is in thiamine transport.

The sideroblastic anemia in Pearson marrow-pancreas syndrome associated with mitochondrial DNA deletions is macrocytic and associated with exocrine pancreatic dysfunction, growth failure, and lactic acidosis. The clinical phenotype may be that of Kearns-Sayre syndrome (Table 34.7).

34.5 Sideroblastic Anemia

Sideroblasts are red cell precursors with iron loaded (Prussian blue staining) mitochondria clustered in a ring around the nucleus ("ringed sideroblasts") seen in the bone marrow. Sideroblastic anemia (SA) is caused by mitochondrial dysfunction which may be primary or acquired. The acquired forms far outnumber the congenital forms; however, a hereditary form should be particularly sought in the young and in those without a history to suggest acquired forms (e.g., alcohol ingestion) (Table 34.7). Four of the eight enzymes necessary for the biosynthesis of heme are located in mitochondria (the other four are cytoplasmic and deficiencies do not result in sideroblasts, such as the porphyrias). Defective mitochondrial heme synthesis causes iron accumulation within the mitochondria resulting in oxidative mitochondrial damage and the formation of sideroblasts. Sideroblastic anemia is usually microcytic or normocytic with hypochromia; megaloblastic sideroblastic anemia suggests Pearson syndrome or thiamine-responsive megaloblastic anemia.

The acquired sideroblastic anemias are caused by inhibitors of 5-aminolevulinate synthase (ALAS), such as isoniazid, cycloserine, and lead. Chloramphenicol and ethanol also inhibit mitochondrial function resulting in sideroblastic anemia.

Partial response to pyridoxine treatment has been seen in some of the congenital and acquired sideroblastic anemias.

Inherited sideroblastic anemia may be syndromic or non-syndromic. The most frequent non-syndromic SAs are X-linked SA (XLSA) and recessive SA caused by mutations in ALAS2

Table 34.6 Key metabolic investigations of macrocytic anemia

Plasma cobalamin, plasma, and red cell folate levels
Plasma total homocysteine, amino acids, lactate, urate
Urinary organic acids
Urinary orotic acid
Urine amino acids

Results from these initial investigations will determine further testing – e.g., Schilling test, folate challenge, mutation analysis, specific enzyme, or complementation studies

Table 34.7 Sideroblastic anemias

Disorder	Gene	Inheritance	Diagnostic tests
Pyridoxine-responsive X-linked SA (XLSA)	*ALAS2*	XLR	Response to high-dose pyridoxine treatment, mutation analysis
Pyridoxine- refractory SA	*SLC25A38*	AR	Mutation analysis
Pearson marrow-pancreas syndrome[a]	*mt*DNA	Maternal (sporadic)	Plasma immunoreactive trypsin (IRT), analysis of mtDNA for deletions
Mitochondrial myopathy, lactic acidosis and SA: MLASA type 1 MLASA type 2	 *PUS1* *YARS2*	 AR AR	Mutation analysis
Anemia, sideroblastic with spinocerebellar ataxia (ASAT)	*ABC7*	XLR	Mutation analysis
SA, B cell immunodeficiency, fever, and + dev delay (SIFD)	*TRNTI*	AR	Mutation analysis

[a]Pancreatic insufficiency often precedes development of anemia

and SLC25A38, respectively. Syndromic sideroblastic anemias include Pearson syndrome and MLASA. Disease-causing mutations are still not identified in many patients with SA suggesting there are as yet unidentified genes that cause SA.

34.6 Cytopenias

Decreased numbers of the formed elements of blood may occur as a result of defects in production or increased destruction. Pancytopenia is an almost constant finding with propionate intoxication, a feature of some of the organic acidurias during acute decompensation which is accompanied by metabolic derangement including high anion gap metabolic acidosis, hyperammonemia and hypoglycemia. Urine organic acid analysis will be key to achieving a diagnosis. The mechanism is not understood, and there is no specific treatment other than restoration of metabolic control in these disorders. During periods of good metabolic control, the hematological picture generally normalizes.

Neutropenia is a chronic finding seen in glycogen storage disease (GSD) 1b/c; patients often develop inflammatory bowel disease later in the course of the disease. The neutropenia responds to granulocyte colony-stimulating factor (G-CSF) treatment. The neutropenia of Barth syndrome (X-linked recessive inheritance) may be mild and may have a cyclical pattern sometimes hinted at by intermittent mouth ulcers. Congenital neutropenia caused by glucose-6-phosphatse, catalytic, 3 (G6PC3) mutations (Dursun syndrome) is usually syndromic and associated with prominent superficial veins or varicosities and congenital cardiac disease but may be isolated.

In some storage disorders, such as Gaucher disease and Niemann-Pick disease, bone marrow infiltration may lead to pancytopenia/neutropenia.

Pancytopenia (Table 34.8)
Neutropenia (Table 34.9)
Thrombocytopenia (Table 34.10)

34.7 Bleeding Tendency

Coagulopathy may be seen in any metabolic disorder associated with severe liver disease, such as galactosemia, fructosemia, tyrosinemia type I, and neonatal hemochromatosis. In untreated classical galactosemia, the coagulopathy often seems disproportionately severe (for the degree of liver dysfunction). This may be due in part to secondary glycosylation abnormalities. With supportive treatment and elimination of galactose from the diet, the coagulopathy and liver disease usually resolve within days.

In congenital disorders of glycosylation, coagulopathy is seen due to deficient coagulation factors (especially XI). Bleeding tendency associated with (hepato)splenomegaly, but without significant hepatic dysfunction, is found in Gaucher disease type 1 and glycogenosis type Ia and Ib/c.

34.8 Hypercoagulability

The classic disorder associated with hypercoagulable states is classical homocystinuria due to CBS deficiency. It has been shown that good metabolic control through dietary and medical intervention reduces the number of vascular events in classical homocystinuria. Some of the congenital disorders of glycosylation may also be associated with hypercoagulable states, likely due to deficiencies in coagulation inhibitors.

34.9 Hyperleukocytosis

Hyperleukocytosis >100,000/mm^3 has been described in SLC35C1-CDG, formerly called CDG IIc (GDP-fucose transporter 1) or leukocyte adhesion deficiency syndrome.

34.10 Hemophagocytosis

Hemophagocytosis is encountered in occasional patients with carnitine palmitoyltransferase I deficiency, propionic aciduria, cobalamin C deficiency, hemochromatosis, lysinuric protein intolerance, Gaucher disease, and Niemann-Pick disease.

Table 34.8 Pancytopenia

Associated finding	Disorder	Diagnostic tests
Splenomegaly	Gaucher disease types 1 and 3	Leukocyte or fibroblast β-glucosidase, *GBA* mutation analysis
	Niemann-Pick disease type B	Leukocyte or fibroblast acid sphingomyelinase, *SMPD1* mutation analysis
+ Neurological abnormalities	Gaucher disease type 3	Leukocyte or fibroblast β-glucosidase, *GBA* mutation analysis
	Niemann-Pick disease type A	Leukocyte or fibroblast acid sphingomyelinase, *SMPD1*mutation analysis
Ketoacidosis, hyperammonemia, hypotonia	PA, MMA, IVA	Urinary organic acids, acylcarnitine profile
Failure to thrive, diarrhea	Folate malabsorption	Plasma and red cell folate, folate challenge test. *SLC 46A1* mutation analysis
	Transcobalamin II deficiency	Plasma transcobalamin II level, plasma vitamin B_{12} (normal), *TCN2* mutation analysis

Table 34.9 Neutropenia

Associated findings	Disorder	Diagnostic tests
Hepatomegaly, hypoglycemia, short stature, inflammatory bowel disease (late)	GSD 1b	Glucose challenge. *G6PT* mutation analysis
Hyperammonemia, failure to thrive, interstitial pneumonia, diarrhea	Lysinuric protein intolerance	Plasma and urine amino acids *SLC7A7* mutation analysis
Ketoacidosis, hypotonia, failure to thrive, developmental delay	PA, MMA, IVA	Urinary organic acids, acylcarnitine profile
Cardiomyopathy and skeletal myopathy, short stature in a male	Barth syndrome	Urinary organic acids (elevated 3 methylglutaconate excretion common but not universal), cardiolipin electrophoresis, *TAZ* mutation analysis
Macrocytic anemia, developmental delay, failure to thrive	Orotic aciduria	Plasma urate, urinary orotic acid, UMPS enzyme assay or *UMPS* mutation analysis
Coarse facies, developmental delay, hepatomegaly, skeletal abnormalities	Aspartylglucosaminuria	Urine oligosaccharides, enzyme studies, *AGA* mutation analysis

Table 34.10 Thrombocytopenia

Associated finding	Disorder	Diagnostic tests
Splenomegaly	Gaucher disease type 1	Leukocyte or fibroblast β-glucosidase, *GBA* mutation analysis
	Niemann-Pick disease type B	Leukocyte or fibroblast acid sphingomyelinase, *SMPD1* mutation analysis
Ketoacidosis, hypotonia, failure to thrive, developmental delay	MMA, PA, IVA	Urinary organic acids, acylcarnitine profile
Cardiomyopathy, ketoacidosis, macrocytic anemia	Cobalamin defects	Plasma homocysteine, amino acids, urinary organic acids, plasma cobalamin, complementation studies on cultured skin fibroblasts, specific gene mutation analysis
Lactic acidosis, hypotonia, dermatosis, alopecia	Holocarboxylase synthetase deficiency	Plasma lactate, ammonia, urinary organic acids, enzyme assay on fibroblasts. *HLCS* mutation analysis

34.11 Methemoglobinemia

Methemoglobinemia is the result of the failure of reduction of oxidized circulating hemoglobin, either as the result of exposure to strong oxidizing agents, such as nitrites, or failure of the normal enzymatic reduction of heme. Congenital methemoglobinemia may occur as a result of deficiency of NADH-cytochrome b5 reductase that is limited to red cells (type I) or a generalized deficiency of the enzyme (type II); both are caused by recessive mutations of the *CYB5R3* gene. In type I, the only clinical abnormality is chronic persistent apparent cyanosis resulting from methemoglobin accumulation; in type II, the methemoglobinemia is associated with a chronic progressive encephalopathy, the mechanism for which is not fully elucidated. Autosomal dominant methemoglobinemia is caused by mutations in variations in the hemoglobin A *(HBA1)* or hemoglobin B *(HBB)* genes.

General Suggestions for Reading

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Immunological Problems

35

Ertan Mayatepek

Key Facts

- Patients with inherited metabolic diseases may have immunological problems resulting in increased susceptibility to infections.
- In most metabolic defects, immunological abnormalities are secondary to the metabolic derangement. Immune dysfunction can affect any of the major components of the immune system: T cells, B cells (including immunoglobulins), and phagocytes, e.g., neutrophils, monocytes, natural killer (NK) cells, and complement.
- Inherited metabolic diseases associated with T cell immunodeficiency mainly feature purine nucleoside phosphorylase deficiency. This disease is characterized by severe viral, bacterial, or fungal infections. Milder impaired T cell function is sometimes seen in lysinuric protein intolerance, Menkes disease, or Zellweger syndrome.
- B cell immunodeficiency has been observed in patients with transcobalamin II deficiency or propionic academia.
- Adenosine deaminase deficiency represents the best characterized inherited metabolic disease with combined T and B cell immunodeficiencies. Recurrent infections caused by a broad variety of microorganisms often become life threatening. Hematopoietic stem cell transplantation is considered the treatment of choice.
- Immunological dysfunction affecting both the B and T cell lines has been reported in acrodermatitis enteropathica, biotinidase and holocarboxylase synthetase deficiencies, hereditary orotic aciduria, deficiency of intestinal folic acid absorption, phosphoglucomutase 3 deficiency, methylenetetrahy-

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drofolate dehydrogenase deficiency, and α-mannosidosis and in some patients with methylmalonic aciduria.

- Phagocytic dysfunction and neutropenia are characteristic findings in glycogen storage disease type I non-a. Dysfunction of phagocytes or neutropenia is also frequently observed in classical galactosemia, X-linked cardioskeletal myopathy (Barth syndrome), glucose-6-phosphatase catalytic subunit 3 deficiency, congenital disorder of glycosylation type IIc, glutathione synthetase deficiency, or Pearson syndrome.
- NK cell immunodeficiency is very rarely associated with inborn errors of metabolism. Besides adenylate kinase 2 deficiency, this immunological dysfunction has been reported in single cases in lysinuric protein intolerance.

Table 35.1 Diagnostic tests in patients with a known metabolic disorder and frequent infection

History
Physical examination
Differential blood count
Sedimentation rate
Urine analysis
Chest X-ray
Immune workup
B cell disorders
Quantitative immunoglobulins
Isohemagglutinin
Specific antibody titers
T cell disorders
Skin tests for tetanus, mumps, and monilia (>3 years of age)
Lymphocyte count/differentiation
Lymphocyte stimulation
Thymus on X-ray studies
Phagocyte disorders
Granulocyte count
Nitro blue tetrazolium test
Neutrophil function tests

35.1 General Remarks

A wide range of immune defects has been identified in association with inherited metabolic diseases, sometimes in single case reports, and in others as a constant manifestation of the defect. In clinical practice, two situations arise: (1) the patient presents with a known metabolic disorder and immunological problems appear as recognized manifestations of the disease; or (2) the patient presents with immunological problems and a metabolic disorder is sought. Some metabolic defects lead to symptoms such as chronic or recurrent infection or infection with unusual agents. An example of an inherited metabolic disease linked to an inflammatory periodic fever syndrome is *mevalonic aciduria*. Mutations and consecutively reduced activity of mevalonate kinase have been found in two different clinical pictures, mevalonic aciduria as well as in hyper-IgD and periodic fever syndrome (HIDS), the less severe form. Besides congenital malformations, hepatosplenomegaly, failure to thrive, developmental retardation, and others, the clinical course of mevalonic aciduria which is more severe than HIDS is also characterized by recurrent crises of fever, vomiting, and diarrhea, suggesting an infectious or autoimmune disease.

Clinically significant immunodeficiency presents with both an unusual history of infection and corresponding confirmatory laboratory tests. Other patients may have milder manifestations or sometimes only laboratory evidence of immunological abnormalities. One or several major components of the immune system can be affected in distinctive metabolic diseases, single or combined T or B cell immunodeficiencies, phagocyte immunodeficiency, or natural killer (NK) cell immunodeficiency (Tables 35.1 and 35.2).

35.2 Inherited Metabolic Diseases Associated with T Cell Immunodeficiency

Purine nucleoside phosphorylase is required for normal catabolism of purines. In *purine nucleoside phosphorylase deficiency*, substrates accumulate which affect the immune and nervous

Table 35.2 Investigations for differential diagnosis of inherited metabolic diseases in patients with immunological problems

Urine
Purines and pyrimidines
Organic acids
Amino acids
Orotic acid
Oligosaccharides
Plasma/serum
Lactate
Copper
Zinc
Very long-chain fatty acids
Folic acid
Transcobalamin II
Enzyme studies
Galactose-1-phosphate uridyl transferase
Biotinidase
Lysosomal enzymes
Mutational analysis (e.g., GSD type I non-a or Pearson syndrome)

systems. The disease is the most important metabolic disease associated with clinically relevant isolated T cell immunodeficiency resulting in severe immunodeficiency. The number of T cells is greatly reduced leading to lymphopenia and cutaneous anergy. Recurrent infections are usually obvious at least by the end of the first year. There is an enhanced susceptibility to viral diseases, such as varicella, measles, cytomegalovirus, and vaccinia. Severe pyogenic or fungal infections also occur. T cell dysfunction may worsen in the course of the disease. There is a great heterogeneity, and affected patients may have autoantibodies, autoimmune hemolytic anemia, failure to thrive, and malignancies. Neurological symptoms include abnormal motor development, ataxia, and spasticity. Hematopoietic stem cell transplantation is the treatment of choice. However, neurological symptoms often progress.

Lysinuric protein intolerance is characterized by defective transport of the dibasic amino acids lysine, arginine, and ornithine in the intestine and renal tubules. Biochemically this defect results in decreased levels of these amino acids in plasma and increased levels in urine interfering with the urea cycle and consecutively hyperammonemia. Intestinal protein intolerance, failure to thrive, hepatosplenomegaly, and osteoporosis as well as progressive encephalopathy develop. Pulmonary involvement is the most threatening complication. Nephropathy may lead to end-stage renal disease. The development of hemophagocytic lymphohistiocytosis (HLH) is one further major complication. Moreover, patients may display autoimmune features, e.g., systemic lupus erythematosus or other vasculitides. Decrease in $CD4^+$ T cell number, lymphopenia, and leukopenia have been reported, as well as decreased leukocyte phagocytic activity. As in purine nucleoside phosphorylase deficiency, varicella infection may be characterized by an especially severe clinical course.

Impaired T cell function has been also described in *Menkes disease*. This disorder is caused by a defect in a membrane copper transport channel which interferes with the absorption of copper from food and its distribution to the cells, resulting in generalized copper deficiency. Clinical symptoms of classical Menkes disease include neonatal hypothermia, unconjugated hyperbilirubinemia, mental retardation, seizures, typical facies, "kinky" hair, and abnormalities of connective tissue and bone. Immunological problems or infections are rare, but pulmonary infection secondary to inhalation may prove lethal.

Thymic hypoplasia and defective T cell function have been noted in some patients with *Zellweger syndrome*, the most severe of the disorders of peroxisome biogenesis. Affected infants exhibit extreme muscular hypotonia, seizures, liver dysfunction, dysmorphic skeletal and eye abnormalities, failure to thrive, and mostly early death due to the progressive encephalopathy. Immunological problems are not usually of high clinical relevance.

35.3 Inherited Metabolic Diseases Associated with B Cell Immunodeficiency

Transcobalamin II is necessary for intestinal absorption of cobalamin and its transport to tissues. *Deficiency of transcobalamin II* leads to severe

megaloblastic anemia with hypocellular bone marrow, leukopenia, thrombocytopenia, vomiting, failure to thrive, diarrhea, and lethargy. A frequent finding in transcobalamin II deficiency is the presence of hypogammaglobulinemia, particularly of IgG. Less often, levels of IgA and IgM are found decreased. Failure to produce specific antibodies against diphteria or poliomyelitis has been found in several patients. Although phagocytic killing is usually normal, a specific impairment of neutrophils against *Staphylococcus aureus* has been reported in a single patient. Immunological abnormalities usually resolve after cobalamin supplementation.

Propionic aciduria is caused by deficiency of propionyl-CoA carboxylase, a biotin-dependent enzyme. In the long-term mental retardation, an extrapyramidal movement disorder and osteoporosis develop in most patients. Decreased levels of IgG and IgM as well as B cell lymphopenia have been observed during periods of metabolic decompensation.

35.4 Inherited Metabolic Diseases Associated with Combined T and B Cell Immunodeficiency

Adenosine deaminase (*ADA1*) *deficiency* represents the best characterized metabolic disease leading to combined immunodeficiency. ADA1 deficiency accounts for up to 50 % of the patients with autosomal recessive severe combined immunodeficiency (SCID) disease. ADA1 converts adenosine and deoxyadenosine to inosine and deoxyinosine, respectively. The resulting accumulation of deoxyadenosine and deoxyadenosine triphosphate exerts toxicity on lymphocyte development and function. Obviously, the severity of the disease correlates with accumulation of toxic metabolites and inversely with residual adenosine deaminase expression. Multiple, recurrent infections are usually more severe than in purine nucleoside phosphorylase deficiency and become rapidly life threatening. Typical laboratory findings include lymphopenia (usually less than 500 total lymphocytes per cubic millimeter), involving both B and T cells as well as hypogammaglobulinemia. While IgM deficiency may be detected early, IgG deficiency manifests only after the age of 3 months, when the maternal supply becomes exhausted. Further immunologic abnormalities include a deficiency of antibody formation following specific immunization and absence or severe diminution of lymphocyte proliferation induced by mitogens. This condition is progressive, since residual B and T cell function that may be found at birth disappears later on. Only a few patients have been reported with delayed (up to 3 years of age) or late (up to 8 years of age) onset. Infections can be caused by a broad variety of microorganisms. Localization of infections is predominantly the skin and the respiratory as well as the gastrointestinal tract, where they often lead to intractable diarrhea and malnutrition. In patients older than 6 months of age, hypoplasia or apparent absence of lymphoid tissue may constitute a diagnostic sign. In about half of the patients, bony abnormalities include prominence of the costochondral junctions. In some patients most often with increasing age, neurologic abnormalities are found, including spasticity, head lag, movement disorders, learning disability, cognitive deficit, hyperactivity, and nystagmus. Hepatic dysfunction is reported in some patients. Hematopoietic stem cell transplantation is considered the treatment of choice. Otherwise, injections of bovine adenosine deaminase conjugated to polyethylene glycol (PEG-ADA) resulting in normalization of T cell number and many cellular and humoral responses as well as gene therapy are other possibilities.

Recently, autosomal recessive ADA2 deficiency has been reported as the cause of a devastating autoinflammatory syndrome. Main clinical feature is a vasculopathy involving the skin and internal organs, e.g., brain, kidneys, and intestine. In some patients polyarteritis nodosa and early stroke are leading symptoms. Affected patients have fewer memory B cells in the peripheral blood and a slight reduction in the terminal differentiation of B cells after T cell stimulation.

Enzyme replacement and bone marrow reconstitution might be considered.

The disturbance of zinc homeostasis in *acrodermatitis enteropathica* results from a partial block in intestinal absorption. Reduced zinc absorption leads to impairment of the function of many zinc metalloenzymes, which are involved in major metabolic pathways. Symptoms usually start in infancy. The most dramatic clinical feature is a characteristic skin rash. In patients with zinc deficiency states, impaired humoral and cell-mediated immune responses can be usually demonstrated. Secondary infections are common, mostly with *Candida* or *Staphylococci*. Mucosal lesions include gingivitis, stomatitis, and glossitis. Further symptoms include diarrhea, failure to thrive, alopecia, irritability, and mood changes. Zinc therapy leads to clinical remission.

Biotin is a cofactor for carboxylation of 3-methylcrotonyl-CoA, propionyl-CoA, acetyl-CoA, and pyruvate. *Biotinidase deficiency* and *holocarboxylase synthetase deficiency* result in multiple carboxylase deficiency. Symptoms include lactic acidosis, muscular hypotonia, seizures, ataxia, psychomotor retardation, skin rashes, hair loss, and immune defects. Immunologic dysfunction affecting both the B and T cell lines has been reported in several children with biotinidase deficiency. Symptoms include mucocutaneous candidiasis, absence of delayed hypersensitivity as assessed by skin test and by in vitro lymphocyte responses to *Candida* challenge, decreased IgA levels, poor antibody formation to pneumococcal immunization, subnormal amounts of T lymphocytes, reduced leukocyte killing against *Candida*, lack of myeloperoxidase activity in neutrophils, impaired lymphocyte suppressor activity, and decreased prostaglandin E_2 production in vitro. One child with biotinidase deficiency was diagnosed initially as having SCID and was treated with bone marrow transplantation, but the symptoms were not ameliorated until biotin was given. In general, immunological findings in biotinidase deficiency are inconsistent. However, biotin treatment corrects the immunologic as well as the metabolic abnormalities.

Hereditary orotic aciduria is an inborn error of pyrimidine metabolism characterized by growth retardation, developmental delay, and megaloblastic anemia unresponsive to cobalamin and folic acid. Lymphopenia and increased susceptibility to infections, including candidiasis, bacterial meningitis, and fatal varicella, have been observed. Immunologic abnormalities are variable and include low T cell number, impaired delayed-type hypersensitivity response, reduced T cell-mediated killing, and decreased levels of IgG and IgA.

Deficiency of hereditary folate absorption leads to megaloblastic anemia, recurrent chronic diarrhea, psychomotor retardation, seizures, and ataxia. Recurrent infections are an occasional clinical feature. Inconstantly found immunologic abnormalities include decreased levels of IgM, IgG, and IgA as well as decreased proliferation to phytohemagglutinin and pokeweed mitogen (PWM).

Methylenetetrahydrofolate dehydrogenase (*MTHFD*) *deficiency*, another inborn error of folate metabolism, can also present with symptoms including megaloblastic anemia or neurological problems as well as life-threatening infections due to severe immunological defects that may mimic SCID. Affected patients are highly susceptible to *P. jiroveci* infections.

Phosphoglucomutase 3 (*PGM3*) *deficiency* belongs to the group of congenital disorders of glycosylation. It is characterized by neurological symptoms including developmental delay, ataxia, dysarthria, or seizures. Prominent immunologic features include severe atrophy, hyper-IgE phenotype, immunodeficiency, and autoimmunity. Lymphopenia is predominantly caused by low $CD8^+$ T cells and $CD27^+$ B cells. Serum levels of IgE, IgG, and IgA as well as specific antibodies to protein and polysaccharide antigens are markedly elevated.

α-Mannosidase caused by deficiency of α-mannosidase leads to the accumulation of mannose-rich oligosaccharides in neural and visceral tissues. This lysosomal storage disease is

characterized by progressive mental retardation, deafness, cataracts, corneal clouding, dysostosis multiplex, progressive ataxia, hernias, and hepatomegaly. Many patients with α-mannosidosis have recurrent infections. Immunologic abnormalities may include decreased IgG levels, impaired lymphoproliferation to phytohemagglutinin, defective chemotaxis, phagocytosis, and bactericidal killing. In one patient pancytopenia resulting from antineutrophil antibodies has been reported.

Leukopenia occurs in about 50 % of patients with *methylmalonic aciduria*, a classical organic aciduria affecting branched-chain amino acid metabolism. Immunologic abnormalities associated with methylmalonic aciduria may include neutropenia, pancytopenia, decreased B and T cell numbers, low IgG levels, and impaired phagocyte chemotaxis. Specific lack of responsiveness to Candida antigen has been observed resulting in extensive dermatosis. In addition, methylmalonic acid inhibits bone marrow stem cell growth in vitro.

35.5 Inherited Metabolic Diseases Associated with Phagocyte Immunodeficiency

Galactosemia results from galactose-1-phosphate uridyl transferase deficiency and is characterized by jaundice, hepatomegaly, nuclear cataracts, mental disability, and feeding difficulties. Neonates with galactosemia are at increased risk for life-threatening sepsis from *Escherichia coli*. Granulocyte chemotaxis is impaired, whereas bactericidal activity is usually not affected. In vitro exposure of neutrophils from affected neonates to galactose results in impaired function.

Glycogen storage diseases (*GSD*) *type I non-a* presents with hepatomegaly, hypoglycemia acidosis, and growth failure. The conditions are clearly distinguished from GSD type Ia by the occurrence of recurrent severe bacterial infections and immunologic abnormalities, resulting from neutropenia and defective leukocyte function. Neutrophil function is variable, in most patients random movement, chemotaxis, microbial killing, and respiratory burst are diminished. In contrast, monocytes have decreased respiratory burst but usually have normal random and directed motility. T cell, B cell, and NK cell functions are normal. In most patients, recurrent or chronic bacterial infections represent a major clinical problem. These infections are underlined by a decreased number of neutrophils (usually below 1,500 per microliter) combined with defective neutrophil and monocyte functions. Bone marrow examination shows hypercellularity. GSD type I non-a is caused by mutations in the glucose-6-phosphate translocase gene. This gene encodes a microsomal transmembrane protein that is expressed in numerous tissues, including monocytes and neutrophils. Frequently observed symptoms, such as inflammatory bowel disease similar to Crohn disease, oral lesions, and perianal abscesses, are presumably related to defective neutrophil function. Neutrophil cell counts, and some but not all neutrophil functions improve after subcutaneous treatment with granulocyte colony-stimulating factor (G-CSF).

In *glucose-6-phosphatase catalytic subunit 3* (*G6PC3*) *deficiency*, there are no signs of glycogen storage or metabolic disorders. However, congenital neutropenia, intermittent or persistent thrombocytopenia, congenital heart defects, and congenital malformations are associated features.

Defects like *GDP-fucose transporter deficiency* or *congenital disorder of glycosylation type IIc* result in immune defects known as leukocyte adhesion deficiency (LAD) disorders characterized by leukocyte rolling defects. Patients suffer from recurrent infections mostly starting in infancy and associated with neutrophilia. Moreover, they display facial dysmorphism and severe psychomotor retardation. Orally fucose might have positive effects in some patients.

X-linked cardioskeletal myopathy (*Barth syndrome*) is characterized by a congenital cardiomyopathy, mitochondrial myopathy, growth failure, as well as exercise intolerance. In most boys moderate to severe neutropenia is an intermittent feature leading to recurrent serious bacterial infections.

Glutathione synthetase deficiency causes severe metabolic acidosis and hemolytic anemia. In the course of the disease, progressive neurological symptoms may develop, including psychomotor retardation, seizures, ataxia, and spasticity. In about 10 % of patients, recurrent bacterial infections have been reported resulting from impaired bacterial killing. The patient's neutrophils fail to assemble microtubules during phagocytosis leading to damage to membranous structures. However, the susceptibility to these infections is relatively mild. Treatment with vitamins E and C can restore abnormal immunologic functions.

Pearson syndrome is caused by large deletions and duplications in the mitochondrial DNA and characterized by exocrine pancreatic and liver dysfunction, failure to thrive, renal tubular defects, chronic diarrhea, lactic acidosis, as well as neuromyopathy. In addition to sideroblastic anemia and thrombocytopenia, neutropenia is a frequent finding.

In *isovaleric aciduria*, as in propionic aciduria or methylmalonic aciduria, neutropenia and pancytopenia can develop during periods of acidosis. Neonatal sepsis can lead to early death. The bone marrow contains large numbers of immature cells, suggesting an arrest of maturation. The underlying mechanism, however, is not clear but probably related to the accumulation of CoA esters of organic acids.

Other inborn errors of metabolism including *acrodermatitis enteropathica*, *methylmalonic aciduria*, *propionic aciduria*, *Gaucher disease*, *lysinuric protein intolerance*, *Niemann-Pick disease*, or *α-mannosidosis* are at least in some patients associated with phagocyte dysfunction or macrophage activating syndrome.

35.6 Inherited Metabolic Diseases Associated with NK Cell Immunodeficiency

Lysinuric protein intolerance is one of the very few inborn errors of metabolism in whom NK cell immunodeficiency has been reported at least in single instances. Other syndromes such as the Chediak-Higashi syndrome, Sutor syndrome, Griscelli syndrome, or xeroderma pigmentosum form this specific subgroup of immunodeficiency disorders.

In addition, *adenylate kinase 2 deficiency* is characterized by absence of NK cells as well as of neutrophils and T cells and sometimes also with low B cell counts. Moreover, affected patients present with anemia, thrombocytopenia, as well as clinically sensorineural hearing loss.

Take-Home Messages

- Patients with inherited metabolic diseases may have immunological problems resulting in increased susceptibility to infections. In addition, infections may be secondary to chronic disease, malnutrition, movement disorders, or poor control of swallowing and resulting aspiration. In most metabolic defects, immunological abnormalities are secondary to the metabolic derangement. Immune dysfunction can affect any of the major components of the immune system: T cells, B cells (including immunoglobulins), phagocytes, e.g., neutrophils, monocytes, natural killer (NK) cells, and complement.
- Primary metabolic immunodeficiency diseases include adenosine deaminase deficiency or purine nucleoside phosphorylase deficiency. Other inherited diseases with often compromised immunity include, beside others,

inborn errors of cobalamin and folate metabolism, organic acidurias, and disorders of carbohydrate metabolism. In most of them, correction of the metabolic defect restores normal immune function.

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Part IV

Investigations for Metabolic Diseases

Newborn Screening for Inherited Metabolic Disease

36

Dietrich Matern and Piero Rinaldo

Routine screening of all newborns for inherited disorders began in the 1960s after Horst Bickel had established an effective dietary therapy for phenylketonuria (PKU) and Robert Guthrie introduced a simple bacterial inhibition assay to detect elevated concentrations of phenylalanine in dried blood spots, a type of specimen universally known as the "Guthrie card." Over time newborn screening was extended to several other treatable metabolic and endocrine disorders including galactosemia, maple syrup urine disease, biotinidase deficiency, hypothyroidism, congenital adrenal hyperplasia, and hemoglobinopathies. Beginning in the late 1990s, the scope of newborn screening has leaped forward exponentially by the introduction of acylcarnitine and amino acid analysis in Guthrie cards by tandem mass spectrometry (MS/MS). This technology has been used in biochemical genetics laboratories for the detection and analysis of acylcarnitines since the 1980s and has become amenable to newborn screening because of the development of automated, high-throughput techniques of sample preparation (typically to butyl esters derivatives) and injection into the instrument, as well as simultaneous quantitative analysis of amino acids and acylcarnitines under 2 min per sample. Amino acids are the building blocks of proteins. Acylcarnitines are formed from free carnitine and acyl-CoA moieties derived from fatty acids and organic acids (which may have been derived from amino acids) through the action of one of several carnitine-acyl-CoA transferases. This multiplex platform therefore enables the concurrent detection of disorders of amino acid, fatty acid, and organic acid intermediary metabolism and thus several of the most prevalent treatable inborn errors of metabolism. The concentrations of amino acids and acylcarnitine species fluctuate in response to many possible metabolic defects leading to complex profiles with more than 60 informative markers that require various degrees of differential diagnosis. In other words, the significant progress achieved at the analytical level has no remedy, or substitute, for the inevitable complexity of post-analytical interpretation. Additional MS/MS applications have been proposed for newborn screening, most notably several enzyme assays for lysosomal storage disorders, and lysophosphatidylcholines for X-adrenoleukodystrophy.

MS/MS has also pushed many countries to formulate more clearly which conditions every newborn should be screened for. For example, in the USA, the Secretary of Health and Human Services regularly updates the "Recommended Uniform Screening Panel" (RUSP) which currently includes 34 primary targets and more than 25 secondary targets (Table 36.1). The secondary targets, including 22 identified by acylcarnitine

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Table 36.1 Summary of the conditions that can be included in newborn screening programs because relevant assays have been described and tested at least in several pilot studies

	Conditions			Name of deficient enzyme or function	Assay (primary marker[c])	Likelihood of identification by NBS using cutoffs based on disease ranges	Detection of carriers by NBS
Group	MIM number	Common name	RUSP code[b]				
AA	**207900**	**Argininosuccinic acidemia**	**ASA**	**Argininosuccinate lyase**	**MS/MS (Asa)**	**Low if Asa not measured**	–
AA	**215700**	**Citrullinemia type I**	**CIT**	**Argininosuccinate synthetase**	**MS/MS (Cit)**	**High**	–
AA	**236200**	**Homocystinuria**	**HCY**	**Cystathionine beta-synthase**	**MS/MS (Met)**	**High (with second-tier test)**	
AA	**248600**	**Maple syrup urine disease**	**MSUD**	**Branched-chain alpha-keto acid dehydrogenase**	**MS/MS (Leu/Ile/Val)**	**High (with second-tier test)**	–
AA	**261600**	**Phenylketonuria (classic)**	**PKU**	**Phenylalanine hydroxylase**	**MS/MS (Phe)**	**High**	**Possible (maternal cases)**
AA	**276700**	**Tyrosinemia type I**	**TYR I**	**Fumarylacetoacetate hydrolase**	**MS/MS (SUAC)**	**Low if SUAC not measured**	**–**
AA	*207800*	*Argininemia*	*ARG*	*Arginase*	*MS/MS (Arg)*	*High*	–
AA	*605814 603472*	*Citrullinemia type II (neonatal/adult)*	*CIT*	*Aspartate glutamate carrier (citrin)*	*MS/MS (Cit)*	*Unknown but likely to be low*	–
AA	*600225 261640*	*Disorders of biopterin biosynthesis*	*BIOPT (BS)*	*GTP cyclohydrolase, 6-pyruvoyl-tetrahydropterin synthase*	*MS/MS (Phe)*	*High*	–
AA	*126090 261630*	*Disorders of biopterin regeneration*	*BIOPT (REG)*	*Dihydropteridine reductase, pterin-4α-carbinolamine dehydratase*	*MS/MS (Phe)*	*High*	–
AA	*180960 250850 606664*	*Hypermethioninemias*	*MET*	*Methionine adenosyltransferase I/III, S-adenosylhomocysteine hydrolase, glycine N-methyltransferase*	*MS/MS (Met)*	*High*	–
AA	*261600*	*Hyperphenylalaninemia*	*H-PHE*	*Phenylalanine hydroxylase*	*MS/MS (Phe)*	*Low if cutoff >150 µmol/L*	–

AA	*276600*	*Tyrosinemia type II*	*TYR II*	*Tyrosine transaminase*	*MS/MS (Tyr)*	*High*	–
AA	*276710*	*Tyrosinemia type III*	*TYR III*	*4-Hydroxyphenyl pyruvate acid oxidase (dioxygenase)*	*MS/MS (Tyr)*	*Unknown but likely to be high*	–
AA	614923	Branched-chain ketoacid dehydrogenase kinase deficiency	(BCKDK)	Branched-chain ketoacid dehydrogenase kinase	MS/MS (Leu/Ile/Val low)	Uncertain	–
AA	237300	Carbamoyl phosphate synthetase deficiency	(CPS)	Carbamoyl phosphate synthetase	MS/MS (Cit low)	Unknown but likely to be low	–
AA	605899	Glycine encephalopathy (GCE; nonketotic hyperglycinemia, NKH)	(NKH)	Mitochondrial glycine cleavage system	MS/MS (Gly)	Low	–
AA	258870	Gyrate atrophy of the choroid and retina (hyperornithinemia)	(H-ORN)	Ornithine aminotransferase	MS/MS (Orn)	Low	–
AA	236250	Homocystinuria due to MTHFR deficiency	(MTHFR)	5,10-Methylenetetrahydrofolate reductase	MS/MS (Met)	High (with second-tier test)	–
AA	237000	Hydroxyprolinemia (of no clinical significance)	(OH-Pro)	Hydroxy-L-proline oxidase	MS/MS (OH-Pro)	Unknown but likely to be high (with second-tier test)	–
AA	238700	Hyperlysinemia (of no clinical significance)	(H-LYS)	Alpha-aminoadipic semialdehyde synthase	MS/MS (Lys)	Low	–
AA	238970	Hyperornithinemia-hyperammonemia-homocitrullinuria (HHH) syndrome	(HHH)	Mitochondrial ornithine transporter (SLC25A15)	MS/MS (Orn)	Low	–
AA	239500	Hyperprolinemia type I	(HP-I)	Proline dehydrogenase (oxidase)	MS/MS (Pro)	Low	–
AA	239510	Hyperprolinemia type II	(HP-II)	Pyrroline-5-carboxylate dehydrogenase	MS/MS (Pro)	Low	–
AA	277100	Hypervalinemia (of no clinical significance)	(H-VAL)	Valine transaminase	MS/MS (Val)	Uncertain	–
AA	311250	Ornithine transcarbamylase (OTC) deficiency	(OTC)	Ornithine carbamoyltransferase	MS/MS (Cit low)	Unknown but likely to be high	–
AA	260005 266130	Oxoprolinuria	(OXO-PRO)	5-Oxoprolinase, glutathione synthetase	MS/MS (pyroglutamic acid)	Low	–

(continued)

Table 36.1 (continued)

	Conditions			Name of deficient enzyme or function	Assay (primary marker[c])	Likelihood of identification by NBS using cutoffs based on disease ranges	Detection of carriers by NBS
Group	MIM number	Common name	RUSP code[b]				
Bedside[a]	**–**	**Critical congenital heart disease**	**CCHD**	**Heart disease**	**Pulse oximetry**	**High**	–
Bedside[a]	**–**	**Hearing loss**	**HEAR**	**Hearing loss**	**Otoacoustic emissions**	**High**	–
CF	**219700**	**Cystic fibrosis**	**CF**	**Epithelial chloride efflux**	**Immunoassay (IRT)**	**High**	**Yes**
Endo	**201910 202010**	**Congenital adrenal hyperplasia**	**CAH**	**21-Hydroxylase, 11-beta-hydroxylase**	**Immunoassay (17-hydroxy progesterone)**	**High (with second-tier test)**	–
Endo	**–**	**Congenital hypothyroidism**	**CH**	**Thyroid function**	**Immunoassay (T4, TSH)**	**High**	–
FAO	**212140**	**Carnitine uptake defect**	**CUD**	**Plasma membrane carnitine transporter (OCTN2)**	**MS/MS (C0)**	**High**	**Possible (maternal cases)**
FAO	**609016**	**Long-chain 3-OH acyl-CoA dehydrogenase deficiency**	**LCHAD**	**Long-chain L-3-Hydroxy dehydrogenase**	**MS/MS (C16-OH)**	**High**	–
FAO	**607008**	**Medium-chain acyl-CoA dehydrogenase deficiency**	**MCAD**	**Medium-chain acyl-CoA dehydrogenase**	**MS/MS (C8)**	**High**	**Possible**
FAO	**609015**	**Trifunctional protein deficiency**	**TFP**	**Mitochondrial trifunctional protein (α,β subunit)**	**MS/MS (C16-OH)**	**High**	–
FAO	**201475**	**Very long-chain acyl-CoA dehydrogenase deficiency**	**VLCAD**	**Very long-chain acyl-CoA dehydrogenase**	**MS/MS (C14:1)**	**Potential false negatives**	**Possible**
FAO	*222745*	*2,4-Dienoyl reductase deficiency*	*DE-RED*	*2,4-Dienoyl-CoA reductase*	*MS/MS (C10:2)*	*Uncertain*	–
FAO	*255120*	*Carnitine palmitoyltransferase Ia deficiency*	*CPT I*	*Carnitine palmitoyltransferase Ia*	*MS/MS (C0/[16+C18])*	*High*	–
FAO	*255110*	*Carnitine palmitoyltransferase II deficiency*	*CPT II*	*Carnitine palmitoyltransferase II*	*MS/MS (C16, C18:1)*	*High*	–
FAO	*255110*	*Carnitine-acylcarnitine translocase deficiency*	*CACT*	*Carnitine-acylcarnitine translocase*	*MS/MS (C16, C18:1)*	*High*	–

FAO	*130410 231675 608053*	*Glutaric acidemia type II*	*GA II*	*α-ETF, β-ETF, ETF-QO*	*MS/MS (various acylcarnitine species)*	*High*	–
FAO	*602199*	*Medium-chain ketoacyl-CoA dehydrogenase deficiency*	*MCKAT*	*Medium-chain ketoacyl-CoA thiolase*	*MS/MS (C8, C8-OH)*	*Uncertain*	–
FAO	*609975*	*Familial hyperinsulinemic hypoglycemia 4*	*HADH*	*3-Hydroxyacyl-CoA dehydrogenase*	*MS/MS (C4-OH)*	*Uncertain*	–
FAO	*231530*	*3-Hydroxyacyl-CoA dehydrogenase deficiency*	*S/MCHAD*	*3-Hydroxyacyl-CoA dehydrogenase*	*MS/MS (C4-OH)*	*Uncertain*	–
FAO	*201470*	*Short-chain acyl-CoA dehydrogenase deficiency*	*SCAD*	*Short-chain acyl-CoA dehydrogenase*	*MS/MS (C4)*	*High*	–
G6PD	300908	Glucose-6-phosphate dehydrogenase deficiency, favism	(G6PD)	Glucose-6-phosphate dehydrogenase	Coupled fluorometric enzyme assay (G6PD)	High[d]	Possible
GAL	**230400**	**Classic Galactosemia (Galactosemia type I)**	**GALT**	**Galactose-1-phosphate uridylyltransferase**	**Coupled fluorometric enzyme assay (GALT)**	**High[d]**	**Possible**
GAL	*230200*	*Galactosemia type II*	*GALK*	*Galactokinase*	*Coupled fluorometric enzyme assay (total galactose)*	*Potential false negatives[d]*	–
GAL	*230350*	*Galactosemia type III*	*GALE*	*UDP-galactose-4-epimerase*	*Coupled fluorometric enzyme assay (total galactose)*	*Potential false negatives[d]*	–
Hb	**613985**	**Beta-thalassemia**	**βTh**	**Hemoglobin A**	**Electrophoresis, HPLC, MS/MS (hemoglobin)**	**High[d]**	**Yes**
Hb	**603903**	**Hemoglobin SC disease**	**Hb S/C**	**Hemoglobin A**	**Electrophoresis, HPLC, MS/MS (hemoglobin)**	**High[d]**	**Yes**
Hb	**603903**	**S,S disease (sickle-cell anemia)**	**HB SS**	**Hemoglobin A**	**Electrophoresis, HPLC, MS/MS (hemoglobin)**	**High[d]**	**Yes**
Hb	*613978*	*Hemoglobin H disease*	*HB H*	*Hemoglobin Barts*	*Electrophoresis, HPLC, MS/MS (hemoglobin)*	*High[d]*	*Yes*

(continued)

Table 36.1 (continued)

	Conditions			Name of deficient enzyme or function	Assay (primary marker[c])	Likelihood of identification by NBS using cutoffs based on disease ranges	Detection of carriers by NBS
Group	MIM number	Common name	RUSP code[b]				
Hb	*Various*	*Various other hemoglobinopathies*	*Var Hb*	*Hemoglobin A*	*Electrophoresis, HPLC, MS/MS (hemoglobin)*	*High*[d]	*Yes*
ID	–	*Human immunodeficiency virus (HIV)*	*(HIV)*	*Infectious disease*	*ELISA (HIV-1)*	*High*	–
LA	Various	Lactic acidosis	(LA)	Energy metabolism	MS/MS (Ala)	Uncertain	–
LSD	**232300**	**Pompe disease (glycogen storage disease type II, GSD II)**	**GSD II**	**Acid alpha-glucosidase (GAA)**	**Enzyme assay by MS/MS or fluorometry (GAA)**	**High**	**Possible**
LSD	301500	Fabry disease	(GLA)	Alpha-galactosidase A	Enzyme assay by MS/MS or fluorometry (GLA)	High	Possible
LSD	230800 230900 231000	Gaucher disease types I, II, III	(GBA)	Acid beta-glucosidase	Enzyme assay by MS/MS or fluorometry (GBA)	High	Possible
LSD	**607014 607015 607016**	**Hurler/Hurler-Scheie/Scheie diseases (MPS I)**	**MPS I**	**Alpha-L-iduronidase (IDUA)**	**Enzyme assay by MS/MS or fluorometry (IDUA)**	**High**	**Possible**
LSD	245200	Krabbe disease	(GALC)	Galactosylceramidase	Enzyme assay by MS/MS or fluorometry (GALC)	High	Possible
LSD	257200 607616	Niemann-Pick type A, Niemann-Pick type B	(NPA/B)	Acid sphingomyelinase (ASM)	Enzyme assay by MS/MS or fluorometry (ASM)	High	Possible
OA	**253260**	**Biotinidase deficiency**	**BIOT**	**Biotinidase**	**Colorimetric enzyme assay (biotinidase)**	**High**[e]	–
OA	**246450**	**3-Hydroxy 3-methyl glutaric acidemia**	**HMG**	**3-Hydroxy-3-methylglutaryl-CoA lyase**	**MS/MS (C5-OH)**	**High**	–
OA	**210200**	**3-Methyl crotonyl-CoA carboxylase deficiency**	**3MCC**	**3-Methylcrotonyl-CoA carboxylase (α,β subunit)**	**MS/MS (C5-OH)**	**High**	**Possible (maternal cases)**

OA	**231670**	**Glutaric acidemia type I**	**GA I**	**Glutaryl-CoA dehydrogenase**	**MS/MS (C5-DC)**	**Potential false negatives**	**Possible (maternal cases)**
OA	**243500**	**Isovaleric acidemia**	**IVA**	**Isovaleryl-CoA dehydrogenase**	**MS/MS (C5)**	**High**	–
OA	**251100 251110**	**Methylmalonic acidemia (Cbl A, CblB, CblD, var 2)**	**CblA, CblB, CblD variant 2**	**Adenosylcobalamin synthesis**	**MS/MS (C3)**	**High (with second-tier test)**	–
OA	**251000**	**Methylmalonic acidemia (Mut)**	**MUT**	**Methylmalonyl-CoA mutase**	**MS/MS (C3)**	**High (with second-tier test)**	–
OA	**253270**	**Multiple carboxylase deficiency**	**MCD**	**Holocarboxylase synthetase**	**MS/MS (C5-OH)**	**High**	**Possible (maternal cases)**
OA	**606054**	**Propionic acidemia**	**PA**	**Propionyl-CoA carboxylase**	**MS/MS (C3)**	**High (with second-tier test)**	–
OA	**203750**	**β-Ketothiolase deficiency**	**BKT**	**β-Ketothiolase**	**MS/MS (C5:1, C5-OH)**	**High**	–
OA	*300256*	*2-Methyl 3-hydroxybutyryl-CoA dehydrogenase deficiency*	*2M3HBA*	*2-Methyl 3-hydroxybutyryl-CoA dehydrogenase*	*MS/MS (C5:1, C5-OH)*	*Unknown but likely to be high*	–
OA	*600301*	*2-Methyl butyryl-CoA dehydrogenase deficiency*	*2MBG*	*2-Methylbutyryl-CoA dehydrogenase*	*MS/MS (C5)*	*High*	–
OA	*250950*	*3-Methyl glutaconic acidemia*	*3MGA*	*3-Methylglutaconyl-CoA hydratase*	*MS/MS (C5-OH)*	*High*	–
OA	*611283*	*Isobutyryl-CoA dehydrogenase deficiency*	*IBG*	*Isobutyryl-CoA dehydrogenase*	*MS/MS (C4)*	*High*	–
OA	*248360*	*Malonic acidemia*	*MAL*	*Malonyl-CoA decarboxylase*	*MS/MS (C3-DC)*	*High*	–
OA	*277400 611935*	*Methylmalonic acidemia (CblC, CblD, CblF, CblJ)*	*CblC, CblD, CblF, CblJ*	*Cobalamin metabolism*	*MS/MS (C3)*	*High (with second-tier test)*	–
OA	246900	Dihydrolipoamide dehydrogenase (DLD) deficiency, E3 deficiency	(DLD)	Dihydrolipoamide dehydrogenase	MS/MS (Cit)	Uncertain	–
OA	602473	Ethylmalonic encephalopathy	(EE)	Mitochondrial sulfur dioxygenase	MS/MS (C4, C5)	Uncertain	–
OA	229100	Formiminoglutamic aciduria	(FIGLU)	Glutamate formiminotransferase	MS/MS (m/z 287)	Uncertain	–

(continued)

Table 36.1 (continued)

	Conditions			Name of deficient enzyme or function	Assay (primary marker[c])	Likelihood of identification by NBS using cutoffs based on disease ranges	Detection of carriers by NBS
Group	MIM number	Common name	RUSP code[b]				
OA	277410 236270 250940	Methylmalonic acidemia (CblD var1, CblE, CblG)	(CblD variant 1, CblE, CblG)	Methylcobalamin synthesis	MS/MS (Met low)	High (with second-tier test)	–
OA	612073	Mitochondrial DNA depletion syndrome 5	(SUCLA2)	Succinate-CoA ligase	MS/MS (C4-DC)	Uncertain	–
OA	266150	Pyruvate carboxylase deficiency	(PC)	Pyruvate carboxylase	MS/MS (Cit)	Uncertain	–
POX	214100 and others	Peroxisome biogenesis disorders (Zellweger spectrum disorders)	(PBD)	Peroxisome biogenesis	MS/MS (LPC)	High	–
POX	**300100**	**X-adrenoleukodystrophy (X-ALD), adrenomyeloneuropathy (AMN)**	**X-ALD**	**ATPase binding cassette protein (ABCD1)**	**MS/MS (LPC)**	**High**	**Possible**
SCID	**Various**	**Severe combined immunodeficiencies**	**SCID**	**T- and B-cell differentiation**	**Quantitative PCR (TREC)**	**Potential false negatives**	–
SCID	*Various*	*T-cell-related lymphocyte deficiencies*	*SCID*	*T-cell-related immune system*	*Quantitative PCR (TREC)*	*Potential false negatives*	–
SCID	Various	X-linked agammaglobulinemia and other agammaglobulinemias	(XLA)	B-cell differentiation	Quantitative PCR (KREC)	Potential false negatives	–

The conditions are listed in groups and ordered within each group based on their status in the current US Recommended Uniform Screening Panel (RUSP): conditions in **bold** face are primary screening targets, those in *italics* are secondary targets, and all others are either also part of the differential diagnosis of primary targets or included in an increasing number of newborn screening programs but not in the RUSP. While primary markers for each condition are given, it is important to note that for many conditions, additional analytes and analyte ratios can markedly improve the specificity of screening, especially when considered in parallel as is the case when using post-analytical tools provided by the R4S Laboratory Performance Database project. Second-tier tests are performed on the original newborn screening sample and determine more disease-specific markers for conditions where there is significant overlap between results for the primary analytes in the healthy and the affected population. When applied, the second-tier results override those of the primary screen

[a]Bedside tests. All other conditions are tested in centralized laboratories using dried blood spot specimens

[b]Codes in parentheses are for conditions that are not included in the US Recommended Uniform Screening Panel (RUSP) as either primary targets or secondary conditions

[c]Participation in R4S (https://www.nbstrn.org/research-tools/lab-performance-database) allows achievement of high sensitivity and specificity

[d]Red blood cell transfusion prior to sample collection can cause false-negative results

and amino acid analysis by MS/MS, are conditions which do not fulfill the requirements for newborn screening based on our understanding of the natural history and severity of the disorders and available evidence of effective modalities of treatment. However, all but one (2,4-dienoyl-CoA reductase deficiency) are identified as part of the differential diagnosis of one or more primary targets, and "elimination" of conditions which are detected based on exactly the same markers is unrealistic and potentially dangerous.

36.1 Hyperphenylalaninemias and Other Inborn Errors of Amino Acid Metabolism

Neonatal hyperphenylalaninemia can be caused by a variety of conditions. The primary genetic deficiency is that of phenylalanine hydroxylase, the enzyme that catalyzes the conversion of phenylalanine to tyrosine. Missing phenylalanine hydroxylase activity is the cause of phenylketonuria (PKU). In addition, abnormalities in the biosynthesis or regeneration of tetrahydrobiopterin (BH_4), the cofactor of this enzyme, are also detected. Secondary causes of an elevated concentration of phenylalanine include parenteral nutrition, drugs (trimethoprim, chemotherapeutic agents), and liver disease. Persistent hyperphenylalaninemia above 360–600 μmol/L is harmful to the developing brain leading to progressive mental retardation, epilepsy, spasticity, and psychiatric problems. It can be prevented by early diagnosis and dietary restriction of phenylalanine intake, ideally beginning as soon as possible after birth and no later than 14 days of age.

Most neonates with significant hyperphenylalaninemia suffer from phenylalanine hydroxylase deficiency; the incidence in most Caucasian populations is between 1:4000 and 1:12,000. For practical purposes, two forms of phenylalanine hydroxylase deficiency are distinguished: PKU which requires treatment and mild hyperphenylalaninemia (phenylalanine levels <360 μmol/L) which does not. Figure 36.1 depicts a flowchart for the work-up of a neonate with hyperphenylalaninemia recognized through newborn screening, according to the relevant ACT sheet established by the American College of Medical Genetics and Genomics (ACMG, available online at http://www.ncbi.nlm.nih.gov/books/NBK55827/). High phenylalanine values should be confirmed through quantitative analysis of plasma amino acids so that concentrations of tyrosine as well as phenylalanine are known. Analysis of pterins in urine or blood spots and the activity of dihydropteridine reductase in blood are required to exclude BH_4 cofactor deficiency. A positive response of the BH_4 loading test can be of immediate therapeutic relevance. Treatment of PKU should be started immediately with a phenylalanine-restricted diet and supplementation of essential amino acids. Additional treatment with a commercially available synthetic BH_4 is indicated for patients who experience at least a 30 % reduction in blood phenylalanine upon a single dose of 10 mg BH_4/kg body weight. Recommended therapeutic phenylalanine values differ between countries but should not be above 240–360 μmol/L in the first 6–10 years of life. Dietary restrictions could be liberalized after reaching adolescence. There is no consensus with regard to whether or not strict treatment is necessary in adulthood, with the exception of pregnancy, when plasma concentrations of phenylalanine must be kept below 360 μmol/L prior to conception and throughout gestation in order to minimize the potential teratogenic effects of phenylalanine on a developing fetus.

The introduction of amino acid analysis by MS/MS replaced traditional screening tests for PKU and other amino acid disorders such as maple syrup urine disease (MSUD). This evolution in technology resulted in a gain of efficiency, expanded the number of amino acid disorders that can be screened for, and improved sensitivity and specificity. For example, to avoid false-negative results, traditional assays that only measured single amino acids required a few days of nutritional protein intake. Guthrie's original bacterial inhibition assays carried a risk of false-negative results when newborns are treated with antibiotics at the time of sample collection. Both of these scenarios were remedied by the application of MS/MS because it measures

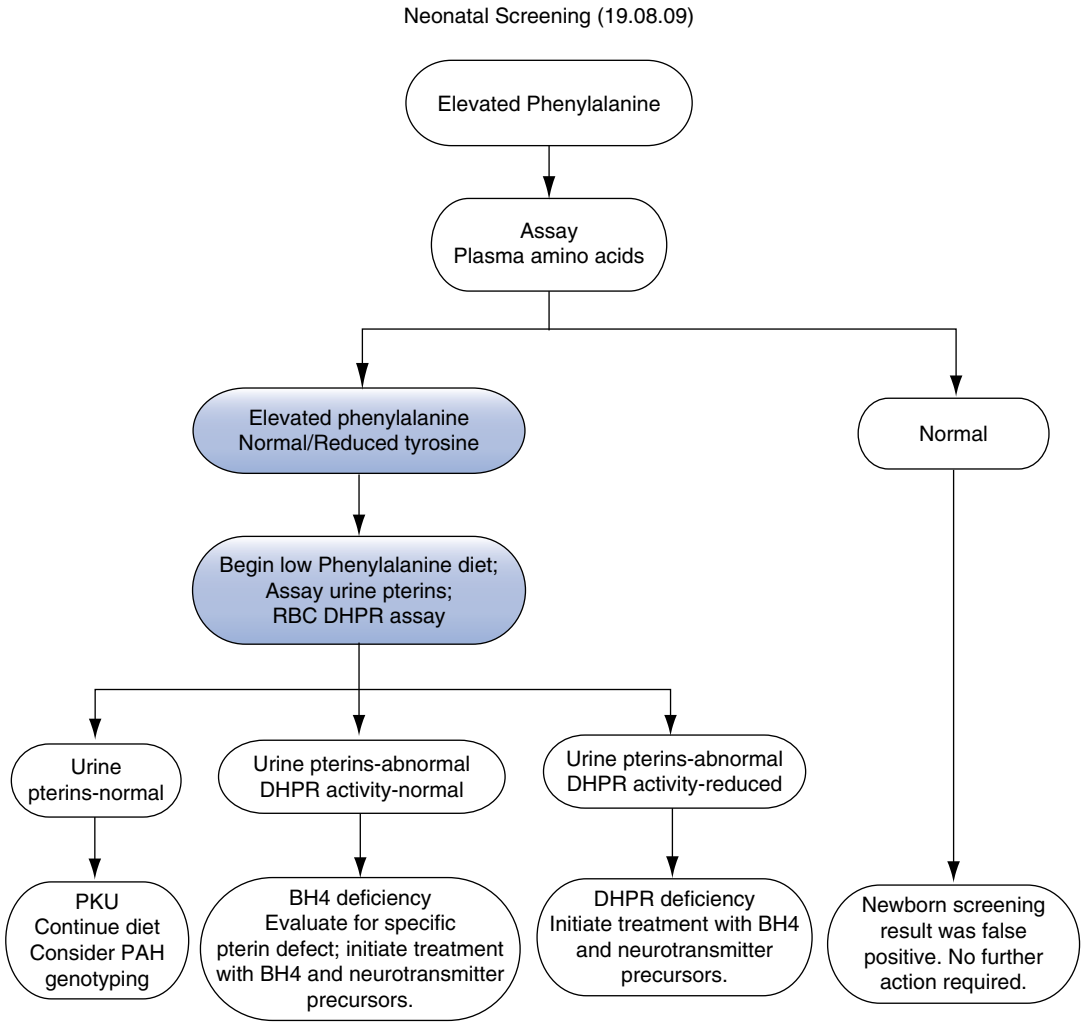

Fig. 36.1 Short-term follow-up algorithm for elevated phenylalanine detected by newborn screening (Reproduced from http://www.acmg.net, with permission)

simultaneously up to 23 amino acids as well as succinylacetone without interference from antibiotics and because it allows for the calculation of helpful amino acid ratios (e.g., the phenylalanine-to-tyrosine ratio for PKU) that are independent of the beginning of regular feedings; an important aspect as the time of blood spot collection has moved in many countries to no later than the third day of life. Furthermore, previously common false-positive results due to total parenteral nutrition can be avoided by the recognition of relevant amino acid profiles. Table 36.1 lists the aminoacidopathies that can be identified in newborn screening by MS/MS.

36.2 Medium-Chain Acyl-CoA Dehydrogenase (MCAD) Deficiency and Other Inborn Errors of Mitochondrial Fatty Acid Metabolism

MCAD deficiency is the most common (ca. 1 in 17,000 live births with fewer cases among Asians) of at least 13 mitochondrial fatty acid oxidation disorders that can be detected by acylcarnitine analysis in newborn screening (Table 36.1). MCAD deficiency, as most other mitochondrial fatty acid oxidation disorders, is asymptomatic at birth and typically presents in

infancy or early childhood during times of negative energy balance. For example, during weaning from nighttime feedings or during febrile infections when energy demand is high often concurrent with loss of appetite, MCAD-deficient patients are unable to utilize fatty acids for energy production via ketogenesis as glycogen stores become depleted. This leads to the characteristic finding of hypoketotic hypoglycemia. Clinically such patients present with vomiting, increasing lethargy, and a Reye-like syndrome that can rapidly lead to coma and death. Up to 20 % of undiagnosed MCAD deficient patients suffer unexpected sudden death during such episodes, which are readily preventable by avoidance of fasting. Given this contrast of severe outcome vs. effective and inexpensive treatment, the ability to identify patients with mitochondrial fatty acid oxidation disorders presymptomatically by acylcarnitine analysis was the major promoter of the introduction of MS/MS into newborn screening. Since then, follow-up studies have documented the benefit of newborn screening for most of the mitochondrial fatty acid oxidation disorders. As described for other screened conditions, however, very early onset of disease can escape the benefit of newborn screening, while the clinical spectrum of many disorders has been expanded by the detection of individuals with seemingly milder disease variants that had gone undetected prior to population screening.

36.3 Biotinidase Deficiency and Other Inborn Errors of Organic Acid Metabolism

The carboxylation of 3-methylcrotonyl-CoA, propionyl-CoA, acetyl-CoA, and pyruvate is biotin dependent. The individual apoenzymes need to be covalently bound to biotin in order to generate the active holoenzymes. This reaction is catalyzed by the enzyme holocarboxylase synthetase. A deficiency of this enzyme causes severe, multiple carboxylase deficiency which usually presents in the neonatal period. Affected children show severe metabolic decompensation typical of an organic aciduria and progressive neurological problems; in addition there are usually skin rashes and alopecia. Another, milder but therefore often insidious form of multiple carboxylase deficiency is caused by an impaired release of covalently bound biotin from dietary and endogenous proteins, a reaction that is catalyzed by the enzyme biotinidase. The deficiency of biotinidase causes a depletion of free biotin and progressive neurological and dermatological symptoms usually starting in infancy. Symptomatology includes seizures, ataxia, hearing loss, optic atrophy, spastic diplegia, and developmental delay as well as skin rash and alopecia. These characteristic symptoms may occur in half of the affected patients, but less specific symptoms like hypotonia, developmental delay, and seizures may occur in some cases. Because of the insidious onset of symptoms, the diagnosis is often delayed or even missed. This is a major concern since this form of multiple carboxylase deficiency can be effectively treated with biotin. A semiquantitative colorimetric or fluorimetric measurement of biotinidase activity in dried blood spots has therefore been included in newborn screening programs in many countries.

False-positive results may occur with improper handling of samples (e.g., exposure to excessive heat). On the other hand, false-negative findings in newborn screening may be observed after blood transfusion. Residual biotinidase activity may vary, depending on the underlying mutations in the biotinidase gene. Mild forms of biotinidase deficiency with relatively high enzyme activity levels occur that do not cause disease and do not require treatment. Nevertheless, it is advisable to start biotin supplementation immediately after an abnormal newborn screening result has been reported, before confirmatory tests are completed. It is also advisable to collect samples for blood ammonia, plasma lactate, and urinary organic acids before commencing biotin supplementation to evaluate the baseline metabolic status. Treatment is simple and does not involve complicated dietary regimens as in some other disorders that are included in newborn screening programs. Temporary initiation of biotin supplementation does not interfere with breastfeeding and parent-child interaction. The recommended starting dose is 10 mg per day. If a reduced biotinidase activity is confirmed in the repeat blood

spot screening test, biotinidase activity should be determined in serum. A residual activity of 0–10 % indicates profound biotinidase deficiency. Long-term treatment with 5–20 mg biotin per day is usually adequate. Treatment can be reevaluated by determining the activity of carboxylases in lymphocytes. Biotinidase activity between 10 and 25 % indicates partial deficiency which may not require long-term treatment. Many centers recommend treatment of these children with 10 mg/day for the first year of life. It is not yet clear what regimens are optimal for control of late complications, particularly to the optic and auditory nerves.

While several acylcarnitines derive from organic acids, biotinidase deficiency is typically not identified by acylcarnitine analysis and therefore requires a dedicated enzyme assay as described above. But at least 20 other organic acidemias can be detected by acylcarnitine analysis in newborn screening (Table 36.1). Many of these are well-known and serious conditions including methylmalonic, propionic, isovaleric, and glutaric acidemias. Others are of uncertain clinical relevance (typically considered secondary targets in the USA) but detected because they are part of the differential diagnosis of markers needed for the core conditions. For example, propionylcarnitine is not only elevated in propionic acidemia but also in the various methylmalonic acidemias due to inherited defects in methylmalonic mutase and cobalamin metabolism and transport, as well as vitamin B_{12} deficiency of the mother. Second-tier tests performed on the original newborn screening sample allow for higher specificity and some degree of discrimination by the measurement of 2-methylcitric acid, methylmalonic acid, and total homocysteine and/or 3-hydroxy propionic acid. Furthermore, MS/MS analysis as applied to newborn screening does not allow for the discrimination of isomers. This is relevant for several amino acids and acylcarnitines. For example, isovaleric acidemia is indicated by elevated isovalerylcarnitine. However, 2-methylbutyrylcarnitine, which is elevated in short/branched-chain acyl-CoA dehydrogenase (SBCAD) deficiency (now considered a non-disease), and medication-derived artifacts (pivalic acid, neopentanoic acid) are not distinguished from isovalerylcarnitine. This problem can also be remedied by second-tier chromatographic separation of these isomers.

36.4 Galactosemia

The activated 1-phosphate metabolites of both galactose and fructose are highly toxic particularly for the liver, kidneys, and brain. In classical galactosemia, galactose-1-phosphate (Gal-1-P) accumulates because of a defective synthesis of UDP-galactose catalyzed by galactose-1-phosphate uridyltransferase (GALT). Affected children show symptoms such as vomiting, diarrhea, and jaundice progressing after the start of milk (lactose) feedings usually from the third to fourth day of life onward. Untreated, the disease usually progresses to hepatic and renal failure and death; there may be progressive bilateral cataracts. The severe acute manifestations of galactosemia can be prevented by exclusion of galactose from the diet. Late complications such as ovarian failure and impaired speech and language development may occur despite good compliance with treatment. The preventive potential of newborn screening may not be fully realized if infants become symptomatic before the newborn screening results are available. The incidence of classic galactosemia in newborn screening programs in the USA has ranged from 1:55,000 to 1:80,000. False-negative findings in newborn screening may be observed after blood transfusion.

Newborn screening is carried out either by measurement of the activity of GALT alone or (less frequently in the USA) in combination with determination of the total galactose concentration. The combined approach allows identification of GALT deficiency and also of galactokinase deficiency and epimerase deficiency. A positive screening result is followed up by measurement of GALT activity in red blood cells. When GALT activity is reduced, further diagnostic characterization is possible by isoelectric focusing of the

GALT protein on a gel which helps to determine either the classic GG variant, the common Duarte variant (DD), compound heterozygotes (DG), or heterozygotes. A large number of different mutations have been identified in the GALT gene. The Duarte variant has an allele frequency of ca. 6 % and is associated with a 50 % reduction in enzyme activity which does not usually require dietary intervention. Even in compound heterozygotes with a severe mutation on the other allele, diet may not be necessary. Therefore, a majority of children with elevated galactose concentrations found in newborn screening may have a mild form of GALT deficiency that does not require treatment. In these children galactose and Gal-1-P concentrations on an ordinary diet often normalize within a few weeks or months.

When galactose is less than 20 mg/dL (1.1 mmol/L), it is sufficient to check the general condition (feeding, vomiting, weight gain, liver size) and to send another blood spot sample for galactose measurements (preferentially taken 60 min after a milk feed) to the newborn screening laboratory.

When *galactose is between 20 and 50 mg/dL* (1.1–2.8 mmol/L) or when it has been determined that the infant has a GG or DG phenotype, determination of plasma galactose and erythrocyte Gal-1-P is carried out in 1–3 mL of EDTA blood shipped at ambient temperature. Molecular genetic testing of the GALT gene is also available to confirm the diagnosis. EDTA blood may be stored at room temperature for a couple of days, if necessary. Lactose-free milk feedings are recommended until final results are available.

Immediate hospital admission is necessary in the presence of *galactose concentrations above 50 mg/dL* (>2.8 mmol/L), laboratory signs of liver disease, or clinical distress. Lactose-free feedings should be commenced as soon as the appropriate blood and urine samples have been taken (amino acids and reducing substances in the urine, determination of plasma galactose and erythrocyte Gal-1-P, complete blood cell count). Coagulation studies and blood cultures (*E. coli* sepsis is common) should be considered in all patients not clinically normal.

Lactose-free feedings must continue until galactosemia has been excluded. The indication for long-term therapy ultimately rests on the degree of GALT deficiency and the concentration of Gal-1-P in erythrocytes which is normally below 0.3 mg/dL (11 μmol/L) but may rise up to 100 mg/dL (~4 mmol/L) in classical galactosemia. It is impossible to obtain completely normal Gal-1-P levels in most GG patients because of an endogenous production of galactose. Therapeutic target concentrations of 2–4 (at most 5) mg/dL are realistic.

36.5 Congenital Hypothyroidism

Congenital hypothyroidism occurs in infants who are born without the ability to produce adequate amounts of thyroid hormone. Thyroid hormone is essential for normal growth and brain development. If untreated, congenital deficiency of thyroid hormone results in mental retardation and stunted growth. Infants with untreated congenital hypothyroidism may appear clinically normal for up to 3 months of age, by which time some brain damage will already have occurred. When symptoms or clinical signs are present, they may include prolonged neonatal jaundice, constipation, lethargy, poor muscle tone, feeding problems, macroglossia, mottled and dry skin, distended abdomen, and umbilical hernia.

The most common causes are total or partial failure of the thyroid gland to develop (aplasia or hypoplasia) or its development in an abnormal location (an ectopic gland). These types of hypothyroidism rarely recur in siblings. Less commonly, the hypothyroidism results from a hereditary inability to synthesize thyroid hormones, maternal medications during gestation (iodine, antithyroid drugs), or maternal antibodies.

The initial screening test is the assay of thyroid-stimulating hormone (TSH) to detect an elevated concentration (usually >20 μU/mL). However, in some laboratories the assay of thyroxine (T_4) is preferred; others measure both.

Newborns with TSH >50 μU/mL are considered highly likely to have congenital hypothyroidism and, therefore, require immediate follow-up testing of plasma and treatment. Those having a less prominent elevation of TSH are evaluated by confirmatory serum tests (free T_4, T_3 resin uptake, and TSH).

Treatment includes oral L-thyroxine at a dosage to maintain blood TSH concentration <4 μU/mL and T_3/T_4 in the age-related range. Dosage and follow-up should be coordinated in consultation with a pediatric endocrinologist.

36.6 Congenital Adrenal Hyperplasia (CAH)

Infants with CAH have a deficiency of one of several adrenal enzymes involved in steroid biosynthesis resulting in limited cortisol production and, in some cases, limited aldosterone production. The pituitary gland senses the cortisol deficiency and produces increased amounts of ACTH. The adrenal glands enlarge but continue to produce inadequate amounts of cortisol. Some of the precursors of cortisol are virilizing hormones. As a result of cortisol deficiency, affected infants are unable to respond adequately to the stress of injury or illness. Because of aldosterone deficiency, sodium and water are lost in the urine, resulting in dehydration. Potassium accumulates in the blood, causing irritability or lethargy, vomiting, and muscle weakness, including cardiac muscle irritability and weakness, leading to shock and death in a salt-wasting crisis.

Male infants with CAH usually appear normal at birth. Female infants usually show the effects of elevated virilizing hormones: an enlarged clitoris and fusion of the labia majora over the vaginal opening. Occasionally the female infant may be virilized so as to appear to have a male penile structure with hypospadia. Such newborns should not have a palpable gonad in the labial/scrotal sac. Their ovaries, uterus, and fallopian tubes are normal.

Several types of genetic defects cause the enzymatic deficiencies of CAH. All are autosomal recessive. The traditional newborn screening test is designed to detect the accumulation of 17-hydroxy progesterone (17-OHP), the result in most cases (>90 %) of underlying 21-hydroxylase deficiency. In clinical practice, however, one should remember that an abnormal newborn screening test can also indicate rarer enzyme deficiencies which cause CAH, for example, 11-hydroxylase deficiency.

The immunoassay for 17-OHP, a precursor of cortisol, is affected by cross-reactivity with other steroids, in particular among premature newborns. If screening indicates the possibility of CAH, a cost-effective approach is to perform on the same blood spot a second-tier test by LC-MS/MS for the determination of 17-OHP, androstenedione, 11-deoxycortisol, 21-deoxycortisol, and most importantly cortisol, the end product of the pathway. This approach can eliminate more than 90 % of potential false-positive results of the primary screening (frequently ca. 1 % of all newborns and a much greater proportion of premature babies). Overall, steroid profiling by LC-MS/MS achieves improved specificity and could potentially evolve into a first-tier screening with clinically validated cut-offs based on birth weight, gender, and comparison with disease ranges.

Effective treatment for CAH is hormone replacement. Decisions about hormonal treatment should be made in consultation with a pediatric endocrinologist and may include hydrocortisone and mineralocorticoids. Medications need to be adjusted as the child grows. Serum adrenal hormone levels and renin are monitored. Female infants who have virilization of the genitalia may need surgical correction. This is usually done in stages, with the first surgery before the age of 2 years. Infants with CAH, if detected early and treated with appropriate doses of medication, can have normal growth, development, and intellectual potential. In addition, fertility is usually normal.

36.7 Other Conditions Included or Proposed for Newborn Screening

36.7.1 Glucose-6-Phosphate Dehydrogenase (G6PD) Deficiency

G6PD deficiency is an X-linked recessive condition. Depending on the degree of enzyme deficiency, the phenotype varies from chronic hemolytic anemia to intermittent hemolysis or no disease. G6PD is expressed in erythrocytes, and the hemolytic episodes are caused by oxidative stress triggered by ingestion of medications or fava beans as well as infections. In newborns, the development of severe hyperbilirubinemia can cause kernicterus, the reason why G6PD has been considered for newborn screening. The frequency of G6PD deficiency is highest among populations originating in the same geographic regions in which plasmodium infection causing malaria is common. Therefore, newborn screening for G6PD is not yet implemented in the USA and Europe but is likely to be included in the future as the ethnic background of northern populations is changing. Treatment of G6PD deficiency only requires avoidance of exposure to triggering agents.

36.7.2 Severe Combined Immunodeficiency (SCID)

SCID is a group of usually fatal conditions with different etiologies causing impaired T-cell production and inability of B cells to produce antibodies. Because it has been shown that early treatment by hematopoietic stem cell transplantation, enzyme replacement therapy (for adenosine deaminase deficiency), or gene therapy markedly improve survival and reduce morbidity, newborn screening becomes desirable. It was made possible by development of assays that measure T-cell receptor excision circles (TREC) in dried blood spots by high-throughput quantitative polymerase chain reaction (PCR) methods. Encouraging results with very low false-positive rates prompted the addition of SCID to the RUSP in the USA. Further improvement of SCID screening to identify more SCID variants, such as agammaglobulinemias, by simultaneous measurement of TREC and kappa-deleting recombination excision circles (KRECs) has been proposed, but the usefulness of KRECs has not yet been fully validated.

36.7.3 Lysosomal Storage Disorders (LSD)

The increased availability of treatment options for a growing number of LSDs and the development of high-throughput assays for several lysosomal enzymes simultaneously have led to some LSDs being proposed for inclusion in newborn screening, specifically Pompe, Hurler, Fabry, Gaucher, Krabbe, and Niemann-Pick A/B diseases. Pompe disease and Fabry disease have been part of newborn screening in Taiwan for several years already, New York State has been screening for Krabbe disease since 2006, and other states in the USA are following suit, mostly because of local pressure on the political establishment put on by disease advocacy groups. Except for Gaucher disease, all of the abovementioned LSDs were reviewed by the ACHDNC which resulted in the inclusion of Pompe disease and mucopolysaccharidosis type I (MPS I, Hurler disease) to the RUSP. The screening experience to date uncovered a gross underestimation of the prevalence of several of these LSDs, mostly mild and late-onset variants that are currently either misdiagnosed or possibly of no clinical significance. For example, newborn screening studies in Europe and Taiwan for Fabry disease revealed surprisingly high incidences of one case in approximately 3000 male newborns predominantly with genotypes suggestive of late-onset disease.

36.7.4 X-Adrenoleukodystrophy (X-ALD)

X-ALD is an X-linked peroxisomal disorder with adrenocortical insufficiency being one of the most frequent symptoms that can be apparent already in the first year of life and is effectively treated by adrenal steroid supplementation. While hormone replacement therapy is mandatory in affected patients, hematopoietic stem cell transplantation is the only proven successful treatment for the cerebral form of X-ALD if performed before neurological symptoms, significant neuropsychological deficits, and cerebral demyelination have occurred. Newborn screening is possible by measurement of very long-chain lysophosphatidylcholines (LPC) which can be multiplexed with lysosomal enzyme assays. New York State has been screening for X-ALD since January 2014 without any false-positive result when including X-ALD carrier females and peroxisomal biogenesis disorders such as Zellweger disease. The Netherlands decided to screen only male newborns for X-ALD and after formal evidence review by the ACHDNC X-ALD was recently added to the RUSP independent of gender.

36.7.5 Sickle Cell Disease and Other Hemoglobinopathies, Congenital Infections, Hearing Loss, and Critical Congenital Heart Disease

Sickle cell disease and other hemoglobinopathies are included as core conditions and secondary targets in the RUSP. In addition, newborn screening for these conditions is provided in many regions where hemoglobinopathies are prevalent.

The only congenital infection currently included in a routine newborn screening program is the human immunodeficiency virus (HIV, specifically HIV-1) that is tested for by ELISA in New York State. The relevant program includes the offer of testing for HIV to expecting mothers so that antiretroviral treatment can ideally be initiated as early as possible before birth and be given to the newborn after birth to avoid pre- and perinatal infection. The effectiveness of newborn screening for HIV for infected newborns whose mothers were not identified prenatally is limited to monitoring and treatment as needed.

Hearing loss and critical congenital heart disease (CCHD) are also included in the RUSP. These are screened for in the nursery by audiology exam and pulse oximetry, respectively. Accordingly this screening mode adds a level of complexity for screening programs to ensure compliance, results reporting to the screening program, and outcome evaluation, all critical responsibilities of a complete population screening system.

The table summarizes the more than 90 conditions that can be included in newborn screening programs. The complexity of this table should be sufficient to call attention to the need to carefully monitor the performance of a screening laboratory and to assess it based on objective metrics. The R4S Laboratory Performance Database is a collaborative effort involving more than 250 laboratories in 69 countries that has set the following targets of adequate performance of amino acid and acylcarnitine analysis in newborn screening by MS/MS: (1) detection rate of at least 1:3000 births (assuming testing for most of the 20 conditions included in the RUSP), (2) false-positive rate <0.3 %, and (3) positive predictive value >20 %. To achieve these targets, the collaborative project collects data to define evidence-based, clinically driven cutoff target ranges for all analytes detected by MS/MS and calculated ratios. The cutoff target range could be either above (high) or below (low) the range of the normal population. The high target range is defined as the interval between the cumulative 99 percentile of the normal population and the lowest 5 percentile of disease ranges, if the analyte is informative for multiple conditions. On the other hand, the low target range is defined as the interval between the highest 99 percentile of disease ranges, if the analyte is informative for multiple conditions, and the 1 percentile of the normal population. When the degree of overlap between normal population and disease range makes it

inapplicable to use the criteria stated above, one or both limits are modified to give priority to the disease range and may require the use of a second-tier test to maintain adequate specificity (low false-positive rate). Second-tier tests are performed on the original newborn screening sample and determine more disease-specific markers for conditions where the primary screen has low specificity. When applied, the second-tier results override those of the primary screen. Implementation of second-tier tests requires additional resources, but cost-effectiveness could be achieved for most conditions by regionalization through collaboration of screening laboratories. The Region 4 Stork (R4S) laboratory quality improvement project is based on cumulative data from approximately 30 million unaffected newborns and more than 18,000 true positive cases. This initiative is being expanded to include all assays applied in newborn screening and to incorporate covariate-adjusted reference intervals and disease ranges to automatically correct for differences in age at collection, birth weight, and gender. More information on current and future state of this effort can be found at the R4S (https://www.clir-r4s.org) and CLIR (Collaborative Laboratory Integrated Reports; https://clir.mayo.edu) websites, respectively.

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Biochemical Studies

37

Miao He and K. Michael Gibson

Key Facts

- Evidence for the presence of an inherited metabolic disease may often be derived from detailed clinical evaluation of the patient and examination of the family history.
- Absence of acute metabolic decompensation (e.g., hyperammonemia, hypoglycemia, overwhelming metabolic acidosis, anion gap) does not rule out an inherited metabolic disease.
- Important stumbling blocks in identifying an inherited metabolic disease include the fact that signs and symptoms are often nonspecific, leading to initial testing to exclude routine childhood illnesses and delaying consideration of metabolic disorders.
- Even when appropriately suspected, ordering physicians may be unfamiliar with important biochemical interrelationships and the appropriate diagnostic tests to order, occasionally leading to inappropriate sample collection and storage.
- Consultation and coordination with a licensed clinical biochemical genetics laboratory helps to insure that appropriate tests are ordered, the correct samples are obtained, and the limitations of the testing scheme are clearly defined prior to metabolic workup.

This chapter draws extensively on a previously published work: Hoffmann GF, Nyhan WL, Zschocke J, Kahler SG, Mayatepek E (2002) *Inherited Metabolic Diseases – Biochemical Studies*. Lippincott Williams & Wilkins, Core Handbooks in Pediatrics, pp. 95–109. The authors acknowledge the use of that material.

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37.1 General Remarks

Most known inherited metabolic diseases are identified via biochemical analyses of various body fluids, predominantly blood and urine, but also cerebrospinal, vitreous, and even bile fluids. Concentrations of physiologically relevant metabolites in plasma or serum are generally tightly controlled, and thus increases/decreases

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of specific intermediates may have Diagnosis relevance. Normative data for many compounds of intermediary metabolism are highly dependent upon the metabolic state at sampling, and appropriate interpretation of assay results requires knowledge of intake and other physiological data, including fasting or postprandial status or postexercise status. Some disorders may only be identified through specific function tests (e.g., loading) that stress metabolic conditions or result in supraphysiological increases in metabolite load (see Chap. 41). Such tests have inherent risks and escalate the potential for metabolic overload and decompensation; accordingly, such tests should only be instituted by experienced clinicians in the appropriate hospital setting, and only when other diagnostic options which carry less patient risk have been exhausted (Table 37.1).

Many inherited metabolic disorders induce the accumulation of substrates which are either metabolized via alternative processes (alternate pathways, liver biotransformation) and/or removed via excretion in the urine. Studies carried out in urine for many such diseases may be more straightforward and sensitive than plasma/serum analyses. Differences in fluid intake and urinary dilution, and their effect on metabolite concentrations, are usually accounted for by correcting urine metabolite levels with creatinine output. Urine analyses are generally less influenced by metabolic and nutritional changes, since the specimen is collected over a time period and often (but not always) there are significant differences between normal and pathological values that are readily recognized. As a general rule, a spot urine sample (morning void to enhance metabolite concentrations) is sufficient for most studies. Exceptions may occur, however, as in the case of some fatty acid oxidation disorders that frequently show urinary abnormalities only under fasting or loading conditions, or in the case of certain disorders (e.g., cystinuria, porphyrias, etc.) where a 24-h urine collection may be required.

Table 37.1 An overview of metabolic investigations described in this chapter

Simple colorimetric evaluations in urine
Amino and organic acids, carnitine, and acylcarnitines
Lactate, pyruvate, nonesterified fatty acids, and ketones
Congenital disorders of glycosylation (CDG)
Purines, pyrimidines, and orotic acid
Sugars and polyols
Glutathione
Mucopolysaccharides and oligosaccharides
Very long-chain fatty and pristanic acids (peroxisomal function)
Creatine and folate
Sterols, bile acids, and porphyrins
Biogenic amines and pterins

The first three categories form the core tests for any patient suspected of having an inherited metabolic disease (e.g., baseline selective screening). The remaining test groups rely more heavily on enhanced clinical suspicion

Laboratory investigations for inherited metabolic diseases are complex and susceptible to technical problems. To maintain acceptable standards, laboratories performing biochemical investigations for inherited metabolic diseases should process a sufficiently high number of samples to maintain diagnostic acumen, and should participate in quality assurance/quality control (QA/QC) processes.

Referring physicians should bear in mind that many analyses are often qualitative and not quantitative (although the expanding implementation of tandem mass spectrometry (MS-MS) is changing this paradigm), thereby leading to a certain level of subjective interpretation. Furthermore, the conditions examined are biochemically heterogeneous in their expression, which can complicate identification of subtle abnormalities. For these reasons, diagnostic laboratories must adhere to accepted practices of internal and external QC schemes, which insure ongoing education of laboratory staff and competence in analytical performance. External schemes are particularly important, providing data on accuracy and bias of results. Participation in external QC schemes (and acceptable performance) is often a requirement for external accreditation of the laboratory. In the USA, the College of American Pathologists (CAP) offers proficiency testing for urine and blood amino acids, urine organic acids, plasma acylcarnitines, and qualitative mucopolysaccharide analyses. The European Research Network

for evaluation and improvement of screening. Diagnosis, and treatment of Inherited Disorders of Metabolism (ERNDIM) offers these and a more extensive menu of special assays in urine and blood, proficiency testing for purine and pyrimidine analysis, lysosomal enzyme analysis in fibroblasts, cystine in white blood cells, and qualitative analysis for congenital disorder of glycosylation in serum. It has also recently instituted a pilot program for neurotransmitters in CSF and pterins in urine and dried blood spots. Depending on the country or region, laboratory accreditation may be optional or mandatory, the latter being the case in the USA (CAP).

37.2 Simple Colorimetric Evaluations of Urine

A number of simple tests are available that may be carried out in nonspecialized hospitals or at the bedside and provide important first clues for the diagnosis of metabolic disorders.

37.3 Dinitrophenylhydrazine (DNPH) Test

Method: Mix 1.0 ml urine with 1.0 ml 0.2 % dinitrophenylhydrazine (DNPH) solution.

The DNPH assay detects urine α-keto acids via formation of hydrazones that precipitate out of solution. Several metabolic disorders may be detected, including phenylketonuria (PKU), maple syrup urine disease (MSUD), tyrosinemia type I, tyrosyluria, histidinemia, and methionine malabsorption syndrome. Acetone also yields a positive result and may suggest ketonuria associated with diabetic ketoacidosis, branched-chain organic acidurias, and glycogen storage disorders. A 2 N HCl test is performed on all DNPH positives to determine the presence of false-positive results. Substances with low acid solubility may cause interference. Mandelamine (methenamine mandelate), an antibacterial medication, and radiopaque contrast material will form a precipitate immediately upon addition of DNPH. The color and immediacy of precipitate formation distinguishes it from the yellowish precipitate of α-keto acid hydrazones. Information on medications is critical prior to use of the DNPH test. The DNPH test may be carried out by caregivers and may be useful in the management of patients with MSUD living at some distance from the Metabolic Center.

37.4 Reducing Substances in Urine

Method: Commercially available test tablets (e.g., Clinitest®, Bayer Corporation)

Numerous disorders lead to the urinary excretion of sugars and other reducing substances, some of which are described in Table 37.2. Urine-reducing substances may be detected with commercially available test tablets which provide a color change in the presence of reducing substances. This test, in conjunction with the Multistix analysis described below, can assist in differentiating between glucose-related and non-glucose-related reducing substances.

Table 37.2 Urine-reducing substances (with associated disorders or conditions)

Galactose (classical galactosemia, galactokinase deficiency, liver disease, and secondary galactose intolerance)
Fructose (fructose intolerance, essential fructosuria)
4-Hydroxyphenylpyruvate (tyrosinemia types I/II, bacterial contamination of urine)
Homogentisic acid (alkaptonuria)
Xylose (pentosuria)
Glucose (diabetes mellitus, Fanconi syndrome)
Oxalate (hyperoxaluria)
Salicylates, ascorbic acid (drugs)
Uric acid (hyperuricosuria)
Hippurate (sodium benzoate treatment of hyperglycinemia and hyperammonemia)

37.5 Rapid Urinalysis

Method: Commercially available reagent test strips (Multistix 10 SG/Multistix PRO 11®, Bayer Corporation)

Multistix 10 SG and Multistix PRO 11 provide qualitative colorimetric analysis of protein, blood, leukocytes, nitrite, glucose, ketones (acetoacetic acid), pH, specific gravity, creatinine, bilirubin, and urobilinogen in urine. Methodology, interpretation, and characteristics of the approximate linearity of these measurements are provided in the package insert. In addition, for each test, expected values, limitations, and interfering substances are described. This screening procedure can provide insight into likelihood of bacterial infection/contamination, kidney and liver function, acid-base balance, and/or carbohydrate metabolism.

A number of interferences can occur for each metabolite estimated. Certain medications (e.g., riboflavin or drugs containing azo dyes, or nitrofurantoin) may lead to urine discoloration and test interference. False negatives for glucose may be encountered with ascorbate levels >50 mg/dL or in the presence of ketones. Ascorbate interferes with bilirubin estimation, as does indican (indoxyl sulfate). Estimation of ketones is hampered by the presence of highly pigmented samples, levodopa metabolites, and sulfhydryls. False positives for heme (blood) may arise from oxidizing contaminants (hypochlorite), or microbial peroxidase (urinary tract infection). Finally, protein in the urine may be overestimated in highly buffered or alkaline samples or in urine contaminated with quaternary ammonium compounds (antiseptics, detergents).

37.6 Cyanide-Nitroprusside Test (Brand Reaction)

Method: Add 0.4 ml 5% NaCN to 1.0 ml urine. After 10 min, add 0.2 ml of 0.5% Na-nitroprusside (Na-nitroferricyanide). Mix and immediately assess the color.

Table 37.3 Intermediates generating a positive nitroprusside reaction (and associated disorder or condition)

Cystine (cystinuria, hyperaminoaciduria)
Homocysteine (homocystinuria, cobalamin disorders, cystathioninuria, urinary tract infection)
Glutathione (disorders of the gamma-glutamyl cycle)
Drugs (see text)

The Brand reaction identifies free sulfhydryl or disulfide compounds in urine. Cyanide reduces any disulfides to free sulfhydryls. In the subsequent reaction, a reddish color results when free sulfhydryl groups complex with nitroprusside. A positive result is usually due to cystine in the urine. Familial cystinuria is among the most common aminoacidurias. Disulfides are also excreted in other metabolic disorders such as homocystinuria and β-mercaptolactate-cysteine disulfiduria. Both will produce a positive result. The urine specimen should be at approximately neutral pH. If the sample has been preserved with acid, a false-positive reaction may occur. Drugs such as N-acetylcysteine, 2-mercaptoethanesulfonate, 2-mercaptopropionylglycine, captopril, penicillamine, and large amounts of synthetic penicillin metabolites and acetoacetate will yield false positives; accordingly, a listing of medications must be obtained. Bacterial contamination may also generate a false positive. Cystathionine, methionine, and taurine are not detected (Table 37.3).

37.7 Ehrlich's Aldehyde Reagent

Method: Add 1.0 ml of urine to 0.1 ml 2% p-dimethylaminobenzaldehyde (p-DABA) in 2 N HCl. Mix and assess color formation after 10 min.

The Ehrlich test detects porphobilinogen (PBG) or urobilinogen in urine. Porphobilinogen yields a red color in urine for the patient with acute porphyria. Urobilinogen, a component of heme degradation, results when bilirubin analogues are secreted into the bile and further degraded by intestinal bacteria. In the normal patient, some urobilinogen is reabsorbed and

transported to the kidneys where it is converted to urobilin (yellow) and excreted. However, the majority of urobilinogen is converted microbially to stercobilin (deep red-brown). Urobilin is responsible for the characteristic color of urine, while stercobilin is a major pigment of feces. Indoles in the urine may also give a positive Ehrlich test.

Individuals with porphyria may present with acute attacks, skin lesions, or both, but rarely prior to the onset of puberty. An attack usually consists of severe abdominal pain and may be associated with neurological findings. Such attacks are associated with excessive amounts of δ-aminolevulinic acid and porphobilinogen in the urine. Porphobilinogen deaminase deficiency (acute intermittent porphyria) is the most common form of porphyria. A positive Ehrlich test, coupled with hepatosplenomegaly, indicates that evaluation of tyrosine metabolites in urine (nitrosonaphthol test) should be pursued.

37.8 Nitrosonaphthol Test (Tyrosine Metabolites)

Method: 3 drops of clear urine are sequentially treated with 1.0 ml 2.6 N nitric acid, 1 drop 2.5 % sodium nitrite, and 0.2 ml 1 mg/ml 1-nitroso-2-naphthol. After 15 min, the color formation is recorded. As blank, water is substituted for urine; N-acetyl-L-tyrosine (0.04 mg/ml 1 M HCl) is employed to develop a qualitative standard curve.

4-Hydroxylated phenolic acids (and to a limited extent hydroxylated indoles derived from tryptophan) conjugate with 1-nitroso-2-naphthol in the presence of nitric acid to yield orange/red chromophores. The corresponding tyrosine analogues include 4-hydroxyphenylpyruvate, 4-hydroxyphenyllactate and 4-hydroxyphenylacetate, and tyrosine itself. A limitation of this method is transient newborn tyrosinemia, a relatively common finding as the hepatic enzymes involved in tyrosine metabolism may develop slowly. 4-Hydroxyphenylacetate may be elevated as a result of intestinal bacterial metabolism, malabsorption, and other disorders and may lead to false positives. Some disorders of carbohydrate metabolism, and liver dysfunction, may also alter tyrosine metabolism and produce positives. Patients undergoing parenteral nutrition are often supplemented with tyrosine analogues, which can lead to difficulties in interpretation. Patients with adrenal tumors may excrete increased homovanillic acid and/or 5-hydroxyindoleacetic acids (end products of dopamine and serotonin metabolism, respectively) which yield pink and purple chromophores with nitrosonaphthol. Any positive should be correlated with clinical history and/or followed up with more specialized testing (blood amino acids/urine organic acids).

37.9 Sulfite Test

Method: Commercially available dipstick (Merckoquant 10013, Merck Darmstadt, Germany); fresh urine

The sulfite test recognizes increased concentrations of sulfite in the urine which may be a marker of primary deficiencies of the enzyme sulfite oxidase or molybdenum cofactor deficiency. Children affected with one of these disorders typically suffer from severe epileptic encephalopathy which usually starts in the neonatal period or infancy. Testing for urinary sulfite should be part of baseline investigations in any child with unexplained psychomotor delay particularly in combination with severe epilepsy. The test may be falsely negative if urine samples left at room temperature for some hours or sent to the laboratory at ambiguous temperature are used for testing. The test should be performed three times at the bedside with fresh urine before a negative result can be ascertained. False-positive results may be caused by various drugs and other conditions that cause increased urinary sulfite concentrations.

37.10 Amino Acids

Physiological amino acids occupy essential positions in intermediary metabolism as the building blocks of proteins but also are involved in numerous other metabolic processes including methylation reactions, neurotransmission, energy production, and others. A long list of inherited metabolic diseases either directly or indirectly affect amino acid metabolism (both catabolic and anabolic processes), and lend themselves to detection through careful analysis of the appropriate physiological fluid.

37.10.1 Plasma

Sample: 1–2 ml heparinized blood, with plasma immediately separated. If shipped, the sample should be sent on dry ice.

Quantitative amino acid analysis in plasma (or serum) by ion-exchange chromatography or High-performance liquid chromatography (HPLC) (ninhydrin or phenylisothiocyanate (PITC) derivatization) provides pertinent information on alterations in amino acid homeostasis and is one of the first-line investigations performed in a patient with suspected inherited metabolic disease. Analysis should be performed promptly in patients with hyperammonemia or with an acute presentation of a suspected amino acid disorder; the results should be available STAT. Routine (fasting) amino acid analysis is also necessary in patients that are protein restricted or receiving specific metabolic dietary therapy, in order to adjust amino acid intake and to identify any deficiencies of essential amino acids. Many amino acids (but not all as yet, especially the dibasic amino acids) can also be quantified reliably in dried blood spots.

Amino acids can be quantified in Guthrie cards by electrospray tandem mass spectroscopy (MS-MS) or fast atom bombardment MS (see below). For some disorders such as phenylketonuria, this is a superior technology (throughput, time savings) to classical chromatographic methods (analysis time from 2 to 3 h) with less invasive approaches for the patient. The newest technological advance for amino acid analysis is the UPLC-MS/MS methodology. This system employs high flow-rate (to 20,000 psi) and small resin particle size to enable extremely rapid analysis and resolution. Analysis of a sample from a suspected MSUD patient can be achieved in minutes with absolute quantification (stable isotope internal standards). An added advantage over current electrospray MS-MS is that stereoisomers (e.g., leucine/isoleucine) are separated on the UPLC system. LC-MS/MS can also be used to separate branched chain amino acids for diagnosis and diet monitoring of MSUD patients, although the run time is longer than UPLC-MS/MS. LC-MS/MS, UPLC, and UPLC-MS/MS technology is rapidly developing, and are replacing the older (yet still robust) ninhydrin-based amino acid analyzers in many laboratories.

Remember

Although amino acid analysis can be quantitatively performed, slight deviations in one (or even a few) intermediates do not necessarily indicate a defect of amino acid metabolism. The entire pattern of physiologically relevant amino acids should be reviewed by an experienced biochemical geneticist whose training has included evaluation of numerous chromatograms over a number of years.

When looking at 20–30 physiologically relevant amino acids in plasma, artifacts are a common occurrence (Table 37.4), linked to other pathological states, infection, nutritional status, and a host of other variables. Plasma amino acid levels are dependent upon metabolic status, and routine sampling should occur 4–6 h after the last meal. Further, it may be useful to quantify amino acids in postprandial (and fasting) samples, particularly when disordered energy metabolism is suspected. For postprandial analyses, it may be advisable to provide a defined meal to achieve standardized substrate intake (see Chap. 41 for more information). Postprandial samples may reveal significant elevations of essential amino acids. For example, an excessive alanine increase indicates impaired pyruvate handling and may

Table 37.4 Facts and artifacts for selected plasma amino acid elevations (and associated conditions)

Glycine (organic acidurias, nonketotic hyperglycinemia, valproate treatment)
Serine (tracks with glycine as a result of metabolic interconversion; decreased in serine biosynthetic defects)
Phenylalanine (phenylketonuria and biopterin disorders, tyrosinemias, liver disease)
Tyrosine (tyrosinemia I, II, and III; liver disease)
Arginine (argininemia; decreased in trauma, shock, hemolysis and most urea cycle disorders)
Methylhistidines, anserine, carnosine (consumption of fowl)
Methionine (homocystinuria, cobalamin disorders, pernicious anemia, liver disease)
Citrulline (argininosuccinic aciduria and citrullinemias, decreased in certain forms of pyruvate carboxylase deficiency and urea cycle disorders (OTC, CPS, NAGS))

suggest a mitochondrial disorder. Conversely, fasting results in a marked elevation of the branched-chain amino acids (leucine, isoleucine, valine), while most other amino acids are low.

For optimal results in amino acid analysis, it is important to separate plasma or serum from cells as soon as possible. Hemolysis or transport of whole blood results in essentially useless values for some amino acids (e.g., arginine or taurine), either through the action of erythrocyte enzymes such as arginase (which converts arginine to ornithine) or through concentration gradients between cells and fluid (e.g., liberation of taurine from platelets). Exact arginine concentrations are essential for diagnosis and treatment monitoring of urea cycle defects. Even after prompt separation, shipment at ambient temperature results in questionable values for several amino acids, e.g., glutamate, aspartate (both artificially elevated), glutamine, asparagine, cysteine, and homocyst(e)ine (reduced). The concentrations of some amino acids (phenylalanine, tyrosine, valine, isoleucine, leucine) are less affected by overnight shipment of room temperature plasma or serum, but frozen samples are optimal to avoid artifactual results.

Certain amino acids, notably homocysteine and tryptophan, require specific methods for exact quantification. Determination of total homocysteine is critical for the evaluation of hyperhomocysteinemias, due to both cystathionine-β-synthase deficiency and cobalamin disorders. For optimal results, immediately centrifuge the blood sample, order total homocysteine, and transport plasma to the laboratory (for short transit time, room temperature is acceptable; for longer transport times, freeze plasma and ship on dry ice). Analytically, homocysteine is released from disulfide conjugates by treatment with reducing agents, and the total homocysteine concentration is determined. Normative data for total homocysteine (fasting) in children <10 years approximates 3.5–9 μmol/L; >10 years, 4.5–11 μmol/L; women (premenopausal) 6–15 μmol/L; and postmenopausal 6–19 μmol/L; men 8–18 μmol/L. Reference ranges, however, must be established in the individual laboratories and include normal and pathological samples.

37.10.2 Urine

Sample: minimum 10 ml urine, preferably a morning void but a random sample is acceptable, without preservatives. If shipped, transport on dry ice.

Quantitation of urine amino acids is usually less revealing than comparable studies in plasma, since amino acids are normally well reabsorbed in the renal tubules; accordingly, subtle to moderate changes in amino acid homeostasis cannot be recognized using urine analyses. Various methodologies are available, including standard quantitative analysis (above) and qualitative assessment by thin-layer or paper chromatography. The qualitative analysis is relatively inexpensive, provides insight into renal tubular function, and has the capacity to identify several amino acidopathies/amino acidurias; it is therefore a key element of routine selective screening for inherited metabolic diseases. Quantitative urine amino acid analysis is appropriate in instances in which a renal tubular reabsorption defect such as cystinuria or Lowe syndrome is

suspected and (in conjunction with plasma analysis) in hyperammonemia when increased urinary excretion of specific amino acids (e.g., homocitrulline, ornithine, argininosuccinate, citrulline and lysine) may be diagnostic for certain urea cycle defects, such as HHH syndrome and lysinuric protein intolerance.

37.10.3 Cerebrospinal Fluid (CSF)

Sample: 1 ml CSF, freeze immediately, in conjunction with a plasma sample obtained at the time of lumbar puncture. If shipped, always transport on dry ice.

Quantitation of amino acids in cerebrospinal fluid may be pursued in patients with suspected neurometabolic disorders, in particular, severe (neonatal) epileptic encephalopathy (see also Table 37.5). It is highly desirable to obtain a concurrent plasma sample for amino acid analysis as calculation of the plasma/CSF ratio of specific amino acids may be required for the diagnosis of nonketotic hyperglycinemia (glycine CSF-plasma ratio >0.08, variants 0.02–0.08) and serine biosynthesis deficiency (3-phosphoglycerate dehydrogenase deficiency; serine CSF-plasma ratio <0.2). An increased glycine CSF-plasma ratio is only meaningful, however, in the presence of elevated CSF glycine. If plasma glycine is abnormally low, elevation of the CSF-plasma ratio in the face of a normal CSF glycine level is not consistent with nonketotic hyperglycinemia. Raised levels of alanine and threonine in CSF are indicators of mitochondriopathies. Again, interpretation of CSF amino acid values beyond normal limits is more reliable if concurrent plasma concentrations are available. Heavily bloodstained CSF samples are unusable; slightly blood-contaminated specimens should be rapidly centrifuged and the supernatant frozen (notify the laboratory). Immediate deep-freezing and special analytical methods are required for the determination of numerous CSF metabolites (free and total GABA, homocarnosine, carnosine, biogenic amines, and pterins).

37.11 Organic Acids

Samples: *Urine*: minimum 10 ml random (morning) urine, without preservatives. If transported, send on dry ice.

Table 37.5 Inherited metabolic diseases requiring CSF investigations for diagnosis

Disorder	Relevant parameter
Glucose transporter deficiency	Glucose and alanine, glucose ratio (CSF/blood)
Selected mitochondrial encephalomyopathies	CSF lactate, pyruvate, alanine, and threonine
Nonketotic hyperglycinemia	Glycine ratio (CSF/plasma)
Serine biosynthesis defects	CSF amino acids (low serine; serine CSF/plasma ratio)
Cohen syndrome	β-Alanine in CSF
Isolated pipecolic oxidase deficiency	Pipecolic acid
Defects of monoamine metabolism	CSF monoamines and metabolites
Autosomal dominant GTP	CSF biogenic amines and pterins cyclohydrolase I deficiency
Sepiapterin reductase deficiency	CSF biogenic amines and pterins including sepiapterin
GABA transaminase deficiency	CSF GABA, β-alanine, homocarnosine
Cerebral folate deficiency	CSF 5-methyltetrahydrofolate
Selected cases of leucine catabolic defects	CSF 3-hydroxyisovaleric acid

Modified from Hoffmann et al. (1998), see also Chapt. 27, Sect. 27.11 Diagnostic Lumbar Puncture

Plasma, *CSF*, *vitreous fluid* (*only limited*, *specific indications*): minimum 1 ml, freeze immediately and send on dry ice.

The major source of organic acids in mammals include the amino acids, lipids and carbohydrates, and to a limited extent nucleic acids and steroids. Urine organic acid analysis is most readily performed by gas chromatography-mass spectroscopy (GC-MS), which provides insight into a wide range of metabolic pathways and is accordingly a mainstay of selective metabolic screening. Urine organic acid by gas chromatography alone is not recommended, as slight elevations of metabolites are missed, and confirmatory identification via fragmentation patterns is not possible. In addition to the classical organic acidurias, urine organic acid analysis is a key diagnostic component in the evaluation of patients with suspected amino acid disorders, fatty acid oxidation defects, or disorders of mitochondrial energy metabolism.

Organic acids may be extracted from urine following acidification with mineral acids and are then converted to derivatives (e.g., trimethylsilyl or methyl esters) for analysis by gas chromatography-mass spectrometry. One or more nonphysiological internal standards are included in the analysis for internal quality control and for retention time standardization (e.g., 2-phenylbutyrate, undecanoic acid). Organic acid analysis in urine is suggested in the patient with systemic intoxication, unexplained metabolic crisis, or unexplained laboratory findings of disturbed intermediary metabolism such as metabolic acidosis, elevated lactate, elevated anion gap, hypoglycemia, ketonemia, neonatal ketonuria, or hyperammonemia. Furthermore, organic acid analysis is indicated in children with unclear hepatopathy, neurological/neuromuscular symptoms including epileptic encephalopathy, and multisystem disorders (particularly when symptoms fluctuate or progress).

Organic acids are optimally analyzed in urine as they are usually efficiently excreted via the kidneys (high water solubility) and show higher concentrations in urine than other body fluids. Nonetheless, exact quantification of specific organic acids in plasma or CSF with isotope dilution analysis (only in selected laboratories) may be useful in selected cases for exclusionary purposes or for therapeutic assessment. Examples include glutaric aciduria type I (glutaric acid and 3-hydroxyglutaric acid), cobalamin defects (methylmalonic acid), tyrosinemia type I (succinylacetone), and various cerebral organic acidurias such as succinic semialdehyde dehydrogenase deficiency (4-hydroxybutyric aciduria) and Canavan disease (N-acetylaspartic acid). Stable isotope dilution assays (either by routine gas chromatography-mass spectrometry or via liquid chromatography-tandem mass spectrometry) are the methods of choice for prenatal diagnoses, when metabolite levels may be low and accurate quantification is required. For postmortem studies, urine should be obtained via bladder puncture; organic acid analysis in postmortem plasma, CSF, or vitreous fluid is much less informative.

Remember

Organic acid analyses in urine are, for the most part, run in a qualitative or only semiquantitative fashion. Exact quantitation of the hundreds of normal (and abnormal) intermediates detected in a human urine sample is laborious, technically challenging, and time consuming but is offered in some laboratories. A key to diagnosis for qualitative analysis is pattern recognition, especially in those instances in which elevations may be only very slight. An experienced diagnostic laboratory, with ample involvement in internal/external QC/QA, is optimal for the best diagnostic outcome.

Remember

Organic acid analysis in plasma is almost never of diagnostic value. Urine is the fluid of choice for analysis. However, in selected instances of fatty acid oxidation defects, increases of C14:0 and C14:1 fatty acids can provide evidence for long-chain hydroxyacyl-CoA dehydrogenase (LCHAD) deficiency. This disorder is readily detected by plasma acylcarnitine analysis (see below).

37.12 Acylcarnitine Analysis

Sample: 3–6 dried blood spots on filter paper (Guthrie card) or 1–2-cc EDTA blood (plasma rapidly separated). Plasma sent on dry ice, blood spots at room temperature.

Acylcarnitine analysis in dried blood spots by electrospray tandem mass spectrometry (MS-MS) or fast atom bombardment MS-MS facilitates diagnosis of most organic acidurias and fatty acid oxidation defects. This method has enabled the massive growth of expanded newborn screening around the world, not only in North America and Europe but Australia, Saudi Arabia, Qatar, and many other countries. The rapidity of analysis (<2 min/sample for newborn screening) lends itself to high throughput analysis. For acylcarnitine analysis, the characteristic daughter ion of all acylcarnitines is *m*/*z* 85 (a fragment of the parent carnitine moiety), an ion selectively monitored for quantitation. Tandem MS-MS analysis of acylcarnitines in plasma is also a primary evaluation for selective screening of inherited metabolic diseases, with diagnostic relevance to organic acidurias, fatty acid oxidation defects, and amino acid disorders (when the appropriate amino acid ion fragment is quantified).

In selected laboratories, acylcarnitine analysis in cultured fibroblasts can provide important diagnostic information on the primary disorder. For example, fibroblasts may be cultured in the presence of [U-^{13}C]leucine, isoleucine, or valine with added L-carnitine. Isolation of cell culture medium and fibroblasts, followed by lysis, enables analysis for ^{13}C-carnitine esters of the corresponding acyl-CoA intermediates which occur in amino acid degradation (L-carnitine transesterifies the acyl-CoA species).

Remember

Acylcarnitine analysis in plasma has become a primary adjuvant for the routine analysis of inherited metabolic diseases. Its rapid throughput and sensitivity makes it the method of choice in emergency situations.

Accumulation of any one acylcarnitine provides evidence for the site of the specific defect in the pathway. Acylcarnitine analysis by tandem MS-MS may also be employed in an emergency in children with acute metabolic crises or hypoglycemia; it is faster than standard organic acid analysis for the diagnosis of organic acidurias, and provides more rapid and reliable identification of the fatty acid oxidation defects. The analysis of many (but not all) relevant amino acids, as well as orotic acid, which are required in the emergency of metabolic decompensation can be quantified simultaneously by MS-MS. Quantification of total and free carnitine by MS-MS using Guthrie cards is inaccurate and requires specific plasma/sera analysis (see below).

37.13 Carnitine Status

Sample: 1 ml serum/plasma, ± 5 ml urine, shipped to the laboratory frozen.

Long-chain fatty acids are transported into the mitochondrion as their respective carnitine esters, a process that requires transesterification of acyl-CoA species at the inner and outer sides of the inner mitochondrial membrane. Similarly, acyl-CoA compounds that accumulate within the mitochondrial matrix may transesterify to L-carnitine esters, with transport out of the mitochondrion and urinary excretion. This process of detoxification induces secondary carnitine depletion in disorders which alter the metabolism of mitochondrial CoA-activated carboxylic acids (e.g., organic acidurias and fatty acid oxidation defects). In the workup of a patient with a suspected inherited metabolic disease, reduced serum carnitine may be regarded as one potential indicator of these disorders. Quantification of total, free, and esterified carnitine in serum or plasma (carnitine status) using MS-MS is mandatory for recognizing carnitine deficiency and

monitoring carnitine supplementation in these disorders, as well as identifying primary carnitine transporter deficiency. Many laboratories still successfully utilize spectrophotometric methods for carnitine analysis on the autoanalyzer.

Free carnitine is efficiently reabsorbed in the renal tubule, while filtered acylcarnitine species accumulate in the urine. Urine carnitine analyses should be performed in conjunction with plasma analyses, and may be indicated if plasma values are abnormal or for monitoring treatment. Increased urinary acylcarnitines are often detected in organic acidurias and fatty acid oxidation defects, and in the context of normal plasma, carnitine values suggest good detoxification capacity. High urine concentrations of free carnitine and a reduced renal tubular reabsorption rate (<90 %) may reveal renal tubular dysfunction as a cause of systemic carnitine depletion or primary carnitine transporter deficiency.

37.14 Lactate, Pyruvate, Ketone Bodies, and Nonesterified Fatty Acids

Sample: 1 ml serum/plasma, ship on dry ice (to avoid lipolysis). If determinations of pyruvate and acetoacetate are requested, rapid deproteinization at the bedside (using perchloric acid) is fundamental for an accurate value.

Lactate, pyruvate, and the ketone bodies (primarily 3-hydroxybutyrate and acetoacetate, but also acetone) are intermediates in blood which provide key information on a number of metabolic processes, including pyruvate metabolism, the citric acid cycle, gluconeogenesis, hepatic glycogenolysis, oxidation of fatty acids, ketogenesis, and the respiratory chain. When quantified in blood in conjunction with glucose and nonesterified fatty acids (NEFA), a wealth of information may be gleaned concerning the function of intermediary metabolism in the patient. All of these intermediates may be determined employing spectrophotometric assays (commercial kits available), generally relying on NAD^+/NADH coupled systems. During fasting, lactate is employed in the liver for glucose production; in the fed state, lactate is a source of energy for the muscle and heart. Pyruvate is a key product of carbohydrate, fat, and protein breakdown that enters the citric acid cycle as acetyl-CoA (catalyzed by pyruvate dehydrogenase). The concentration of ketone bodies in the circulation is regulated by the interplay between hepatic ketogenesis and peripheral consumption as fuel source. Especially during the fasting state, ketone bodies are critical for brain energy needs. Also in the fasting state, free fatty acids are oxidized in the mitochondrion to acetyl-CoA species, which are interconverted in the liver (hepatic 3-hydroxy-3-methylglutaryl-CoA lyase activity) to ketone bodies (acetoacetate primarily).

Remember

Lactate, pyruvate, and the ketone bodies may be quantified in plasma, urine, and cerebrospinal fluid. Nutritional status must be known (fasting, postprandial, or post-loading with triglyceride or other diets). To accurately quantify the keto species (acetoacetate and pyruvate), blood should be immediately deproteinized with perchloric acid at the bedside.

The analysis of nonesterified fatty acids (NEFA) in plasma or serum, in addition to the studies outlined above, provides important information on lipid catabolism and ketogenesis and is essential for any patient with acute metabolic coma or hypoglycemia. NEFA quantification is a critical investigation at the end of a fasting test. Normal values vary greatly according to the metabolic state. High insulin levels in the fed state inhibit lipolysis, resulting in low concentrations of both NEFA (<300 μmol/l) and ketone bodies (<100 μmol/l). Conversely, increased lipolysis during fasting leads to a continuous rise of NEFA levels up to 2–3 mmol/l within 24 h and a corresponding increase of ketone bodies to concentrations that generally exceed those of NEFA. Interpretation of the results at an early stage of the fast may be inconclusive, since NEFA may exceed 1 mmol/l prior to elevations

of ketone bodies. A molar concentration of ketone bodies < NEFA after a 24-h fast suggests a disorder of fatty acid oxidation or ketogenesis; a molar concentration of ketone bodies <50 % of NEFA is strongly suggestive of such disorders. The determination of NEFA without simultaneous determination of ketone bodies has little diagnostic utility.

37.15 Investigations for Congenital Disorders of Glycosylation (CDG)

Sample: 1 ml serum (plasma unsuitable) shipped to the laboratory on dry ice.

In the discipline of inherited metabolic diseases, no other area has grown at such a staggering rate as that of the CDG disorders. To date, over 100 different forms of CDG diseases have been described. CDG type I and type II are two traditional subcategories differentiated by the pattern of serum transferrins following isoelectric focusing or LC-ESI-MS, still the common 1st tier approach for the diagnosis of the CDG disorders. However the older nomenclature can no longer keep up with the growth of new subtypes of discovered CDGs. Current nomenclature uses the gene name and a hyphen with CDG, such that CDG-Ia is now stated as PMM2-CDG, and new subcategories include N-linked, O-linked protein glycosylation, glycolipid synthesis, and multiple protein glycosylation disorders. The majority of the traditional CDG subtypes are under the current subcategories of N-linked or multiple protein glycosylation disorders. In addition to transferrin analysis, N-linked glycan qualitative analysis by MALDI-TOF and O-linked glycan quantitative analysis by LC-MS/MS in serum or plasma has been used to diagnose N-linked and multiple protein glycosylation disorders. In the subcategory of glycolipid synthesis defects, the genetic defect in glycosylphosphatidylinositol (GPI) anchor synthesis has grown rapidly, and diagnosis of this group of CDGs could be achieved by measuring the decrease of surface expression of GPI anchor proteins commonly including CD16, CD59, and FLAER employing leukocytes, erythrocytes, or cultured skin fibroblasts by FACS (fluorescence-activated cell sorting) methods. To date, at least nine different GPI anchor synthesis disorders have been discovered in order to identify subtypes of CDGs that are associated with the glycosylation process in endoplasmic reticulum, metabolic-labeling studies on dolichol-linked oligosaccharides with [2–^{3}H]mannose are carried out in fibroblasts of patients, who present with a characteristic CDG type I transferrin pattern in the IEF but normal PMM2, MPI, and PGM1 activities, thereby excluding PMM2-CDG, MPI-CDG, and PGM1-CDG. Investigations consist of extraction and analysis of dolichol-linked oligosaccharides by HPLC. To further narrow some of the very rare CDG types, a broad spectrum of biochemical methods are performed, which include, e.g., enzyme and transporter activity measurements, lectin, and Western blots for different glycoprotein markers. Since most of these investigations are nonroutine diagnostics with huge experimental efforts behind and therefore extremely time consuming, these assays should be performed in specialized laboratories.

The phenotypic spectrum of the CDGs is broad and covers every system; many of the disorders have a neurological component, but some may have only visceral (gastrointestinal) involvement, while others may feature mainly immune dysfunction. One CDG disorder features accumulation of a specific free tetrasaccharide (Glucose3Mannose1) in the urine of affected patients and glucosylated N-glycan species in N-glycan profile from total serum glycoproteins. In addition, a group of mannose-devoid small N-glycans were recently discovered in N-glycan profile of total serum glycoproteins from patients with PMM2-CDG (CDG-Ia), ALG1-CDG (CDG-Ik), and MPI-CDG (CDG-Ib), which allows a fast screen for these common CDG subtypes that comprises about 80 % of CDG patients with type I transferrin pattern. Thus, evaluation

of serum transferrin and N-glycan analysis has become common analyses in selective screening for N-linked CDG.

- Not a single biochemical analysis can screen for all the CDG subtypes.
- The combination of serum transferrin and N-glycan analysis provides a better screening strategy than transferrin analysis alone although it is still not 100 % sensitive for CDG detection.

In humans, the glycosylation process may involve more than 900 glycosyltransferases, glycosidases, and sugar or protein transporters, all residing in the cytosolic as well as endoplasmic reticulum and Golgi compartments. More than 60 different enzymes are involved in the production of oligosaccharide side chains that may be either lipid or protein linked. Diagnosis and differentiation of several subtypes of CDG involves the demonstration of pathological glycosylation patterns or quantities on the glycoproteins, such as transferrin or apolipoprotein C or total serum glycoproteins. Once an abnormal glycoprotein pattern or glycan profile is detected, additional studies may include enzymology, immunocytochemistry, and mutational studies. Secondary disturbances of glycosylation may be linked to chronic alcoholism, classic galactosemia, or fructose intolerance (deficient mannose-6-phosphate synthesis).

37.16 Purines and Pyrimidines

Sample: minimum 10 ml urine, preferably morning void or 24 h urine collection (refrigerate and avoid light). Sample must be sent refrigerated or on dry ice (especially for diagnosis of adenylosuccinase deficiency).

The spectrum of clinical features in the disorders of purine and pyrimidine metabolism is exceptionally broad, encompassing immunodeficiency, seizures, nephrolithiasis, developmental delay, autistic features, and growth abnormalities. Purine/pyrimidine nucleotides are involved in nucleic acid synthesis and formation of phospholipids and glycogen and are critical for glycosylation reactions that use nucleotide sugar as the building block. The HPLC analysis of purines and pyrimidines in urine may be particularly indicated in patients with renal calculi and related problems, neurological problems, or both. Other symptoms may include arthritis, muscle cramps, and muscle wasting. Purine and pyrimidine excretion is significantly influenced by diet and may vary considerably during the day; thus, a 24 h urine collection may be optimal for analysis, but purines are a favorite food for microorganisms, and thus 24 h collections are accurate only when each voided sample is added to a container in a freezer. For diagnostic work, a spot sample assayed promptly is preferable. Urinary tract infections result in a markedly reduced concentration of purines or pyrimidines in the urine. Foodstuffs such as coffee, black tea, cocoa, or licorice contain methylxanthines and should be avoided during urine collection and 24 h prior.

Remember

Patients with dihydropyrimidine dehydrogenase (DHPD) deficiency and other pyrimidine degradation defects can manifest a life-threatening response to treatment with the antitumor agent 5-fluorouracil, making DHPD deficiency one of the classical pharmacogenetic disorders.

Succinylaminoimidazole carboxamide ribonucleoside (SAICAR), the marker metabolite of adenylosuccinase deficiency, is very unstable at room temperature; thus, if this disorder is suspected, the urine specimen must be maintained frozen and shipped on dry ice. For the diagnosis of disorders of pyrimidine breakdown, urine needs to be examined for dihydropyrimidines via GC-MS (as in organic acid analysis), since these substances are not recognized by HPLC alone. Some groups have begun to extend screening for inherited metabolic diseases in blood spots to the

use of filter papers soaked with urine for analysis of purines and pyrimidines using HPLC coupled to electrospray MS-MS methodology.

37.17 Orotic Acid

Sample: minimum 10 ml urine, sent on dry ice.

Pyrimidine biosynthesis, in which orotic acid is a major intermediate, is regulated at the level of carbamoyl phosphate synthetase II (CPS II), the enzyme catalyzing the first reaction in this pathway. The isoenzyme CPS I (a mitochondrial enzyme) catalyzes the production of carbamylphosphate during the process of urea synthesis. Under conditions in which mitochondrial carbamylphosphate accumulates (e.g., urea cycle disorders), cytosolic carbamylphosphate levels rise and urine orotic acid excretion increases. This may be recognized in organic acid analysis, but orotic acid is a charged nitrogen species which has very limited solubility in organic solvents, and the amount detected by routine GC-MS is variable and at best a fraction of the actual concentration. Thus, accurate quantification of orotic acid requires HPLC or electrospray tandem mass spectroscopy (MS-MS) methodology. Urine orotic acid analysis is one of the key investigations in significant hyperammonemia for the diagnosis of ornithine transcarbamylase (OTC) deficiency. In the past, heterozygous female carriers for OTC deficiency have been detected via urine orotic acid quantification following ingestion of allopurinol, a compound that blocks the conversion of orotic acid to uridine monophosphate. However, the allopurinol loading test is no longer widely employed, because mutational analysis provides more accurate information. Uracil can occasionally be increased in manifesting heterozygous female carriers for OTC deficiency in the absence of increased orotic acid. Also, the allopurinol test may be normal in the presence of some OTC variants. Increased urine orotic acid may be found in other disorders as well, including hereditary orotic aciduria (uridine 5'-monophosphate synthase deficiency) at the very beginning, mitochondrial disease, Rett syndrome, and Lesch-Nyhan syndrome. Low levels of orotic acid are observed in CPSI and CAD (CPSII, aspartate transcarbamylase (ATCase) and dihydroorotase (DHOase)) deficiencies.

37.18 Disorders of Galactose Metabolism

Samples: *Screening* (*galactose*, *galactose-1-phosphate uridyltransferase* (*GALT*) *activity*): 3–6 dried blood spots on filter paper (Guthrie card), routine mail.

Specific analyses (*galactose*, *galactose-1-phoshate*, *galactitol*, *enzyme studies*, *mutation analysis*): EDTA whole blood (2 cc) preferably 30 min post milk feeding, send at ambient temperature. Red blood cells are used for enzyme studies, plasma for metabolite determination; leucocytes may be employed for isolation of genomic DNA for mutation screening. Store only at room temperature (no refrigeration or freezing), since hemolysis leads to significant artifact and galactokinase is a membrane bound protein lost with RBC hemolysis)

Inherited metabolic defects of galactose metabolism are recognized through determination of galactose concentration and GALT activity in dried blood spots (Guthrie card), a core part of newborn screening in many countries. More specific analyses are needed for those newborns with pathological screening tests (Chap. 36). Several laboratories quantify galactose in plasma (normal <3 mg/dl). To decide on necessary intervention in patients with suspected galactosemia (GALT deficiency), galactose-1-phosphate is measured in erythrocytes (normal <0.3 mg/dl). Further, some laboratories quantify urine galactitol (a further metabolite of galactose) using HPLC or stable-isotope dilution methodology with gas chromatography-mass spectrometry (normal <100 mmol/mol creatinine, age 0–1 year; <25 mmol/mol creatinine, age >1 year).

37.19 Sugars

Sample: minimum 10 ml urine, which may be transported refrigerated or on dry ice.

The detection of pathological urinary excretion of sugars is routinely assessed by thin-layer chromatography, although not technologically advanced, is still sufficient. This methodology has utility for the characterization of renal tubular defects and to provide evidence for selected disorders of carbohydrate metabolism. These include (but are not limited to) galactokinase deficiency, GALT deficiency (see above), UDP galactose-4-epimerase deficiency (rare), fructoaldolase (aldolase A) deficiency, fructose-1,6-bisphosphatase (aldolase B) deficiency, and essential fructosuria.

Remember

Urinary accumulation of glycerol may suggest X-linked heritable glycerol kinase deficiency and fructose-1,6-bisphosphatase deficiency and may also be an artifact from the use of glycerin gels/ointments.

The sugars routinely tested using thin-layer methodology include glucose, galactose, fructose, and lactose (laboratory specific), though recent advances in mass spectrometry allow galactose quantification to be measured by UPLC-MS/MS in a few laboratories. The determination of liver glycogen (the key storage form of liver glucose), when a disorder of glycogen storage is suspected, is more complex and requires liver biopsy. Glycogen is quantified only in a limited number of specialized laboratories. Gas chromatography (with and without mass spectrometry) method by which a number of sugar and polyols have been measured (e.g., C4-polyols threitol and erythritol; C5-sugars/polyols arabinose, xylulose, ribose, xylose, ribulose, xylitol, arabitol, and ribitol; C6-sugars/polyols fucose, fructose, glucose, mannitol, sorbitol, and galactitol; and some C7-sugars/polyols including sedoheptulose and sedoheptitol) was crucial in the discovery of two defects in the pentose phosphate pathway (see Sect. 37.20).

37.20 Polyols

Sample: minimum 10 ml urine, shipped on dry ice or refrigerated if transit time is not extensive.

As in the case of the CDG, defects in polyol (sugar alcohols) metabolism represent an expanding area of interest for screening of a patient with suspected inherited metabolic disease. The polyols include C4 (erythritol and threitol), C5 (ribitol, arabitol, and xylitol), C6 (galactitol, sorbitol, and mannitol), and C7 (e.g., sedoheptitol and perseitol). These intermediates may be quantified utilizing GC, GC-MS, or electrospray MS-MS with internal standards. Two discovered disorders in the pentose phosphate pathway have driven the expansion of this area of screening. The first is transaldolase deficiency, which features primarily hepatic dysfunction and the elevated excretion of erythritol, arabitol, ribitol, and the C7 compounds sedoheptitol and sedoheptulose. The other defect, ribose-5-phosphate isomerase deficiency, is very rare (a single case). In that patient, a progressive leukoencephalopathy was associated with considerably increased urine concentrations of arabitol and ribitol, with considerably higher concentrations of these species in cerebrospinal fluid and brain.

37.21 Glutathione (and Analogues)

Sample: 3 ml EDTA whole blood. Centrifuge, remove, and freeze plasma immediately. Deproteinize erythrocyte fraction with 5 % sulfosalicylic acid (ratio approx. 1:1), shake/vortex thoroughly (until homogenous brown color), centrifuge twice at 5,000 × g, remove, and freeze clear supernatant. Send plasma and erythrocyte extract on dry ice.

Defects of the γ-glutamyl cycle lead to a range of clinical problems including neonatal metabolic acidosis, hemolytic anemia, electrolyte disturbances, and a progressive neurological syndrome. All four enzyme defects are inherited in autosomal-recessive fashion; glutathione synthetase deficiency is the most prevalent disorder of this group. Initial investigations include the analysis of organic acids (elevated 5-oxoproline, i.e., pyroglutamic acid), in conjunction with quantification of glutathione and its metabolites in urine, erythrocytes, leukocytes, and/or fibroblasts. Enzyme studies are performed in erythrocytes or other nucleated cells (leukocytes, fibroblasts), but erythrocytes lack γ-glutamyl transpeptidase and 5-oxoprolinase activities.

37.22 Glycosaminoglycans (Mucopolysaccharides)

Sample: 10 ml urine, preferable morning void sent on dry ice or refrigerated if transit time is not extensive

Glycosaminoglycans (GAGs; mucopolysaccharides) are protein-bound oligosaccharides. Mucopolysaccharidoses (MPS) are the result of defective degradation of certain glycosaminoglycans (GAG) which accumulate in the lysosomes and are excreted in the urine. All MPS represent progressive diseases with considerable variability in phenotypic presentation.

Remember

Only MPS II is an X-linked disorder (the remaining MPS disorders are transmitted as autosomal-recessive traits), which may often (but not always) be identified in relation to the creamy-colored skin lesions which are unique to this disease.

These disorders may be detected using quantitative analysis of total urinary GAG concentration (in relation to creatinine), usually through determination of total uronic acid via alcian blue or the carbazole reaction. MPS disorders are more reliably detected through electrophoretic separation of different GAG species, usually on cellulose acetate plates. For example, increased dermatan sulfate (associated with skeletal and organ changes) is found in MPS types I, II, VI, and VII; elevated heparan sulfate (associated with mental retardation and, especially behavioral disturbances in MPS III (Sanfilippo syndromes)) is detected in MPS types I, II, III, and VII.

Remember

Morquio syndrome cannot be identified by quantitation of urine GAGs, as the characteristic metabolite (keratan sulfate) does not react with reagents typically employed to quantify uronic acids.

The increased presence of keratan sulfate (primarily associated with skeletal changes, including scoliosis and/or kyphoscoliosis) is pathognomonic for MPS type IV (the Morquio syndrome). Increased chondroitin sulfate is characteristically found in MPS type VII and less consistently in MPS type IV, but chondroitin sulfate is a normal constituent of urine. Quantification of total urinary GAGs may produce borderline or false-normal results, particularly in MPS types III and IV. In those instances, electrophoretic GAG separation is the method of choice for identification. The determination of quantitative GAG levels is amenable to MS-MS methodology by measuring disaccharides from breakdown of GAG employing enzymatic or chemical reactions, and some laboratories are employing this platform.

37.23 Oligosaccharides

Sample: 10 ml urine shipped refrigerated or on dry ice. When possible, 24 h urine collections are optimal, but not necessary.

Many lysosomal storage disorders result in accumulation of specific oligosaccharides that accumulate and are excreted in the urine. This

is most readily recognized through separation of individual oligosaccharides by thin-layer chromatography (TLC). The chromatographic separation is generally on silica gel, and the plates are developed with solvent systems in a single dimension. For detection, oligosaccharides are reacted with the orcinol reagent followed by heating at 100 °C, and Bial's reagent may be necessary to detect sialylated oligosaccharides. Quantitative analysis is available only for free neuraminic acid (sialic acid) which accumulates in sialic acid storage disease (Salla disease). Oligosaccharide analysis provides evidence for the following disorders: fucosidosis, α- and β-mannosidoses, aspartylglycosaminuria, Schindler disease, sialidosis, GM_1 and GM_2 gangliosidoses (Sandhoff only), galactosialidosis, Gaucher disease, Pompe disease, mucolipidosis types II and III, and Salla disease. However, pathological oligosaccharide patterns are often variable and may be a challenge to recognize by TLC-based methodology. Recently, MALDI/TOF-based qualitative analysis of oligosaccharides provides a much better resolution, and the majority of the oligosaccharidosis can be readily detected by this method. In some patients, clinical suspicion may be developed on the basis of coarse facial features, dysostosis multiplex, and/or ocular involvement (also findings in the MPS disorders). If the phenotype is strongly suggestive of one of these disorders, it is preferable to perform enzyme studies even in the face of normal oligosaccharide findings. On the other hand, also be aware that secondary increase in levels of sialylated oligosaccharides in urine is common in majority of the MPS disorders. Enzyme analysis is generally required for lysosomal storage disorders. However, lysosomal enzyme panels offered by many clinical laboratories do not cover all the common lysosomal storage conditions, and pseudo-enzyme deficiency is present in multiple conditions. Metabolite-based analysis still needs to be considered as part of the first-tier screen, and laboratories with extensive experience in this analysis should be considered and consulted.

37.24 Peroxisomal Function: Very Long-Chain Fatty, Phytanic, and Pristanic Acids

Samples: 1 ml serum/plasma for very long-chain fatty acids (VLCFA), shipped on dry ice to avoid lipolysis (hemolysis is to be avoided as RBCs contribute extensively to VLCFA levels, and the patient should be fasted at draw); 1 ml EDTA whole blood for the analysis of plasmalogens in erythrocytes, express delivery, room temperature

As cellular organelles, the peroxisomes catalyze a number of important processes. These include β-oxidation of very long-chain fatty acids, bile acids, biosynthesis of ether phospholipids, and α-oxidation of branched fatty acids such as phytanic acid. The methodological procedure of choice is quantification of VLCFA and phytanic and pristanic acids using isotope dilution gas chromatography-mass spectrometry, with deuterium-labeled internal standards.

Remember

Combined analysis of VLCFA and pristanic and phytanic acids has great utility in diagnosing peroxisomal disorders. However, diseases such as rhizomelic chondrodysplasia types 2 and 3 and hyperoxaluria type I require additional studies (e.g., erythrocyte plasmalogens, urine glyoxylate and oxalate, etc.)

Quantification of VLCFA via gas chromatography alone relies on peak area and retention time, and is not recommended. Peroxisome biogenesis disorders manifest multisystem pathology in affected patients. Most diseases of peroxisomal biogenesis result in defective peroxisomal beta-oxidation, and result in accumulation of VLCFA species in serum or plasma. Additional studies to pinpoint the exact biochemical defect may include bile acid analysis, plasmalogens, and other species.

Remember

Circulating VLCFA, phytanic acid, and pristanic acid are essentially present in esterified form. Accordingly, they are not filtered by the kidney and cannot be detected in urine.

An isolated elevation of serum phytanic acid is detected in adult Refsum disease. Disorders of etherphospholipid biosynthesis, such as rhizomelic chondrodysplasia punctata (RCDP) or its variants, entail erythrocyte plasmalogen determination in more specialized laboratories.

37.25 Creatine Disorders

Samples: *AGAT and GAMT deficiency*: 10 cc urine (preferably morning void) and 1–2 cc heparinized plasma, for analysis of creatine and guanidinoacetate in both fluids

SLC6A8 (*creatine transporter*) *defect*: 10 cc urine (preferably morning void), for creatine determination expressed in relation to creatinine

Much like the CDG, the defects in creatine biosynthesis and transport have become an additional emerging field in the selective screening for inherited metabolic disorders. The biosynthetic disorders include L-arginine:glycine amidinotransferase (AGAT) deficiency (featuring low guanidinoacetate in urine and plasma), guanidinoacetate methyltransferase (GAMT) deficiency (elevated guanidinoacetate in urine and plasma), and the X-linked creatine transporter (SLC6A8) defect (increased creatine in urine, expressed in relation to creatinine). The latter represents an emerging focus of research into X-linked mental retardation. The nonspecific clinical features in all disorders may include developmental delay, language and speech disorders, autistic behavior, and seizures, occasionally associated with an extrapyramidal movement disorder. A sensitive investigation for all disorders is in vivo proton magnetic resonance spectroscopy (MRS) of the brain, which reveals a significant reduction of creatine.

Remember

X-linked mental retardation syndromes should give rise to a high clinical suspicion for fragile X syndrome as well as the creatine transporter (SLC6A8) defect.

The method of choice for determining guanidinoacetate and creatine in body fluids is isotope dilution gas chromatography-mass spectrometry, although liquid chromatography-mass spectrometry (LC-MS/MS) methods are becoming more widely available. In addition, all creatine disorders can be investigated through brain proton magnetic resonance spectroscopy (^{1}H-MRS), a noninvasive measurement of the decrease in the creatine signal.

37.26 Folate Metabolites

Samples: 1–2-cc EDTA plasma (hemolysis must be avoided, as it artificially increases 5-methyltetrahydrofolate levels). Plasma must be deep-frozen immediately at – 80 °C. Cerebrospinal fluid (any blood must immediately be removed by centrifugation), 1 cc, also deep-frozen immediately at – 80 °C.

The active and principle form of circulating folate is 5-methyltetrahydrofolate (5-MTHF), one-carbon donor in a number of reactions. Biochemical processes including serine and glycine interconversion, homocysteine and methionine interconversion, as well as purine biosynthesis, depend heavily on 5-MTHF as co-substrate. Serum folate deficiency leads to megaloblastic anemia, hyperhomocysteinemia, and neural tube defects in the newborn. Two disorders (dihydrofolate reductase deficiency and cerebral folate transport deficiency caused by pathogenic mutations in the FOLR1 gene) can only be diagnosed by demonstrating

severely reduced 5-MTHF in CSF. Quantitation of 5-MTHF is readily achieved in a number of laboratories employing HPLC separation with electrochemical detection (see Table 37.5).

37.27 Cholesterol Biosynthetic Defects

Sample: 1–2-cc EDTA/heparin serum/plasma, sent on dry ice

Cholesterol is a key membrane component, and the precursor for numerous intermediates such as oxysterols, hormones, vitamins, and bile acids. Cholesterol is fundamental for membrane fluidity, and with sphingomyelin forms the lipid rafts/caveolae, sites at which intracellular signaling molecules aggregate. The predominance of disorders of cholesterol formation occurs at the post-squalene step, a step at which isoprenes formed in the biosynthetic pathway diverge to form either ubiquinone, dolichol, or cholesterol. Two disorders (severe mevalonic aciduria and hyper-IgD syndrome) represent defects of pre-squalene synthesis, and can be identified via routine urine organic acid analysis under most circumstances. However, mevalonate excretion in the hyper-IgD syndrome can be low, and a high degree of clinical suspicion (periodic fever, rash) should lead to specific quantitation of mevalonic acid by stable isotope dilution assay or directly to molecular or enzyme analysis of mevalonate kinase (leukocytes and fibroblasts).

Remember

Immune dysfunction and skin abnormalities (rash, psoriasis) may, in conjunction with other clinical features, suggest a disorder of cholesterol.

With the exception of the Smith-Lemli-Opitz syndrome (SLOS), the remaining post-squalene disorders are exceedingly rare. Plasma analysis of 7-dehydrocholesterol (7-DHC) and (in some laboratories) 8-dehydrocholesterol (8-DHC), employing stable isotope dilution GC-MS, is the first-line investigation for identification of SLOS. Other suspected cholesterol defects (e.g., lathosterolemia, 4-methylsterol oxidase deficiency, lanosterol demethylase deficiency, Antley-Bixler syndrome – defect in P450 oxidoreductase – Greenberg dysplasia, desmosterolosis, congenital hemidysplasia with ichthyosiform erythroderma and limb defects (CHILD) syndrome, Conradi-Hünermann-Happle syndrome) require detailed sterol profiling in specialized laboratories, generally employing gas chromatography-mass spectrometry with the appropriate internal standards. Sterol profile in skin scales or cultured skin fibroblast may be necessary as confirmatory tests. Certain medications such as aripiprazole and trazodone may lead to mild increase of 7-DHC. In all of these disorders, clinical suspicion is raised by patients with abnormalities in morphogenesis, such as hand/toe polydactyly, internal organ defects, and skeletal and/or skin abnormalities.

37.28 Bile Acids

Sample: 10 ml urine, 2 ml EDTA/heparin plasma or 2 ml bile fluid, shipped express delivery on dry ice. Stool may also be evaluated by specialist laboratories.

The primary bile acids, cholic acid (CA) and chenodeoxycholic acid (CDCA) are the end products of cholesterol metabolism that conjugate with glycine or taurine to form the excreted bile salts. A large group of enzymes, located in the endoplasmic reticulum, mitochondria, and peroxisomes of hepatocytes, is involved in the pathway of bile acid synthesis. Therefore, alterations in concentrations of specific bile acids may be observed in various peroxisomal disorders, and also as specific defects of bile acid biosynthesis. Defective side-chain oxidation of

cholesterol is detected in patients with cerebrotendinous xanthomatosis (CTX), characterized by mitochondrial 27-hydroxylase deficiency.

Remember

Serum bile acid levels in healthy (fasting) individuals are generally <2 ug/ml, but may climb artifactually in liver cirrhosis, obstructive jaundice, and viral hepatitis.

CTX results in accumulation of cholestanol and cholesterol in most tissues, whereas serum cholic and chenodeoxycholic acids are significantly decreased. Although a number of methodological approaches are utilized to quantify bile acids in different fluids, the most reliable include GC-MS, LC-MS-MS, and fast atom bombardment (FAB)-MS. Older methodologies, including HPLC, radioimmunoassay, enzymatic analysis, and/or GC alone are more time consuming, laborious, and less sensitive than MS or MS-MS methodologies.

37.29 Porphyrins

Samples: 20 ml urine (random sample), 5 ml feces, 5–10 ml heparinized whole blood; no additives; store cool and dark, may be sent overnight at ambient temperature. In many instances, a 24-h urine collection is desirable.

Porphyrias are inherited abnormalities in heme biosynthesis. Heme formation is highest in the erythrocyte progenitors of marrow, but also high in hepatocytes. Accordingly, the heritable porphyrias manifest significant hepatic involvement. The clinical presentation of the porphyrias facilitates their subdivision into two broad categories, including the acute- and non-acute porphyric syndromes. The acute disorders feature debilitating abdominal pain, nausea, vomiting, constipation, and psychiatric findings. Urine levels of the heme precursor δ-aminolevulinic acid (ALA) and porphobilinogen (PBG) are significantly elevated. Conversely, the non-acute disorders feature dermatological findings primarily in sun-exposed body regions. Congenital erythropoietic porphyria (Günther disease) features discoloration of the urine (brown, red-fluorescent spots in the diapers). Generally, ALA and PBG are readily detected in urine. They may be separated from other contaminants by ion-exchange chromatography. ALA in urine may be derivatized to a pyrrole analogue, and both ALA and PBG may be derivatized as dimethylaminobenzaldehyde derivatives (see Sect. 37.7), which, in the presence of mineral acids, yield strongly fluorescent analogues for both compounds ($Ex = 405$ nm; $Em = 615$ nm). Less polar porphyrins (e.g., coproporphyrin and protoporphyrin) are often only detected in bile secretions. Commercially available kits can be utilized for determination of ALA and PBG.

37.30 Biogenic Amines and Pterins (Specialized CSF/Urine Analyses)

CSF: Collect several samples (age <1 year, 0.5 ml fractions, use fractions 2–4 for metabolic investigations; age >1 year, 1 ml fractions, use fractions 3–5). Freeze samples immediately at the bedside (dry ice or liquid nitrogen), store at –70 °C. The analysis of pterins requires the addition of specific antioxidants (contact the referring laboratory for explicit instructions). If blood stained, centrifuge before freezing. It is strongly recommended that two CSF samples (of approx. 1 ml each) remain in storage at −70 °C for follow-up studies that may become indicated.

Urinary pterins: 10 ml random urine sample, keep cool and dark (dark urine collection bag), ship on dry ice. Alternatively, 5 ml urine, add 6 M HCl to pH 1.0–1.5, add 100 mg MnO_2, shake for 5 min (ambient temperature), centrifuge for 5 min (4,000 rpm), and send supernatant protected against light (aluminum foil) by

express mail. Recently, a reliable method to differentiate and quantify pterins form a filter paper card has been successfully established. Sepiapterin is not detected in routine investigations of biogenic monoamine metabolites in CSF but can be determined reliably in a few specialized laboratories.

For the suspected neurometabolic disorder, a lumbar puncture should only be pursued after basic analyses have been performed in blood and urine, and following neuroimaging studies (see Sect. 27.15). The use of cerebrospinal fluid (CSF) is not warranted in selective screening, and there must be a high degree of clinical suspicion to suggest a lumbar puncture. Nonetheless, several neurometabolic disorders can only be diagnosed by specific CSF studies (Table 37.5), especially the disorders of monoamine metabolism. As a rule with any CSF investigation, the analysis should include quantitative determination of lactate, pyruvate, amino acids, cell count, glucose, protein, immunoglobulin classes, and specific immunoglobulins and an evaluation of the integrity of the blood-brain barrier.

Remember

The analysis of monoamines in CSF requires analysis in only well-skilled, specialized laboratories with the appropriately derived collection protocols and age-related control ranges necessary for correct evaluation of the findings.

Remember

CSF monoamine concentrations show diurnal variation and a rostrocaudal gradient of concentration. Exact collection protocols must be followed for collection of CSF to ensure meaningful data are obtained.

Remember

Many specialized laboratories provide collection tubes for CSF samples, and detailed instructions. Selected tubes in the collection set will contain pertinent antioxidant compounds (e.g., for tetrahydrobiopterin) that will insure accurate measurements.

Generally speaking, the term biogenic amine is equivalent to monamines, but in the case of inherited metabolic disorders encompasses catecholamines (dopamine, noradrenaline) and serotonin. These derive from amino acid precursors tyrosine and tryptophan. The end products of dopamine and serotonin metabolism include homovanillic (HVA) and 5-hydroxyindoleacetic acids (5-HIAA). Defects in these pathways have a significant component of neurological dysfunction. Species including HVA, 5-HIAA, dopamine, 3,4-dihydroxyphenylacetate, 5-hydroxytryptophan, and 3-O-methyldopa are quantified by HPLC with electrochemical detection. Normative data vary extensively with age and sex, and thus it is fundamentally important to only send samples to a highly experienced, specialized laboratory. Dopamine is the natural inhibitor of prolactin secretion, and occasionally high serum prolactin may be an important sign for dopamine-dependent dystonia.

Quantifying pterins in all body fluids (urine, CSF, plasma, etc.) is the most common and reliable method for diagnosing the inborn errors of tetrahydrobiopterin (BH_4) metabolism. BH_4 is a mandatory cofactor in the phenylalanine hydroxylase reaction, and its intracellular level regulates tyrosine and tryptophan hydroxylases, and nitric oxide synthase. Pterin analysis is mandatory in all patients identified with hyperphenylalaninemia through newborn screening and should be included in any study quantifying monoamines in CSF as well. A number of defects have been demonstrated in the biosynthetic pathway of BH_4 synthesis, including GTP cyclohydrolase deficiency, 6-pyruvoyl-tetrahydropterin synthase (PTPS) deficiency, and sepiapterin reductase (SR) deficiency. Quantitation of pterin levels is a valuable tool for identification of these disorders. The analysis of neopterin, biopterin, 5-MTHF, 5-HIAA, and HVA serves to differentiate between mild and severe forms of BH_4 deficiencies. Because of its key role in phenylalanine metabolism, selected screening for BH_4 deficiency must be pursued in newborns whose plasma phenylalanine exceeds

120 μmol/L. Notably, neopterin is also employed as a marker of T-helper cell-derived cellular immune activation. Pterin analogues are generally present in physiological fluids in reduced forms, but may be artificially oxidized to highly fluorescent species prior to HPLC analysis with fluorescent detection. Neopterin, biopterin, and primapterin are blue-fluorescing species, whereas sepiapterin is a yellow-fluorescing compound.

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38 Enzyme Diagnostics in a Changing World of Exome Sequencing and Newborn Screening as Exemplified for Peroxisomal, Mitochondrial, and Lysosomal Disorders

Ronald J.A. Wanders, Ben J.H.M. Poorthuis, and Richard J.T. Rodenburg

Key Facts

- Enzymes are the key players of metabolism since they are the true catalysts of biological reactions.
- Flux through metabolic pathways at any given substrate concentration is determined by the kinetic properties of the individual enzymes in the pathway as reflected in their Michaelis constants (K_m, K_i) and turnover numbers (V_{max}, k_{cat}).
- Mutations in genes coding for metabolic enzymes may either affect the Michaelis constants (K_m, K_i), turnover numbers (V_{max}, k_{cat}), and/or the amount of enzyme protein.
- In the classical approach, aimed to identify the defect in a patient suspected to suffer from an inborn error of metabolism (IEM), enzymatic analysis has been the obligatory step following metabolite analysis.
- With the introduction of rapid and highly reliable DNA sequencing techniques, the order of events in the diagnostic algorithm of IEMs is rapidly changing with enzymology and metabolite analysis often following whole exome and/or whole genome sequencing.
- Enzymatic analysis will remain of key importance in order to ascertain the functional consequences of the mutation(s) found with whole exome and/or whole genome sequencing and the consequences for the metabolic pathway in which the mutant enzyme is involved.
- The importance of enzymatic analysis both in the classical and current approach is especially clear for mitochondrial, peroxisomal, and lysosomal disorders.

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38.1 Introduction

Until only recently, the classical approach toward the identification of patients affected by an inborn error of metabolism (IEM) would start with a patient in whom a physician, including a pediatrician, clinical geneticist, (child) neurologist, or otherwise, suspects an IEM based on a certain set of clinical signs and symptoms strengthened by additional information coming from routine clinical chemistry, imaging studies, and family history, among others. Different scenarios can be discerned in this classical approach. In one scenario the clinical signs and symptoms of a patient directly point to a certain IEM so that the laboratory diagnosis can be simple and directly focused on measurement of a characteristic biomarker associated with the disease followed by enzyme studies and/or direct molecular analysis. Smith-Lemli-Opitz (SLO) syndrome in its classical form, with 7-dehydrocholesterol as characteristic biomarker, is a good example. If abnormal, the SLO gene is usually sequenced directly without enzyme testing (Fig. 38.1a).

A second scenario is when the clinical signs and symptoms combined with results from other studies are not immediately suggestive for a specific IEM but rather point to a group of related IEMs which requires laboratory analysis of a selected panel of metabolites. The mucopolysaccharidoses, peroxisomal disorders, and mitochondrial fatty acid beta-oxidation deficiencies are good examples of this category. Indeed, in any patient presenting with drowsiness, lethargy, and listlessness, with or without cardiac features, especially when combined with the finding of hypoketotic hypoglycemia, a defect in mitochondrial beta-oxidation would be a logical option to consider which requires acylcarnitine analysis as first-line diagnostic test, followed by enzymatic and molecular studies if an abnormal acylcarnitine profile is detected (Fig. 38.1a). In this scenario, the importance of enzyme studies is obvious since it allows not only determination of the true enzyme deficiency and the gene involved but also allows accurate assessment of the severity of the enzyme deficiency in terms of residual activity (see, for instance, Wanders et al. (2010)).

In clinical practice, however, the most frequently observed scenario is that of a patient showing clinical signs and symptoms which are not immediately suggestive for a specific IEM or group of IEMs. In such cases, a broader panel of metabolites needs to be analyzed in the blood, urine, and/or cerebrospinal fluid (CSF). Most laboratories use targeted analysis of groups of metabolites for this purpose such as the analysis of amino acids, organic acids, acylcarnitines, mucopolysaccharides (MPS), and a range of other metabolites with the aim to cover as much of the total metabolome as possible using these assays. Based on the type of metabolic abnormalities identified, the activity of one or more particular enzymes needs to be determined, followed by DNA testing (Fig. 38.1a).

At least two new developments in the field of IEMs have changed this classical diagnostic algorithm as depicted in Fig. 38.1a. The *first* is the inclusion of an increasing number of IEMs in newborn screening programs around the world. In the USA, >50 different IEMs are now being screened for in newborns, at least in some states. The expansion of the number of IEMs in newborn screening programs in the USA, the Netherlands, and other countries, has certainly changed the diagnostic process and allows identification of patients before clinical signs and symptoms have become apparent. Unfortunately, newborn screening is not perfect and is inevitably associated with the identification of so-called false positives. Subsequent clinical and laboratory investigations need to be performed in these neonates to resolve whether the newborn identified by newborn screening is truly affected or not. At the laboratory level, this requires metabolite analyses and/or enzymatic analyses. For virtually all these disorders as included in the Dutch neonatal screening program, rapid and unequivocal enzyme testing can now be done in plasma, erythrocytes, and/or lymphocytes, to resolve this question (see Wanders et al. (2010), for instance). Taken together, newborn screening for IEMs has not only considerably changed the role of physicians in the identification of IEM patients but also that of the laboratory, especially with respect to the role of enzyme diagnostics in the diagnostic

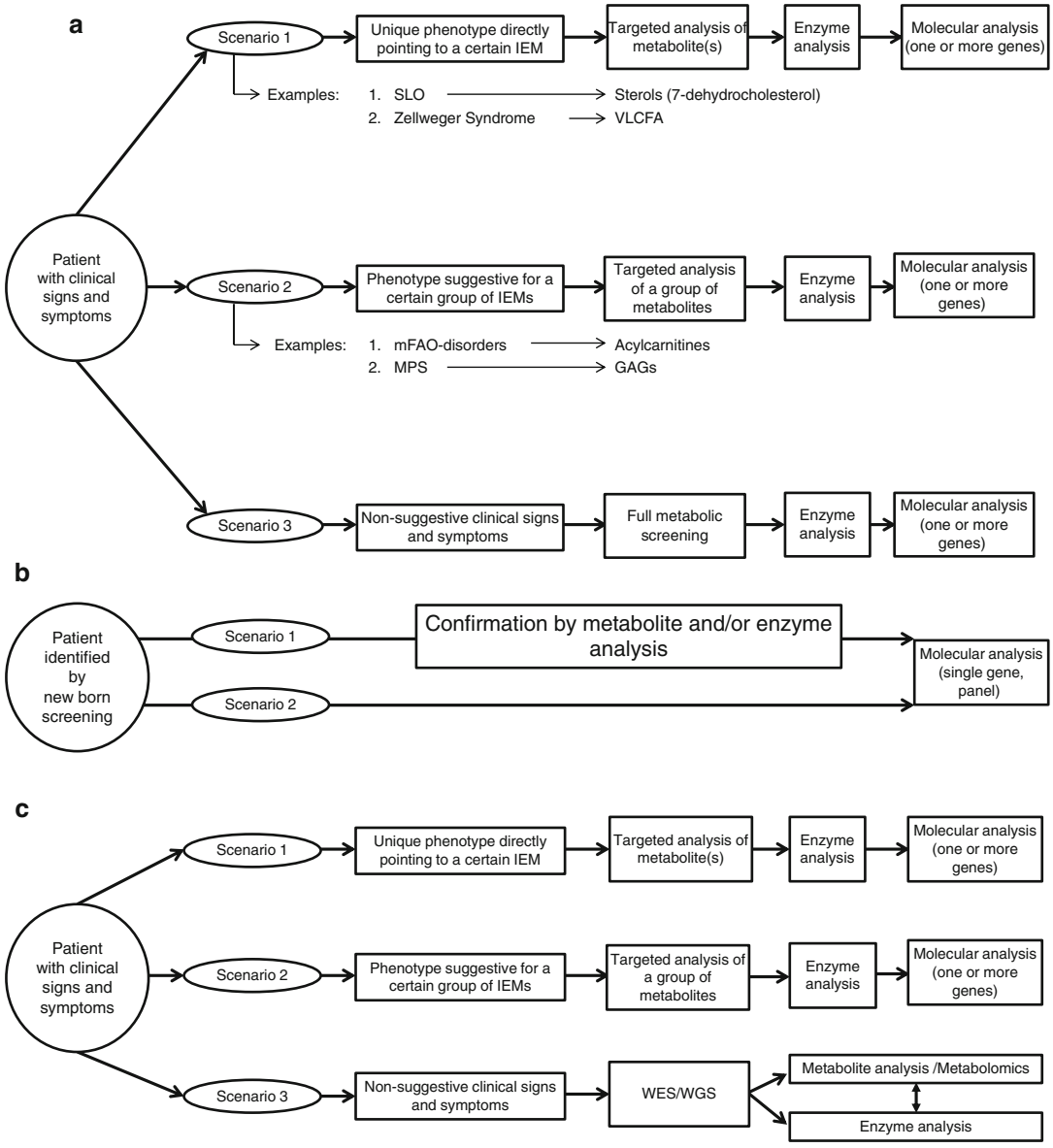

Fig. 38.1 Schematic diagrams depicting the classical (**a**) and current (**b**, **c**) algorithms for the diagnosis of patients suspected to suffer from an IEM (See text for background information)

process in case a newborn has been identified with an abnormal screening result as described above (Fig. 38.1b).

A *second* major development which has revolutionized the diagnostic process is the introduction of different DNA sequencing methods such as panel, whole exome, and whole genome sequencing. Based on the success of these new technologies, whole exome and/or whole genome sequencing in patients suspected to suffer from a genetic disease – metabolic or not – has gained increasing popularity as first-line diagnostic test to the extent that analysis of metabolites in body fluids is no longer performed first, at least in several centers around the world. For a number of reasons, we strongly believe that this is definitely not the best way forward. Firstly, metabolite analysis either targeted or not, still gives the most

direct information about the metabolic status of a particular patient and, if abnormal, usually paves the way to the identification of the enzyme deficiency and the genetic defect plus the rapid institution of therapeutic measures. On the other hand, when whole exome sequencing/whole genome sequencing (WES/WGS) analysis is performed as a first-line diagnostic test, the results are often difficult to interpret since in many instances genetic variants are encountered that are of unknown significance. Furthermore, patient databases are filled with supposedly pathogenic variants that have not been validated experimentally and in fact may turn out to have no functional consequences. In addition, prediction programs like Polymorphism Phenotyping (PolyPhen) and Sorting Intolerant From Tolerant (SIFT) are far from optimal with correct predictions limited to some 60–70 % at most. Another point of concern is that existing WES/WGS analyses do not cover the exome or genome completely. Based on all these arguments, we strongly believe that a combined approach which consists of both proper biochemical (targeted analysis of metabolites and/or (untargeted) metabolomics) and high throughput molecular genetic testing, either in parallel or one after the other, is the method of choice for the proper diagnosis of patients especially when the clinical signs and symptoms are not directly pointing toward a certain IEM or a group of IEMs (Fig. 38.1c). In case of patients showing clinical signs and symptoms suggestive for a specific IEM or group of IEMs, the diagnostic approach remains identical to that shown in Fig. 38.1a in the classical approach. Regardless of whether the diagnosis has been based on metabolite abnormalities or is derived from high throughput molecular genetic testing, metabolite studies and enzymatic analyses remain of key importance. Indeed, if a metabolic abnormality has been identified, enzymatic studies are required to pinpoint the enzymatic defect. On the other hand, when WES or WGS has identified mutation(s) in a certain gene, subsequent studies at the metabolite, protein, and enzymatic levels are usually necessary to ascertain whether the mutation(s) truly affect(s) enzyme function (Fig. 38.1c). Apart from single enzyme testing, flux studies in intact organelles including mitochondria and/or preferably whole cells (lymphocytes, fibroblasts, biopsies) are of key importance since such studies can provide direct information on the effect of the dysfunctional enzyme protein on the functional capacity of the metabolic pathway in which the enzyme is involved. In this chapter, we will describe the basic features of enzymes, how to measure their activities, and the importance of biochemical studies including enzyme testing as exemplified for peroxisomal disorders, OXPHOS deficiencies, mitochondrial beta-oxidation defects, and lysosomal storage disorders.

38.2 Basic Principles of Enzymes and Enzyme Diagnostics Relevant for IEMs

Enzymes are the key players of metabolism since they are the true catalysts of biological reactions. These remarkable molecular devices convert a particular substrate into a specific product, a process called catalysis. Nearly all known enzymes are proteins, although proteins do not have an absolute monopoly on catalysis, since the discovery of catalytically active RNA molecules (ribozymes). Proteins are built from a repertoire of 20 different amino acids, whereby the amino acids are linked by amide bonds, formed between the carboxyl group of 1 amino acid and the amino group of the next amino acid, thus giving the primary structure of the protein. In reality, proteins adopt a folded structure and are often part of large, multi-subunit structures either in the form of homo- or heteromultimers.

A remarkable feature of enzymes is that they usually show a high level of specificity, which means that the enzyme only converts one or at most a few substrates into the corresponding products. The underlying basis for this specificity is that the active site at which catalysis takes place generally takes the form of a three-dimensional cleft or crevice formed by groups that usually come from different parts of the

amino acid sequence. The three-dimensional active site shields the substrate(s) from the solvent and creates a unique microenvironment, which allows catalysis to take place.

Enzymes may accelerate reactions by factors of as much as a million or more. Indeed, most reactions in biological systems do not take place at any perceptible rate in the absence of enzymes. Even a reaction as simple as the hydration of CO_2 to H_2CO_3 is catalyzed by an enzyme, i.e., carbonic anhydrase. The identification of IEMs in which one of the carbonic anhydrases is deficient shows the importance of such enzymes in human metabolism (e.g., see van Karnebeek et al. (2014)). The transfer of CO_2 into the blood and then to the alveolar air would be less efficient in the absence of carbonic anhydrase.

In order to understand how a certain enzyme functions in a particular metabolic pathway, it is important to know the properties of the enzyme in terms of its ability to convert a certain substrate (*S*) into a particular product (*P*). These properties are best described in the K_m and V_{max} values of that particular enzyme for a certain substrate. The K_m, also called Michaelis constant, is defined as the concentration of substrate at which the enzyme operates at 50% of its maximal velocity, whereas V_{max} is defined as the turnover number of an enzyme, which is the number of substrate molecules converted into product by an enzyme molecule in a unit time under conditions such that the enzyme is fully saturated with substrate. Figure 38.2a shows a plot of the reaction velocity (v_i) as a function of the substrate concentration (*S*) for an enzyme that obeys Michaelis-Menten kinetics, which clearly illustrates that the maximal velocity (V_{max}) is approached asymptotically. The Michaelis constant (K_m) can best be determined with the aid of a double-reciprocal or Lineweaver-Burk plot (Fig. 38.2b). A simple, direct measure describing the efficiency of a particular enzyme is the catalytic efficiency of an enzyme defined as k_{cat}/K_m or, alternatively, as V_{max}/K_m whereby k_{cat} and V_{max} are essentially similar parameters for the same property of each individual enzyme which is its turnover number.

When localized within the cell, the activity of an enzyme may be changed by several distinct mechanisms. One key mechanism is allosteric control, in which the activity of an enzyme is stimulated or decreased by effectors of the enzyme which bind at an allosteric site. Examples of allosteric enzymes are (1) carbamoyl phosphate synthase (allosteric effector (positive), N-acetyl glutamate); (2) pyruvate carboxylase (allosteric effector (positive), acetyl-CoA); (3) aspartate transcarbamoylase (allosteric effector (negative), cystidine triphosphate (CTP)); and (4) carnitine palmitoyltransferase 1 (CPT1) (allosteric effector (negative), malonyl-CoA).

A second level of control is exerted by the reversible covalent modification of enzymes. Many modifications are known at present, ranging from glycosylation, hydroxylation, acylation,

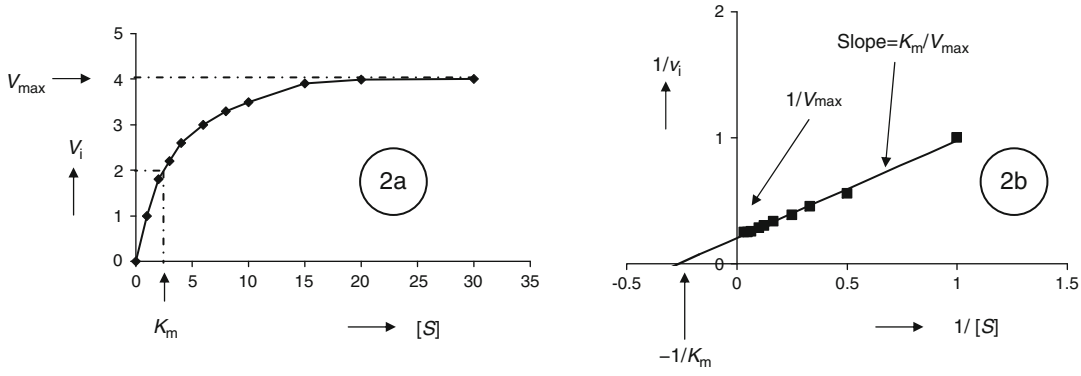

Fig. 38.2 Effect of the concentration of substrate (*S*) on the initial velocity (*v*) of an enzyme-catalyzed reaction represented in a *V* versus *S* plot (**a**) or a double-reciprocal, Lineweaver-Burke plot of 1/*v* versus 1/*S* (**b**)

farnesylation, phosphorylation, ubiquitination, acetylation, et cetera. Phosphorylation of proteins is achieved by protein kinases, which catalyze the transfer of the terminal phosphoryl group of adenosine triphosphate (ATP) to the hydroxyl groups of seryl, threonyl, or tyrosyl residues, thus forming O-phosphoseryl, O-phosphothreonyl, and/or O-phosphotyrosyl residues. Typical examples of mammalian enzymes undergoing phosphorylation/dephosphorylation are acetyl-CoA carboxylase, glycogen synthase, pyruvate dehydrogenase, and HMG-CoA reductase.

The activity of enzymes can also be regulated by changing its quantity. Indeed, whereas some enzymes are constitutive in nature, which implies that the concentration remains essentially constant over time, other enzymes are inducible. Examples of the latter category are (1) the enzymes of the urea cycle, as induced by high-protein diets; (2) the enzymes of the mitochondrial fatty acid oxidation system as induced by high-fat diets, prolonged fasting, and fibrates; and (3) HMG-CoA reductase and the other enzymes of the cholesterol biosynthesis pathway as induced by low cholesterol.

38.3 Enzyme Diagnostics

Enzyme diagnostics requires the accurate and specific analysis of enzyme activities in cells from an individual patient either isolated from blood (erythrocytes, lymphocytes, platelets, etc.), obtained by biopsy (muscle and/or liver), or grown from a skin biopsy (fibroblasts). Preferably, enzyme activities should be measured under physiological conditions taking into account parameters like pH, ionic composition of the medium, and type of substrate(s), among others, as discussed below in more detail:

1. *pH*: The activity of an enzyme should be measured at the actual physiological pH. This implies that the activity of cytosolic enzymes should be measured at pH~7.0, whereas the activity of mitochondrial matrix enzymes, for instance, should be measured at pH~8.0. In reality, enzymes are often measured under conditions at which the enzyme shows the highest activity which may be far away from the physiological pH. For example, the activity of the urea cycle enzyme arginase, which is a cytosolic enzyme, is usually not measured at pH~7.0 but at pH > 9.0 because the pH optimum of the enzyme is 9.5.
2. *Enzyme activity measurements in the forward or backward mode*: Enzymes are not always measured in the true physiological direction but actually in the reverse direction as several enzymes are more active in the reverse, nonphysiological direction. This is true, for instance, for the mitochondrial enzyme 3-hydroxyacyl-CoA dehydrogenase which is preferably measured in the nonphysiological direction from 3-ketoacyl-CoA to 3-hydroxyacyl-CoA (see Wanders et al. (2010)).
3. *Enzymes are not always measured with the true physiological substrate(s) but instead some derivative thereof.* The lysosomal enzymes are a good example in this respect. Indeed, these enzymes are often measured using artificial substrates including the 4-methylumbelliferyl- and dinitrophenyl-conjugated substrates.
4. *Enzymes are often measured at concentrations of the substrate(s) which are far away from the physiological concentrations of the substrate(s) and, in fact, are often measured at concentrations exceeding the Km value(s).* Under such conditions the enzyme runs at maximal velocity whereas under in vivo conditions, the enzyme often runs at a fraction of its maximal velocity. Ideally, one should measure enzyme activities at different substrate concentrations and calculate the *catalytic efficiency* of an enzyme. This is especially relevant for enzyme diagnostics in inborn errors of metabolism in which mutations are often missense mutations causing amino acid substitutions which may well leave the V_{max} intact but not the *K*m. Measurement of the catalytic efficiency of an enzyme would identify such abnormalities, whereas in the classical approach when enzymes are measured at substrate concentrations far exceeding the Km, these abnormalities would remain undetected.

38.4 Methods Involved in Enzyme Diagnostics

In principle there are a large variety of different methods allowing the activity of enzymes to be measured. This includes spectrophotometric and fluorometric methods in which enzyme reactions are either coupled to the consumption or generation of NAD(H) or NADP(H) or generate a light absorbing or fluorescent product. Ideally enzyme activities should be measured by separating the substrate(s) and product(s) using high-performance liquid chromatography (HPLC) or ultra performance liquid chromatography (UPLC) with UV or fluorescence detection or using other methods of detection including gas chromatography/mass spectrometry (GC/MS) or tandem mass spectrometry (MS/MS). These practical details will not be discussed here further.

38.5 The Role of Enzyme Studies in the Diagnostic Work-up of Peroxisomal, Mitochondrial, and Lysosomal Disorders

38.5.1 Peroxisomal Disorders

38.5.1.1 Background

The peroxisomal disorders (PDs) represent a group of inherited diseases in man in which there is an impairment in either the biogenesis of peroxisomes or one of the metabolic functions of peroxisomes (Sacksteder and Gould 2000; Weller et al. 2003; Wanders 2004, 2014; Braverman and Moser 2012; Fujiki et al. 2014). Accordingly, the peroxisomal disorders are usually subdivided into two subgroups, including (1) the peroxisome biogenesis disorders (PBDs) and (2) the single peroxisomal enzyme deficiencies. The group of peroxisome biogenesis disorders is again subdivided into two categories, including (1) the Zellweger spectrum disorders with Zellweger syndrome, neonatal adrenoleukodystrophy (NALD), and infantile Refsum disease (IRD) as representative disorders and (2) rhizomelic chondrodysplasia punctata (RCDP) type 1.

The principal features of the biogenesis of peroxisomes have been elucidated in recent years. In many respects the biogenesis of peroxisomes resembles that of mitochondria, although peroxisomes, unlike mitochondria, do not possess their own DNA. This implies that the genetic information for *all* peroxisomal proteins irrespective of their localization in the peroxisomal membrane or the peroxisomal matrix is nuclear DNA encoded (Platta and Erdmann 2007).

Peroxisomal proteins are targeted to peroxisomes by virtue of distinct peroxisomal targeting signals (PTS) which are recognized by the peroxisomal protein uptake machinery, followed by their incorporation into either the peroxisomal membrane or the peroxisomal matrix. Multiple so-called peroxins encoded by PEX genes are involved in this process. This immediately explains why the group of PBDs is genetically heterogeneous. Indeed, the molecular basis of the PBDs is remarkably diverse and involves mutations in 12 different PEX genes (Waterham and Ebberink 2012; Fujiki et al. 2014). Interestingly, mutations in *PEX7* which codes for a specific peroxin involved in the import of a distinct set of peroxisomal proteins carrying the so-called PTS2 signal are only found in patients with rhizomelic chondrodysplasia punctata but not in patients having a Zellweger spectrum disorder-like phenotype.

With respect to the metabolic role of peroxisomes, peroxisomes are known to be involved in various metabolic processes, including (1) fatty acid beta-oxidation, (2) ether phospholipid biosynthesis, (3) fatty acid alpha-oxidation, and (4) glyoxylate metabolism as most important peroxisomal functions, at least from the perspective of human disease (Wanders and Waterham 2006). Accordingly, the group of single peroxisomal enzyme deficiencies can be subdivided into subgroups based on whether the enzyme defect affects beta-oxidation, alpha-oxidation, ether phospholipid biosynthesis, or glyoxylate metabolism. Table 38.1 lists the peroxisomal disorders as identified until now with details on the mutated gene and dysfunctional enzyme/transporter protein in each of the diseases.

Table 38.1 List of peroxisomal disorders plus information on the enzyme and gene defects, the metabolite abnormalities in blood, and the first-line test for each of the disorders

				Plasma					Erythrocytes
		Enzyme defect	Gene defect	VLCFA	THCA/DHCA	Phyt[a]	Pris[a]	Pi	Plasmalogens
Peroxisome biogenesis disorders	Zellweger spectrum disorders	*Deficiency of most but not all peroxisomal enzyme activities*	*PEX1,2,3,5,6,10,12, 13,14,16,19,26*	**↑**	↑	↑	↑	↑	↓
	Rhizomelic chondrodysplasia punctata type 1	PTS2-protein deficiency (ADHAPS, PHYH, peroxisomal thiolase 1)	*PEX7*	**N**	**N**	↑	**N**	**N**	**↓**
Single peroxisomal enzyme deficiencies	*Peroxisomal beta-oxidation defects*								
	X-linked adrenoleukodystrophy	ALDP	*ABCD1*	**↑**	**N**	**N**	**N**	**N**	**N**
	Acyl-CoA oxidase deficiency	ACOX1	*ACOX1*	**↑**	**N**	**N**	**N**	**N**	**N**
	D-bifunctional protein deficiency	DBP	*HSD17B4*	**↑**	↑	↑	↑	**N**	**N**
	SCPx deficiency	SCPx	*SCP2*	**N**	**↑**	↑	↑	**N**	**N**
	AMACR deficiency	AMACR	*AMACR*	**N**	**↑**	↑	↑	**N**	**N**
	70-kDa peroxisomal membrane (PMP70) deficiency	PMP70	*ABCD3*	**N**	**↑**	**N**	**N**	**N**	**N**
	Plasmalogen biosynthesis defects								
	RCDP Type 2	DHAPAT	*GNPAT*	**N**	**N**	**N**	**N**	**N**	**↓**
	RCDP Type 3	ADHAPS	*AGPS*	**N**	**N**	**N**	**N**	**N**	**↓**
	RCDP Type 4	FAR1	*FAR1*	**N**	**N**	**N**	**N**	**N**	**↓**
	RCDP Type 5	PTS2-proteins (ADHAPS, PHYH, peroxisomal thiolase 1)	*PEX5L*	**N**	**N**		**N**	**N**	**↓**
	Phytanic acid alpha-oxidation defects								
	Refsum disease	PHYH (phytanoyl-CoA2-hydroxylase)	*PHYH*	**N**	**N**	**↑**	**N**	**N**	**N**
	Bile acid synthesis defects								
	Bile acid CoA:amino acid N-acyltransferase (BAAT) deficiency[b]	BAAT	*BAAT*	**N**	**N**	**N**	**N**	**N**	**N**
	Glyoxylate metabolism defects								
	Hyperoxaluria type 1[c]	AGT	*AGTX*	**N**	**N**	**N**	**N**	**N**	**N**

The first-line test for each individual disorder is encircled

VLCFA very long-chain fatty acids, *BA* bile acid intermediates, *Phyt* phytanic acid, *Pris* pristanic acid, *Pi* pipecolic acid

[a]Accumulation of phytanic acid and pristanic acid is diet- and age-dependent

[b]In BAAT deficiency there is accumulation of the primary bile acids in their unconjugated form

[c]In hyperoxaluria type 1, there is accumulation of oxalate, glycolate, and glyoxylate. For each of the disorders, the first-line diagnostic test is marked in bold

38.5.1.2 Laboratory Diagnosis of Peroxisomal Disorders

Once a patient is suspected to suffer from one of the peroxisomal disorders on clinical grounds, metabolite analysis of specific (sets of) metabolites should be initiated which differs for each of the peroxisomal disorders (see Table 38.1). In general, most of the peroxisomal disorders known until now can be screened for reliably by analyses in plasma and/or erythrocytes. This is true for the Zellweger spectrum disorders plus acyl-CoA oxidase (ACOX) deficiency and D-bifunctional protein (DBP) deficiency by performing plasma VLCFA analysis. This is also true for the different forms of rhizomelic chondrodysplasia punctata if plasmalogen analysis is performed in erythrocytes and also for Refsum disease if plasma phytanic acid is measured. The same applies to alpha-methylacyl-CoA racemase (AMACR) and sterol carrier protein X (SCPx) deficiency if the plasma bile acid intermediates are measured. Finally, plasma very-long-chain fatty acid (VLCFA) analysis is also very reliable for the identification of X-linked adrenoleukodystrophy (X-ALD), especially in case of hemizygote detection. Identification of the true defect in each of these cases warrants detailed enzymatic studies in fibroblasts as described below.

38.5.1.3 Enzymology of the Peroxisomal Disorders

Following the analysis of metabolites as described above, enzymatic studies are required for most of the peroxisomal disorders with some exceptions as described below in order to identify the ultimate genetic defect. The latter is especially important if prenatal diagnosis is required in the future. Below we will describe the enzymology of the different groups of peroxisomal disorders.

1. In any patient with a Zellweger spectrum disorder-like phenotype in which VLCFAs have been found abnormal in plasma plus or minus abnormalities in any of the other peroxisomal parameters measured, including the bile acid intermediates, phytanic acid, pipecolic acid, and erythrocyte plasmalogens, a full enzymatic study in fibroblasts is warranted in order to resolve whether the patient has a defect in the biogenesis of peroxisomes or is affected by a single peroxisomal enzyme deficiency, notably at the level of acyl-CoA oxidase (Ferdinandusse et al. 2007) or D-bifunctional protein (Ferdinandusse et al. 2006a). Such a study includes (1) measurement of C26:0 and pristanic acid beta-oxidation, (2) plasmalogen biosynthesis, (3) phytanic acid alpha-oxidation, and (4) immunofluorescence microscopy analysis of peroxisomes using antibodies raised against catalase. If all parameters including immunofluorescence microscopy analysis are abnormal, a PBD is very likely and subsequent complementation analysis, followed by molecular analysis of the relevant PEX gene is necessary to identify the gene which is defective (see Waterham and Ebberink 2012). If the abnormalities as observed in fibroblasts are restricted to the peroxisomal beta-oxidation system only, one is probably dealing with either acyl-CoA oxidase (Ferdinandusse et al. 2007) or D-bifunctional protein deficiency, which can be resolved by direct measurement of the activity of these enzymes in fibroblasts, followed by analysis of either ACOX1 or hydroxysteroid (17-beta) dehydrogenase 4 (HSD17B4) (see Wanders 2004).
2. In any patient with a rhizomelic chondrodysplasia punctata-like phenotype, plasmalogens should be analyzed in erythrocytes and, if abnormal, it is fully certain that one is dealing with one of the peroxisomal forms of rhizomelic chondrodysplasia punctata, albeit type 1, 2, 3, or 4 as caused by mutations in either *PEX7*, *GNPAT*, *AGPS*, or *FAR1*. The latter three genes code for the three peroxisomal enzymes involved in etherphospholipid biosynthesis, i.e., dihydroxyacetone phosphate acyltransferase (DHAPAT), alkyl-DHAP synthase (ADHAPS), and fatty acyl-CoA reductase 1 (FAR1). Resolution between these different forms of rhizomelic chondrodysplasia can be done via enzymatic studies in fibroblasts, which includes direct measurement of DHAPAT and alkyl-DHAP synthase, followed by molecular analysis of the different

genes. Importantly, erythrocyte plasmalogen analysis has turned out to be an extremely powerful initial screening method since all patients with RCDP, which have been analyzed by us, have shown clearly deficient plasmalogen levels in erythrocytes independent whether it was RCDP type 1, 2, 3, or 4 with only one exception, which concerns a patient with a very mild Refsum-like phenotype (Horn et al. 2007). Recently, a new type of RCDP has been described caused by mutations in *PEX5* (Baroy et al. 2015) which codes for two different proteins including PEX5S and PEX5L. Interestingly, the mutations in the patient with this new type of RCDP only affect the synthesis of PEX5L but not PEX5S which explains why the PTS2- but not PTS1-pathway is disrupted (Baroy et al. 2015).

3. In patients with a Refsum-like phenotype in which plasma phytanic acid levels are elevated, fibroblasts studies should be done with prime emphasis on phytanic acid alpha-oxidation, which, if abnormal, should be followed up by measurement of phytanoyl-CoA hydroxylase (PHYH) activity and molecular analysis of the *PHYH* genes. In some atypical patients, these studies have failed to show mutations in the *PHYH* gene. Instead mutations in the *PEX7* gene were identified (van den Brink et al. 2003). Apparently, mutations in the PEX7 gene can give rise to two different phenotypes including a Refsum-like phenotype in case of mild mutations (van den Brink et al. 2003) and rhizomelic chondrodysplasia punctata in case of severe mutations (Braverman et al. 2002; Motley et al. 2002). In a few other patients with some signs and symptoms reminiscent of Refsum disease, but, in addition, showing signs and symptoms not found in Refsum disease, the defect has turned out to be at some other level including SCPx (Ferdinandusse et al. 2006b) and AMACR (Ferdinandusse et al. 2000).
4. Analysis of plasma VLCFA is also the first line of testing for the identification of X-ALD. Especially for hemizygote detection, plasma VLCFA analysis has turned out to be fully conclusive (Bezman et al. 2001). For X-ALD, detailed enzymatic studies in fibroblasts may not be warranted, and instead VLCFA analysis may be followed up right away by molecular analysis of the X-ALD gene, i.e., *ABCD1*. At present >700 different mutations have been identified in the *ABCD1* gene (see www.XALD.nl). Heterozygote detection is much less straightforward especially if the patient is from a family having no family history of X-linked adrenoleukodystrophy. In such cases, a full study should be done: (1) in plasma which should include VLCFA analysis (which is only abnormal in 80 % of obligate heterozygotes) and (2) in fibroblasts, which should include immunofluorescence microscopy analysis of adrenoleukodystrophy protein (ALDP). If the presumed mutation affects the stability of ALDP as is the case in about 65 % of the mutations found in the *ABCD1* gene until now, one will see a *mosaic* pattern upon immunofluorescence microscopy analysis, when an antibody raised against ALDP is used, with some cells showing a punctate fluorescence pattern, whereas in other cells no punctate fluorescence is observed.
5. Hyperoxaluria type 1 is also a peroxisomal disorder and is caused by a functional deficiency of the peroxisomal enzyme alanine glyoxylate aminotransferase (AGT) encoded by the *AGXT* gene. Interestingly, a functional deficiency of AGT can be caused either by a deficient activity of the enzyme or by the mistargeting of the enzyme to the wrong organelle, i.e., the mitochondrion (Danpure et al. 1993). This leads to the bizarre situation that the detoxification of glyoxylate in the peroxisome is impaired thus leading to hyperoxaluria, not because the AGT enzyme is catalytically inactive, but because of a functionally active AGT enzyme which is not able to detoxify glyoxylate in the peroxisome, due to its aberrant localization in the mitochondrion. This immediately suggests that there is no place for enzymatic analysis of AGT in liver biopsies in the absence of parallel studies to determine the subcellular localization of AGT. For this reason direct analysis of the

AGXT gene by sequence analysis of all exons and flanking introns is the method of choice now for the identification of hyperoxaluria type 1 in any patient suspected to suffer from hyperoxaluria type 1, based on clinical grounds and/or laboratory findings including elevated oxalic acid, glycolic acid, and/or glyoxylic acid in urine (van Woerden et al. 2004).

38.5.2 Mitochondrial Fatty Acid Oxidation Disorders

38.5.2.1 Background

Fatty acids (FAs) are an important source of energy for human beings, and most organs are able to oxidize fatty acids with some exceptions, notably erythrocytes and the brain. Especially the heart is very much dependent on mitochondrial fatty acid oxidation with 60–90 % of the energy equivalents being generated by means of mitochondrial fatty acid beta-oxidation. Although both mitochondria and peroxisomes are able to oxidize fatty acids, mitochondria are responsible for the oxidation of the bulk of fatty acids, derived from our daily diet. FAs destined for mitochondria enter the cell via a mechanism which has remained ill-defined until now. Inside the cell, the FAs are rapidly esterified with coenzyme A (CoA) by one of several acyl-CoA synthetases to generate the corresponding acyl-CoA esters. Acyl-CoA esters cannot traverse the mitochondrial membrane and first need to be converted into the corresponding acylcarnitine esters by the enzyme carnitine palmitoyltransferase 1 (CPT1), whereby the carnitine required for this reaction enters the cell via a coupled carnitine/Na^+-importer called organic cation/carnitine transporter 2 (OCTN2). The acylcarnitine generated in the CPT1 reaction then enters the mitochondria via the mitochondrial carnitine/acylcarnitine translocase (CACT), which exchanges acylcarnitines for free carnitine. Once inside the mitochondrion, the acylcarnitine ester is reconverted into the corresponding acyl-CoA ester via the enzyme carnitine palmitoyltransferase 2 (CPT2). The acyl-CoA ester can then be broken down via subsequent rounds of beta-oxidation, and the acetyl-CoA units generated in each cycle of beta-oxidation can subsequently be degraded to CO_2 and H_2O in the Krebs cycle or used for the synthesis of the ketone bodies acetoacetate and 3-hydroxybutyrate, which occurs primarily in the liver (Rinaldo et al. 2002; Houten and Wanders 2010).

38.5.2.2 Laboratory Diagnosis of Fatty Acid Oxidation (FAO) Disorders

The introduction of tandem mass spectrometry in laboratories has revolutionized the diagnosis of inborn errors of metabolism in general and FAO disorders in particular. This is due to the fact that acylcarnitine species, which were previously known to be notoriously difficult to measure, are relatively easy to quantify by means of tandem MS techniques. For this reason, acylcarnitine analysis is a first-line diagnostic test in patients suspected to suffer from a FAO disorder. In fact, the same technique is also used in newborn screening programs designed to identify neonates affected by a certain FAO disorder. If abnormal, enzyme studies should be done to resolve the underlying enzymatic defect as specified below.

38.5.2.3 Enzymatic Analysis of FAO Disorders

In principle there are two scenarios for the enzymatic workup of patients suspected to suffer from a FAO disorder. The *first* scenario is that plasma or blood spot acylcarnitine analysis has been done and has shown a characteristic abnormal profile, which immediately suggests a specific defect in the FAO system. Inspection of Fig. 38.3 reveals that many of the currently known FAO disorders may show characteristic acylcarnitine profiles. This is true for (1) OCTN2 deficiency (primary carnitine deficiency), (2) CPT1 deficiency, (3) CPT2/CACT deficiency, (4) very long-chain acyl-CoA dehydrogenase (VLCAD) deficiency, (5) medium-chain acyl-CoA dehydrogenase (MCAD) deficiency, (6) short-chain acyl-CoA dehydrogenase (SCAD) deficiency, (7) long-chain 3-hydroxyacyl dehydrogenase/ mitochondrial trifunctional protein (LCHAD/MTP) deficiency, and (8) short-chain 3-hydroxyacyl-

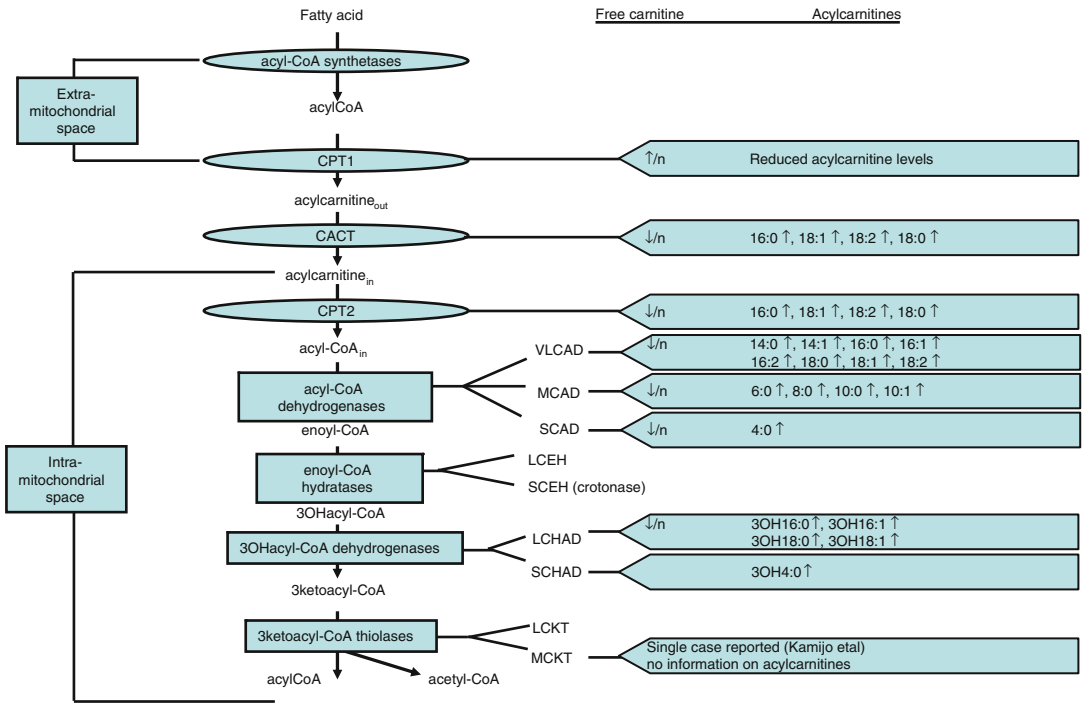

Fig. 38.3 Plasma acylcarnitine profiling and its importance for guiding the way toward the enzyme defect

CoA dehydrogenase (SCHAD) deficiency. In case of such a diagnostic acylcarnitine profile, the enzyme which is supposed to be deficient should be measured right away, preferably in lymphocytes, in order to identify the enzyme defect as soon as possible. The activities of CPT2, VLCAD, MCAD, SCAD, LCHAD/MTP, and SCHAD can all be measured reliably and reproducibly in peripheral blood mononuclear cells (PBMCs) (Wanders et al. 2010). Although OCTN2 and CPT1 are also expressed in lymphocytes, the activity of these two gene products is relatively low at least compared to fibroblasts so that fibroblasts remain the best material for enzymatic analysis of OCTN2 and CPT1 (see Table 38.2).

In contrast to the clear-cut situation described above in which a patient may show a characteristic abnormal acylcarnitine profile, patients may also show an acylcarnitine profile which is not immediately indicative of a certain enzyme defect. In addition there are patients with signs and symptoms suggestive for a FAO defect such as hypoketotic hypoglycemia, cardiomyopathy, and/or muscle weakness, in the absence of any characteristic plasma acylcarnitine abnormalities. In such cases, a full enzymatic study should be done in cultured skin fibroblasts, which first involves whole cell beta-oxidation analysis, to establish whether flux through the mitochondrial beta-oxidation machinery is defective or not. Two different tests are advisable here. The first test is the tritium release assay in which intact fibroblasts are incubated with one of several tritium-labeled fatty acids, notably myristic acid (C14:0), palmitic acid (C16:0), and oleic acid (C18:1). Use of such FAs has proven very helpful in the identification of disturbances in the mitochondrial FAO system (Olpin et al. 1997).

The *second* test, originally developed by Nada and coworkers (Nada et al. 1995), involves loading of intact fibroblasts with deuterated FAs and notably deuterated palmitic acid, followed by acylcarnitine analysis of the suspending medium (Ventura et al. 1999). If one of the two different tests is abnormal, additional enzymatic analyses must be done to try to identify the underlying defect. In the Amsterdam laboratory, we have

Table 38.2 List of mitochondrial fatty acid beta-oxidation disorders plus information on the deficient enzyme and the availability of enzyme testing in fibroblasts versus lymphocytes

	Mitochondrial FAD disorders	Deficient enzyme	Mutated gene	Enzyme assay in fibroblasts	Enzyme assay in lymphocytes
1.	Primary carnitine deficiency	OCTN2	*OCTN2*	+	(+)[a]
2.	Carnitine palmitoyltransferase 1 deficiency	CPT1	*CPT1*	+	(+)[a]
3.	Carnitine/acylcarnitine translocase deficiency	CACT	*CACT*	+	(+)[a]
4.	Carnitine palmitoyltransferase 2 deficiency	CPT2	*CPT2*	+	+
5.	VLCAD deficiency	VLCAD	*ACADVL*	+	+
6.	MCAD deficiency	MCAD	*ACADM*	+	+
7.	SCAD deficiency	SCAD	*ACADS*	+	+
8.	LCHAD/MTP deficiency	LCHAD/MTP	*HADHA/HADHB*	+	+
9.	SCHAD deficiency	SCHAD	*HADH*	+	+

[a]In principle feasible, but not yet validated

methods available for the measurement of all individual enzyme activities, and also molecular analysis of all the different genes involved has been established in our laboratory (see Wanders et al. 2010).

38.5.3 Mitochondrial OXPHOS Deficiencies

38.5.3.1 Background

Mitochondrial disorders can be defined as disorders caused by a defect in the mitochondrial energy-generating system (MEGS). In "classical" mitochondrial disorders, the primary defect leading to a reduced mitochondrial energy-generating capacity is located in the oxidative phosphorylation system, the citric acid cycle, or pyruvate dehydrogenase. In addition, there is a growing number of mitochondrial disorders in which the reduced mitochondrial energy-generating capacity is caused by other defects, for example, in mitochondrial metabolite transporters (Molinari et al. 2005; Mayr et al. 2007). A major substrate for mitochondrial energy production is pyruvate, which is transported into the mitochondria and subsequently converted into acetyl-CoA by the pyruvate dehydrogenase complex (PDHc), a key enzyme in energy metabolism. The activity of PDHc is regulated allosterically by its products acetyl-CoA and NADH and by specific kinases and phosphatases, which are activated depending on the energy demands of the cell. Acetyl-CoA formed by PDHc and from other sources (e.g., oxidation of fatty acids) is taken up by the tricarboxylic acid (TCA) cycle. In the TCA cycle, the reducing equivalents NADH and $FADH_2$ are formed, which are the substrates for the oxidative phosphorylation (OXPHOS) system. The TCA cycle is a dynamic cycle of enzyme reactions, and metabolites can enter or leave at various steps of the cycle. Levels of intermediates of the TCA cycle can increase due to defects in subsequent steps of the MEGS and can often be detected in urine of mitochondrial patients. The products of the TCA cycle and the mitochondrial beta-oxidation, NADH and $FADH_2$, are oxidized by respiratory chain complexes I and II, respectively, and electrons are transferred via coenzyme Q, complexes III, cytochrome c, and IV to molecular oxygen. The electron transfer is accompanied by proton translocation by respiratory chain complexes I, III, and IV. In this way, a mitochondrial inner-membrane potential is maintained, which provides the energy for complex V to convert ADP into ATP, the final product of the mitochondrial energy-generating system. The five enzyme complexes of the OXPHOS system are multisubunit assemblies of proteins, iron-sulfur clusters, flavins, cupper ions, and heme groups. The largest OXPHOS enzyme is complex I, consist-

ing of 44 different subunits of which 7 are encoded by the mtDNA and 37 by the nuclear genome. There is evidence for a higher degree of organization of respiratory chain enzymes into supercomplexes, containing different combinations of respiratory chain enzyme complexes. Some of these can function as respirasomes with NADH/O_2 oxidoreductase activity (Acin-Perez et al. 2008). It has been suggested that supercomplexes can form even larger structures, so-called respiratory strings (Wittig et al. 2006), although this remains to be established.

The clinical spectrum of mitochondrial disorders is very broad, and moreover, the genotype – phenotype correlation of mitochondrial disorders can be very poor. One major reason for the clinical heterogeneity is due to (tissue-specific) differences in heteroplasmy levels of mtDNA mutations. It seems likely that the complexity of the mitochondrial energy-generating system, as described above, is another reason for the clinical heterogeneity. There are hundreds of proteins involved, and it can be envisioned that the effect of a mutation in one gene can be modulated or enhanced by polymorphisms in genes encoding other proteins involved in the MEGS. For mtDNA mutations, evidence has been found for a role of the mtDNA haplotype in the pathogenicity of mtDNA mutations (Pulkes et al. 2000). Striking examples of clinical heterogeneity due to mutations in a single gene are disorders caused by mutations in POLG, a gene encoding a subunit of the mtDNA polymerase. Originally described as a gene involved in progressive external ophthalmoplegia, and later in Alpers' syndrome, which seem quite different clinical phenotypes, it is now recognized that the phenotype associated with POLG mutations is extremely diverse (Chinnery and Zeviani 2008).

38.5.3.2 Laboratory Diagnosis of Mitochondrial Disorders

Given the complexity of the MEGS, reaching a diagnosis is not always straightforward, and often an in-depth evaluation of clinical, biochemical, and genetic information is necessary. High throughput sequencing technology is becoming more and more a standard diagnostic approach for screening large numbers of candidate genes of patients, and this approach is very suitable to examine suspected mitochondrial patients, as there are often a large number of candidate genes (Wortmann et al. 2015). This has led to a debate about the role of the biochemical and genetic tests in the diagnostic workflow of suspected mitochondrial patients. The molecular genetic analysis of "all" genes by whole exome sequencing (WES) is a relatively noninvasive procedure and can give a clear answer without the need for a more invasive procedure, such as a muscle biopsy. However, WES will detect in any individual a large number of molecular genetic variants, and the interpretation of these data is in many cases ambiguous. The genetic data interpretation benefits from proper phenotyping of the patient. The more information is available, the better the interpretation of molecular genetic variants. Knowledge about a possible mitochondrial biochemical defect, such as an OXPHOS deficiency, can greatly improve the outcome of the data interpretation of whole exome sequencing. This means that the biochemical and molecular genetic tests are complementary and when performed in combination, it is a very powerful approach to diagnose patients.

Biochemical diagnostics of presumed OXPHOS deficiencies usually involves a broad screening of mitochondrial enzyme activities and analysis of fluxes through the citric acid cycle and oxidative phosphorylation system. The analysis of fluxes can only be performed in freshly obtained tissue samples (usually muscle), although assays for cultured fibroblasts have also been described. There are three types of assays to determine fluxes through the MEGS, namely, the analysis of the (1) ATP production rate, (2) substrate oxidation rate, and (3) oxygen consumption rate. A reduced rate of any of these parameters can be indicative of a defect in the MEGS. Moreover, by using different combinations of substrates, indications for the localization of the defect within the MEGS can be obtained. For example, a reduced oxidation rate of pyruvate in the presence of malate and a normal oxidation rate of pyruvate in the presence of carnitine are indicative of an OXPHOS defect

(Janssen et al. 2006). Also defects in the citric acid cycle and in a number of mitochondrial carriers can be detected in this way (Mayr et al. 2007). In addition to the analysis of fluxes, the activity of individual enzymes can be measured. Biochemical assays for the determination of enzyme activities of pyruvate dehydrogenase, citric acid cycle enzymes, respiratory chain, and complex V are available. These can be performed both in freshly obtained tissue samples and also in frozen tissue samples, cultured cells, and blood cells. Given the tissue-specific character of part of the mitochondrial disorder spectrum, it is very important to examine enzyme activities in clinically affected tissues. Often muscle is the tissue of choice, but in certain cases a liver or heart biopsy should be considered. All of the enzymes mentioned above can be determined by spectrophotometric assays. This is also true for pyruvate dehydrogenase for which spectrophotometric analysis is possible. However, often a more sensitive radiochemical assay with ^{14}C-labeled pyruvate is used.

Once MEGS assays and individual enzyme assays have been performed in a patient muscle biopsy, there are a number of possible outcomes: (1) all parameters are normal; (2) MEGS capacity is reduced and one or more enzymes are deficient; (3) MEGS capacity is reduced, but no enzyme deficiency is observed; and (4) all parameters are normal. In case all parameters are normal, a mitochondrial disorder is unlikely, especially if both enzyme activities and fluxes through the MEGS have been examined in affected tissue. However, if this is not the case, e.g., in patients with central nervous system impairment, a mitochondrial disorder should not be completely excluded. In those cases, additional diagnostic tests may still be worthwhile, such as examination of the mtDNA, as tissue-specific expression of the disease may be due to differences in heteroplasmy levels in different tissues. When only the MEGS capacity is reduced, there are several possible explanations. First, it could be a primary mitochondrial dysfunctioning due to a defect in a mitochondrial protein/enzyme that was not examined. Examples of previously unidentified mitochondrial protein defects other than the "classical" mitochondrial defects are the substrate carrier deficiencies at the level of the glutamate, phosphate, and pyruvate carriers (Molinari et al. 2005; Mayr et al. 2007). In these cases, detailed analysis of the MEGS in the muscle and in cultured cells provides important biochemical clues that can help to identify (or validate) the primary defect. Second, as it has been shown that MEGS capacity assays are more sensitive to detect certain mitochondrial defects, e.g., mutations in the mtDNA (Janssen et al. 2008), in case of a reduced MEGS capacity sequence, analysis of the mtDNA should always be considered. Third, it could be a reflection of secondary mitochondrial dysfunctioning, e.g., due to a muscle dystrophy, a very poor clinical status of the patient, or other reasons. In the case of a reduced MEGS capacity and a deficiency of one or more enzymes, there are several possibilities for subsequent biochemical diagnostic analysis. In many cases, biochemical analysis of fibroblasts is useful. Both positive and negative fibroblasts results are informative. In case of normal enzyme activities in fibroblasts, in particular in cases with reduced activities of complex I, III, and/or IV in muscle, analysis of the mtDNA is indicated. If mtDNA mutations have been excluded, variants in genes involved in mtDNA maintenance should be investigated. In the case of clearly reduced activities in fibroblasts, mtDNA depletion is less likely, and defects in either mtDNA or in nuclear genes encoding either structural components of the respiratory chain or assembly factors should be considered. A blue native polyacrylamide gel electrophoresis (BN-PAGE) analysis can be informative as well, as certain defects give a characteristic pattern in (two-dimensional) BN-PAGE.

Thanks to the implementation of whole exome sequencing as a diagnostic tool to sequence DNA from suspected mitochondrial patients, the number of genes in which defects have been described is already approaching 300 and still increases each year. As WES is being performed as a routine diagnostic test, often genetic variants are encountered that are difficult to interpret. It is important to realize that for many mitochondrial protein encoding genes, it is possible to validate

these by functional tests. As already mentioned above, databases are filled with supposedly pathogenic variants that unfortunately have not been validated, and research papers with such variants are still being published on a regular basis. Cell model systems, such as patient-derived fibroblasts and myoblasts can be invaluable to establish the pathogenicity of newly identified mutations. This can be a relatively simple test, such as the measurement of an enzyme. But also complementation studies, e.g., by using lentiviral or transient transfections of patient fibroblast (Hoefs et al. 2008; Jonckheere et al. 2013), can be done to proof the pathogenicity of molecular genetic variants. The technological possibilities to perform such an analysis within a relatively short time span and with limited costs are increasing steadily which renders a diagnostic application feasible in the near future. This illustrates that the combination of proper biochemical and high throughput molecular genetic tests provides the most optimal opportunity to reach a diagnosis in suspected mitochondrial patients.

38.5.4 Lysosomal Storage Disorders

38.5.4.1 Biochemical Background

Lysosomes play a very important role in the turnover of the cell. Turnover is the constant state of breakdown and resynthesis of macromolecules leading to renewal of these molecules, while their cellular concentration remains constant. The main function of lysosomes is the breakdown of complex macromolecules by lysosomal enzymes to simple monomers and the transport of these monomers by specific membrane transporters out of the lysosomal compartment into the cytosol, where they can be reutilized for biosynthesis. This function is achieved by the presence of a large set of hydrolytic enzymes with an acidic pH optimum capable of hydrolyzing all cellular components including DNA, RNA, complex carbohydrates like glycolipids, glycoproteins, and glycosaminoglycans, proteins, neutral lipids, and phospholipids. With few exceptions the reactions catalyzed by lysosomal hydrolases are irreversible.

The pH within the lysosome is maintained around 5 by an ATP-driven proton pump. Most lysosomal enzymes are glycoproteins and are present in the matrix of the lysosome. They are synthesized as larger precursors (pre-proenzymes) and glycosylated during transport in the endoplasmic reticulum and the Golgi apparatus. Some of the free mannose groups on the lysosomal precursor enzymes are phosphorylated to mannose-6-phosphate groups in the trans-Golgi, which are recognized by the mannose-6-phosphate receptors present in acidic endosomes. These mannose-6-phosphate receptors are essential for the lysosomal targeting of matrix enzymes to the lysosomes and are also present on the plasma membrane. After dissociation of the lysosomal precursor enzymes from the mannose-6-phosphate receptor in the acidic environment, they finally reach the endosomal/lysosomal compartment (Kornfeld 1987). Once they have reached the endosomal/lysosomal compartment, the precursor enzymes may be further processed by proteolytic cleavage to smaller molecular weight mature enzymes. Lysosomal enzymes can be active as monomers, homodimers (e.g., α-galactosidase, α-galactosaminidase, and β-hexosaminidase B), heterodimers (β -hexosaminidase A), or other polymers and are mostly present as soluble enzymes. Like all other proteins lysosomal enzymes are also degraded in the lysosome and have a finite half-life. Until recently it was assumed that lysosomal enzymes are constitutively expressed. However, recent evidence suggests the concerted regulation of the expression of genes encoding lysosomal enzymes, of the number of lysosomes, and of autophagy by a new gene network named coordinated lysosomal expression and regulation (CLEAR) network, which is under the control of a central regulator transcription factor EB (TFEB). This leads to an increased ability of cells to degrade lysosomal substrates, for example, during starvation (Settembre et al. 2013).

Besides glycosylation, which is essential for the activity of all soluble lysosomal enzymes, another posttranslational modification is necessary for all sulfatases including the lysosomal sulfatases. This modification involves

the oxidation of an essential cysteine residue to a formylglycine group by the formylglycine-generating enzyme, which is present in the endoplasmic reticulum (Dierks et al. 2009). There is no evidence that the activity of lysosomal enzymes is regulated by covalent modifications once they are in their mature form.

The enzymes β-galactosidase and α-neuraminidase are present in an enzyme complex together with the so-called protective protein, which is actually an enzyme – cathepsin A – with proteolytic activity. The presence of this protein is essential for the stability of both β-galactosidase and α-neuraminidase.

In general lysosomal enzymes have to work in concert in order to efficiently catalyze the stepwise breakdown of macromolecules like glycosaminoglycans, glycoproteins, and glycolipids. However, with the exception of the example discussed above, there is no evidence that they are actually organized in enzyme complexes.

When the substrate is not water soluble as is the case with glycolipid molecules destined for degradation, which are present in internal membranes within the endosomal/lysosomal compartments, the lipid-hydrolyzing enzymes need an activator protein for full activity (Kolter and Sandhoff 2005). A unique late endosomal/lysosomal phospholipid, bis(monoacylglycero) phosphate, provides a negative charge on the internal vesicles which appears to be indispensable for the catalytic process. Examples of activator proteins are the GM2-activator protein, which is specifically needed for the breakdown of GM2 gangliosides by β-hexosaminidase A and the saposins A-D. The latter activator proteins are proteolytic cleavage products of the precursor protein prosaposin and are needed for the hydrolysis of complex glycolipids like sulfatide, globotriaosylceramide, galactosylceramide, glucosylceramide, and ceramide.

Substrates for lysosomal hydrolysis usually do not enter the lysosome by diffusion or transport of single molecules, but are delivered to the lysosome by endocytosis, by autophagy when they are of intracellular origin or by phagocytosis when they are of extracellular origin. They may be taken up as single molecules or may be part of a larger molecular complex like a lipoprotein or even an organelle or a microorganism. Uptake by endocytosis is a very efficient process when it is facilitated by the presence of a receptor such as the uptake of low-density lipoprotein (LDL), which is catalyzed by the LDL receptor. Also lysosomal enzymes carrying a mannose-6-phosphate group can be very efficiently taken up by most cell types because of the presence of mannose-6-phosphate receptors on the plasma membrane.

Lysosomal storage disorders are characterized by the presence of nondegraded material in endosomal/lysosomal compartments. Any process that interferes with the lysosomal degradation or endosomal/lysosomal transport of molecules can give rise to storage. The cause may be genetic of nature or environmental as is the case in drug-induced lipidoses or when nondegradable materials are present. Here we will discuss the genetic lysosomal storage disorders.

38.5.4.2 Genetic Causes of Lysosomal Storage Diseases

Single Enzyme Deficiencies (See Tables 38.3 and 38.4)

The majority of the inherited lysosomal storage disorders are caused by mutations in genes encoding a lysosomal enzyme. These mutations may give rise to the complete absence or a severe reduction in activity of the enzyme. Since many lysosomal enzymes are exo-hydrolases, the stepwise breakdown of macromolecules stops at the step which is normally catalyzed by the deficient enzyme. These deficiencies give rise to the storage of specific macromolecules, which causes disease. The storage is a slow but continuous process, which explains why lysosomal storage disorders are chronic, progressive diseases. Most lysosomal storage diseases are autosomal recessive disorders with the exception of Fabry disease and mucopolysaccharidosis type II (Hunter's disease), which are X-linked. Since carriers of autosomal recessive lysosomal storage disorders are not affected, enzyme levels of 50 % of normal are sufficient for normal lysosomal functioning. In fact, a reduction of enzyme activity to less than

Table 38.3 Mucopolysaccharidoses (MPS, single enzyme deficiencies)

Disease	Synonym	OMIM	Locus/gene symbol	Gene product	Storage products
MPS I	Hurler	607014		α-L-Iduronidase	DS, HS
	Hurler/Scheie	607015	4p16.3/*IDUA*		
	Scheie	607016			
MPS II	Hunter	309900	Xq28/*IDS*	Iduronate -2-sulfatase	DS, HS
MPS IIIA	Sanfilippo A	252900	17q25.3/*SGSH*	Heparan N-sulfatase	HS
MPS IIIB	Sanfilippo B	252920	17q21.2/*NAGLU*	α-N-Acetyl-glucosaminidase	HS
MPS IIIC	Sanfilippo C	252930	8p11.21/*HGSNAT*	AcetylCoA α-glucosamine N-acetyltransferase	HS
MPS IIID	Sanfilippo D	252940	12q14.3/*GNS*	N-Acetylglucosamine- 6-sulfatase	HS
MPS IVA	Morquio A	253000	16q24.3/GALNS	N-Acetylgalactosamine-6-sulfatase	KS, CS
MPS IVB	Morquio B	253010	3p21.33/*GLB1*	β -Galactosidase	KS, oligosaccharides
MPS VI	Maroteaux-Lamy	253200	5q14.1/*ARSB*	Arylsulfatase B	DS
MPS VII	Sly	253220	7q11.21/*GUSB*	β-Glucuronidase	DS, HS, CS
MPS IX	Hyaluronidase deficiency	601492	3p21.31/*HYAL1*	Hyaluronidase 1	HA
Glycoproteinoses (single enzyme deficiencies)					
Aspartylglucosaminuria		208400	4q34.3/*AGA*	Aspartylglucosaminidase	Aspartylglucosamine
					Oligosaccharides
Fucosidosis		230000	1p36.11/*FUCA1*	α-Fucosidase	Oligosaccharides
α-Mannosidosis		248500	19q13.2/*MAN2B1*	α-Mannosidase	Oligosaccharides
β-Mannosidosis		248510	4q24/*MANBA*	β-Mannosidase	Oligosaccharides
Sialidosis	Mucolipidosis type I	256550	6p21.3/*NEU1*	Sialidase,	Oligosaccharides
				α-Neuraminidase-1	
N-Acetyl-α-glucosaminidase deficiency	Schindler disease,	609241			
	Kanzaki disease	609242	22q13.2/*NAGA*	N-Acetyl-α-galactosaminidase	Oligosaccharides

DS dermatansulfate, *HS* heparansulfate, *KS* keratansulfate, *CS* chondroitinsulfate, *HA* hyaluronan

Table 38.4 Sphingolipidoses/lipidoses (single enzyme defects and activator protein deficiencies)

Disease	Synonym	OMIM	Locus/gene symbols	Gene product	Storage products
Farber	Lipogranulomatosis	228000	8p22/*ASAH1*	Acid Ceramidase	Cer
Fabry	Anderson-Fabry	301500	Xq22.1/*GLA*	α-Galactosidase A	Gb3, lysoGb3
Gaucher		230900		β-Glucocerebrosidase	GlcCer, lysoGlccer
		231000	1q21/*GBA1*		
		230800			
GM1-gangliosidosis		230500	3p22.3/*GLB1*	β-Galactosidase	GM1, KS, oligosaccharides
		230600			
		230650			
GM2 gangliosidosis B	Tay-Sachs	272800	15q23/*HEXA*	β-Hexosaminidase α-subunit	GM2
GM2 gangliosidosis O	Sandhoff	268800	5q13.3/*HEXB*	β-Hexosaminidase β-subunit	GM2, oligosaccharides
GM2 gangliosidosis AB	Tay-Sachs AB variant	272750	5q33.1/GM2A	GM2-activator protein	GM2
Krabbe	Globoid cell leukodystrophy	245200	14q31/*GALC*	β-Galactocerebrosidase	GalCer
Metachromatic leukodystrophy		250100	22q13.33/*ARSA*	Arylsulfatase A	Sulfatide
Niemann-Pick types A and B		257200	11p15.4/*SPMPD1*	Acid sphingomyelinase	SM
		607616			
Wolman	Cholesteryl ester storage disease	278000	10q23.31/*LIPA*	Acid lipase	Cholesterolester, triglycerides
Prosaposin deficiency		611722	10q21-q22/*PSAP*	Prosaposin	Multiple (glyco)lipids
Saposin A deficiency	Krabbe variant		10q21-q22/*PSAP*	Saposin A	GalCer
Saposin B deficiency	Metachromatic leukodystrophy variant	249900	10q21-q22/*PSAP*	Saposin B	Sulfatide
Saposin C deficiency	Gaucher variant, glucosylceramidosis	610539	10q21-q22/*PSAP*	Saposin C	GlcCer
Glycogen storage disease (single enzyme deficiency)					
Glycogen storage disease type II	Pompe	232300	17q25.3/*GAA*	α-Glucosidase	Glycogen

Cer ceramide, *GlcCer* glucosylceramide, *Gb3* globotriaosylceramide, *GM1 and GM2* GM1 and GM2 gangliosides, *SM* sphingomyelin

10 % of normal is usually necessary to cause disease. A wide spectrum of disease expression is usually found, and it is customary to classify the individual lysosomal storage disorders according to age of onset in (late) infantile, juvenile, and adult forms. In practice there is often a continuum of clinical phenotypes varying from very severe to attenuated forms. In several lysosomal disorders, the residual activity expressed by the mutant enzyme correlates with the severity of the disease (phenotype). Examples are metachromatic leukodystrophy (arylsulfatase A deficiency), GM2-gangliosidosis type Tay-Sachs (β-hexosaminidase A deficiency), and mucopolysaccharidosis type I – Hurler and Scheie syndromes (α-iduronidase deficiency). In patients with severe and attenuated phenotypes of GM2 gangliosidosis and metachromatic leukodystrophy, a good correlation was found between the residual enzyme activities, the residual turnover rates of the substrate, and the clinical phenotype (Leinekugel et al. 1992).

Although lysosomal enzymes are present in all cell types except red blood cells, cells and tissues may be differentially affected depending on the enzyme deficiency involved. It is the flux of substrate through the endosomal/lysosomal system that determines which cells and tissues will be affected and the clinical phenotype that emerges. Thus it can be understood that the deficiency of alpha-glucosidase in Pompe disease will primarily affect the skeletal muscle, the heart, and the liver, which are most active in glycogen metabolism. Similarly, the defect in the turnover of sulfatide – an important component of myelin – due to the deficiency of arylsulfatase A will affect normal myelin function and cause white matter disease in metachromatic leukodystrophy, while a defect in the turnover of gangliosides due to deficiencies in β-hexosaminidase A and β-galactosidase in GM2 and GM1 gangliosidosis, respectively, will lead to severe neuronal dysfunction. The lysosomal accumulation of the primary storage products is the determining factor in the onset of disease, but the pathophysiology is usually more complex and also involves secondary processes such as secondary storage, disturbances in intracellular trafficking and vesicle maturation (Walkley 2007), disturbance of autophagy (Lieberman et al. 2012; Settembre et al. 2013), calcium homeostasis (Vitner et al. 2010), and inflammation (Simonaro et al. 2008).

Activator Protein Deficiencies (See Table 38.4)

Mutations in genes encoding for the activator proteins give rise to a functional deficiency of glycolipid hydrolases, while the enzymes themselves are normally present. The phenotypes of the sphingolipidoses caused by activator protein deficiencies are similar to the phenotypes caused by the primary enzyme deficiencies. Examples are metachromatic leukodystrophy caused by saposin B deficiency, Gaucher disease caused by saposin C deficiency, and GM2 gangliosidosis caused by GM2-activator protein deficiency.

Multiple Enzyme Deficiencies (See Table 38.5)

A deficiency of the enzyme UDP-N-acetylglucosamine:lysosomal enzyme N-acetylglucosamine-1-phosphotransferase leads to mistargeting of lysosomal enzymes, which cannot be delivered to lysosomes, but are instead excreted into the plasma because they lack the mannose-6-phosphate targeting signal. Interestingly, although the phosphotransferase deficiency is present in all cell types, only cells of mesenchymal origin appear to be affected, suggesting that alternative routes of delivering lysosomal enzymes to lysosomes must exist in other cell types. The phosphotransferase enzyme consists of three subunits ($\alpha_2\beta_2\gamma_2$) encoded by two genes, one for the α and β subunits and one for the γ subunits.

Mutations in the gene for the α and β subunits give rise to mucolipidosis II (I-cell disease) and its attenuated form mucolipidosis III (pseudo-Hurler polydystrophy), while mutations in the γ subunit only lead to mucolipidosis III (Kollmann et al. 2010).

A defect in the formylglycine-generating enzyme leads to a disease called multiple sulfatase deficiency in which all sulfatases including

at least seven lysosomal sulfatases are deficient. In its most severe form, the disease has features of a mucopolysaccharidosis, metachromatic leukodystrophy, and ichthyosis (due to steroid sulfatase deficiency), but the clinical presentation is very variable (Dierks et al. 2009).

A defect in the protective protein/cathepsin A leads to a deficiency of both β-galactosidase and alpha-neuraminidase and is the cause of the lysosomal storage disorder galactosialidosis.

Lysosomal/Endosomal Trafficking Defects (See Table 38.6)

Lysosomal storage diseases can also be caused by abnormalities in lysosomal/endosomal trafficking or vesicle fusion due to defects in lysosomal membrane proteins.

Niemann-Pick type C is caused by two different gene defects *NPC1* and *NPC2*. NPC1 is a membrane protein with 13 transmembrane domains and a cholesterol-sensing domain and is

Table 38.5 Multiple enzyme deficiencies

Disease	Synonym	OMIM	Locus/gene symbol	Gene product	Storage products
Mucolipidosis type II α/β	I-cell disease	252500	12q23.2/*GNPTAB*	UDP-GlcNac phosphotransferase α/β subunits	Lipids and oligosaccharides
Mucolipidosis type III α/β	Pseudo-Hurler polydystrophy	252600	12q23.2/*GNPTAB*	UDP-GlcNac phosphotransferase α/β subunits	Lipids and oligosaccharides
Mucolipidosis type III γ	Pseudo-Hurler polydystrophy	252605	16p13.3/*GNPTG*	UDP-GlcNac phosphotransferase γ-subunit	Lipids and oligosaccharides
Multiple sulfatase deficiency	Austin disease	272200	3p26.1/*SUMF1*	Formylglycine-generating enzyme	Sulfatides, glycosaminoglycans
Galactosialidosis		256540	20q13.12/*CTSA*	Protective protein, cathepsin A	Oligosaccharides

Table 38.6 Lysosomal membrane and transport defects

Disease	Synonym	OMIM	Locus/gene symbol	Gene product	Storage product
Cystinosis		219800	17p13.2/*CTNS*	Cystinosin	Cystine
		219900			
		219750			
Sialic acid storage disease	Salla disease	269920	6q13/*SLC17A5*	Sialin	Sialic acid
		604369			
Cobalamin F transporter deficiency	Methylmalonic aciduria and homocystinuria (CblF type)	277380	6q13/*LMBRD1*	Cobalamin F transporter	Vitamin B12
Lysosomal membrane and endosomal/lysosomal trafficking defects					
Niemann-Pick type C1		257220	18q11.2/*NPC1*	NPC1	Cholesterol, phospholipids, glycosphingolipids
Niemann-Pick type C2		607625	14q24.3/*NPC2*	NPC2	Cholesterol, phospholipids, glycosphingolipids
Mucolipidosis type IV		252650	19p13.2/*MCOLN1*	Mucolipin-1	Phospholipids, glycosphingolipids, mucopolysaccharides
Danon disease	Pseudoglycogenosis II	300257	Xq24/*LAMP2*	LAMP2	Glycogen

present in late endosomes, whereas NPC2 is a soluble lysosomal protein that is targeted to lysosomes by the mannose-6-phosphate receptor pathway. Despite numerous studies the function of NPC1 is still poorly understood. It may function as a transmembrane efflux pump. NPC2 serves probably as a lysosomal lipid transfer protein with a high specificity for cholesterol. Both defects give rise to a similar clinical phenotype. Biochemically both defects lead to a perturbed flow of substrate, primarily lipids, through the endosomal/lysosomal pathway and to a complex pattern of lipid storage consisting of cholesterol, glycosphingolipids, sphingoid bases, and phospholipids. Mucolipidosis IV is a lysosomal storage disease with a heterogeneous storage pattern consisting of phospholipids, glycosphingolipids, and mucopolysaccharides. The disease is caused by a mutation in the MCOLN1 gene encoding the mucolipin-1 protein, a lysosomal membrane protein with six transmembrane domains. There is evidence that this protein functions as a cation channel. This defect may lead to a reduced capacity in the fusion and fission of lysosomal membranes with endosomal and plasma membranes.

Danon disease is an X-linked glycogen storage disorder caused by a defect in the endosomal/lysosomal membrane protein lysosome-associated membrane protein (LAMP2). There is a failure of autophagic vacuoles to fuse with endosomes/lysosomes or of autophagolysosomes to mature into digestive organelles.

Transport Defects (See Table 38.6)

Lysosomal storage diseases due to a defect in the transport of small molecules out of the lysosome are caused by defects in lysosomal membrane proteins facilitating transport across the lysosomal membrane. Examples are et cetera cystinosis due to the cystine transport protein cystinosin and sialic acid storage disease due to a defect in the lysosomal sialic acid transporter sialin.

38.5.4.3 Laboratory Diagnosis of Lysosomal Storage Disorders

The laboratory diagnosis of lysosomal storage diseases consists of four different approaches including histology, metabolite analysis, enzymatic analysis, and mutation analysis that are complementary to each other.

Histology

Lysosomal storage can be demonstrated by light or electron microscopy in easily accessible cells like white blood cells and/or in skin biopsies. In some diseases biopsies are taken from affected organs such as the bone marrow in Gaucher disease and a kidney biopsy in Fabry disease to establish a diagnosis. However, it is recommended to perform enzymatic analysis first. Abnormal findings in the biopsy should always be confirmed by enzymatic analysis. In some cases histology is the first choice to obtain evidence for a lysosomal storage disorder, for example, when mucolipidosis IV or a neuronal ceroid lipofuscinosis is suspected.

Metabolite Analysis

Lysosomal storage can be demonstrated by the analysis of plasma and urine for storage products such as glycosaminoglycans, oligosaccharides, and glycosphingolipids. The combined qualitative and quantitative analyses of glycosaminoglycans yield important information on the type of mucopolysaccharidosis involved and limit the number of enzymes that need to be tested to definitively establish the diagnosis (see Table 38.3). It should be kept in mind, however, that the sensitivity (and specificity) of urinary glycosaminoglycan determinations is not 100 % (Gray et al. 2007). Especially in more attenuated or adult cases of mucopolysaccharidoses (MPS III and MPS IV), it can be difficult to diagnose the disease if only quantitative screening tests are used. The use of combined quantitative and qualitative screening tests and the generous application of enzyme assays even when the results of metabolite testing appear normal are recommended in those cases. Traditional quantitative and qualitative tests for the detection of glycosaminoglycans based on colorimetry and electrophoresis will soon be replaced by tandem mass spectrometry-based tests. These tests yield both quantitative and qualitative information and have a high sensitivity and specificity (Langereis et al.

2015). Each lysosomal defect in the degradation of oligosaccharides shows a specific pattern of oligosaccharides in urine (see Table 38.3). Traditionally oligosaccharide analysis was performed by thin layer chromatography, but recently mass spectrometric techniques with great promise to provide fingerprint analysis for each glycoproteinosis have been developed (Xia et al. 2013; Bonesso et al. 2014). Glycosphingolipid analyses in plasma and urine can be performed to obtain evidence for a sphingolipidosis. However, in the case of the sphingolipidoses, enzymatic tests are usually performed first based on the clinical information (see Table 38.4). The analysis of the specific storage product in plasma or urine serves three purposes: (1) to confirm the diagnosis; (2) to exclude pseudo-deficiencies, for example, in metachromatic leukodystrophy; and (3) to obtain evidence for an activator protein deficiency in cases with strong clinical suspicion for Gaucher disease, metachromatic leukodystrophy, or Krabbe disease with normal results of enzyme activity measurements determination (see Table 38.4). The determination of specific lipid storage metabolites such as lysoglobotriaosylsphingosine in Fabry disease is an important adjunct in determining the pathogenicity of mutations in cases with nonclassical presentations, for example, when the symptomatology is restricted to a single organ (Aerts et al. 2008; Smid et al. 2015). These cases are increasingly found in screening studies (van der Tol et al. 2014).

38.5.4.4 Enzymatic Analysis

Single Enzyme Deficiencies

(See Tables 38.3 and 38.4)

Determination of the activity of lysosomal enzymes in leukocytes, plasma, or dried blood spots prepared from whole blood or in fibroblasts is the core of the laboratory diagnosis of lysosomal storage disorders. Most lysosomal storage disorders due to single enzyme deficiencies can be diagnosed reliably and definitively by enzyme assays using artificial substrates. Although the K_m of the enzymes measured with these substrates is usually much higher than with the natural substrate, the specificity of the substrates is sufficiently high to allow reliable diagnostic performance.

Multiple Enzyme Deficiencies

(See Table 38.5)

Mucolipidoses II and III are diagnosed by the strongly increased levels of multiple lysosomal enzymes in plasma and their deficiency in fibroblasts. Multiple sulfatase deficiency is diagnosed by the deficiency of several lysosomal sulfatases in leukocytes and/or fibroblasts. Galactosialidosis is diagnosed by the deficiency of both β-galactosidase and α-neuraminidase in leukocytes and/or fibroblasts. Confirmation can be obtained by measuring cathepsin A in leukocytes or fibroblasts.

Lysosomal Transport Defects

(See Table 38.6)

Cystinosis is diagnosed by measuring increased cystine levels in leukocytes or granulocytes. The sialic acid storage disorders are diagnosed by the increased excretion of sialic acid in urine or increased levels of sialic acid in fibroblasts. A very rare defect in the release of Vitamin B_{12} from lysosomes gives rise to methylmalonic aciduria. The initial diagnosis can be established by organic acid analysis in urine.

Lysosomal/Endosomal Trafficking Defects

(See Table 38.6)

Niemann-Pick type C disease is diagnosed by showing cholesterol storage in fibroblasts followed by mutation analysis to discriminate between NPC1 and NPC2. Mutation analysis has superseded the laborious biochemical test used to show decreased cholesterol esterification in fibroblasts. Oxysterols have been identified as promising plasma biomarkers for Niemann-Pick type C (Jiang et al. 2011). Their use in a routine clinical setting and the sensitivity and specificity of the test are currently being evaluated by several laboratories (Klinke et al. 2015; Pajares et al. 2015). Mucolipidosis IV may be diagnosed by a combination of electron microscopy of the skin and mutation analysis. Danon disease may be diagnosed by electron microscopy of cardiac biopsies and by mutation analysis.

Neuronal Ceroid Lipofuscinoses (CLN Diseases) (See Table 38.7)

Four of the neuronal ceroid lipofuscinoses (NCLs) are single lysosomal enzyme defects (Boustany 2013) (see Table 38.7), of which CLN1, CLN2, and CLN10 disease can be diagnosed by enzyme assays with artificial fluorescent substrates. Since these enzyme deficiencies are not restricted to the classical presentations of CLN1, CLN2, and CLN10, a generous application of these simple assays in patients with more attenuated phenotypes of the CLN diseases is recommended (van Diggelen et al. 2001; Ramadan et al. 2007; Sun et al. 2013). For the remaining NCLs, a combination of electron microscopy of blood cells or skin biopsy and mutation analysis is recommended.

38.5.4.5 Mutation Analysis

The molecular basis of most of the lysosomal storage diseases is known (see Tables 38.3, 38.4, 38.5, 38.6, and 38.7), and many mutations have been characterized. However, with few exceptions, mutation analysis is rarely used as the initial step in the diagnostic workup of patients suspected of suffering from a lysosomal storage

Table 38.7 Neuronal ceroid lipofuscinoses (CLN disease)

Disease	Eponym	OMIM	Locus/gene symbol	Gene product	Storage product
CLN1	Haltia-Santavuori	256730	1p34.2/*CLN1 (PPT1)*	Palmitoyl protein thioesterase 1 (PPT1)	Lipofuscin, SAPs
CLN2	Janský-Bielschowsky	204500	11p15.4/*CLN2(TPP1)*	Tripeptidyl peptidase I (TPP1)	Lipofuscin, SCMAS
CLN3	Spielmeyer-Sjögren, Batten	204200	16p11.2/*CLN3*	CLN3 protein (battenin)	Lipofuscin, SCMAS
CLN4	Parry disease, dominant adult NCL	162350	20q13.33 *CLN4/(DNAJC5)*	DNAJ homologue subfamily C member 5	Lipofuscin, SAPs
CLN5	Variant late infantile NCL (Finnish)	256731	13q22.3/*CLN5*	Protein CLN5	Lipofuscin, SCMAS
CLN6	Early juvenile Lake-Cavanagh, adult Kufs type A	601780	15q23/*CLN6*	Protein CLN6	Lipofuscin, SCMAS
CLN7	Variant late infantile NCL (Turkish)	610951	4q28.2/*CLN7/(MFSD8)*	Major facilitator superfamily domain-containing protein 8	Lipofuscin, SCMAS
CLN8	Northern epilepsy, progressive epilepsy with mental retardation (EPMR)	600143	8p23.3/*CLN8*	CLN8	Lipofuscin, SCMAS
CLN9	Variant Batten disease	609055	Unknown	Unknown	Lipofuscin, SCMAS
CLN10	Congenital NCL	610127	11p15.5/*CLN10 (CTSD)*	Cathepsin D (CTSD)	Lipofuscin, SAPs
CLN 11	Adult variant	614706	17q21.31/*CLN11/(GRN)*	Progranulin	Lipofuscin
CLN12	Juvenile variant, Kufor-Rakeb syndrome	606693	1p36.13/*CLN12/(ATP13A2)*	–	Lipofuscin
CLN13	Adult Kufs type B	615362	11q13.2/*CLN13/(CTSF)*	Cathepsin F	Lipofuscin
CLN14	Infantile variant, EPM3	611726	7q11.21/*CLN14/(KCTD7)*	Potassium channel tetramerization domain-containing protein 7	–

SAPs sphingolipid activator proteins, *SCMAS* subunit c of mitochondrial ATP synthase

disorder. Rather mutation analysis follows after enzymatic analysis. It is employed when enzyme determinations cannot be used to reach a diagnosis (see the examples discussed above), for carrier testing, which is not possible with certainty by enzymatic analysis and – increasingly – in prenatal diagnosis, especially in cases where enzymatic analysis is difficult. Sometimes there is a relation between the genotype and the phenotype, and mutation analysis can help in making a prognosis about the course of the disease. However, this situation may change due to the rapid development of next-generation sequencing techniques such as whole exome sequencing and the possibilities to screen packages of mutations in a targeted way, for example, mutations related to (all) genes involved in lysosomal metabolism and autophagy. It could well be that mutation analysis will precede enzymatic and metabolite analysis in the future, but the latter will retain their important positions in the diagnostic workup of patients with lysosomal storage disease as functional tests. This will be especially important in cases when the phenotype is not very specific as it is often the case in adult patients with lysosomal storage disorders.

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Molecular Genetic Analyses

39

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Key Facts

- Mutation studies have become an important component in the diagnostic work-up of patients, but their use should be balanced with other (phenotypic) diagnostic methods.
- The growing availability of massively parallel sequencing approaches for diagnostic purposes will make DNA tests the first-line diagnostic test for many patients with inherited metabolic diseases, in particular those not covered by standard selective screening tests (amino acids, organic acids, acylcarnitines). This will increase the need for high competence of the center that offers molecular tests.
- Molecular analyses have a limited sensitivity as some disease-causing mutations may be missed by standard methods, and failure to identify a diagnostic genotype may not necessarily exclude a diagnosis. Sensitivity depends on both genetic characteristics and the method employed.
- Identification of disease-causing mutations confirms the diagnosis, may provide prognostic information, and allows simple testing of other family members including prenatal diagnosis.
- The functional impact of novel genetic variants identified in a patient should be assessed with great care. They should be denoted "unclassified variants" unless they are proven to be clinically irrelevant or to cause disease.
- Mutation analyses (like all specialized tests) are prone to errors. Confirmatory repeat analyses (either on a new sample or by analysis at another laboratory) may be considered when the results of molecular studies are important for patient management but do not seem to fit the clinician's assessment of the case.

Molecular genetic analyses have become a central component in the identification, understanding, and diagnosis of inherited metabolic diseases. Localization and structure of most genes involved in recognized monogenic metabolic disorders have been characterized. The identification of various disease-causing mutations in the individual conditions has not only greatly enhanced the diagnostic options but also led to an improved understanding of molecular

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disease mechanisms and sometimes novel therapeutic options. Genome-wide molecular genetic approaches, in particular autozygosity mapping and exome sequencing, have led to the identification of a large number of "new" diseases particularly in the mitochondrial disease group and the congenital disorders of protein modification where the biochemical diagnostic options are limited. The advent of massively parallel (next-generation) sequencing methods for diagnostic purposes is now causing a major change in diagnostic algorithms. The new genetic methods have major advantages for the rapid and reliable disease identification in affected patients, but the wealth of generated data and the often underestimated difficulties in interpreting novel genetic variants is causing new challenges.

39.1 From Genotype to Phenotype

The basic principle underlying diagnostic molecular genetic analyses is a proposed causal relationship between the genetic *information* and the development of clinical symptoms (Zschocke 2008). This statement may sound rather banal; however, the true correlation between the *genotype* (i.e., the constellation of the genetic "text" as well as epigenetic alterations in the diploid genome) and the *phenotype* (i.e., the resulting physical features that are strongly influenced by nongenetic factors) may be difficult to determine and is sometimes poorly understood. This can lead to complete misinterpretation of genetic data. In order to better assess genotype–phenotype correlations, it is useful to distinguish different phenotype levels that may be recognized in the individual patient (with phenylketonuria, PKU, as an example):

- The *molecular or cellular phenotype* describes the status of proteins and cells, including the immunohistochemically assessed presence or absence of a protein, or the activity of an enzyme. This phenotype is mostly independent from external factors but often restricted to specific organs. In the example PKU, this is the phenylalanine hydroxylase (PAH) activity in the liver.
- The *functional or structural phenotype* describes the normal or abnormal appearance and function of the different organs including the concentration of metabolites in body fluids or electrophysiological findings. This phenotype tends to vary considerably depending on the external factors including treatment. In the example PKU, this is the blood or CSF concentration of phenylalanine.
- The *clinical phenotype* describes what matters most, the symptoms and signs in the affected patient which result from the combined effects of genetic and external factors. In the example PKU, this is the severe intellectual disability of an untreated individual, but it can also encompass the need to maintain a phe-restricted diet in order to avoid the development of neurological damage.

There are a large number of examples also with regard to inherited metabolic diseases where an assumed correlation between genotype and clinical phenotype turned out to be completely wrong. Histidinemia (OMIM 235800), for example, is an inborn error of metabolism in which mutations in the *HAL* gene cause reduced or absent function of the enzyme histidine ammonia-lyase and highly elevated histidine concentrations in the blood. For many years histidinemia was included in newborn screening programs in several countries as it was assumed that it causes intellectual impairment, and many children received a histidine-restricted diet, until it became clear that the condition is a non-disease (Brosco et al. 2010). Methylglutaconic aciduria type I caused by a deficiency of 3-methylglutaconyl-CoA hydratase is included in newborn screening programs in some countries although it has become clear that it is probably a non-disease in most children but may cause late-onset progressive leukoencephalopathy in adults (Wortmann et al. 2010). In a high-ranking publication, mutations in *ACSF3* were reported to cause of a wide range of symptoms in patients with combined malonic and methylmalonic aciduria (CMAMMA) (Sloan et al. 2011). The correlation of genotype and metabolic phenotype is clearly correct, but only the lucky availability of a population urine newborn screening program without ascertainment bias showed that prospectively iden-

tified affected individuals are clinically normal and that CMAMMA is probably a benign condition at least in most individuals (Levtova et al. 2015). On an individual basis, similar misdiagnoses may happen when genome-wide sequencing approaches are used to find the diagnosis in a patient with nonspecific or poorly characterized clinical problems, and rare or novel variants in one of the >20,000 protein-coding genes are wrongly assumed to be disease-causing. Next-generation sequencing demands next-generation phenotyping (Hennekam and Biesecker 2012) including thoughtful evaluation of genetic findings and presumed pathomechanisms. One should not assume that attractive (novel) genetic variants in a patient explain a particular problem unless this has been proven.

Remember

It is often difficult to predict the functional effects of genetic alterations, and the impact of novel variants identified in a patient should be discussed with great care.

39.2 Indications for a Molecular Genetic Analysis

In many countries molecular analyses are now routinely performed in most patients with inherited metabolic diseases. In order to gain maximum benefit from the results, it is helpful to specify the exact aim of the DNA test and the knowledge that is expected from the analysis. Indications for genetic investigations include:

- *Identification or confirmation of a* (*suspected*) *diagnosis*: The diagnostic value of genetic tests should always be compared to phenotype-based investigations such as metabolic or enzymatic studies with regard to availability, cost, and reliability.
- *Information on disease course*, *prognosis*, *and treatment options*: The identification of well-characterized genetic variants can provide information on the severity and likely course of a disease as well as the possible response to treatment. Prerequisite for this type of information is an excellent understanding of genotype–phenotype correlations in the respective disease.
- *Predictive information on individual disease risks including prenatal diagnosis*: Most inherited metabolic diseases are autosomal recessive traits with a recurrence risk of 25 % in later siblings of an affected child. Mutation analyses are the method of choice for prenatal analyses and are a prerequisite for polar body analyses or preimplantation genetic diagnosis (see Chap. 40). Genetic tests may also allow predictive diagnosis of a late-onset or latent condition (such as heterozygous ornithine transcarbamylase deficiency) and start of preventive measures when available. The individual value of this type of information must be discussed with the person concerned in the setting of genetic counseling before a predictive genetic test is ordered.

Remember

Mutation analyses in children may only be performed if there is an important medical consequence in childhood. Carrier analyses in healthy siblings of children with metabolic disorders are not indicated and should not usually be performed even when requested by the parents.

Biochemical and enzymatic analyses, when available, are preferable for the diagnosis of metabolic disorders as they reflect phenotypical parameters ("enzymatic" or "metabolic phenotype") and are therefore closer to the patient's clinical phenotype and disease expression. Molecular analyses do not have complete sensitivity as some disease-causing mutations may be missed by standard methods, and failure to identify a diagnostic genotype may not necessarily exclude a diagnosis. Also, technical and interpretative difficulties may be underestimated by clinicians and laboratories, and quality assessment schemes even for a common condition such as phenylketonuria have given rather disconcerting results (Zschocke et al. 2008). Nevertheless, there are various circumstances in which molecular studies are cheaper, faster, more convenient, or the only reliable method for diagnosis or confirmation of diagnosis of an inherited disorder. Many metabolic defects involve enzymes that are only expressed in specific organs such as the liver or the brain, necessitating invasive procedures (if at all possible) for enzymatic confirmation. Other

disorders involving structural, receptor, or membrane proteins that do not cause metabolic alterations are not open for enzyme testing and therefore may be difficult to confirm by traditional methods. Molecular studies may be fast and efficient and therefore the method of choice for the confirmation of disorders that are caused by single prevalent mutations in the patient's population.

The growing availability of panel/exome sequencing for diagnostic purposes and the rapid reduction in costs for this type of analyses will make DNA tests the first-line diagnostic test in many patients with inherited metabolic diseases, in particular those not covered by standard selective screening tests (amino acids, organic acids, acylcarnitines). This will increase the need for high competence of the institute that offers molecular tests. Laboratory-based physicians and scientists need to have good knowledge of the tested disorder, the spectrum of mutations, genotype–phenotype correlations, alternative diagnostic approaches, as well as the sensitivity and specificity of mutation analysis in the individual patient. Like any other medical investigation, genetic tests must provide an answer to a clinical question, and the laboratory needs to understand which information the referring clinician would like to obtain.

39.3 Relevant Methods

Most inherited metabolic diseases are caused by point mutations or small deletions/insertions in the coding region or intronic splice sites of a gene. Molecular methods for the identification of such mutations are mostly based on the polymerase chain reaction (PCR). Different approaches may be used to test for specific known variants, to examine a small gene for unknown mutations, or to investigate large or multiple genes with a high-throughput method. Large deletions and genomic rearrangements that destroy the integrity of a gene are rare causes of metabolic diseases and usually require specific methods for their identification. Mutation scanning methods such as single-strand confirmation polymorphism (SSCP) analyses that were previously used for cost-efficient mutation analysis particularly in large genes have become obsolete. There is no uniform strategy that is suitable for all applications, and usually a combination of methods is used. The exact approach depends on gene characteristics, type and frequency of mutations, and the sensitivity required to answer the clinical question. For the ordering of tests, and the interpretation of results, it is important that the referring clinician is familiar with the sensitivity, specificity, and indication of the most frequently used mutation detection strategies.

39.3.1 Testing for Common Mutations

Because of the reduced costs of DNA sequencing, specific methods for the identification of individual prevalent mutations are now rarely used in diagnostic practice. Nevertheless, testing for common mutations, usually through sequencing of selected exons, may be an efficient first step of molecular analyses in some conditions. Examples include long-chain hydroxyacyl-CoA dehydrogenase deficiency (mutation c.1528G>C, p.E510Q in the *HADHA* gene) or medium-chain acyl-CoA dehydrogenase deficiency (mutation c.985A>G, p.K329E in the *ACADM* gene). Homozygosity for a well-characterized common mutation is diagnostic in the investigated patients and provides reliable information on the clinical relevance. On the other hand, failure to identify a common mutation does not exclude the disorder as rare or novel mutations in the non-examined gene sequencing will not be covered. If analyses are restricted to common mutations, the sensitivity of the test with regard to the population background of the proband must be stated, and the probability that a relevant mutation was missed must be calculated taking the clinical and metabolic findings in the patient into consideration.

39.3.2 Sanger Sequencing of Whole Genes

DNA sequencing of the whole coding region of relevant genes is currently still the method of choice for the majority of indications. In the standard approach based on the invention of the British biochemist Frederick Sanger, the genomic target region is first amplified by PCR. The PCR products are purified, and labeled copies of specific segments are generated through "cycle sequencing" with unidirectional primers and ddNTP nucleotides that are attached to a fluorescent marker dye. The copying process starts at the primers and is randomly terminated by the ddNTPs at specific nucleotides, resulting in a large number of fragments with variable length and base-specific label. These fragments are separated by capillary electrophoresis on a semiautomated capillary DNA sequencer that detects fluorescently labeled DNA fragments with a laser system. The results are converted into graphical images in which each nucleotide of the investigated PCR product is represented by a distinct "peak" with a base-specific color. There is as yet no 100 % reliable system for the automatic evaluation of the results, and skilled interpretation is required particularly for the detection or exclusion of heterozygous mutations. In order to reduce errors, each target region is sequenced in both directions (forward and reverse) in individual reactions. It is important to note that direct sequencing of coding exons detects neither large genomic rearrangements (deletions, duplications) nor intronic mutations outside splicing regions.

39.3.3 Massively Parallel ("Next-Generation") Sequencing

In traditional Sanger sequencing, a separate reaction tube is required for each PCR product, and sequencing of a gene with, e.g., 24 exons requires 48 different analyses in as many reaction tubes (24 PCR products that are sequenced in forward and reverse directions). Sequencing large genes or many different genes thus used to be tedious, time consuming, and expensive. This problem was solved with the advent of massively parallel "next-generation" sequencing methods that allow individual analyses of many different sequencing reactions in parallel, e.g., on a microchip slide or array (Tucker et al. 2009). Millions or even billions of reactions are carried out and captured in parallel, each reaction representing a single DNA strand that was used as template. Individual steps in massively parallel sequencing approaches for diagnostic purposes include:

1. Library creation
   - Fragmentation of the patient's/proband's DNA, usually genomic DNA extracted from a blood sample
   - If appropriate, enrichment of specific target regions in the DNA sample, e.g., all protein-coding sequences or a particular gene
   - Linkage of adaptors to the DNA fragments (= creation of a "library")
2. Sequencing reaction
   - Fixation of the DNA fragment to an array/slide, in situ amplification
   - Sequencing reaction which generates base-specific signals that represent the sequence of the DNA fragment
3. Bioinformatic analysis and genetic evaluation
   - Quality control, trimming, filtering, etc. of the generated sequences
   - Comparison of the results with reference sequences, alignment of the individual sequences to the likely respective locus
   - Variant identification and annotation, quantitative analyses, etc.
4. Confirmation of relevant variants by Sanger sequencing from genomic DNA or other methods
5. Clinical interpretation and reporting

Several different instruments for massively parallel sequencing are available, and the technology is advancing rapidly. Different methods have different *error rates* which partly depend on sequencing characteristics (e.g., if there is a

stretch with the same nucleotide in a row, it can be difficult to determine the exact number) and obviously affect the reliability of the results. In practice, the most relevant factor is the *coverage*, i.e., how often a particular nucleotide in the sequence of interest (the target sequence) was read in the analysis. Each generated sequence represents a single DNA fragment used by chance from the millions of potential templates in the extracted DNA sample. Considering that most genes have two copies in the diploid genome, and there is a certain error rate that causes calling of the wrong nucleotide, a minimum coverage of 15–30× is required to make sure that correct genotype (heterozygous and homozygous variants) has been determined. Genetic mosaicism, i.e., the presence of variants in only a proportion of cells, is relevant for some diseases, and a much higher coverage is required to reliably detect low-level mosaicism. There are two possibilities to increase coverage:

- Increase the power of the instrument, measured as throughput in gigabases (Gb, billion nucleotides) or now terabases (Tb) per sequencing run.
- Enrich the target sequences in the sample that is used as template for massively parallel sequencing.

It is likely that in some years the sequencing instruments will be so powerful and cheap that sequencing the whole genome in centralized high-throughput facility will be the most cost-efficient approach for most indications. For the time being, however, enrichment of specific analysis targets in the template DNA is the cost-efficient method of choice for most research and clinical diagnostic purposes (Mamanova et al. 2010). In principle any DNA or RNA target can be enriched, mostly either through multiplex PCR amplification or hybridization methods. The most frequently used approaches include:

- Exome sequencing: Enrichment of the coding sequences and adjacent introns of all protein-coding genes; this allows great flexibility for rare diagnostic indications or when there is a large number of potentially disease-causing genes although the coverage is still incomplete, and some relevant sequences must be reanalyzed, e.g., by Sanger sequencing.
- Panel sequencing: Enrichment of a panel (selection) of relevant genes for specific diagnostic indications; this results in a high coverage of all predefined target genes and is used for frequent indications such as in familial cancer disposition syndromes or suspected lysosomal storage diseases.

Remember

Different enrichment strategies such as exome sequencing or panel sequencing are used to increase coverage of target sequences relevant for different clinical indications when massively parallel sequencing is used for diagnostic purposes.

The technical costs for reading a DNA sample has now dropped so much that the effort required for the correct interpretation of the generated sequences and identified variants has become the limiting factor with regard to resources and costs. Considering that an average exome contains several 10.000 missense variants and more than a hundred rare or novel nonsense mutations that are predicted to destroy the function of a gene, it is difficult to prove which of them (if any) is clinically relevant. Interpretation of variants involves several steps, only some of which can be automized with bioinformatics methods:

1. Biological prediction:
   (a) What is the predicted effect of the variant on the protein sequence or structure (e.g., missense or nonsense effect)?
   (b) Could there be an effect of splicing or other transcript modification processes?
   (c) What are the predicted effects on structure, stability, or function of the protein?
2. Statistical database analysis:
   - Has the variant been previously observed, i.e., is it listed in in-house or international databases?
   - If it has been observed, what is the *minor allele frequency* (MAF, the frequency of

the variant in different populations)? Variants are unlike to be responsible for a dominant disease with a MAF >0.1 % and for a recessive disease with a MAF >1 %.
   - Is the affected nucleotide conserved in other species?
3. Clinical information:
   - Has the variant been previously linked to a disease?
   - Does the variant segregate with a disease in the investigated family?
4. Interaction with other variants:
   - Could the variant contribute to the development of disease in conjunction with other genetic changes?

As discussed above (Sect. 39.1), the correct interpretation of genetic variants can be difficult even when only single well-characterized genes are sequenced, and sometimes functional studies (expression analyses) are required to confirm a clinically relevant effect. Dealing with the wealth of molecular data generated by massively parallel sequencing now creates major challenges for human geneticists who must be familiar with a large number of different genes and often have to wade through several databases and various original research articles to write a dependable report. In order to avoid irrelevant or confusing results including secondary findings unrelated to the clinical question, many laboratories restrict molecular diagnostic analyses to the genes that are clinically relevant and do not visualize the other sequences even if they have been technically read by the instrument. Thus panel sequencing is not merely a technical strategy but also an analytical concept where, e.g., the evaluation of exome sequencing is restricted to a few target genes; if necessary, other genes may be unblinded on later request (Rehm 2013).

It is important to realize that exome or panel sequencing methods used in clinical practice are not suitable or less reliable for the determination of some types of clinically relevant genetic alterations, such as:

- Genomic regions not covered by the enrichment process, such as most of the introns
- Epigenetic changes
- Trinucleotide repeat expansions, e.g., in fragile X syndrome or myotonic dystrophy
- Mutations in genes with very similar pseudogenes
- Large deletions or duplications, e.g., exon deletions or microdeletion syndromes (inquire with laboratory)
- Balanced chromosomal translocations with breakpoints that destroy a single gene

Remember

Massively parallel sequencing has fundamentally changed molecular genetic diagnostics by dramatically reducing technical costs but increasing the required knowledge, skill, and clinical understanding of human geneticists that produce the reports. Considering the complexity and limitations of the analyses, and the possibility of unexpected findings that may be unclear or unrelated to the clinical question, care must be taken to inform the patient about the planned investigation and possible results through genetic counseling.

39.3.4 Autozygosity Mapping

Most inherited metabolic diseases are inherited as autosomal traits and are thus more likely to occur in consanguineous marriages. First cousins share at least one-eight of their genetic material, and children from first cousin parents have a 1–2 % probability of being homozygous for a disease-causing mutation that was heterozygous in one of the shared maternal/paternal grandparents. Disease-causing autosomal recessive mutations in children from consanguineous marriages are usually (but not always!) in extended chromosomal areas (limited by recombination events) in which not only the mutation but also adjacent markers are homozygous. Such areas are called autozygous when they trace back to the same ancestor through the maternal and paternal lines. Autozygous regions in the genome are easily recognized with quantitative single nucleotide polymorphism (SNP) arrays used for molecular karyotyping, and putative candidate genes in these regions may be selected, e.g., for exome analysis (Alkuraya 2013). This

approach is even more powerful when there are several affected persons in the family and has been an important tool in the recent identification of novel disease genes. Autozygosity mapping also recognizes incest that may explain intellectual disability in a child but may cause unexpected challenges in clinical practice.

39.3.5 Identification of Large Deletions and Duplications

Genomic rearrangements such as the deletion of several exons of a gene are not usually recognized by PCR-based methods including Sanger sequencing. Elaborate algorithms in some massively parallel sequencing approaches, e.g., panel sequencing with high coverage of target regions, allow the detection not only of sequence variants but also of quantitative differences that may indicate large deletions or duplications. Nevertheless, specialized quantitative molecular genetic methods are still used for the detection of such changes. The most relevant is the multiple ligation-dependent probe amplification (MLPA) technique; the reader is referred to the medical literature for an exact description of this method and its application (Schouten et al. 2002; Sellner and Taylor 2004).

Remember

Large genomic deletions or duplications are not usually recognized by standard sequencing analyses although they may be detectable through some massively parallel sequencing tests.

39.4 Samples

Remember

Sample for most applications

5–10 ml EDTA full blood, shipped at ambient temperature. For long distances it may be preferable to extract the DNA and send that.

Mutation analysis can be performed in any human sample that contains cellular nuclei. The exact sample depends on the type of DNA that needs to be investigated. Routine diagnostic mutation analyses are usually performed in genomic DNA which is most conveniently extracted from 5 to 10 ml of anticoagulated full blood (usually EDTA blood). Smaller amounts of blood down to a few 100 µl may be acceptable but less satisfactory as DNA extraction from small samples is less reliable, less DNA is available, and the extraction method may be more expensive (discuss with laboratory). The sample should not be centrifuged but shipped as native full blood by normal (overnight) mail at ambient temperature. DNA is quite stable, and the sample may be stored at room temperature or in the refrigerator for 1–2 days if necessary (e.g., on weekends). Alternatively, whole blood may be stored frozen for several weeks or may be sent on dry ice; inquire with the molecular laboratory whether frozen blood is accepted. If EDTA blood is not available, other materials including dried blood spots on filter paper cards, coagulated blood, hair roots, buccal swabs, or even serum, urine, or feces may be used for extraction of genomic DNA or for polymerase chain reaction (PCR) amplification of specific sequences, but these methods are less reliable and may be considerably more expensive.

An alternative template for genetic analysis is mRNA obtained from cells in which the target gene is expressed. As a single-stranded molecule, mRNA is quite unstable and in the laboratory is converted into double-stranded complementary DNA (cDNA) which can be stored indefinitely. cDNA analysis has certain advantages over genomic DNA. It does not have an intron–exon structure but contains the uninterrupted sequence that is translated into protein. The detection of unknown mutations may be more convenient from cDNA since fewer fragments are required for PCR-based analysis methods. Splicing mutations that cause the removal of whole exons from the translated sequence are easily recognized, thus confirming the pathological relevance of DNA variants in the introns. On the other hand, splicing variants that are observed under physiological or cell culture conditions are sometimes not likely to cause disease and may be difficult to interpret; RNA fragments that contain premature stop codons may be eliminated by nonsense-

mediated decay, and the preparation of cDNA is more tedious than that of genomic DNA. While cDNA analysis may be the method of choice in conjunction with enzyme studies if organ tissue such as liver or skin fibroblasts is available for investigation, genomic DNA analysis remains the method of choice for most applications.

39.5 Pitfalls

Remember

Mutation analyses, like all laboratory techniques, have a certain error rate which is impossible to eliminate completely.

Incorrect results or interpretations may be generated either through analytical or sampling errors, methodological limitations, insufficient knowledge of the types of mutations causing a particular disease, or inadequate consideration of family or population information. The following factors should be considered in the choice of mutation analysis methods and the interpretation of the results:

- What type of mutation usually causes the disease? Different approaches need to be chosen for different mutations such as point mutations, trinucleotide repeats, or large deletions.
- Are there certain prevalent disease-causing mutations in particular populations? Screening for such mutations may be a cost-efficient method for confirming the disease or reducing the likelihood of its presence in a patient. It is essential to take the ethnic origin of a patient into consideration when such an approach is chosen.
- What is the sensitivity of comprehensive mutation analysis with PCR-based methods such as DNA sequencing? For some disorders, this may approach 100 %, while for others only a proportion of mutations is recognized. PCR-based methods are usually restricted to coding exons and adjacent intron sequences of the particular gene and may fail to detect, e.g., large gene deletions spanning several exons.
- Could there be more than one mutation on the same chromosomal strand? Double mutants have been identified in many genes. It is not justified to conclude that there is compound heterozygosity when two mutations are identified in a patient with a recessive disease. This constellation is only one of the reasons why inheritance of mutations on separate chromosomes should be confirmed in samples from parents or other relatives.
- What is the diagnostic specificity of mutation identification? Only a proportion of variants in a gene affect protein function and cause disease. Criteria that may be used to estimate pathogenetic relevance include type of mutation (missense or nonsense, predicted impact on amino acid sequence), extent of DNA analyzed, segregation with the disease in a family, prevalence of the mutation in the general population, and functional assessment through expression analysis.
- How important are nongenetic factors of pathogenesis? Disease penetrance may vary considerably even within single families. Strict genotype–phenotype correlations are observed only in a proportion of metabolic disorders, and the clinical picture in a patient may be insufficiently explained by the mutations in a single gene.

Clinicians who request mutation analyses should treat the results with care, just like all other laboratory tests. Quality assessment schemes, available only for very few conditions anyway, show that significant errors occur even in laboratories with good technical facilities and accreditation for diagnostic molecular genetic services. Confirmatory repeat analyses (either on a new sample or by analysis at a second independent laboratory) may be considered when the results of molecular studies are important for patient management but do not seem to fit the clinician's assessment of the case. Adequate interpretation of the results requires good knowledge of the respective disease and the underlying mutations that determine its pathogenicity. It is prudent to request molecular diagnostic services only from laboratories that are familiar with the respective disorders and the underlying genotype–phenotype correlations.

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Family Issues, Carrier Tests and Prenatal Diagnosis

40

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Key Facts

- Many inherited metabolic diseases are inherited as autosomal recessive traits and have a high recurrence risk in subsequent siblings of an affected child.
- The relevance of an inherited metabolic disease for other family members should be discussed in the setting of genetic counselling.
- Carrier tests should not be performed in asymptomatic children unless there is a medical consequence of the result for the tested individual in childhood.
- Standard invasive procedures required for prenatal analysis of inborn errors of metabolism have risk of abortion of 0.5–1 %.
- Chorionic villus biopsy is the method of choice for DNA-based prenatal tests. It can usually be carried out from the 11th to the 12th week of pregnancy onwards.
- Preimplantation genetic diagnosis requires in vitro fertilisation and is illegal in many countries. An alternative is polar body analysis, which is more limited in its applications.

40.1 General Remarks

Most inborn errors of metabolism are inherited as autosomal recessive traits. Diagnosis in a child thus implies a high recurrence risk for subsequent pregnancies in the family. Although clinicians caring for the child usually explain the genetic basis of the condition at an early stage (text box), many parents do not really appreciate all the implications, and formal genetic counselling including an explanatory letter to the parents is usually beneficial even in disorders with an apparently simple autosomal recessive inheritance (Claustres et al. 2014). Genetic counselling also involves taking a standardised family tree and may help to identify other relatives with a high risk for an affected child, such as consanguineous partners in the same extended family. Genetic counselling should be recommended to these couples as well.

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Text Box: The Human Genome as a Shelf of Cook Books

A simple way to explain the human genome and the molecular basis of inherited disorders to patients is a comparison with a kitchen shelf of books. The genetic information is stored in each cell in a collection of 46 books (chromosomes) that are comprised of a total of more than 20,000 recipes (protein-coding genes). Each gene contains the information for a particular function that the cell may need to fulfil. Quite importantly, there are no 46 different books but 23 pairs of the same books with the same recipes, and each person thus has two copies of most recipes. The only exceptions are the sex chromosomes: females have two X chromosomes, whereas males have only one and an additional Y chromosome. The Y chromosome used to be an X chromosome several hundred million years ago but over time has lost most of its genes, with the exception of genes that are important for male sex development and approx. 30 homologous genes in the pseudoautosomal regions of the X and Y chromosomes. When people have children, they pack one of the two copies of each book into the parcel for the child, which thus contains only 23 different exemplars. As a child gets a parcel from each parent, it again receives a total of 46 books. The text of the recipes consists of four letters ACGT, and one set of books has more than three billion letters. Because the books are copied by hand from generation to generation, they contain the occasional spelling mistake or typing error, and there may be different variants (alleles) of the same recipes. Polymorphisms are variants that are quite common (>1 % of gene copies in a population), while mutations are variants that change the information content and in consequence change or remove the normal function. It may be useful to point out that, for example, humans do not have two PAH genes (PAH codes for the enzyme phenylalanine hydroxylase deficient in phenylketonuria) but have two copies of the one PAH gene; just like having two copies of the same recipe for chocolate cake does not mean that one has two chocolate cake recipes. Any genetic abnormality may be explained with this example, from X chromosome inactivation (only one book is used; the other exemplar is sealed) to genomic imprinting (a recipe can only be read on either the paternal or the maternal copy of the book; the other version is sealed) or balanced translocations (two books have fallen apart and have been wrongly glued together again).

40.2 Carrier Tests

Once disease-causing mutations have been identified in a patient, it is easily possible to test other family members for the familial mutation(s). In autosomal recessive conditions, both the parents are normally carriers for the disease, and mutation testing may not be necessary for confirmation. Sometimes, however, it is advisable to confirm the results in the child through carrier testing in both parents. In a child with homozygosity for a mutation, for example, it may be advisable to exclude the presence of a large deletion on one allele that may lead to PCR non-amplification on the other gene copy. When a child appears to be compound heterozygous for two mutations, it may be important to confirm that the two mutations are indeed in trans, i.e. on the two different chromosomal strands and not on the same chromosomal strand/in the same gene copy. In the latter case, the child is heterozygous for a double mutation, and the genotype does not confirm the disease. It should be kept in mind, however, that carrier tests in the parents may also lead to the identification of non-paternity which may be embarrassing (or worse) both for the couple involved.

Carrier analyses in siblings of affected children or in more distant relatives may appear harmless on first sight. Nevertheless, there have been cases where the presence of mutations in completely healthy persons was misunderstood by the affected individual or others as a kind of disease and at worst had an adverse impact on relationship, insurance or employment. It is thus generally agreed that carrier tests should not be performed in underage siblings of patients with recessive disorders. This is true for all kinds of genetic tests. These should not be carried out in asymptomatic children unless there is a medical consequence of the result for the tested individual already in childhood. It is much wiser to let the child grow up to an age where she or he understands the implications and may request carrier testing in the course of genetic counselling. Growing up with the knowledge of the result may rob the child of the option to later actively work on this issue in the process of making a decision on testing (Borry et al. 2009; Botkin et al. 2015; European Society of Human Genetics 2009).

40.3 Prenatal Diagnosis

Many parents who experienced a severely debilitating or fatal inherited disease in a child and who have a high risk for the same disease in future pregnancies request prenatal diagnosis with the option of termination of pregnancy. The associated ethical, social and legal issues differ markedly between countries and are not discussed here. There is generally a window of opportunity between the 11th and 12th week of pregnancy when the placenta and foetus are sufficiently large for invasive testing and the 22nd–23rd week when the foetus may survive outside the womb and termination of pregnancy is often regarded as much more problematic for ethical reasons. Termination of pregnancy in the second trimester involves the induction of labour and delivery of a foetus which dies through the procedure, in contrast to the first trimester when curettage is usually sufficient. After the 22nd–23rd week, the foetus is killed in the womb through injection of potassium into the heart (foeticide) in order to avoid that the child is born alive, a procedure that is emotionally and ethically very challenging or unacceptable for many parents and doctors. Parents should be offered genetic and/or psychosocial counselling when termination of pregnancy is considered. The decision for or against termination of pregnancy rests solely with the couple or the mother. Non-directive counselling is mandatory and is possible even when termination is requested by the parents but is rejected by the doctors or is deemed incompatible with legal regulations in the specific circumstances.

Prenatal diagnosis should be organised by a clinical geneticist in close contact with the gynaecologist who performs both the invasive procedure and possibly the termination. Parents should be advised to contact the geneticist as early as possible once a pregnancy has been confirmed. Traditionally, there are different approaches to prenatal diagnosis including metabolic analyses in amniotic fluid and enzyme tests in chorionic villus cells or amniocytes, but direct mutation analysis is now mostly regarded as the method of choice. Because of the enormous progress in the understanding of inherited disorders over the last years, it is now possible to test a foetus for almost all inborn error of metabolism once a disease has been recognised in a family.

Metabolic conditions are not usually recognised through abnormal ultrasound scans, and invasive sampling of foetal cells or fluid is necessary for prenatal testing. There are three main approaches (Besley 2005):

1. *Chorionic villus sampling* (CVS) is usually possible from the 11th week of pregnancy onwards. Small fragments of placental tissue are taken with a moderately large needle either through the abdomen or the cervix. Chorionic villi have both foetal and maternal components, and it is essential to carefully remove the latter after the sample has been taken. Cells may be either cultured, e.g. for chromosome analysis or enzyme studies, or may be used for DNA extraction. Chorionic cells are derived from the trophoblast and the extraembryonic mesoderm, and chromosomal abnormalities found in these cells (particularly

when the abnormality is found only in a proportion of cells) do not necessarily reflect the chromosomal status of the foetus. For karyotype analysis, therefore, two different cell culture types are used. This is not necessary for molecular analyses, but it is mandatory to exclude maternal contamination when chorionic villi are used for genetic tests as the presence of maternal cells may lead to false-negative results. For this purpose, highly polymorphic microsatellite markers in DNA extracted from chorionic villi and maternal blood are compared; maternal contamination is excluded when only one of the two maternal alleles and one paternal allele are identified in the foetal sample. CVS has a risk for miscarriage of approximately 1 %, i.e. 1 in 100 women loses a healthy child after the procedure. Because of the early availability of foetal DNA, CVS is the method of choice for prenatal diagnosis through mutation analysis.

2. *Amniocentesis* is usually possible from the 14th to 15th week of pregnancy onwards. Amniotic fluid contains foetal cells derived from the urinary tract or other sources and is obtained transabdominally with a long, relatively small needle. There is not usually sufficient cellular material for DNA extraction, and amniocytes have to be cultured for 7–10 days both for chromosomal and molecular analyses. DNA is thus available for testing at least 4 weeks later than after CVS. In contrast to CVS, there is smaller risk of maternal contamination unless there is a significant amount of maternal blood in the amniotic fluid (which may be recognised, e.g. through a reddish colour). Compared with CVS, amniocentesis has a slightly lower risk for miscarriage of approximately 0.5 %, although these figures also depend on the expertise of the gynaecologist who performs the procedure.
3. *Foetal blood sampling* is generally possible after the 20th week of pregnancy and has a risk of miscarriage of approximately 2 %. Blood is taken transabdominally with a long needle from an umbilical vein at the insertion of the umbilicus into the placenta. As this foetal sampling approach becomes possible only relatively late in pregnancy, it is usually restricted to special indications.

Prenatal diagnosis through examination of free foetal (placental) DNA in maternal blood (non-invasive prenatal testing, NIPT) is an emerging method which is now routinely available for chromosome aberrations (Daley et al. 2014). It is expected that this method will also be adapted for the diagnosis of monogenic disorders in the future.

40.4 Preimplantation Genetic Diagnosis and Polar Body Analysis

As an alternative to prenatal diagnosis after several months of pregnancy, disease status may also be determined in the course of in vitro fertilisation (IVF) prior to implantation of the embryo into the womb. For preimplantation genetic diagnosis (PGD), one or two cells are taken from the embryo 2–3 days after fertilisation (in the four- or eight-cell stadium). PCR-based mutation analysis is performed after DNA extraction and amplification (Dahdouh et al. 2015; Dreesen et al. 2014). This procedure requires a highly controlled laboratory setting, and the analyses have to be specifically established for the individual case. PGD has now been legalised in an increasing number of countries as it can be regarded as preferable to invasive prenatal diagnosis and termination of pregnancy. However, regulations are rather strict in many countries, partly because of a perceived risk of a "slippery slope" of easy selection of preferable characteristics in a child conceived through IVF. As an alternative to PGD, mutation status may be determined in DNA extracted from the polar bodies of an egg prior to fertilisation (polar body analysis). This method only allows testing for mutations carried by the mother and not the father, again may need to be specially established for the individual case and is offered by few specialised laboratories only. Both approaches require IVF, which may be stressful and carries additional risks, and are not necessarily covered by health insurances.

However, they are options for parents who for religious or ethical reasons object to termination of pregnancy.

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Function Tests

41

Johannes Zschocke and Stefan Kölker

Key Facts

- In general, in vivo function tests have been partly superseded by improved diagnostic methods. A few of these tests, however, are still used for specific diagnostic indications or to tailor therapy. In vivo function tests that have a high risk of harming patients such as oleic acid loading in patients with suspected fatty acid oxidation disorder are obsolete.
- Remaining indications for the fasting test include the differentiation of disorders of gluconeogenesis from those with defective oxidation of pyruvate in patients with lactic acidemia; diagnostic work-up in patients with recurrent episodes of symptomatic ketonemia, recurrent cyclic vomiting, recurrent intermittent metabolic acidosis, or the suspicion of fluctuating neurometabolic disease; controlled determination of fasting tolerance to fine-tune the therapy of metabolic disorders; and the assessment of the duration of safe intervals between feedings, e.g., in patients with mitochondrial disorders.
- The standardized determination of metabolic parameters before and after meals is particularly useful in the diagnosis of mitochondrial disorders, which typically show pathological postprandial increases of lactate, alanine, and other small amino acids. In addition, it is useful for the diagnosis of patients with ketolysis and ketogenesis defects and for the differentiation of glycogen storage disease type I and III.
- A controlled glucose challenge is useful to assess cellular respiration in patients with suspected disorders of energy metabolism, in whom lactate values have been repeatedly normal.
- The phenylalanine challenge test may be useful for the identification of disorders of pterin metabolism in patients with unclear dystonic movement disorders, in particular when Segawa syndrome or sepiapterin reductase deficiency is suspected.
- The tetrahydrobiopterin (BH_4) test is intended to identify individuals with primary BH_4 deficiency or BH_4-sensitive phenylketonuria.
- The vitamin B_{12} test aims at identifying responsiveness to pharmacological doses of vitamin B_{12}, found in some defects of 5-deoxyadenosylcobalamin metabolism.

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- The allopurinol test may be used for the diagnosis of heterozygous or mild ornithine transcarbamylase deficiency in cases of unclear transient or intermittent hyperammonemia, unclear comatose or encephalopathic episodes in both sexes, or stepwise regressing neurodegenerative disease in girls or women, especially those who show epilepsy and ataxia as prominent symptoms. The test, however, is less sensitive and specific than initially reported.
- The glucagon stimulation test is used as a provocative test for the diagnosis of glycogen storage disease type I.

41.1 General Remarks

Many metabolic disorders show biochemical abnormalities only intermittently under metabolic stress, and normal findings in the interval may not reliably exclude a diagnosis in a particular patient. It is often possible to pinpoint the site or at least the area of a patient's metabolic abnormality by the use of a properly chosen challenge. A variety of in vivo function tests are used to create conditions that allow the assessment of metabolism in a controlled manner. Frequently, this entails ingestion of specific substances that give rise to diagnostic metabolites in certain disorders. Most tests are fairly safe; many are inconvenient, but some (including the frequently performed fasting test) can lead to potentially serious complications and should only be carried out by experienced pediatricians after other diagnostic options including mutation analysis of candidate genes have been exhausted. It is essential to carefully plan the collection of samples during the test and to prepare emergency measures in case complications occur.

Rapid advances in enzymatic, molecular, and other diagnostic techniques permit an increasing number of diagnoses to be obtained without tests of tolerance. However, functional studies may provide phenotypic information that more closely reflects the metabolic risk situation in the individual patient and will continue to have their place in the diagnostic work-up as well as in tailoring therapy (Touati et al. 2012).

41.2 Tests for the Utilization of Energy Substrates

41.2.1 Monitored Prolonged Fast

Monitored fasting is a powerful tool in unraveling the nature of metabolic disorders of energy metabolism. It must, however, be emphasized that fasting is potentially dangerous and should only be carried out after other, less risky investigations have been completed without a clear diagnosis. Fasting can cause life-threatening cardiac complications particularly in patients with long-chain fatty acid oxidation defects, severe hypoglycemia, hyperammonemia, and metabolic acidosis. Sudden cardiac arrest may occur even under controlled conditions in an intensive care unit, and a fasting test therefore is almost contraindicated in all patients with cardiomyopathy. Following a careful selection of patients and under careful observation, it can be nevertheless carried out safely (Bonnefont et al. 1990; Morris et al. 1996).

Remember

A fasting may cause life-threatening complications and should only be carried out with careful monitoring in a specialized hospital setting and only after other, less risky investigations have been completed without a clear diagnosis and exclusion of, e.g., defined fatty acid oxidation disorders.

Fasting results in a series of hormonal and metabolic responses to assure an endogenous supply of energy after cessation of exogenous intake (Cahill 1983). After each feeding, nutrients are supplied via gastrointestinal absorption. Depending on the amount and composition of the food, this absorption period can last for up to 6 h in adults. It is more often <4 h in infants and small children. With diminishing exogenous supply of glucose, plasma glucose concentrations fall and insulin levels decrease in parallel. The decrease of insulin

and increase of counteracting hormones diminish glucose consumption in the muscle and peripheral tissues. The body begins to utilize glycogen reserves by glycogenolysis.

In principle, hypoglycemia may be the consequence of endocrine or metabolic disease. The whole picture can therefore only be obtained if insulin, cortisol, and growth hormone as well as metabolic parameters are measured simultaneously in patients who develop hypoglycemia. Patients with hyperinsulinism often have the shortest and sometimes variable fasting tolerance.

After 8–10 h of fasting, free fatty acids begin to substitute glucose as the primary energy source in the muscle, while it often takes 17–24 h to deplete ordinary stores of glycogen. Two central metabolic adaptations to prolonged fasting are initiated in the liver: (1) glucose is synthesized via glyconeogenesis from alanine and oxaloacetate derived from amino acids, as well as from glycerol resulting from fatty acid oxidation; (2) fatty acid oxidation funnels into ketone body production providing an alternative (and most important) source of energy.

Remember

Patients with glycogen storage diseases often become hypoglycemic directly after the absorption period. Patients with defects of gluconeogenesis may become hypoglycemic after 10–20 h of fasting, while those with defects of ketogenesis or ketolysis develop hypoglycemia at 15–24 h.

Infants and small children may have shorter tolerance of fasting. In prolonged fasting, the body finally draws selectively on its lipid resources to spare vitally needed proteins. Depending on the nutritional state, an adult with a defect in fatty acid oxidation may not become symptomatic until fasting has been prolonged for 36 h. In general, hypoglycemia following a short period of fasting signifies disordered carbohydrate metabolism or hormonal disorders; hypoglycemia that occurs only after prolonged fasting signifies disordered fatty acid oxidation or ketolysis. Some patients with electron transport defects follow the prolonged pattern, because fatty acid oxidation may be impaired secondarily.

Free fatty acids can be oxidized in most body tissues as an energy source. As they do not cross the blood–brain barrier, homeostasis of the brain energy supply depends on adequate production of ketone bodies. Defects of fatty acid oxidation, in which ketone production may be impaired and toxic intermediates are produced, may cause acute encephalopathy accompanied by hepatocellular dysfunction, resulting in a Reye-like syndrome.

The fasting test has lost some importance with the advent of acylcarnitine analyses in dried blood spots or plasma and is now largely irrelevant if not contraindicated for the diagnosis of fatty acid oxidation defects. These disorders frequently show clinical symptoms only at times of fasting when there may be marked hypoketotic hypoglycemia and massive excretion of dicarboxylic acids without appropriate ketones in the urine. The single most important investigation is the determination of free fatty acids (elevated) and ketone bodies (no sufficient rise) in a serum or plasma sample at the time of symptomatic hypoglycemia. Diagnostic problems arise when the acute hypoglycemic illness is treated without prior collection of at least a serum sample for analyses, as biochemical abnormalities may completely disappear with restoration of glucose homeostasis.

Remember

Fatty acid oxidation defects and other metabolic diseases that are identifiable by analysis of amino acids and carnitine status in plasma, organic acids in urine, and acylcarnitine profile in dried blood spots or plasma should be ruled out before a diagnostic fast. In fatty acid oxidation disorders, acylcarnitine profiles usually remain abnormal in the nonfasting state.

Determination of fasting tolerance through a monitored fast may be considered in patients with recurrent episodes of apparently fasting-related symptoms, such as episodes of decreased consciousness or especially recurrent documented hypoglycemia or Reye-like disease in whom the above described analyses

were inconclusive. For example, patients with a deficiency of mitochondrial 3-hydroxy-3-methylglutaryl-(HMG-) CoA synthase, an isolated disorder of ketogenesis, may show a normal acylcarnitine profile even during hypoglycemic episodes. A fasting test may reveal the typical, hypoketotic hypoglycemia and a unique spectrum of urinary organic acids. Primary mutation analysis is the method of choice to confirm the diagnosis.

A fasting test is also useful in patients with lactic acidemia to distinguish those with disorders of gluconeogenesis from those with defective oxidation of pyruvate (see Chap. 7). Other indications for controlled fasting include the following:

- Recurrent episodes of symptomatic ketonemia
- Recurrent cyclic vomiting
- Recurrent intermittent metabolic acidosis
- Suspicion of fluctuating neurometabolic disease

Controlled determination of fasting tolerance to fine-tune the therapy of metabolic disorders, as in patients with glycogen storage diseases and nesidioblastosis, but also to judge the duration of safe intervals in between feedings, e.g., in patients with fatty acid oxidation or mitochondrial disorders

41.2.1.1 Preparations

A fasting test should be performed only if the patient is in a stable, steady-state condition. Caloric intake for the last 3 days should have been adequate for age. The test must be postponed in case of even minor intercurrent illness. What is especially important is a detailed history with respect to the individual fasting tolerance and events and time courses of adverse reactions to previous fasting. With this information and that of the presumptive diagnoses, the timing of the fast can be optimally planned. The duration of the fasting period may be scheduled according to the age of the child (Table 41.1) unless a shorter period is indicated by the clinical history. In general, it is usually safe to allow fasting at night as long as the child usually goes without eating, but the period beyond should take place during the daytime. If hypoglycemia occurs, it should be at a time of optimal staffing.

A monitored fast should be undertaken only in settings in which the entire staff is experienced with the procedure, with close clinical supervision and well set out guidelines as to response to hypoglycemia or other adverse events. Necessary details must be explained to everybody involved in advance. Special care must be taken to ensure complete collection of samples. It is advisable to fill out the forms and assemble and label all the tubes including the day and timing of the samples prior to the start of the test.

The aim, nature, and possible adverse effects of the fasting test must be explained in detail to the patient and family to ensure optimal cooperation as well as early notification of symptoms. Informed consent must be obtained from the parents prior to the test.

An intravenous line must be established in order to provide immediate access for the treatment of hypoglycemia.

41.2.1.2 Procedure

After baseline studies of plasma free fatty acids and ketone bodies (acetoacetate and 3-hydroxybutyrate), blood sugar, and urinary organic acids, fasting is conducted with careful bedside monitoring of the concentrations of glucose in blood and ketones in urine. The patient is allowed to drink water and unsweetened tea, but no juices or soft drinks including "diet" beverages. Heart rate may be monitored by ECG.

An example of a fasting test of 24 h is given in Table 41.2. Under normal conditions,

Table 41.1 Suggested time periods for fasting in different age groups

Age	<6 months	6–8 months	8–12 months	1–7 years	>7 years
Starting time	4 a.m.	Midnight	7 p.m.	5 p.m.	3 p.m.
Duration	8 h	15 h	20 h	24 h	24 h

Table 41.2 Twenty-four-hour fast in a 6-year-old child

Begin fast at time T = 0 h at 4 p.m.
T (time) = 0 h (4 p.m.)
End of last meal with intake documented
T = 1 h (5 p.m.)
Serum *glucose*, electrolytes, creatine kinase. Blood gases. Lactate, pyruvate, *3-hydroxybutyrate*, acetoacetate from perchloric acid tube. *Plasma amino acids*, *free fatty acids*, *and carnitine*. Acylcarnitines
Start collection of urine in 8-h aliquots. Monitor ketones in urine at the bedside
T = 1 h until T = 12 h
Blood glucose concentration is monitored 3-hourly at the bedside
From *T = 12 h (4 a.m.)* onward
Collect glucose levels hourly. Heart rate may be monitored by ECG
T = 15 h (7 a.m.)
Serum glucose, electrolytes. Blood gases. Lactate, pyruvate, 3-hydroxybutyrate, acetoacetate. Plasma amino acids, free fatty acids
T = 20 h (noon)
Serum glucose, electrolytes. Blood gases. Lactate, pyruvate, 3-hydroxybutyrate, acetoacetate. Plasma amino acids, free fatty acids
T = 24 h (4 p.m.) or at the time of hypoglycemia
Serum *glucose*, electrolytes, creatine kinase. *Insulin*, *cortisol*, *growth hormone*, and additional specimen for follow-up hormonal investigations. Blood gases. Lactate, pyruvate, *3-hydroxybutyrate*, acetoacetate. *Plasma amino acids*, *free fatty acids*
Acylcarnitines
Quantitative analysis of *organic acids* is performed on the first and last aliquots. The middle collection is to ensure a sample for analysis in a patient developing hypoglycemia during that period

In this sample case, no adverse effect to overnight fasting was to be anticipated from the history
Parameters that are indispensible for evaluation and interpretation are highlighted in italics (see also Table 41.3)

ketogenesis is brisk, and especially after 15–17 h, concentrations of acetoacetate and 3-hydroxybutyrate rise sharply, and gluconeogenesis occurs preserving normoglycemia. The fast is stopped at any time for the development of hypoglycemia, and with close monitoring it is usually possible to avoid symptoms of hypoglycemia.

Blood samples should be obtained at 15, 20, and 24 h and always at a time of hypoglycemia when the fast is stopped. The following basic laboratory parameters should be measured: blood sugar, free fatty acids and ketone bodies (acetoacetate, 3-hydroxybutyrate), lactate, electrolytes, blood gases, transaminases, and creatine kinase. In addition, dried blood spots must be obtained for the analysis of the acylcarnitine profile. Serum carnitine status and amino acids may be considered, and a spare serum sample should be obtained in case additional tests may be indicated. Hormone studies including insulin, glucagon, thyroid hormones, and growth hormone must be carried out during hypoglycemia. Urine is collected in 8-h aliquots for the quantitative determination of organic acids. Hourly glucose determinations are made after the first missed feeding and are repeated more frequently if the blood sugar falls <50 mg/dL. All urine passed should be checked for ketones. Comprehensive investigation of intermediary metabolites and hormones in blood and urine is especially important at the scheduled end of the fast or at the time of developing hypoglycemia, when the fast is terminated.

In the investigation of lactic acidemia, fasting is employed to distinguish disorders of gluconeogenesis from defects of oxidative phosphorylation (see Chap. 14). For this purpose, it is useful to give glucagon at 6 h into the fast (not in the evening or the night, e.g., during a 24-h fast). This may provide a presumptive diagnosis in a patient with glycogen storage disease type I. In addition, it depletes the liver of glycogen derived from exogenous glucose, and, as the fast continues, gluconeogenesis is required to keep from hypoglycemia. A schedule is shown in Table 14.1. If the suspicion for a defect of oxidative phosphorylation is especially high, it is possible to combine the fasting test with a glucose and/or protein load as the last feed.

41.2.1.3 Treatment of Adverse Reactions

The fasting test must be terminated if the intravenous line is lost and cannot be immediately replaced or if the patient develops symptoms due

to hypoglycemia or ketoacidosis, such as irritability, sweating, and drowsiness. It should also be discontinued at any sign of cardiac arrhythmia. The test is also terminated if hypoglycemia (blood glucose <40 mg/dL or 2.2 mmol/L) or significant metabolic acidosis (bicarbonate <15 mmol/L) is documented.

For treatment of hypoglycemia, 2 mL of 20 % or 4 mL of 10 % glucose/kg b.w. is given intravenously as a bolus followed by 3–5 mL of 10 % glucose/kg b.w./h until normoglycemia is restored and the patient retains oral food and fluid.

41.2.1.4 Interpretation

If the patient develops hypoglycemia, the fasting test is abnormal. Accurate interpretation requires knowledge of age-dependant hormonal and metabolic responses to fasting as well as reference values from control individuals. Table 41.3 is based on experience with close to 100 control subjects from two published series and additional control subjects, which were selected after extensive metabolic investigations failed to give any indication of an inherited metabolic disease.

In older children and adults, ketone body production may not be maximal until the second or third day of fasting because of greater stores of glycogen and efficient gluconeogenesis. Prolonged fasting extended up to 36 or even 48 h may be justified in adults. Infants and small children have much lower glycogen stores and higher capacities to form and utilize ketone bodies. Significant ketone body production occurs before 24 h. Interestingly, infants show an intermediate response to fasting as compared to toddlers and older children. This can be explained by larger glycogen stores in infants because of high-calorie meals at regular intervals. Ketone body production for children older than 7 years was variable and, in some, only moderate even after 24 h (see Table 41.3). Metabolic defects of ketogenesis or ketolysis would still be expected to be diagnosed after 24-h fast.

Reliable quantification of acetoacetate and pyruvate is difficult. For the evaluation of the fasting response, ketone bodies (in particular 3-hydroxybutyrate) may be measured in a plasma or serum sample. However, the ratio of 3-hydroxybutyrate to acetoacetate is important in the work-up of a suspected defect of pyruvate metabolism or oxidative phosphorylation, and analysis should be carried out in deproteinized blood samples (perchloric acid extraction) if such a disorder is suspected. Additional clues to a defect of pyruvate carboxylase are bouts of hyperammonemia and elevated levels of citrulline and lysine on amino acid analysis.

A good indicator of blood pyruvate concentrations is the simultaneously determined level of alanine in plasma. An alanine level of 450 µmol/L corresponds to a blood pyruvate of 100 µmol/L, i.e., the upper limit of the normal range. Other amino acids to be evaluated at the end of a fasting test are the branched-chain amino acids isoleucine, leucine, and valine that are physiologically elevated during acute starvation. However, if the increase becomes excessive and alloisoleucine detectable, a variant of maple syrup urine disease may be the cause for recurrent episodes of hyperketotic hypoglycemia.

A diagnostic algorithm of differential metabolic responses to fasting in disorders of carbohydrate and energy metabolism is elaborated in Table 41.4. In general, a high level of free fatty acids and low levels of 3-hydroxybutyrate and acetoacetate indicate a disorder of fatty acid oxidation. In defects of ketolysis, an elevated product of blood glucose times ketones during fasting is the most suggestive parameter. A summary of metabolic and hormonal disorders in which pathological responses are elucidated by fasting is given in Table 41.5.

Congenital hyperinsulinism (previously described as nesidioblastosis or persistent hyperinsulinemia of infancy), the most common cause of persistent symptomatic hypoglycemia in neonates and small infants, leads to hypoketotic hypoglycemia with low levels of free fatty acids. This disorder can be due to defects of the sulfonylurea receptor. The same constellation of neonatal hypoglycemia and impaired lipolysis can be caused by hyperproinsulinemia. In such patients, insulin levels are low and proinsulin grossly elevated.

Diagnosis of hormonal disorders depends on the correct collection of specimens during fasting

Table 41.3 Control ranges of metabolites of energy metabolism in response to controlled prolonged fasting

	Glucose mmol/L mg%	Ketones mmol/L	3-OH-BA mmol/L	3-OH-BA/acetoacetate	FFA mmol/L	FFA/ketones	FFA/3-OH-BA	Glucose × ketones	Lactate	L/P
Infants										
15-h fasting	3.8–5.3 68–95	0.1–1.6	0.1–1.0	1.4–2.7	0.4–1.6	0.6–5.4	0.9–4.4	0.5–6	0.9–2.3	11–21
20-h fasting	3.5–4.7 63–85	0.6–3.5	0.4–2.5	1.7–3.1	0.6–1.4	0.3–1.7	0.5–2.1	3–12	0.8–2.0	12–19
24-h fasting	2.6–4.6 47–83	1.4–4.1	1.0–2.9	2.2–2.9	1.1–1.7	0.3–0.7	0.5–0.9	7–12	0.8–2.1	11–20
1–7 years										
15-h fasting	3.5–5.1 63–92	0.1–2.2	<0.1–1.0	1.2–3.2	0.6–1.8	0.6–4.0	0.9–11	0.7–8	0.6–1.7	12–18
20-h fasting	2.8–4.5 50–81	1.0–3.8	0.6–2.9	2.3–3.3	0.8–2.6	0.4–1.7	0.4–2.1	4–12	0.5–1.8	10–19
24-h fasting	2.8–3.9 50–70	2.1–6.1	1.5–3.4	2.5–3.5	1.0–2.9	0.4–0.9	0.4–1.3	8–13	0.6–1.8	10–18
7–18 years										
15-h fasting	3.8–5.3 68–95	<0.1–0.8	<0.1–0.5	0.5–2.6	0.2–1.3	1.7–8	3.3–22	0.2–2.1	0.6–1.0	11–15
20-h fasting	3.5–4.7 63–85	0.1–1.5	<0.1–1.2	1.3–3.0	0.6–1.4	0.7–4.1	1.5–7.8	0.4–5.1	0.6–1.1	10–17
24-h fasting	2.6–4.6 47–83	0.7–4.1	0.5–1.6	1.5–3.1	0.9–1.8	0.5–2.0	1.1–2.4	2.4–8.1	0.4–1.0	8–18

The table has been modified from the work of Bonnefont et al. (1990) following the grouping of subjects and parameters considered and expanding the ranges from experiences with an additional 25 control subjects studied at the authors' departments

Glucose concentration is converted from mg% into mmol/l by division by 18 and vice versa

FFA free fatty acid, *ketones* sum of 3-hydroxybutyrate and acetoacetate, *L/P* lactate/pyruvate, *3-OH-BA* 3-hydroxybutyrate

Table 41.4 Response to fasting in disorders of carbohydrate and energy metabolism

Presumptive diagnosis	Glucose	Blood ketones	3-OH-butyrate/ acetoacetate	FFA	FFA/ketones	Lactate	L/P
Glycogenosis I	↓↓	N – ↓	N	N	N – ↑	↑↑	N
Glycogenosis III, VI, and 0	↓	↑	N	N	N	N	N
Defects of gluconeogenesis	↓↓	N – ↓	N	N	N – ↑	↑↑	N
Defects of fatty acid oxidation	↓↓	↓↓	N – ↓	N	↑↑	↑	N
Defects of ketolysis	↑ – N – ↓	↑↑	N	N	↓	N	N
Defects of pyruvate carboxylase	↓	↑	↓	N	N	↑↑	⇑
Defects of pyruvate dehydrogenase	↓	N – ↓	N	N	N	↑ – ↑↑	N
Defects of oxidative phosphorylation	N – ↓	↑	↑ – ↑↑	N	N	N – ↑↑	↑ – ↑↑

↑ pathologically elevated and ↓ decreased values
Parameters of specific diagnostic value are highlighted in bold and larger font
FFA free fatty acid, *L/P* lactate/pyruvate, *N* normal values (see Table 41.3)

and interpretation in connection with the blood glucose concentrations. Diagnosis needs to be ascertained by repeated determinations of insulin or detailed studies of pituitary function and additional investigations in patients with insufficiency of one or more of the counteracting hormones. Single growth hormone determinations in response to hypoglycemia are of little value for the diagnosis of growth hormone deficiency, and different provocative tests should be employed if the diagnosis is clinically suspected. Catecholamine deficiency with a tendency to hypoglycemia on the basis of dopamine-β-hydroxylase deficiency, aromatic L-amino acid decarboxylase deficiency, or tyrosine hydroxylase deficiency is exceedingly rare, but an important constellation for such patients. Dopamine-β-hydroxylase deficiency can be ascertained in patients primarily suffering from severe orthostatic hypotension by analysis of catecholamines in urine and biogenic amines in CSF.

A presumptive diagnosis of glycogen storage diseases is usually made before the diagnostic fast on the basis of significant hepatomegaly and repeated bouts of hypoglycemia. Fatty acid oxidation requires the coordinated action of at least 17 different enzymes and one additional transport protein. In each metabolic center, there are patients with definitive diagnoses of defective fatty acid oxidation, in whom the exact enzymatic defect could as yet not be determined. In addition, there are a number of enzymes involved in fatty acid oxidation and ketolysis for which no human defects have yet been discovered.

There are otherwise completely healthy children who can develop severe metabolic decompensation with excessive ketosis with or without hypoglycemia during intercurrent illnesses. In these children, similar reactions can be provoked by prolonged fasting. Although this is not a homogenous group of patients, an exaggerated uncoordinated production of ketone bodies and significant metabolic acidosis leading to nausea and protracted vomiting appears to be a common pathogenetic mechanism. The susceptibility to these reactions slowly diminishes with age but may persist into adolescence and young adulthood. True hypoglycemia is uncommon in these children, who appear to have a defect in ketone utilization. This condition is difficult to distinguish from abdominal migraine. Relatively low stores of fat and glycogen appear to be another common denominator for such exaggerated ketogenesis. Affected children are often

Table 41.5 Differential diagnosis of pathological responses to fasting

Disorder	Response to fasting	Diagnostic markers (in addition to hypoglycemia)
Fatty acid oxidation disorders	Hypoketotic hypoglycemia	Free fatty acids/ketones >2
		Carnitine deficiency, except for CPT I with elevated free carnitine. Dicarboxylic aciduria
		Variable elevations of lactate
		Acute illness – increased creatine kinase and uric acid
Glycogenoses I, III, and VI	Hypoglycemia	Massive hepatomegaly. Variable elevations of lactate, urate, cholesterol, triglycerides, creatine kinase, and transaminases. Hypophosphatemia
Defects of gluconeogenesis	Hypoglycemia	Hepatomegaly Elevations of lactate
Defects of ketolysis/ketone body utilization	Hyperketotic hypoglycemia	Persistently elevated free fatty acids and ketones[a]. Ketones × glucose >15 (fasting)
Mitochondrial disease	Hyperketotic hypoglycemia	Multisystem disease Elevations of lactate and ketones as well as of L/P and 3-OH-BA/acetoacetate ratios
Maple syrup urine disease (intermittent variant)	Hyperketotic hypoglycemia	Maybe ataxia Elevations of branched-chain amino acids including alloisoleucine
Congenital hyperinsulinism (nesidioblastosis)	Hypoketotic hypoglycemia	Increased insulin levels >5 mU/L at glucose <30 mg% or >8 mU/L at glucose <40 mg%. Insulin (mU/L)/glucose (mg%)/>3. Low-free fatty acids and ketones
Hypocortisolism, growth hormone deficiency, panhypopituitarism	Hypoketotic hypoglycemia, but sometimes significant ketosis in patients with Addison's disease	Cortisol <400 nmol/L Deficiency of growth and/or thyroid hormone
Catecholamine deficiency	Hypoketotic hypoglycemia	Decreased catecholamines
Cyclic vomiting (diminished glycogen and protein stores)	Hyperketotic hypoglycemia	No specific abnormalities

CPT I carnitine palmitoyl transferase I deficiency, *ketones* sum of 3-hydroxybutyrate and acetoacetate, *L/P* lactate/pyruvate, *3-OH-BA* 3-hydroxybutyrate

[a]In normally fed children, the concentration of ketone bodies in the steady state is always <0.2 mmol/L

slim and have relatively low muscle mass. Children with pathological muscular wasting, such as patients with spinal muscular atrophy, who have severely diminished glycogen and protein stores, are at especially high risk for metabolic decompensation during fasting. Similar metabolic constellations occur in milder forms of succinyl-CoA:3-oxoacid-CoA transferase deficiency. Recently, inherited deficiency of monocarboxylate transporter 1 has been described as another genetic cause of potentially lethal ketoacidosis resulting from deficient utilization of ketone bodies.

41.2.2 Preprandial/Postprandial Analyses (Protein/Glucose Challenge)

Remember

The standardized analysis of metabolic parameters in the preprandial and postprandial state may provide important functional clues for the diagnosis of metabolic disorders.

Many biochemical parameters show marked variation with food intake and should be routinely examined under preprandial conditions in order to determine baseline values. Reduced

concentrations of amino acids or other metabolites may be diagnostically relevant but are sometimes only found in preprandial samples. On the other hand, some metabolic disorders that affect substrate utilization give rise to abnormal metabolite concentrations only in the postprandial state. In order to reliably detect relevant abnormalities, it is often necessary to examine metabolic parameters before and after defined food intake. In practice, this may be a normal meal enriched with protein and carbohydrates, the exact composition of which is less important. This test is particularly useful in the diagnosis of mitochondrial disorders which typically show pathological postprandial increases of lactate, alanine, and other small amino acids. Alanine, in contrast to lactate, is not affected by cuffing or crying and when elevated is a more reliable indicator of disturbed energy metabolism. This test is also able to recognize amino acidemias and urea cycle defects but may trigger or aggravate acute neurological symptoms.

41.2.2.1 Procedure

- Preprandial samples should reflect a neutral metabolic state (not a fasting reaction) and are best obtained 5–6 h (up to 8 h in older children) after the last meal. Measure blood gases, blood sugar, amino acids, lactate, and ammonia; obtain a deproteinized blood sample (perchloric acid extraction) for pyruvate and ketone bodies in case that lactate is elevated. In urine, check for ketones (Ketostix) and measure lactate and/or organic acids and orotic acid if not previously normal.
- Give normal meal enriched with protein and sugar to attain a total amount of ≈1 g/kg b.w. each of protein and carbohydrate/sugar.
- Postprandial blood sample should be obtained 90 min after the meal; urine should be collected for 2 h. Measure the same parameters as in the preprandial samples.

41.2.2.2 Interpretation

Blood lactate should not rise by >20 % over baseline values and should not reach pathological values (>2.1 mmol/L). When lactate is elevated, measure pyruvate, acetoacetate, and 3-hydroxybutyrate in the perchloric acid extract to determine the redox ratio. Acid–base status should remain normal. Most amino acids will be elevated in the postprandial sample, but the plasma concentration of alanine should stay under 600–700 μmol/L, and the alanine/lysine ratio should stay below three.

41.2.3 Glucose Challenge

Remember

A controlled glucose challenge is useful to assess cellular respiration in patients with suspected disorders of energy metabolism in whom lactate values have been repeatedly normal.

For aerobic generation of energy, glucose is catabolized to pyruvate, transferred into the mitochondrion, and fully oxidized via the Krebs cycle and the respiratory chain. High lactate (the reduced form of pyruvate) is the most valuable diagnostic marker of disturbed mitochondrial energy metabolism in respiratory chain defects and other disorders that affect cellular respiration. However, frequently lactate is elevated only after intake of glucose or glucogenic amino acids; single normal lactate values do not exclude a primary mitochondriopathy (see Chap. 14). A controlled glucose challenge is useful to assess cellular respiration in patients with suspected disorders of energy metabolism in whom lactate values have been repeatedly normal. It is relatively inexpensive as lactate can be measured in all general and pediatric hospitals, but it requires frequent venipunctures, and lactate concentrations may be affected by cuffing or crying of the child. The measurement of pyruvate is not necessary when lactate is normal, but deproteinized blood samples (perchloric acid extraction) should be obtained to determine the redox ration (lactate/pyruvate) when lactate is high. A glucose challenge should not be carried out when lactate has been consistently elevated or when a significant postprandial increase of lactate has already been demonstrated as it may cause acute metabolic decompensation.

In such cases, appropriate enzyme studies (muscle biopsy) and possible molecular analyses should be undertaken immediately upon completion of the basic biochemical analyses. Other indications include unclear hypoglycemic episodes and suspected glycogen storage disease (see Chaps. 14 and 15).

41.2.3.1 Preparations

- Basic investigations including amino acids and organic acids should have been completed; blood lactate should have been normal in repeated measurements before and after meals at different times of the day.
- Glucose challenge should be carried out after overnight fasting in the morning and in younger infants at least 4–5 h after the last meal.
- Secure intravenous access.

41.2.3.2 Procedure

- Measure baseline blood lactate, blood sugar, and acid–base status; obtain 10-mL urine for lactate and/or organic acids before the test.
- Give glucose 2 g/kg (max. 50 g) as 10 % oral solution. The solution may be administered through a nasogastric tube (flush with water) in small children; for administration in older children, the solution may be stored in the refrigerator as it is more pleasant to drink when cool.
- Measure blood lactate, blood sugar, and acid–base status 15, 30, 45, 60, 90, 120, and 180 min after the test; collect urine for 2 h for lactate and/or organic acids. Obtain deproteinized blood samples (perchloric acid extraction) for pyruvate and ketone bodies in case lactate is elevated.

41.2.3.3 Interpretation

Serum glucose should be elevated after the test, but lactate should not rise by >20 % over baseline values and should not reach pathological values (>2.2 mmol/L). Acid–base status and urine measurements should remain normal.

41.2.4 Glucagon Stimulation

Glucagon is a counterregulatory hormone whose secretion is normally stimulated by hypoglycemia. It acts to stimulate hepatic glycogenolysis. It does this by stimulating phosphorylase, but *glucose-6-phosphatase* activity is required for the release of free glucose into the blood. Thus, the glucagon stimulation test is an excellent provocative test for glycogen storage disorder type I in which the hepatic activity of this enzyme is in most patients absent or nearly absent (see also Chap. 14).

The test is usually done following a fast for at least 6 h. An overnight fast is usually employed in normal individuals. The duration of fasting in an infant with glycogenosis I depends on *tolerance* and may have to be 4 h or less. The dose of glucagon we generally employ is 0.5 mg intramuscular. Doses of 0.03–0.1 mg/kg have been used up to a maximum of 1 mg. We measure blood concentrations of glucose at time 0- and 15-min intervals after injection for 60 min, at 90 min, and at 120 min. Lactate and alanine may also be measured in each sample in a patient of sufficient size. In a young infant, these determinations could be done on every other sample.

In control individuals, glucagon administration is followed by a prompt glycemic response, and the concentrations of lactate and alanine do not increase. In a patient with glycogenosis I, the curve for glucose may be flat or there may be a decline. In some patients, there may be a small elevation of serum glucose, because 6–8 % of the glucose residues of glycogen are released as free glucose by the debranching enzyme. However, the increase is usually not prompt and does not exceed 50 % of the fasting level within 30 min. In glycogenosis type I, levels of lactate and alanine increase after glucagon. The level of lactate rises rapidly and may go very high, even over 15 mM.

In *glycogenosis type III* or debrancher deficiency, a presumptive diagnosis can be made by determining the response to glucagon in the fed and fasted state. Administration of glucagon after a 12–14-h fast is followed by little or no increase in blood glucose. The patient is then fed and the glucagon test repeated 2–6 h later at which time the glycemic response is normal. These patients do not have lactic acidemia, and concentrations of lactate do not increase following glucagon. Their concentrations of alanine are low.

In patients with *glycogen synthase deficiency*, glucagon administration in the fasting state is followed by no elevation of glucose, lactate, or alanine. In the fed state, glucagon is followed by a glycemic response. These patients can be distinguished from those with glycogenosis III by the elevation of lactate that occurs with a glucose tolerance test.

41.3 Tests of Protein Metabolism

41.3.1 Phenylalanine Loading Test

The hydroxylation reactions of phenylalanine, tyrosine, and tryptophan require tetrahydrobiopterin (BH_4) as a cofactor. A deficiency in the biosynthesis or recycling of BH_4 may cause reduced synthesis of monoamine neurotransmitters. Frequently, this is not noticeable in plasma amino acid concentrations but can be demonstrated in the kinetics of phenylalanine hydroxylation after oral challenge. The phenylalanine challenge test may be useful for the identification of disorders of pterin metabolism in patients with unclear dystonic movement disorders presenting without hyperphenylalaninemia, in particular when Segawa syndrome or sepiapterin reductase deficiency is suspected (Hyland et al. 1997; Opladen et al. 2010, 2013).

Remember

The phenylalanine challenge has little use in the diagnostic work-up of patients with phenylketonuria (PKU).

41.3.1.1 Procedure

The phenylalanine challenge should be carried out at least 1 h after a light breakfast (minimal protein). No food is permitted until the end of the test. Obtain two separate samples of 1 mL EDTA blood for the determination of basal values of phenylalanine, tyrosine, and plasma pterins. For pterin analysis, it is important to immediately centrifuge the samples and freeze the plasma in two portions. Plasma should be stored at −70 °C or sent on dry ice to the metabolic laboratory. Alternatively, all metabolites can be determined from dried blood spots (DBSs).

- Give 100-mg/kg L-phenylalanine in orange juice, if necessary through a nasogastric tube. Do not use drinks containing protein or aspartame to mix. Phenylalanine does not dissolve well; therefore, stir the mix just before drinking, and rinse the residual phenylalanine with additional juice.
- Obtain blood samples or DBS 1, 2, 4, and 6 h after phenylalanine ingestion; again centrifuge and freeze plasma immediately in two portions for the analysis of phenylalanine/tyrosine and pterins.

41.3.1.2 Interpretation

Plasma phenylalanine levels should rise sharply after ingestion with a maximum around 60 min and then decline continuously through conversion of phenylalanine into tyrosine.

Plasma concentrations of phenylalanine should not exceed tyrosine more than fivefold after loading. A protracted rise and slow decrease of phenylalanine together with a delayed rise of tyrosine indicate a reduced hydroxylation capacity, which may be caused either by the presence of a (heterozygous) mutation in the phenylalanine hydroxylase (PAH) gene or by reduced availability of BH_4. Further differentiation is possible by examination of pterins in the blood, or mutation analysis of the *PAH* gene. Biopterin concentrations should rise under phenylalanine loading severalfold above baseline (to >18 nmol/L), also in heterozygotes of PAH deficiency. In partial BH_4 deficiency, conversion of Phe to Tyr is compromised. In addition, the physiological stimulation of BH_4 biosynthesis via GTPCH feedback regulatory protein (GFRP) by Phe is absent, and biopterin concentrations remain at a low level under Phe loading.

In childhood age-related normal ranges need to be applied (Opladen et al. 2010), and only the combined analysis of the Phe/Tyr ratio and biopterin concentration is reliable in children. In a pediatric population, the most precise identification of dopa-responsive dystonia patients is achieved by the use of the Phe/Tyr ratio after 2 h and biopterin concentration in dried blood 1 h after Phe challenge. False results may be found in a PKU heterozygotes (see above) and in patients during substitution with BH_4. Thus, the

test should not be performed during substitution with BH_4 (Opladen et al. 2013).

Modified Phenylalanine Challenge This time BH_4 (20 mg/kg b.w.) is administered 1 h before or alternatively 3 h after phenylalanine (100 mg/kg b.w.). In case of reduced availability of BH_4, administration of BH_4 prior to phenylalanine results in a complete normalization of the test. If BH_4 is given after 3 h into the test, an immediate decrease of phenylalanine occurs together with a rise of tyrosine leading to normalization of metabolite levels (see later). In the latter setting, plasma amino acids are determined after another 1, 3, and 5 h, i.e., amino acids are determined prior to and 1, 2, 4, 6, and 8 h after phenylalanine loading.

41.3.2 BH_4 Test

Newborn screening in most Western countries includes the diagnosis of PKU, the severe deficiency of the hepatic enzyme PAH, which untreated causes highly elevated phenylalanine (Phe) concentrations in blood (hyperphenylalaninemia) and mental retardation. More than 900 variants or mutations in the *PAH* gene with variable effect on enzyme function have been identified, and depending on dietary Phe tolerance, severe, moderate, and mild forms of PKU have been distinguished. The attenuated form of PAH deficiency that does not require treatment is denoted "mild hyperphenylalaninemia" (MHP). PAH requires BH_4 as a cofactor, and hyperphenylalaninemia is occasionally due to a primary defect of BH_4 metabolism. Oral administration of BH_4 does not significantly reduce plasma phenylalanine concentrations in newborns with classical PKU but may rapidly normalize phenylalanine in patients with a primary defect of pterin synthesis or recycling. Also in many patients with mild or moderate PKU, oral administration of BH_4 enhances PAH activity and thus reduces plasma Phe concentrations. The BH_4 test is intended to identify individuals with either of these conditions, i.e., primary BH_4 deficiency or BH_4-sensitive PKU (Blau et al. 2011).

As responsiveness to BH_4 may be of immediate therapeutic relevance, a BH_4 test should be carried out prior to the introduction of a Phe-reduced diet in all the neonates with plasma phenylalanine concentration above 400 µmol/L (6.5 mg/dL). For the reliable exclusion of cofactor deficiency, it is also necessary to determine pterin concentrations in urine or dried blood spot (DBS) and to measure dihydropteridine reductase (DHPR) activity in DBS, which is usually sent together with the samples of the BH_4 test to the metabolic laboratory. The BH_4 test is not reliably performed when phenylalanine levels are <400 µmol/L as the therapeutic effect in cofactor deficiency is more difficult to recognize, and most MHP patients show BH_4 sensitivity, which, anyway, is not of therapeutic relevance due to the harmless nature of the condition.

Remember

A BH_4 test in conjunction with Phe loading is not recommended since interpretation is unreliable unless results are compared with the results of Phe loading without BH_4 administration.

41.3.2.1 Procedure

- Collect 5–10-mL urine for pterin analysis, protect against light (urine may need to be collected in a dark bag), and freeze. Obtain 2-mL EDTA full blood, centrifuge, and freeze plasma for amino acid analysis. Collect a few drops of blood on a filter paper card (Guthrie card) for the analysis of Phe and tyrosine (Tyr) as well as DHPR activity. Plasma and urine samples should be sent on dry ice to the laboratory. Alternatively, urine may be oxidized with MnO_2 and sent at ambient temperature by Express Mail (for this purpose, acidify 5-mL urine with 6 M HCl up to a pH of 1.0–1.5, add 100-mg MnO_2, shake for 5 min at ambient temperature, centrifuge for 5 min at 4,000 rpm, and mail supernatant protected against light in aluminum foil or in a dark container).
- Give 20-mg/kg BH_4 dissolved in water or orange juice with a normal meal, if necessary

through a nasogastric tube. Beware of the photosensitivity of BH_4.
- Obtain dried blood spots or plasma samples 4, 8, 12 and 24 h after BH_4 administration for Phe and Tyr analysis. Collect urine 4–8 h after administration of BH_4 for pterin analysis (samples should be prepared in the same way as the baseline samples).
- This procedure should be repeated for another 24 h with exactly the same protocol setup for a 48-h BH_4 challenge.
- Protein intake should be constant during the entire test.
- Note that for pterins analysis, DBS can be collected instead of urine sample (before and 4–8 h after BH_4 administration). The same DBS sample can be used for both DHPR activity and pterins. DBS can be sent at room temperature

The infant or older patient may be fed or may eat regularly throughout the test.

41.3.2.2 Interpretation

The BH_4 test is regarded as positive if Phe concentrations fall by at least 30%; this should usually correspond with a transient rise of Tyr concentrations. In primary BH_4 deficiency, Phe concentrations rapidly normalize within the first few hours after BH_4 administration. In GTPCH-, PCD-, and PTPS-deficient patients, blood Phe normalizes (<120 μmol/L) within 4–8 h after the BH_4 challenge, while patients with DHPR deficiency only show a moderate Phe reduction (51% of initial blood levels) during the same time period (Opladen et al. 2012). The differential diagnosis should be clarified through pterin analysis and investigation of DHPR enzyme activity (in urine or DBS):

- GTP cyclohydrolase I deficiency: both neopterin and biopterin absent (or very low, might be normal in DBS!).
- 6-Pyruvoyl-tetrahydropterin synthase deficiency: elevated neopterin but absent (or very low) biopterin.
- Pterin-4a-carbinolamine dehydratase deficiency: elevated concentrations of neopterin and primapterin and subnormal biopterin (not in DBS!).
- DHPR deficiency: elevated biopterin, normal or mild elevation of neopterin, low DHPR activity. In some patients urinary or DBS pterins may be completely normal.

Patients with (severe) PKU also show increased urinary excretion of biopterin and neopterin, with neopterin usually more markedly elevated than biopterin. A positive BH_4 test in PKU patients may indicate BH_4 sensitivity.

41.3.3 Vitamin B_{12} Test

Methylmalonic acidurias comprise a heterogeneous group of diseases with accumulation of methylmalonic acid (MMA) in urine and other body fluids as the common denominator. They are caused by a defect of the mitochondrial enzyme methylmalonyl-CoA mutase (MCM, EC 5.4.99.2) or by one of the many defects in the uptake, transport, or synthesis of 5-deoxyadenosylcobalamin, the cofactor of MCM, the active metabolite of vitamin B_{12}. Primary deficiencies of MCM are further subdivided into defects without residual activity (mut^0) and defects with residual activity (mut^-), caused by mutations in the apomutase locus or in genes coding for the biosynthesis of cobalamin: *cblA*, *cblB*, *cblC*, *cblD*, *cblD* (variant 1 and 2), *cblE*, *cblF*, *cblG*, *and cblJ*. MCM situated in the mitochondrion is part of the catabolic pathways of the amino acids isoleucine, valine, methionine, and threonine as well as of odd chain fatty acids, propionic acid coming from gut bacteria, and cholesterol linking the degradation of these metabolites to the Krebs cycle.

Some defects of 5-deoxyadenosylcobalamin metabolism respond to pharmacological doses of vitamin B_{12}. While *cblC* and *cblD* defects are routinely treated by vitamin B_{12}, only some patients with cblA and *cblB* or even with mut^- disease will respond. Although response to vitamin B_{12} has been identified in many studies as an important prognostic factor and hallmark of therapy, there is a large variation of practice at least in European centers. Therefore, Fowler et al. (2008) proposed the following protocol to test the response to vitamin B_{12} in a standardized way.

41.3.3.1 Procedure

- The patient should be clinically stable on the same treatment for at least 1 month. The intakes of protein and energy should be specified and recorded.
- If the patient is already receiving cobalamin, this should be stopped for at least 1 month before the test. If the patient appears to deteriorate, restart vitamin B_{12} and defer the test. Note a general rule that patients with MMA excretion >10,000 mmol/mol creatinine and those who are clinically unstable rarely respond to vitamin B_{12}.
- Baseline urine collections: at least three specimens should be collected on different days. Plasma concentrations may only be used if a sensitive assay (stable-isotope dilution assay) is available.
- Give hydroxocobalamin 1 mg intramuscularly on three consecutive days.
- After the cobalamin injection, collect urine (and/or plasma) specimens on alternate days for 10 days.
- The urine or plasma samples should be analyzed in the same run in a laboratory participating in a recognized quality control scheme for MMA using gas chromatography–mass spectrometry, in Europe ERNDIM EQA (http://www.erndim.unibas.ch).

41.3.3.2 Interpretation

A decrease of the mean urine and plasma MMA concentrations of >50 % should be regarded as indicative of a beneficial response.

In parallel to this test, the enzymology including in vitro response to adenosylcobalamin and if possible the complementation group should be determined.

41.3.4 Allopurinol Test

Various genetic disorders especially urea cycle defects show increased urinary excretion of the pyrimidine uridine or its precursor orotic acid. In the case of urea cycle defects, this is thought to be caused by mitochondrial accumulation of carbamoyl phosphate, which is transferred into the cytosol and channeled into pyrimidine biosynthesis, thus bypassing the rate-limiting first step in that pathway catalyzed by the cytosolic enzyme carbamoyl phosphate synthase II (CPS II) (see Fig. 17.1). CPS II is different from the mitochondrial isoenzyme CPS I, which is required for the detoxification of ammonia. Orotic aciduria is a diagnostic feature particularly in ornithine transcarbamylase (OTC) deficiency which is helpful for the differential diagnosis of hyperammonemic diseases with high or increased plasma glutamine and low or decreased citrulline concentrations. Nevertheless, orotic acid may be normal in the interval in mild forms of OTC deficiency and particularly in heterozygous carrier women. In these cases, it may be possible to demonstrate an increased throughput in pyrimidine biosynthesis through an excessive rise of orotic acid and its metabolite orotidine after allopurinol-mediated blockage of uridine monophosphate synthase, the enzyme that converts orotic acid to uridine monophosphate (Arranz et al. 1999). The allopurinol test may be used for the diagnosis of heterozygous or mild OTC deficiency in cases of unclear transient or intermittent hyperammonemia, unclear comatose or encephalopathic episodes in both sexes, or stepwise regressing neurodegenerative disease in girls or women, especially those who show epilepsy and ataxia as prominent symptoms. Although the allopurinol test is less sensitive and specific than initially reported (Grunewald et al. 2004), it is still helpful particularly for OTC carrier identification if mutational analysis and enzyme assays are unavailable or negative.

41.3.4.1 Procedure and Interpretation

Avoid caffeine (decaffeinated coffee is acceptable), tea, coffee, cocoa, chocolate, chocolate biscuits, any cola drink, or benzoate-containing beverages 24 h before the test. Otherwise, there is no need for a special diet prior or through the test. Women should be 7–12 days after their last menstrual period if possible. The test is usually started in the morning.

- Collect 10-mL urine for baseline measurement of orotic acid and orotidine, which should be done by high-pressure liquid chromatography and not by a colorimetric method.

- Give allopurinol orally in a dose of 100 mg for preschool children, 200 mg for children between 6 and 10 years of age, or 300 mg for older children and adults.
- Collect urine over 24 h in four 6-h fractions (0–6 h, 7–12 h, 13–18 h, and 19–24 h); send 10 mL of each fraction. The samples should be sent together frozen or, after conservation with three drops of chloroform, at ambient temperature by Express Mail. It is important to label sample tubes accurately and to inform the laboratory of all medication taken during the test as well as on the preceding days.

Remember

Both false-positive and false-negative results of the allopurinol test have been reported. Excessive rise of orotic acid and/or orotidine indicates increased throughput in pyrimidine synthesis as typically caused by mild (heterozygous) OTC deficiency. Positive tests can also be found in other genetic disorders including Rett syndrome, amino acid transport defects, creatine synthesis disorders, and mitochondrial disorders. A negative allopurinol test (or normal orotic acid after protein challenge) does not fully exclude heterozygous OTC deficiency as mosaicism in the liver (lyonization) may be skewed in favor of normal hepatocytes to a degree that renders the detection of metabolic effects impossible. *OTC* gene mutation analysis should be considered if OTC deficiency remains a possibility.

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Diagnostic Workup of Patients with Mitochondrial Diseases

42

Shamima Rahman and Nicole I. Wolf

Key Facts

- Mitochondrial diseases, also known as respiratory chain disorders, disorders of energy metabolism or mitochondriopathies, are genetic conditions that cause direct or indirect impairment of the oxidative phosphorylation (OXPHOS) system.
- Mitochondrial diseases can involve any organ at any age; most commonly affected are the muscle, brain, retina, extra-ocular muscles, heart, liver, kidney, pancreas, gut and bone marrow.
- The biochemical hallmarks of mitochondrial diseases are lactate elevations in blood, CSF or urine or frank lactic acidosis.
- Diagnosis of mitochondrial diseases requires extensive multidisciplinary investigations which may include examination of blood, urine and CSF as well as tissue biopsies. Genome-wide genetic analyses may become primary diagnostic tests for many patients in the future.

42.1 Background

Within a cell, mitochondria and the mitochondrial respiratory chain are at the centre of all energy-related processes. Over the last 20 years, the term mitochondrial disease has come to be understood to describe a heterogeneous group of diseases with the common underlying pathogenic feature of impairment of the oxidative phosphorylation (OXPHOS) system, either directly or indirectly. As a group mitochondrial disorders are probably the most common metabolic disorders, affecting ~1: 5,000 live births. These disorders can involve any tissue at any age with any degree of severity. Table 42.1 lists some of the most important symptoms associated with mitochondrial dysfunction.

The OXPHOS system is a series of five multimeric enzyme complexes that are embedded in the inner mitochondrial membrane, and whose collective function is to synthesise the majority of cellular ATP from ADP and inorganic

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Table 42.1 Important symptoms and findings in respiratory chain deficiency

Organ or tissue	Signs and symptoms
Brain and nervous system	Seizures, stroke-like episodes, myoclonus, ataxia, dystonia, developmental delay +/− regression, parkinsonism, migraine, dementia, leukoencephalopathy, peripheral neuropathy
Eye	Optic atrophy, pigmentary retinopathy, cataracts
Extraocular muscles	Ophthalmoplegia, ptosis
Ear	Sensorineural hearing loss
Skeletal muscle	Myopathy, exercise intolerance, rhabdomyolysis
Bone marrow	Pancytopaenia or failure of specific cell lineages (sideroblastic anaemia, neutropaenia, thrombocytopaenia)
Heart	Cardiomyopathy (most commonly hypertrophic), conduction defects
Liver	Hepatic dysfunction, liver failure, cirrhosis, bile stasis
Intestine	Pseudo-obstruction, enteropathy, diarrhoea, exocrine pancreatic insufficiency
Testes, ovaries	Gonadal failure
Kidney	Tubulopathy, Fanconi syndrome, steroid-resistant nephrotic syndrome
Pancreas	Diabetes mellitus, exocrine failure, pancreatitis
Endocrine organs (thyroid, parathyroid, adrenal, pituitary, pancreatic β cells)	Failure of hormone secretion
Skin/hair	Hypertrichosis
Growth	Faltering growth

phosphate (Fig. 42.1). The complexes are generally known by Roman numerals, and their specific functions are as follows: complex I or NADH/ubiquinone oxidoreductase accepts electrons from the Krebs cycle and transfers them to coenzyme Q_{10}, whilst simultaneously pumping protons across the inner mitochondrial membrane into the intermembrane space to generate an electrochemical gradient across the inner mitochondrial membrane. Complex II or succinate/ubiquinone oxidoreductase accepts electrons from the Krebs cycle and also passes them on to coenzyme Q_{10}. Coenzyme Q_{10} also links mitochondrial fatty acid β-oxidation to the respiratory chain by accepting electrons from the electron transfer flavoprotein dehydrogenase. Electrons are transferred from coenzyme Q_{10} to complex III (ubiquinone/cytochrome *c* oxidoreductase) which passes them on to another mobile electron carrier in the inner mitochondrial membrane, cytochrome *c*, whilst pumping protons across the inner mitochondrial membrane. Cytochrome *c* passes electrons to complex IV (cytochrome *c* oxidase), the final proton pump in the respiratory chain, which then transfers the electrons to molecular oxygen to form water. Complex V (ATP synthase) then harnesses the energy in the electrochemical gradient to synthesise ATP from ADP and inorganic phosphate.

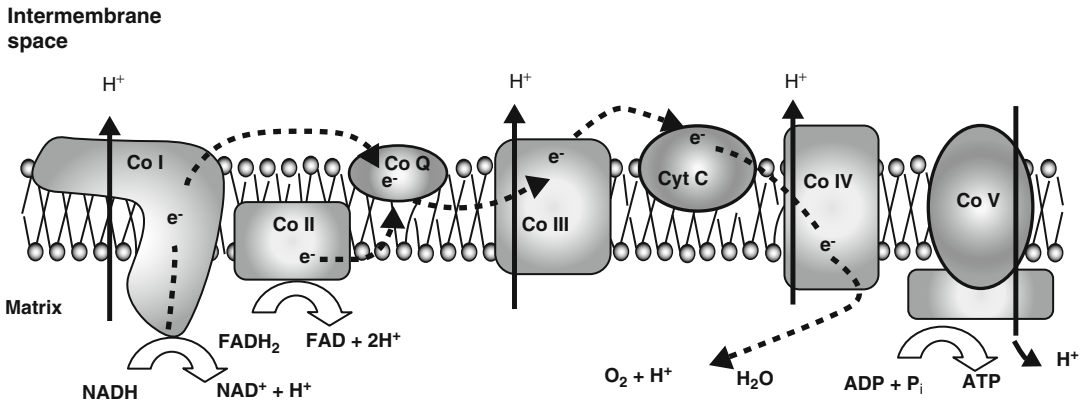
Secondary respiratory chain dysfunction is common in many disease processes, including disorders affecting intermediate metabolism, e.g. organic acidurias or fatty acid oxidation defects, and also many other inherited diseases not primarily affecting metabolic pathways. These will not be discussed further in this chapter.

42.2 Mitochondrial Genetics

Primary respiratory chain defects are disorders that directly involve OXPHOS and the electron transfer chain. Inheritance can be maternal or Mendelian, and a myriad of genes is involved, so that making a specific genetic diagnosis of a mitochondrial disorder remains challenging.

Mitochondria are unique amongst subcellular organelles in containing their own genetic material: the mitochondrial DNA (mtDNA), a circular structure of 16,569 bp coding for 13 peptide components of the respiratory chain and 2 ribosomal RNAs (rRNAs) and 22 transfer RNAs (tRNAs) needed for protein synthesis within the mitochondria. MtDNA is maternally inherited; the result of maternal inheritance is that familial mitochondrial disorders typically affect all the children of affected women, but not children of affected men.

Despite the importance of mtDNA, most subunits of the five OXPHOS complexes are



Complex I:	Complex II:	Complex III:	Complex IV:	Complex V:
7 subunits mtDNA		1 subunit mtDNA	3 subunits mtDNA	2 subunits mtDNA
37 subunits nDNA	4 subunits nDNA	10 subunits nDNA	11 subunits nDNA	14 subunits nDNA

Fig. 42.1 Mitochondrial respiratory chain. Complex I (NADH/ubiquinone oxidoreductase), complex II (succinate/ubiquinone oxidoreductase), complex III (ubiquinol/cytochrome *c* oxidoreductase), complex IV (cytochrome c oxidase), complex V (ATP synthase)

encoded by nuclear genes. The same is true for all transport proteins, proteins for mtDNA synthesis and maintenance, proteins required for mitochondrial transcription and translation and assembly factors of the five complexes (Fig. 42.1). This means that at least 1,000 nuclear genes are involved in mitochondrial biogenesis, maintenance and functioning. It is therefore not surprising that most mitochondrial disease is caused by mutations in nuclear DNA and follows Mendelian inheritance patterns of inheritance (dominant, recessive, or X-linked), as detailed in Table 42.2.

42.3 Clinical Recognition of Mitochondrial Disease

Because mitochondria are present in all cells except mature red cells, mitochondrial dysfunction can theoretically result in abnormal function of any organ or system of the body in any combination, leading to a multitude of clinical presentations. Some clinical syndromes with characteristic constellations of symptoms are recognised, and these are summarised in Table 42.3. However, most paediatric patients do not have classical syndromes, and the most common symptoms and signs of mitochondrial disease are highlighted in Table 42.1. Tissues with highest energetic requirements are generally preferentially affected, leading to neurological (encephalopathy, seizures, developmental regression, stroke-like episodes), cardiac (cardiomyopathy, conduction defects), renal (tubulopathy) and liver (acute liver failure) disease. An otherwise unexplained combination of symptoms in different organ systems is the strongest indicator of a mitochondrial disease.

Leigh syndrome is one of the most severe and frequent manifestations of a mitochondrial disorder in infancy and childhood and illustrates the difficulties in the diagnosis of mitochondrial disorders. Affected patients present with developmental delay or a neurodegenerative course including extrapyramidal movement disorder, ataxia, strabismus and swallowing difficulties. MRI reveals symmetric hyperintensities of basal ganglia, mesencephalon and brain stem (Fig. 42.2). Additional symptoms such as cardiomyopathy or renal tubulopathy may occur and may help to pinpoint the genetic

Table 42.2 Examples of nuclear genes involved in mitochondrial disorders

Biochemical defects	Nuclear genes involved	Gene function	Additional abnormalities
Deficiency of single enzymes			
OXPHOS subunit mutations			
Complex I deficiency	*NDUFS1*, *NDUFS2*, *NDUFS3*, *NDUFS4*, *NDUFS6*, *NDUFS7*, *NDUFS8*, *NDUFV1*, *NDUFV2*, *NDUFA1*, *NDUFA2*, *NDUFA9*, *NDUFA10*, *NDUFA11*, *NDUFA12*, *NDUFB3*, *NDUFB9*	Structural subunits of complex I	
Complex II deficiency	*SDHA*, *SDHB*, *SDHC*, *SDHD*	Structural subunits of complex II	Succinate elevated in cerebral ^{1}H-MRS
Complex III deficiency	*CYC1*, *UQCRB*, *UQCRC2*, *UQCRQ*	Structural subunits of complex III	
Complex IV deficiency	*COX4I2*, *COX6A1*, *COX6B1*, *COX7B*, *NDUFA4*	Structural subunits of complex IV	
Complex V deficiency	*ATP5A1*, *ATP5E*	Structural subunits of complex V	
OXPHOS assembly factor mutations			
Complex I deficiency	*NDUFAF1*, *NDUFAF2*, *NDUFAF3*, *NDUFAF4*, *NDUFAF5*, *NDUFAF6*, *ACAD9*, *FOXRED1*, *NUBPL*	Assembly factors of complex I	Typical neuroimaging in NDUFAF2 and NUPBL deficiencies
Complex II deficiency	*SDHAF1*, *SDHAF2*	Assembly factors of complex II	Succinate elevated in cerebral ^{1}H-MRS
Complex III deficiency	*BCS1L*, *HCCS*, *LYRM7*, *TTC19*, *UQCC2*	Assembly factor of complex III	
Complex IV deficiency	*SURF1*, *COA5 (C2orf64)*, *COX14 (C12orf62)*, *COX20 (FAM36A)*, *FASTKD2*, *PET100*, *CEP89*	Assembly factors of complex IV	
	SCO1, *SCO2*, *COA6 (C1orf31)*	Copper incorporation	
	COX10, *COX15*	Haem synthesis	
	LRPPRC	Complex IV mRNA stabilisation	
	TACO1	Translational co-activator of complex IV subunit I	
Complex V deficiency	*ATP12*, *TMEM70*	Assembly factors of complex V	
Deficiency of multiple enzymesa			

Table 42.2 (continued)

Biochemical defects	Nuclear genes involved	Gene function	Additional abnormalities
Defects of mtDNA maintenance			
Impaired intergenomic communication (activity of respiratory chain enzymes may be normal; depletion or multiple deletions of mtDNA common)	*POLG*, *POLG2*, *C10orf2*, *MGME1*, *DNA2*	mtDNA replication	
	SLC25A4	ADP/ATP translocator	
	TK2	Nucleotide salvage	CK strongly elevated
	DGUOK	Nucleotide salvage	Elevated plasma tyrosine
	SUCLA2, *SUCLG1*	Succinate-coenzyme A ligase	Elevated excretion of methylmalonic acid
	TYMP	Thymidine phosphorylase	Elevation of thymidine and deoxyuridine in plasma and urine
	RRM2B	p53-controlled ribonucleotide reductase subunit	
	MPV17, *FBXL4*, *ABAT*	Mitochondrial proteins with unknown function in mtDNA maintenance	
Defects of mitochondrial gene expression			
Mitochondrial ribosomal defects	*MRPS7*, *MRPS16*, *MRPS22*, *MRPL3*, *MRPL12*, *MRPL44*	Mitochondrial ribosomal proteins	
Impaired mitochondrial RNA modification	*PUS1*	Pseudouridine synthase 1	Sideroblastic anaemia
	ELAC2, *MTO1*, *TRMU*, *GTPBP3*, *TRIT1*, *TRNT1* *MTFMT*, *MTPAP*, *PNPT1*, *HSD17B10*	Enzymes required for mitochondrial RNA metabolism	
Impaired mitochondrial tRNA aminoacylation	*DARS2*	Mitochondrial aspartyl-tRNA synthetase	Lactate elevated in ^{1}H-MRS, typical neuroimaging (LBSL)
	EARS2	Mitochondrial glutamyl-tRNA synthetase	Lactate elevated in ^{1}H-MRS, typical neuroimaging (LTBL)
	RARS2	Mitochondrial arginyl-tRNA synthetase	Pontocerebellar hypoplasia type 6
	SARS2	Mitochondrial seryl-tRNA synthetase	Hyperuricaemia
	YARS2	Mitochondrial tyrosyl-tRNA synthetase	Sideroblastic anaemia
	AARS2, *CARS2*, *FARS2*, *HARS2*, *IARS2*, *LARS2*, *MARS2*, *NARS2*, *PARS2*, *TARS2*, *VARS2 GARS*, *KARS*	Other mitochondrial and cytosolic aminoacyl tRNA synthetases	
Impaired translation elongation	*GFM1*, *TSFM*, *TUFM*	Mitochondrial translation elongation	
Other mitochondrial translation factors	*C12orf65*, *RMND1*	Other proteins required for mitochondrial gene expression	

(continued)

Table 42.2 (continued)

Biochemical defects	Nuclear genes involved	Gene function	Additional abnormalities
Defects of coenzyme Q_{10} biosynthesis			
Impairment of coenzyme Q_{10} biosynthesis (decreased activity of complex I + III and II + III)	*COQ2*, *COQ4*, *COQ6*, *COQ9*, *PDSS1*, *PDSS2*, *ADCK3*, *ADCK4*	Coenzyme Q_{10} biosynthesis	Decreased ubiquinone content in muscle
Defects of lipoic acid and/or iron sulphur cluster biosynthesis			
Lipoic acid biosynthesis	*LIAS*	Lipoic acid biosynthesis	Elevated glycine concentration in plasma and urine
	LIPT1	Required for lipoylation and activation of 2-ketoacid dehydrogenases	
Iron sulphur cluster biosynthesis	*BOLA3*, *GLRX5*, *NFU1*	Iron sulphur cluster biosynthesis	Hyperglycinaemia
	FDX1L, *FXN*, *IBA57*, *ISCU*, *LYRM4*, *ABCB7*, *NFS1*	Iron sulphur metabolism	
Toxic damage to respiratory chain enzymes			
	ETHE1	Sulphur detoxification	Ethylmalonic aciduria
	HIBCH, *ECHS1*	Valine degradation	Elevated 4-hydroxybutyryl carnitine, characteristic urinary metabolites
Normal activity of respiratory chain complexes			
Defects of mitochondrial import			
Components of the mitochondrial protein import apparatus	*TIMM8A*, *GFER*	Mitochondrial protein import	
	DNAJC19	Mitochondrial inner membrane translocase subunit TIM14	Elevated excretion of 3-methylglutaconic acid
Mitochondrial solute translocases	*SLC25A3*, *SLC25A12*, *SLC25A13 SLC25A19*, *SLC25A22*	Mitochondrial solute carriers	
	SLC25A38	Mitochondrial solute carrier required for haem biosynthesis	Sideroblastic anaemia
Defects of mitochondrial membrane lipids			
	TAZ	Tafazzin	Elevated excretion of 3-methylglutaconic acid, cardiolipin deficiency
	AGK, *SERAC1*	Enzymes involved in mitochondrial lipid biosynthesis and/or remodelling	Elevated excretion of 3-methylglutaconic acid
Defects of mitochondrial membrane dynamics			
Mitochondrial fusion proteins	*OPA1*, *MFN2*	Required for mitochondrial fusion	
Mitochondrial fission factors	*DNM1L*	Dynamin-like protein, deficient fission of mitochondria and peroxisomes	Lactic acidosis and mild elevation of VLCFA
	MFF, STAT2	Required for mitochondrial fission	

Table 42.2 (continued)

Biochemical defects	Nuclear genes involved	Gene function	Additional abnormalities
Miscellaneous defects			
	AIFM1	Apoptosis-inducing factor	
	APOPT1	Mitochondrial apoptogenic protein 1	Cavitating leukodystrophy
	CLPB	AAA ATPase (chaperonin)	Elevated excretion of 3-methylglutaconic acid
	SPG7	Paraplegin (mitochondrial AAA metalloprotease)	
	AFG3L2	AAA ATPase	
	HSPD1	Chaperonin (heat shock protein 60)	
	CLPP	Mitochondrial matrix peptidase proteolytic subunit	
	PMPCA	Mitochondrial processing peptidase alpha	
	SFXN4	Sideroflexin 4	Macrocytic anaemia
	NADK2	Mitochondrial NAD kinase 2	Hyperlysinaemia
	NNT	Nicotinamide nucleotide transhydrogenase	
	TRAP1	TNF receptor-associated protein 1	
	TMEM126A	Mitochondrial membrane protein of unknown function	

[a]Normal respiratory chain enzyme activities reported for some of these defects

Table 42.3 Recognised mitochondrial syndromes

Syndrome	Genes involved	Inheritance	Important clinical features
Alpers-Huttenlocher syndrome	*POLG*, mtDNA (various mutations), *FARS2*, other unknown nuclear genes	AR, Mt (rarely)	Neurodegenerative disease with onset in childhood: status epilepticus, epilepsia partialis continua, liver failure (may be triggered by valproic acid), gastrointestinal dysmotility
Barth syndrome	*TAZ*	XL	Cardiomyopathy, cyclical neutropaenia, myopathy, characteristic facies
Coenzyme Q_{10} biosynthesis defects	*COQ2*, *COQ4*, *COQ6*, *COQ9*, *PDSS1*, *PDSS2*, *ADCK3*, *ADCK4*	AR	Heterogeneous presentations, including encephalopathy, cerebellar ataxia, steroid-resistant nephrotic syndrome, rhabdomyolysis
Ethylmalonic encephalopathy	*ETHE1*	AR	Encephalopathy, diarrhoea, acrocyanosis, petechiae, ethylmalonic aciduria
Growth retardation, aminoaciduria, cholestasis, iron overload, lactic acidosis and early death (GRACILE)	*BCS1L*	AR	Intrauterine growth retardation, cholestasis, lactic acidosis, aminoaciduria; allelic with *Björnstad syndrome* = congenital sensorineural hearing loss, pili torti

(continued)

Table 42.3 (continued)

Syndrome	Genes involved	Inheritance	Important clinical features
Kearns-Sayre Syndrome (KSS)	Large mtDNA deletions (most common 4,977 bp) of mtDNA	Mt (usually sporadic)	Progressive external ophthalmoplegia, pigmentary retinopathy, progressive ataxia, dementia, cardiac conduction defect, deafness, elevated CSF protein and lactate, typical neuroimaging
Leber hereditary optic neuropathy (LHON)	Missense mutations in different mtDNA genes, especially complex I subunits	Mt, usually homoplasmic	Acute or subacute mid-life painless visual loss, male preponderance, additional mild neurological symptoms possible, also pre-excitation syndromes
Leigh syndrome	>75 genes, especially *MT-ATP6* (m.8993T>G/C mutations) and *SURF1*	Mt, AR, XL	Neurodegenerative disease with onset in infancy or early childhood: movement disorder, swallowing difficulties, strabismus. Variable extra-neurological features. MRI with symmetric abnormalities in basal ganglia and brainstem
Mitochondrial DNA depletion syndrome (MDDS)	Encephalomyopathic: *TK2*, *SUCLA2*, *SUCLG1*, *RRM2B*, *FBXL4*	AR	Encephalomyopathic: myopathy, seizures, developmental delay/ regression, variable other features
	Hepatocerebral: *POLG*, *DGUOK*, *MPV17*, *C10orf2*		Hepatocerebral: hepatic failure, seizures, roving eye movements, myopathy
3-Methylglutaconic aciduria, deafness, encephalopathy, and Leigh-like disease (MEGDEL)	*SERAC1*	AR	Neonatal hypoglycaemia, lactic acidosis, hyperammonaemia, hepatic dysfunction, 3-methylglutaconic aciduria; later poor feeding, faltering growth, progressive deafness, dystonia, spasticity, profound developmental delay, behavioural disturbance. MRI: bilateral basal ganglia lesions with a so-called putaminal eye
Mitochondrial encephalomyopathy, lactic acidosis and stroke-like episodes (MELAS)	Most common mutation m.3243A>G in *MT-TL1*, other mtDNA mutations, *POLG*	Mt	Stroke-like episodes, epilepsy, migraine, cognitive decline, short stature, diabetes mellitus, deafness
Myoclonic epilepsy, myopathy, sensory ataxia (MEMSA)	*POLG*	AR	Juvenile/young adult onset of ataxia, seizures (often focal, starting in right arm, with subsequent generalisation), myoclonus
Myoclonic epilepsy with ragged-red fibres (MERFF)	Most common mutation m.8344A>G in *MT-TK*, other mtDNA mutations, *POLG*	Mt	Progressive myoclonic epilepsy, ataxia, dementia, encephalopathy, myopathy, deafness, optic atrophy
Mitochondrial neurogastrointestinal encephalopathy (MNGIE)	*TYMP*	AR	Ptosis, progressive external ophthalmoplegia, gastrointestinal dysmotility with pseudo-obstruction, peripheral neuropathy and myopathy. MRI: diffuse leukoencephalopathy

Table 42.3 (continued)

Syndrome	Genes involved	Inheritance	Important clinical features
Myopathy, lactic acidosis, sideroblastic anaemia (MLASA)	*PUS1*, *YARS2*	AR	Myopathy, lactic acidosis, sideroblastic anaemia
NARP (neuropathy, ataxia and retinitis pigmentosa)	*MT-ATP6* (m.8993T>G/C, lower mutant load than in Leigh syndrome)	Mt	Neuropathy, ataxia and retinitis pigmentosa.
Pearson marrow-pancreas syndrome	Large mtDNA deletions (identical to PEO and KSS)	Mt (usually sporadic)	Transfusion-dependent sideroblastic anaemia, exocrine pancreatic insufficiency, faltering growth, enteropathy, evolves into KSS
Progressive external ophthalmoplegia (PEO)	Large mtDNA deletions; *POLG*, *C10ORF2*, *ANT1*, *POLG2*,)	Mt (also sporadic); AD; AR	Progressive external ophthalmoplegia, myopathy and additional symptoms may occur
Reversible infantile respiratory chain deficiency (RIRCD)	*MT-TE* (m.14674T>C/G), *TRMU*	Mt, AR	Severe reversible myopathy or reversible liver failure
Sengers syndrome	AGK	AR	Congenital cataracts, cardiomyopathy, lactic acidosis, 3-methylglutaconic aciduria

AD autosomal dominant, *AR* autosomal recessive, *Mt* mitochondrial, *XL* X-linked

defect. The causes of Leigh syndrome are extremely heterogeneous and include mutations in the mtDNA (especially m.8993T>G/C in *MT-ATP6* coding for one of the complex V subunits) and mutations in more than 50 different nuclear genes, including mutations of subunits and assembly factors of complexes I, II, III and IV as well as mutations in genes required for mtDNA maintenance and expression and mutations in the *PDHA1* gene encoding the E1alpha subunit of the pyruvate dehydrogenase complex. To establish a specific genetic diagnosis of Leigh syndrome, muscle biopsy is often needed in order to guide genetic investigations.

42.4 General Approach to a Patient with Suspected Mitochondrial Disorder

It is still the case that there is no gold standard blood biomarker of mitochondrial disease, and so definitive diagnosis continues to require extensive multidisciplinary investigations which may include examination of blood, urine and CSF as well as tissue biopsies, as detailed below. The consideration of mitochondrial disease proceeds along three axes – clinical symptoms, metabolic investigations and functional assays. Rapid advances in genetic diagnostic techniques will likely change this approach, and in the near future, next-generation sequencing of genes involved in mitochondrial disorders (either a panel of genes such as the 'Mito-exome' or whole exome or even whole genome sequencing) may be performed before an invasive muscle biopsy with assessment of respiratory chain function in many cases, although functional confirmation of genetic changes identified by next-generation sequencing will be essential.

In daily clinical work, there are three different clinical scenarios where mitochondrial disorders are suspected (Fig. 42.3). First, clinical symptoms are so suggestive of a specific mitochondrial disease that further investigations are warranted. Second, in the diagnostic workup of a patient with relatively nonspecific symptoms, results of either laboratory or other, e.g. neuroradiological, investigations are suggestive of a mitochondrial disorder. Third, in patients with unexplained symptoms and signs, a mitochondrial

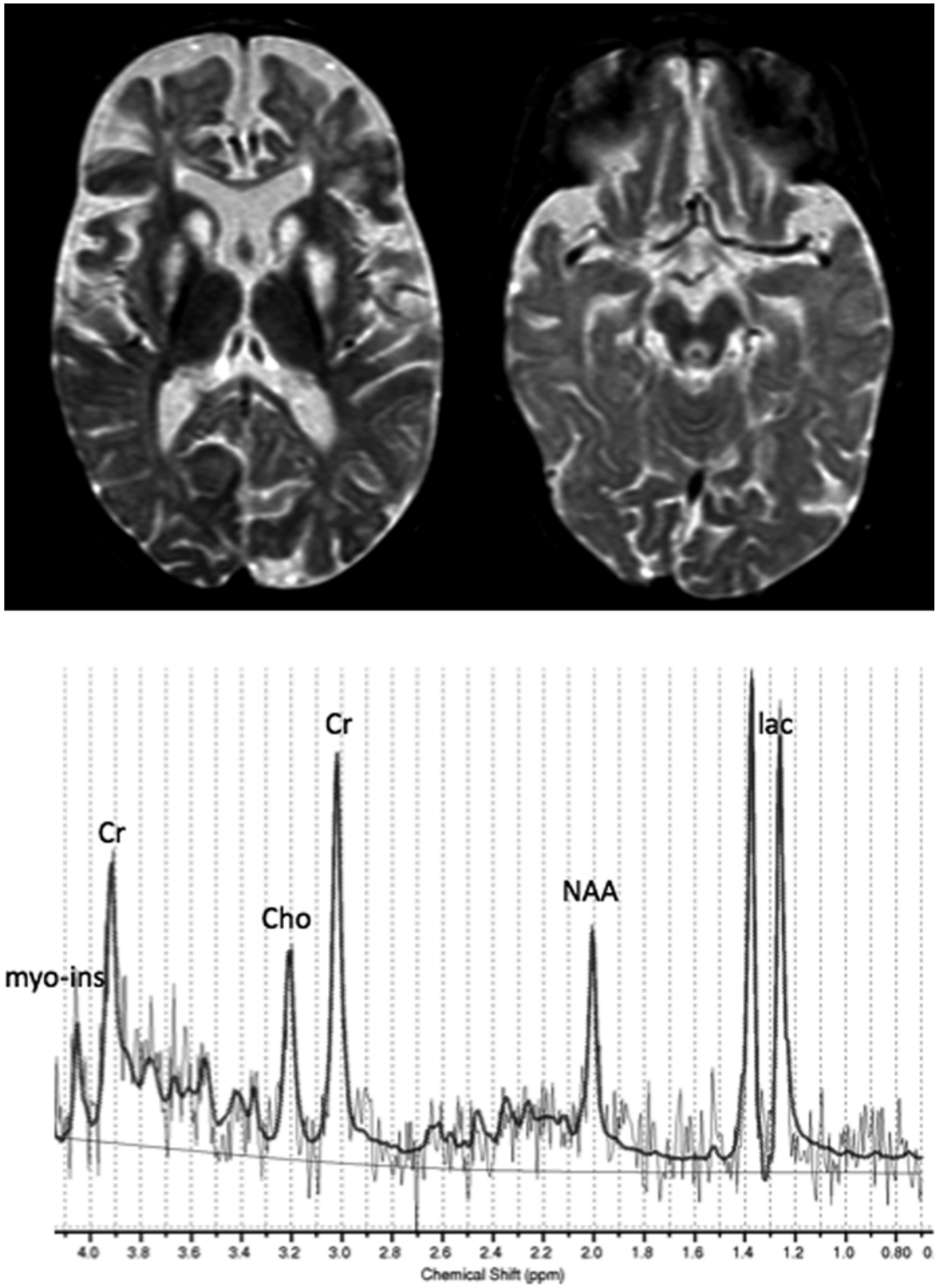


Fig. 42.2 T2w axial images of a child with Leigh syndrome. Nucleus caudatus, pallidum and the periaqueductal area in the mesencephalon show elevated signal. Additionally there is atrophy and white matter changes. ^{1}H-MRS of basal ganglia displays a strongly elevated lactate and decreased NAA (Courtesy of Dr. Inga Harting, Dept. of Neuroradiology, University Hospital Heidelberg)

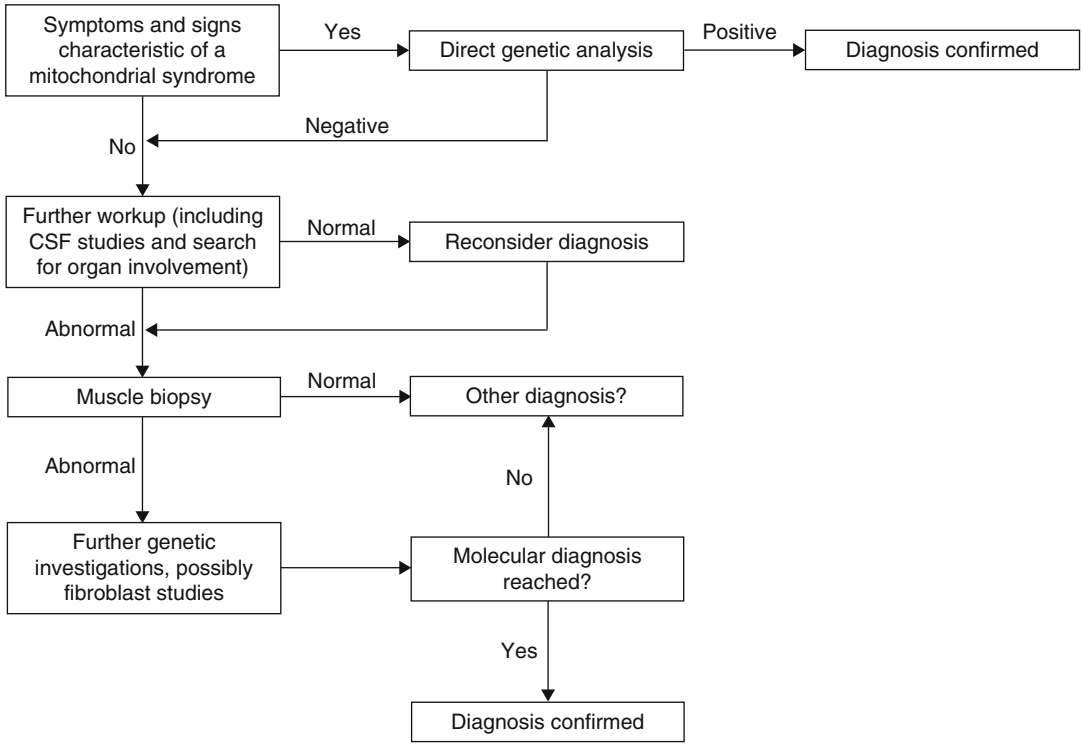


Fig. 42.3 Flowchart for the diagnostic approach in a patient with a suspected mitochondrial disorder

disorder is considered as a possible differential diagnosis, even without typical clinical or laboratory hallmarks supporting this idea. In the first scenario, it is possible to proceed directly to DNA analysis if the symptoms are typical of a certain syndrome such as MELAS or Alpers-Huttenlocher disease; if this turns out to be negative, muscle biopsy is necessary. In the second case, muscle biopsy is the first step, and, depending on the results, tailored genetic investigations would follow (e.g. in complex I deficiency sequencing of genes coding for its subunits and known assembly factors), followed by research genetic investigations such as whole exome or whole genome next-generation sequencing if the initial candidate gene screen is negative. The third scenario is the most difficult one, since it is virtually impossible to exclude a mitochondrial disorder even with the most sophisticated workup (unless another firm non-mitochondrial diagnosis can be established) and difficult to decide to what extent one should pursue invasive and expensive diagnostic procedures such as muscle biopsy.

42.5 Neuroimaging

Brain magnetic resonance imaging may reveal characteristic lesions in certain mitochondrial syndromes, for example, bilateral symmetrical lesions in the basal ganglia, variably extending into midbrain and brainstem in Leigh syndrome, and parieto-occipital lesions, which do not correspond to vascular territories, in MELAS or Alpers-Huttenlocher syndromes. The lesions in Leigh syndrome appear hyperintense in T2-weighted and FLAIR sequences and often hypointense on T1-weighted images, with appearances of swelling in the acute stage. Cavitating leukoencephalopathies are increasingly recognised in various forms of mitochondrial disease including some types of Leigh syndrome and other subgroups of complex I deficiency. Some patients may be surprisingly stable, with even an improvement of imaging findings during the disease course. Some of the mitochondrial translation defects have characteristic MRI brain ‘signatures’, e.g. leukoencephalopathy with

brain stem and spinal cord involvement and lactate elevation (LBSL) in patients with *DARS2* mutations, leukoencephalopathy with thalamus and brainstem involvement and high lactate (LTBL) in patients with *EARS2* mutations and pontocerebellar hypoplasia type 6 in patients with *RARS2* mutations. Magnetic resonance spectroscopy may be helpful in revealing a lactate peak. An elevated succinate peak is typical for succinate dehydrogenase (complex II) deficiency.

42.6 Metabolic Investigations

There are no universally abnormal blood metabolites in patients with mitochondrial disease, but several molecules have been suggested as potential blood biomarkers. These include lactate and the lactate/pyruvate ratio, but it has become clear that normal blood lactate levels do not exclude the possibility of mitochondrial disease. Furthermore lactate levels may fluctuate between being elevated and normal in the same individual at different times. For this reason it may be worth measuring the blood lactate levels on several occasions, and some clinicians advocate determination of postprandial lactate levels (see Sects. 41.2.2 and 41.2.3). Lactate/pyruvate ratios are classically low in patients with pyruvate dehydrogenase deficiency and elevated in those with respiratory chain defects, but can be normal in both groups. A final caveat is that lactate can be elevated for a multitude of other reasons, including artefactual elevation caused by squeezing and/or struggling during venepuncture, hypoxia, hypovolaemia, sepsis and other metabolic disorders (e.g. glycogen storage diseases, Krebs cycle defects, long-chain fat acid oxidation disorders and organic acidurias). Plasma amino acid analysis may be helpful, particularly if alanine is elevated; this suggests persistent lactic acidosis, since pyruvate is transaminated to alanine. Other abnormalities which may be seen in the plasma amino acid profile include high proline or low citrulline or arginine in some mitochondrial disorders and elevated glycine in certain defects of lipoid acid biosynthesis (Table 42.2). More recently fibroblast growth factor 21 (FGF21), also known as a 'starvation response hormone', has been suggested as a better blood biomarker for mitochondrial disease, particularly in patients with muscle involvement. However other disease processes, which may lead to elevation of FGF21 levels, include diabetes, obesity and non-alcoholic fatty liver disease. More specific blood biomarkers are clearly needed in order to identify patients with mitochondrial disease without the need for invasive tissue biopsy.

42.7 Muscle Biopsy

When a muscle biopsy is performed, care must be taken that all necessary investigations are conducted. Every muscle biopsy in a patient with suspected mitochondrial disease should be examined morphologically (if possible including electron microscopy to look at mitochondrial ultrastructure) as well as functionally. Histopathological examination may reveal strongly suggestive abnormalities such as ragged-red or cytochrome oxidase negative fibres, whilst electron microscopy may demonstrate abnormal mitochondrial size, number or shape or crystalline inclusions within the mitochondrial matrix, but these appearances are not sufficient of themselves to establish a specific genetic diagnosis of mitochondrial disease. Biochemical and genetic investigations are also needed.

Complete biochemical assessment of mitochondrial function (including measurement of global OXPHOS function and activity of individual respiratory chain enzyme complexes) can only be performed wholly in fresh muscle. However, for the sake of expediency, many centres measure respiratory chain enzyme complex activities in snap-frozen muscle tissue, with the caveat that some defects may be missed by this approach. Patients may have a defect of an individual respiratory chain complex (suggesting a defect in a structural subunit or assembly factor) or a more global defect affecting several complexes (suggesting a defect in mtDNA maintenance or expression).

Biochemical techniques measuring other aspects of mitochondrial function include measurement of CO_2 production from ^{14}C-labelled substrates and polarographic measurement of oxygen consumption using different substrates. Traditionally the latter was determined using an oxygen electrode which required up to 1 g of fresh muscle, but in recent years, new techniques have allowed oxygraphy to be performed on a miniaturised scale, for example, using the Oroboros oxygraph or the Seahorse bioanalyser. Blue native gel electrophoresis can also be used to measure in gel activity and assess assembly of the OXPHOS complexes. Other parameters of mitochondrial function which can be measured include coenzyme Q_{10} levels, reactive oxygen species, glutathione levels and mitochondrial ATP synthesis. These biochemical assays are complex and difficult to interpret and should ideally be performed in a specialist centre.

42.8 Genetic Investigations

Genetic diagnosis of mitochondrial disease is complicated since >150 mtDNA mutations and defects in >200 different nuclear genes have already been linked to mitochondrial disease (Table 42.2). Molecular defects can be present in the mitochondrially encoded tRNAs, mitochondrial and nuclear-encoded subunits of the OXPHOS complexes and many other proteins including factors needed for mtDNA maintenance and expression, cofactor biosynthesis, mitochondrial import and mitochondrial membrane function and dynamics (Table 42.2). There are many areas of overlap – the same mutation can give rise to different syndromes, and the same syndrome can be caused by different functional impairments or mutations in different genes, so investigations are necessarily wide ranging. It is therefore difficult, if not impossible, to provide guidelines which can be applied to all patients in all settings. Functional deficiencies of a single respiratory chain complex may be due to a mutation involving one of the subunits or assembly factors for that enzyme, whilst mutations in genes required for mtDNA maintenance or expression usually give rise to multiple complex deficiencies. However, respiratory chain activity can also be normal in some of these defects (see Table 42.2).

As discussed above, in a few rare instances, initial genetic investigations can be directed by the clinical phenotype, e.g. screening for the m.3243A>G mutation in *MT-TL1* in MELAS, for *POLG* mutations in Alpers-Huttenlocher syndrome and for *PUS1* or *YARS2* mutations in MLASA. However, for most other cases, a broader approach will be needed, encompassing mtDNA and nuclear gene testing.

Unless there is obvious recessive inheritance (e.g. suggested by parental consanguinity), initial genetic investigations should target the mtDNA, ideally in a muscle sample. Three types of analyses may be performed to assess the integrity of the mitochondrial genome: long-range PCR to screen for large-scale rearrangements of the mtDNA, sequence analysis to determine the presence of sequence variants and real-time PCR to quantitate the amount of mtDNA.

In infants and children, nuclear gene defects are much more common than mtDNA mutations which cause only 10–20 % of respiratory chain disorders in this age group. Next-generation sequencing methods have revolutionised nuclear gene testing for mitochondrial disorders in recent years. Strategies for nuclear gene testing include using a candidate gene approach (e.g. *SURF1* analysis in complex IV-deficient Leigh syndrome, complex I subunits and known assembly factors in cases with isolated complex I deficiency, etc.), usually in the form of a targeted gene panel or a genome-wide approach such as whole exome or whole genome sequencing (Fig. 42.3). It is probable that genome-wide approaches will become first-line tests in the future, as discussed above, leading to ambiguous results in some patients and also to the discoveries of the mildest (and most severe) ends of a spectrum, at least for some conditions. Functional assays will still be necessary in patients with negative or ambiguous results of genetic testing.

Remember

In every patient diagnosed with or suspected to have a mitochondrial disorder, organs must be systematically screened for possible involvement. This includes cerebral MRI (and, if possible, ^{1}H-MRS to assess intracerebral lactate); CSF studies for lactate, alanine, protein, 5-methyltetrahydrofolate and neurotransmitter levels; ECG and echocardiography to detect rhythm abnormalities and cardiomyopathy; urine studies to screen for renal tubulopathy and pathological elevations of organic acids; liver function tests; and assessment of the retina, optic nerve and hearing. Diabetes mellitus should be excluded and thyroid function and cortisol levels measured. If these investigations remain normal and no other final diagnosis has been reached, they need to be repeated at regular intervals.

Remember

In patients with a clinical presentation suggesting a well-defined mitochondrial syndrome, direct genetic testing is possible. Next-generation sequencing techniques might also become the test of choice in patients with less well-defined clinical entities, but a muscle biopsy is necessary for functional validation of mutations of uncertain pathogenicity identified by next-generation sequencing to confirm the diagnosis of a mitochondrial disorder and to help guide further genetic investigations in some patients. Functional analysis of respiratory chain function in fresh muscle tissue remains the gold standard in diagnosis. Morphological studies alone are not sufficient.

42.9 Prenatal and Preimplantation Genetic Diagnosis and Novel Reproductive Options

Prenatal diagnosis in mitochondrial disorders is straightforward if inheritance is Mendelian and the genetic defect is known. For mtDNA mutations, prenatal diagnosis and prediction of likely disease severity in the offspring are challenging, owing to the random segregation of mtDNA, but have been reliably performed for some mutations known to have highly skewed mutation loads in oocytes, notably the m.8993T>G/C mutations associated with maternally inherited Leigh syndrome. Preimplantation genetic diagnosis may be available for some mtDNA mutations and nuclear-encoded mitochondrial defects, subject to local regulations and expertise. These investigations are performed only in a few highly specialised laboratories. Recently the mitochondrial 'donation' techniques of pronuclear transfer and maternal spindle cell transfer have been approved for further development in the United Kingdom, as a potential method for preventing transmission of mutated mtDNA from the mother to the embryo.

Remember

Prenatal diagnosis of mitochondrial disorders is straightforward if nuclear genes are affected and the mutation is known. With mtDNA mutations, prenatal diagnosis and risk assessment remain difficult and are very much dependent on the specific mutation and the mutation load in the mother.

42.10 Differential Diagnosis

There are many possible differential diagnoses for primary respiratory chain disorders. Metabolites accumulating in propionic and methylmalonic acidurias directly affect respiratory chain function and can thereby give rise to 'mitochondrial' symptoms. The clinical presentation of other inherited metabolic disorders, including congenital disorders of glycosylation, the glycogenoses and fatty acid oxidation defects, can also mimic mitochondrial disorders. Biotinidase deficiency causes lactic acidosis, deafness and optic atrophy and must be sought for in every child with elevated lactic acid, since it is treatable. Deficiency of pyridox(am)ine 5-phosphate oxidase (encoded by the *PNPO* gene) may lead to markedly increased CSF lactate. Thiamine deficiency also results in severe lactic acidosis, as does thiamine transporter deficiency due to *SLC19A3* mutations, which is treatable except for its most severe forms. Hypoxia, sepsis and low cardiac output are systemic causes of lactate

elevation. In several neurological disorders of childhood, lactate has been found to be intermittently increased in some patients, including patients with Rett and Angelman syndromes. Respiratory chain enzyme deficiencies and/or decreased ATP production has been demonstrated in patients with variable other non-mitochondrial diagnoses. Since correct diagnosis in these cases is important for prognosis and accurate genetic counselling, clinical suspicion of other disorders must be high, and these should be actively investigated prior to making a definitive diagnosis of mitochondrial disease. Again, increased use of whole exome or whole genome sequencing will facilitate alternative diagnoses in patients with metabolic abnormalities pointing to a possible mitochondrial disorder.

Remember

Besides respiratory chain defects, there are many differential diagnoses for lactate elevation/lactic acidosis including non-metabolic inherited and acquired disorders. The most common situation in which the concentration of lactic acid in blood is elevated is factitious, the result of improper technique, the use of a tourniquet or difficulty in drawing the blood. Depending on the clinical setting, treatable disorders such as biotinidase, PNPO and thiamine deficiency must be considered first.

42.11 Treatment

The vast majority of mitochondrial disorders lack curative therapies. The exceptions are disorders of CoQ_{10} biosynthesis (most cases respond to pharmacological doses of CoQ_{10}), complex I assembly defect caused by *ACAD9* mutations (respond to riboflavin) and reversible infantile respiratory chain disorders caused by *MTTE* or *TRMU* mutations (recover spontaneously with supportive therapy which may include the need for artificial ventilation for several months). Some patients (particularly those with Kearns-Sayre syndrome) have cerebral folate deficiency and clinical benefit from folinic acid supplementation has been documented in some cases. There is some evidence for efficacy of L-arginine or L-citrulline in prevention and amelioration of stroke-like episodes in MELAS syndrome. The mainstay of treatment for most patients with mitochondrial disorders, however, is symptom management, for example, with gastrostomy feeding, antiepileptic drugs, hormone replacement (insulin, growth hormone, cortisol, thyroxine), surgery for ptosis, hearing aids or cochlear implantation for SNHL, pacemakers for heart block, medical management of cardiomyopathy, blood transfusions for sideroblastic anaemia in Pearson syndrome and fluid and electrolyte replacement for renal tubulopathy. Experimental therapies currently at a preclinical stage include various antioxidants aimed to counteract the effects of reactive oxidative species accumulation (some are currently being evaluated in clinical trials), methods to stimulate mitochondrial biogenesis and innovative gene therapy strategies to tackle defects of the mitochondrial genome.

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Pathology: Biopsy

43

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Key Facts

- Neurometabolic single-organelle-multi-organ disorders, i.e., lysosomal, peroxisomal, polyglucosan, and mitochondrial disorders, warrant biopsies for morphological (and often biochemical by direct tissue and/or indirect fibroblast culture) studies.
- Tissues suitable for biopsies are the lymphocytes, skin, conjunctiva, skeletal muscle, rectum, and liver.
- Different neurometabolic disorders show different patterns of morphological expression in different tissues.
- Brain and peripheral nerves need not be biopsied because any neurometabolic disease morphologically expressed in the nervous system may also be encountered in any of the other tissues. Only rectum, among noncerebral tissues, may show exclusive lysosomal neuronal storage.
- Accessibility and morphological manifestation determine the target of biopsy in an individually suspected neurometabolic disorder.
- Preferential sites of biopsy are the lymphocytes in vacuolar and certain non-vacuolar lysosomal diseases; the skin in many lysosomal and Lafora diseases; the skeletal muscle in many lysosomal, polyglucosan, and mitochondrial diseases; and the liver in peroxisomal and lysosomal diseases.

43.1 General Remarks

Pathology, i.e., pathomorphology concerning individual groups of metabolic diseases, as summarized in Chaps. 1, 2, and 3, may be divided into disease-specific or pathognomonic pathology. Recognition allows precise nosological diagnosis. Examples are lysosomal inclusions in certain lysosomal diseases or group-specific pathological features, e.g., lysosomal vacuoles in certain lysosomal diseases or abnormally structured mitochondria in mitochondrial diseases. Lesions nonspecific for any disease may be characteristic of a particular class of metabolic diseases, e.g., sponginess of cerebral tissue in amino acid disorders. Secondary pathological

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phenomena may also be encountered, for instance, reactive cellular and fibrillar astrocytosis or demyelination in the brain following loss of nerve and glial cells and fibrosis in the liver or heart following atrophy or loss of hepatocytes or cardiomyocytes.

Moreover, one may distinguish disease-related pathomorphology of organ-specific parenchymal cells, e.g., myofibers in the skeletal muscle, hepatocytes in the liver and eccrine sweat gland epithelial cells in the skin, and common interstitial cell elements such as mural vascular cells, fibroblasts, and peripheral nerve twigs.

Inherited metabolic diseases are defined by biochemical and molecular criteria, whereas morphological investigations of tissues express pathomorphology (Table 43.1). While not always disease-specific the pathological picture may pave the way for relevant biochemical and molecular studies. Postbiochemical and postmolecular morphological investigations may confirm findings.

The methodological spectrum of the anatomic pathologist, the neuropathologist, and the paidopathologist may encompass histological studies with a wide range of structural and histochemical stains; enzyme histochemical preparations requiring unfixed frozen tissue, of lysosomal, mitochondrial, and peroxisomal enzymes; and electron microscopy. When antibodies against enzyme substrates or enzyme proteins are available, immunohistochemistry is employed.

Table 43.1 Tissues biopsied in neurometabolic disease

Frequently biopsied
Lymphocytes
Skeletal muscle
Skin
Infrequently biopsied
Bone marrow
Brain
Conjunctiva
Intestine
Kidney
Liver
Lymph nodes
Peripheral nerves
Tonsils

These techniques require different approaches, immediately after having obtained the biopsied tissue, e.g., fixation in formalin for regular histological studies and frozen unfixed tissue for certain histochemical and enzyme histochemical methods. Immunohistochemical preparations depend on the suitability of antibodies for fixed, paraffin-embedded, or frozen tissues. Immediate proper fixation, i.e., of small tissue fragments in the specific fixative glutaraldehyde, is required for electron microscopy.

Biopsy obtains tissues of living patients, while autopsies may confirm a diagnosis and document the distribution of the disease and the intensity of the pathology. Clinicopathological and clinicoradiological correlations allow for tissue-specific biochemical and molecular studies, e.g., muscle tissue in postinfantile type II glycogenosis or in mitochondrial diseases. In many fatal inherited metabolic diseases, the diagnosis has been established by the time of autopsy.

Biopsies in patients with metabolic diseases are invasive, diagnostic surgical procedures. The skin, conjunctiva, rectum, and skeletal muscle may be investigated by open biopsy, allowing proper orientation and removal of tissue as well as tissue for different morphological – and other, e.g., biochemical – preparations. Tissues of visceral organs and sometimes skeletal muscle may also be obtained by needle biopsies. A limited number of lysosomal disorders may morphologically be recognized in blood lymphocytes, requiring only venipuncture. It is essential to know that the biopsied organ will contain pathology of the disease, which is suspected or already ascertained by the clinician. Many inherited metabolic diseases are multiorgan diseases, and respective pathology may be recognized in more than one tissue or organ, allowing biopsy of variously available tissue, such as the lymphocytes, skin, or conjunctiva (Table 43.2).

With the enormous expansion of biochemical and molecular assays in metabolic diseases, the significance of a diagnostic biopsy or morphological study has considerably decreased. The number of biopsies is shrinking as is knowledge of postsurgical morphological findings. Hence,

earlier comprehensive reviews are important (Goebel 1997, 1999; Goebel and Jänisch 1995; Goebel and Warlo 1990a, b; Anderson et al. 2013).

Biopsies of extracerebral tissues in patients with metabolic diseases while often pathognomonic may be considered optional, whereas an earlier category of "essential" biopsies (Goebel 1999) has lost its value. Considering the diversity of tissues involved in metabolic diseases, each condition or group of conditions may require different approaches to different tissues or possibly a "sequential" order, such as in the neuronal ceroid lipofuscinoses (NCL) commencing with lymphocytes and, if unyielding or equivocal, proceeding to the skin and other tissues such as the muscle and rectum.

Disease-specific morphological evaluation and advice as to respective biopsy procedures will therefore be provided according to tissues and organs.

Table 43.2 Metabolic diseases with morphological pathology in various tissues suitable for biopsy (single-organelle-multi-organ disorders)

Lysosomal diseases
Lymphocytes
Bone marrow
Brain
Liver
Rectum
Skeletal muscle
Skin
Peripheral nerves
Mitochondrial diseases
Brain
Liver
Skeletal muscle
Peroxisomal diseases
Brain
Kidney
Liver
Adrenal glands
Peripheral nerves
Polyglucosan diseases
Brain
Liver
Skeletal muscle
Skin
Peripheral nerves

Remember

Biopsies are rewarding for morphological diagnosis when disease-typical lesions are present in the tissue. Metabolic single-organelle-multi-organ disorders are such conditions. Lesions may be identified by electron microscopy. The tissue target of biopsy is dictated by the morphological manifestation of the disease and the accessibility of the tissues.

43.2 Circulating Blood Cells

In certain metabolic disorders, particularly lysosomal diseases (Table 43.3), the easiest cells to obtain are circulating white blood cells. A blood smear may show vacuolated lymphocytes in certain lysosomal disorders marked by lysosomal vacuoles, e.g., mucopolysaccharidoses (MPS), oligosaccharidoses, and GM_1 gangliosidosis. However, the procedure may only be considered supportive rather than proving since swollen mitochondria may give the spurious light

Table 43.3 Lysosomal pathology in lymphocytes

Vacuoles
Aspartylglucosaminuria
GM_1 gangliosidosis
Mannosidosis
Fucosidosis
Juvenile neuronal ceroid lipofuscinosis (NCL), CLN3
Mucolipidosis (ML) I and II
Mucopolysaccharidoses I–VII
Salla disease
Type II glycogenosis (when glycogen is autolytically dissolved)
Solid non-vacuolar
Infantile NCL, CLN1
Late-infantile NCL, CLN2
Late-infantile variants of NCL, CLN5–CLN8
Mucolipidosis IV
Niemann-Pick disease
Type II glycogenosis (when glycogen is well preserved)

microscopic impression of lysosomal vacuoles. A PAS stain may show carbohydrate-containing intralysosomal contents. The lysosomal nature of vacuoles may be further confirmed by enzyme histochemical demonstration of increased activity of acid phosphatase, the marker enzyme for lysosomes. In most lysosomal diseases, vacuoles only appear in lymphocytes, but in certain mucopolysaccharidoses, there are granules in polymorphonuclear leukocytes known as Alder bodies.

At the electron microscopic level, non-vacuolar lysosomal residual bodies may be ascertained. Although lymphocytes are present in the buffy coat, numerical predominance of granulocytes may impede careful electron microscopic studies of such a specimen. Therefore, isolation of circulating lymphocytes, using the Ficoll technique, greatly facilitates ultrastructural investigation. For this reason, heparinized blood requires immediate isolation of the lymphocytes before fixation as a pellet in buffered glutaraldehyde and, then, routine embedding in resin for regular electron microscopic work-up. Only at the electron microscopic level, may swollen mitochondria be distinguished from membrane-bound lysosomal vacuoles; the mitochondrial double membrane and marginal remnants of ruptured cristae provide clear distinction.

When disease-specific lysosomal inclusions of vacuolar or non-vacuolar nature are present, they should be photographed for permanent documentation. When absent, one may safely count in the electron microscope 200 consecutive properly identified lymphocytes to be sure that lysosomal storage is not present in the preparation.

Among metabolic diseases, lysosomal storage or residual bodies are vacuolar in mucopolysaccharidoses, oligosaccharidoses, mucolipidoses, GM_1 gangliosidosis (Fig. 43.1), and juvenile or CLN3 NCL. In the latter, vacuoles may occasionally contain fingerprint profiles, but their number is considerably lower than the number of vacuoles. Thus, empty lysosomal vacuoles usually predominate in juvenile NCL. On the other hand, non-vacuolar, i.e., granular or lamellar inclusions, often of disease-specific nature, are seen in type II glycogenosis, Niemann-Pick disease, and Fabry disease. They are granular in infantile NCL/CLN1, curvilinear bodies in classical late-infantile NCL/CLN2, and usually few but regularly shaped solid non-vacuolar lipopigments with a granular component and fingerprint profiles in late-infantile variants of NCL, i.e., CLN5, CLN6, CLN7, and CLN8. Fingerprint profiles in lysosomal vacuoles may also be seen in mucopolysaccharidoses (Goebel

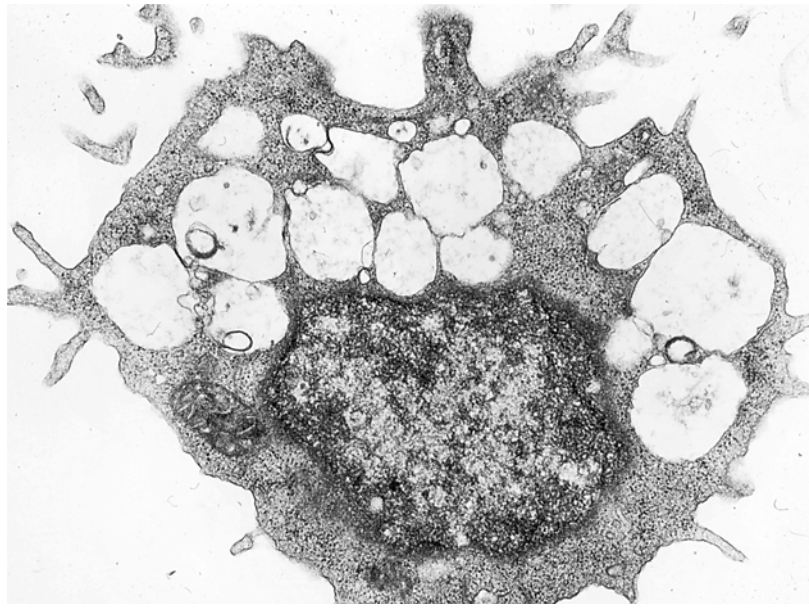

Fig. 43.1 Electron microscopic abundant lysosomal membrane-bound vacuoles in a blood lymphocyte, GM_1 gangliosidosis

et al. 1981). Jordans anomaly is marked by lipid droplets in white blood cells in neutral lipid storage disease.

Remember

Lymphocytes are only suitable for morphological investigations in lysosomal diseases, not in mitochondrial or peroxisomal disorders. Electron microscopy – not light microscopy – may show lysosomal vacuoles or compact lysosomal inclusions which vary in ultrastructure, according to the type of lysosomal disease.

43.3 Skin

The skin is widely used for morphological investigations (Table 43.4). It offers easy accessibility by punch biopsy and a large diversity of cell types, i.e., epithelial, mesenchymal, and neuroectodermal, allowing metabolic conditions to be differently expressed in these cell types. In addition, fibroblast cultures may be obtained from separate biopsies of the skin.

A prerequisite for gainful skin biopsy is that the clinically suspected disease manifests itself morphologically in the skin or in cultured fibroblasts. A rewarding biopsy requires a full-thickness skin biopsy, as diagnostically crucial eccrine sweat gland epithelial complexes are often located in the cutis-subcutis region, while the epidermis is of limited reward (Kaesgen and Goebel 1989).

Table 43.4 Pathology of metabolic diseases in the skin

Vacuolar lysosomal diseases
MPS, ML, oligosaccharidosis
Non-vacuolar lysosomal disorders
Globoid cell leukodystrophy
GM_1 gangliosidosis (also vacuolar)
GM_2 gangliosidosis (Sandhoff type)
Farber disease
Fabry disease
Metachromatic leukodystrophy (when nerves are present)
Neuronal ceroid lipofuscinosis (NCL)
Niemann-Pick disease
Mucolipidosis IV (also vacuolar)
Type II glycogenosis
Peroxisomal diseases
Adrenoleukodystrophy (when nerves are present)
Polyglucosan diseases
Lafora disease

The catalogue of morphologically diagnosable metabolic diseases affecting the skin in children includes lysosomal and peroxisomal diseases as well as Lafora disease (Table 43.4). Mitochondrial disorders – even those associated with abnormally structured mitochondria seen predominantly in skeletal muscle fibers – are not convincingly expressed in the skin. Amino acid and other small-molecule disorders do not display meaningful pathomorphological findings in the skin.

In the skin obtained by diagnostic biopsy, morphological investigations are largely to be performed with the electron microscope because it is only at the ultrastructural level that lesions appear distinct and sometimes recognizably disease-specific. Light microscopic studies may give spurious results even when employing special techniques, such as enzyme histochemistry, immunohistochemistry, or fluorescence microscopy. However, Lafora disease may be recognized at the light microscopic level by strongly PAS-positive diastase-resistant polyglucosan bodies in ductal cells of eccrine sweat glands and in axillary apocrine glands. Lysosomal vacuolation may be excessive in respective lysosomal disorders, such as mucopolysaccharidoses, but confirmatory electron microscopy remains desirable. Another hint at electron microscopic lysosomal lesions may be increased enzyme histochemical activity of acid phosphatase at unusual sites, e.g., smooth muscle cells of arrectores pilorum muscle or mural cells of vessels. The secretory granules in secretory cells of eccrine sweat glands are PAS positive, a nonspecific physiological feature, which is not to be taken as evidence of pathological lysosomal storage of glycolipids, glycoproteins, or glycosaminoglycans. Even semi-thin, 1-µm thick, plastic sections stained with toluidine or methylene blue serve to identify various cytological components in the skin specimen for subsequent electron microscopic examination rather than to ascertain unequivocal pathology.

Among the biochemically heterogeneous lysosomal disorders, different entities are morphologically expressed in different cytological components of the skin. Some, as purely neuronal forms, Tay-Sachs disease, or Gaucher disease, do not express any pathology in the skin.

As in lymphocytes, dermal cells in lysosomal disorders may be characterized by lysosomal vacuolation or by the formation of non-vacuolar compact lysosomal residual bodies. Hence, known lysosomal disorders marked by lysosomal vacuolation, e.g., mucopolysaccharidoses, mucolipidoses, and oligosaccharidoses, show lysosomal vacuoles in mesenchymal cells, such as fibroblasts and mural cells of vessels. Certain lysosomal disorders, such as sialidosis and mucolipidosis IV, may show both lysosomal vacuolation and compact lysosomal residual bodies of lamellar type in different cell types of the skin.

Among the lysosomal diseases, type II glycogenosis and the NCL show ubiquitous lysosomal pathology affecting epithelial, mesenchymal, and neuroectodermal cells. In type II glycogenosis, there is intralysosomal glycogen accretion and a lysosomal vacuolar appearance when glycogen is badly preserved in tissue handling after biopsy. In NCL, there are ultrastructurally distinct lipopigments. Granular lipopigments without any lipid droplets, the latter a conspicuous component of regular lipofuscin, may be encountered in genetic CLN1 or its infantile, late-infantile, juvenile, and adult clinical forms and in CLN10; curvilinear bodies are found in genetic CLN2 or classical late-infantile form, while other late-infantile variant forms, genetic CLN5, CLN6, CLN7, and CLN8 types, show a mixture of curvilinear profiles and fingerprint patterns. In genetic CLN3 or classic juvenile NCL, fingerprint profiles or fingerprint bodies prevail, while, occasionally, vacuolation of lysosomes is also encountered – as in CLN3 in lymphocytes.

Dermal nerves contain myelinated and unmyelinated nerve fibers, covered by Schwann cells and, when still situated in fascicles, surrounded by perineurium or perineurial cells. Schwann cells of both myelinated and unmyelinated axons and perineurial cells may contain pathological lysosomes of different lamellar types, such as in mucolipidosis IV, sialidosis, Niemann-Pick disease, Sandhoff disease, and foremost the lysosomal leukodystrophies with involvement of the peripheral nervous system, such as metachromatic (MLD) and globoid cell (GCL) leukodystrophies. When myelinated nerves are encountered in the skin specimen, metachromasia of stored sulfatides in MLD may be demonstrated as brownish material in acid cresyl violet-stained fixed frozen sections and brownish material in toluidine blue-stained semi-thin sections. Disease-specific lysosomal residual bodies accrue in Schwann cells, particularly of myelinated nerve fibers, including prismatic or tuffaceous bodies in MLD and needle-like inclusions in GCL. In addition, similar compact lysosomal residual bodies may be encountered in macrophages within the endoneurium, derived from damage to and breakdown of myelinated nerve fibers. The ultrastructure of lysosomal inclusions in GCL differs in the infantile and non-infantile forms in that needle-like structures are largely seen in infantile GCL, and rather vacuolar lysosomes filled with indistinct lamellae are seen in non-infantile GCL (Goebel et al. 1990). A remarkable distinction in dermal morphological manifestation between MLD and GCL is the involvement of secretory cells of eccrine sweat glands with similar needle-like inclusions in GCL, but no abnormal lysosomal storage in MLD.

While mural cells of vessels are affected by lysosomal storage in a large number of lysosomal diseases, they are particularly involved in Fabry disease. They are even demonstrable in disease manifesting female carriers of this X-linked inherited disorder.

Important tissue components of the skin are the eccrine sweat glands, the secretory cells of which display a wide variety of lysosomal residual bodies both vacuolar and non-vacuolar in different diseases. These lysosomal residual bodies can often be distinguished from secretory granules, both compact and electron lucent. Sometimes, there are a disturbingly large number of membrane-bound vacuoles in secretory eccrine

sweat gland epithelial cells, not associated with any lysosomal disorder, a nonspecific morphological feature of unknown connotation. While the ductal cells of eccrine sweat glands are not affected by lysosomal storage in lysosomal disorders, they are the main cell type involved in formation of polyglucosan bodies, not limited by a unit membrane, in Lafora disease (Fig. 43.2) (Goebel and Bönnemann 2004). Similarly, polyglucosan bodies may be encountered in apocrine sweat gland epithelial cells of the axilla.

Skin biopsy in the axillar region is not advisable when suspecting a lysosomal disorder because there are physiological inclusions.

Both epithelial cells of apocrine glands and ductal cells of eccrine sweat glands may contain nonspecific normal inclusions, the former rather uniform and compact and the latter of a lamellar ultrastructure. These cytoplasmic inclusions should not be confused with pathological storage bodies. Melanin- and melanosome-containing cells as well as mast cells also harbor cell type-specific inclusions, which should not be confused with true pathological lysosomal inclusions.

Not infrequently, axons, usually unmyelinated in their terminal course, are nonspecifically enlarged by mitochondria and dense bodies, the latter possibly degenerating mitochondria (Dolman et al. 1977; Walter and Goebel 1988), but hardly ever by disease-specific lysosomal residual bodies. These have only been seen in the gangliosidoses and in mucolipidosis IV. When encountering such enlarged axons, a lysosomal disorder may be suspected. The enlargement of the axons may result from impaired axoplasmic transport as a result of lysosomal storage in respective neuronal perikarya.

Diagnostically informative cytological components in the skin are often widely spaced and scarce, and there is an abundance of noninformative collagen fibril aggregates. Disease-specific lesions in affected patients may not be present in the individual skin specimen biopsied. A second skin biopsy or biopsy of another tissue may be required. This may occasionally happen in the NCL.

Among the peroxisomal disorders, those forms marked by needle-like inclusions (Fig. 43.3) in Schwann cells, such as adrenoleukodystrophy and infantile Refsum disease, may show group-specific pathology, while other cell types, such as epithelial and mesenchymal cells, do not seem to be involved in morphological pathology.

Cultures of dermal fibroblasts provide valuable sources of biochemical and molecular investigations, while morphological studies of cultured fibroblasts are largely unrewarding, except in mucolipidosis II or I-cell (inclusion cell) disease. Nonspecific lysosomal residual bodies may accrue over time in cultured fibroblasts giving

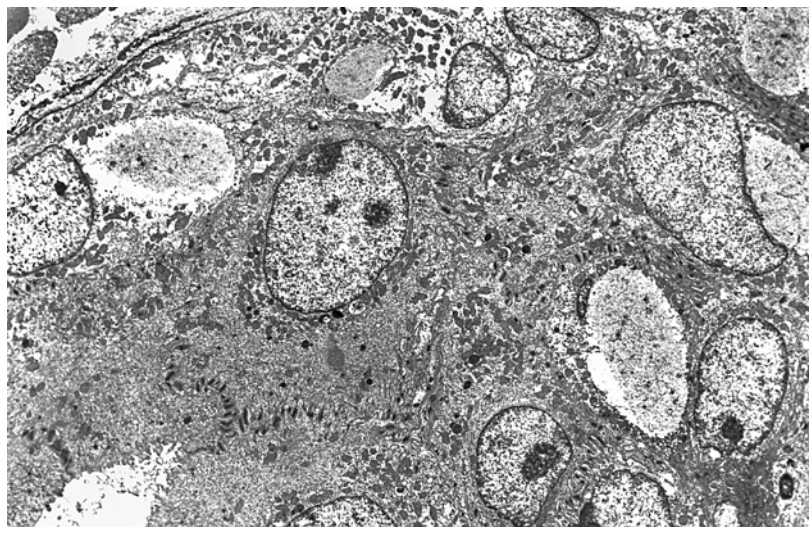

Fig. 43.2 Several electron microscopic non-membrane-bound polyglucosan bodies in ductal sweat gland epithelial cells of the skin, Lafora disease

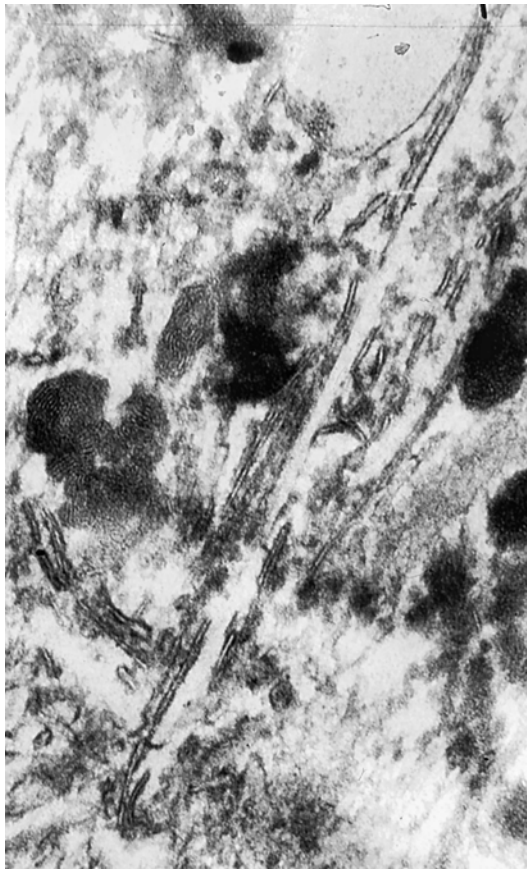

Fig. 43.3 Electron microscopic appearance of needle-like structures in Schwann cell in adrenoleukodystrophy

cause to erroneous interpretations. Hence, performing a skin biopsy solely to produce tissue cultures may be considered incomplete in disorders in which meaningful morphological investigations can be made.

Remember

The skin is an important biopsy target in lysosomal diseases both of vacuolar and non-vacuolar forms because of its diverse cell types and accessibility. Electron microscopy is required for proper morphological diagnosis. When nerves are present, electron microscopy may permit the recognition of lysosomal leukodystrophies (metachromatic and globoid cell forms) and peroxisomal disorders (adrenoleukodystrophies and infantile Refsum disease). Mitochondrial diseases are not morphologically reliably expressed in the skin.

43.4 Conjunctiva

The conjunctiva has occasionally been biopsied in children as a target alternative to the skin. This may reflect a preference of the clinician, e.g., an ophthalmologist. It is seldom preferred by the pathologist. Sweat glands are absent from the conjunctiva; vessels and nerves are, however, more abundant than in the skin and are informative. Mural cells of vessels and Schwann cells of both myelinated and unmyelinated axons may harbor disease-specific lysosomal residual bodies, both vacuolar and non-vacuolar. Axons usually do not show any pathology in metabolic disorders except for nonspecific loss or regeneration. When axons are myelinated, their Schwann cells may harbor very typical disease-specific lysosomal bodies in lysosomal leukodystrophies, so-called prismatic or herring-bone inclusions in MLD. Examples are the needle-like inclusions in GCL or Krabbe leukodystrophy and similar but not identical needle-like inclusions in peroxisomal adrenoleukodystrophies.

43.5 Peripheral Nervous System

Because peripheral nerves are often encountered in dermal and conjunctival biopsy specimens, the biopsy of peripheral nerves as an invasive, purely diagnostic procedure will seldom provide more information on metabolic diseases than the skin and conjunctiva may yield. In mitochondrial disorders, peripheral nerves are equally unsuitable as biopsy targets since they may give ambiguous and, thus, unreliable results. However, in specific constellations biopsy of nerves can yield useful information (Fig. 43.4, Table 43.5). Polyglucosan bodies within axons may be an occasional nonspecific finding, but they may be increased in number in conditions associated with polyglucosan body formation and polyglucosan diseases, such as type IV glycogenosis.

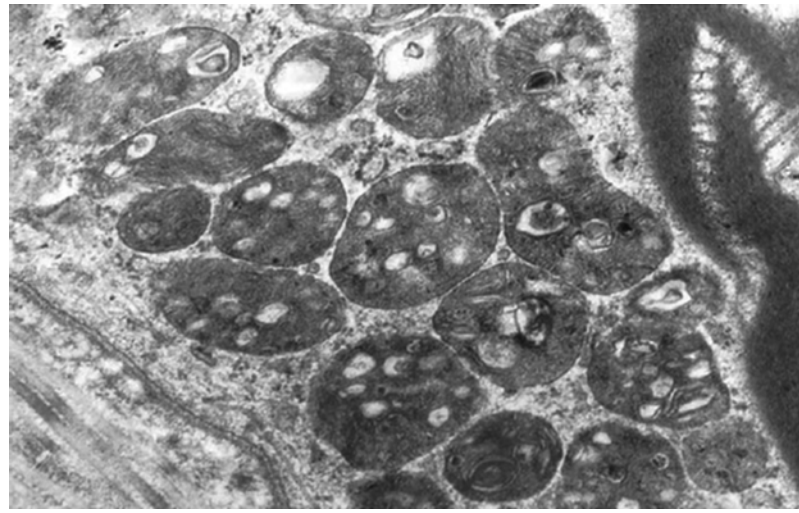

Fig. 43.4 An electron microscopic aggregate of membrane-bound lysosomal lamellar bodies in the Schwann cell cytoplasm of a myelinated peripheral nerve fiber of a patient with metachromatic leukodystrophy

Table 43.5 Morphological expression of neurometabolic diseases in peripheral nerves

Lysosomal diseases
Vacuolar forms
MPS, ML, oligosaccharidoses
Non-vacuolar forms
Fabry disease
Globoid cell leukodystrophy
Type II glycogenosis
GM_1 gangliosidosis
Metachromatic leukodystrophy
Neuronal ceroid lipofuscinosis (NCL)
Vitamin E deficiency
Peroxisomal disorders
Adrenoleukodystrophies
Infantile Refsum disease
Polyglucosan diseases
Polyglucosan body neuropathy

Table 43.6 Morphological expression of neurometabolic diseases in rectum

Lysosomal diseases
Vacuolar forms
MPS, ML, oligosaccharidoses
Non-vacuolar forms
Fabry disease
Farber disease
Gaucher disease
Globoid cell leukodystrophy
Type II glycogenosis
GM_1 gangliosidosis
GM_2 gangliosidosis
Mucolipidosis IV
Neuronal ceroid lipofuscinoses (NCL)
Niemann-Pick disease
Metachromatic and globoid cell leukodystrophies
Peroxisomal disorders
Adrenoleukodystrophies
Infantile Refsum disease
Polyglucosan diseases
Lafora disease
Type IV glycogenosis

43.6 Rectum

Neuronal perikarya or nerve cell bodies of the peripheral nervous system, largely situated in the rectum (Table 43.6), may serve as biopsy substitutes for neurons of the brain, because both are affected by lysosomal storage in many lysosomal diseases. Certain neuronal lysosomal disorders, e.g., mucolipidosis IV or Sandhoff disease, may also involve non-neuronal cell types and tissues as well, while GM_2 gangliosidosis of Tay-Sachs type, as a purely neuronal lysosomal disease, can safely be ascertained by morphological studies on nerve cells only, i.e., neurons of the rectum and other tissues. Other nerve cell-containing regions of the peripheral nervous system located in the dorsal root and autonomic ganglia are hardly ever a target of biopsy. In GM_2 gangliosidosis of all biochemi-

cal and molecular forms and in GM_1 gangliosidosis, membraneous cytoplasmic bodies are the characteristic intraneuronal lysosomal inclusions.

Remember

As nerves are widespread in the skin and conjunctiva, peripheral nerve biopsies in metabolic disorders, especially in lysosomal diseases, are unnecessary. Likewise, when peroxisomal disorders affect peripheral nerves in the skin and conjunctiva as seen in the adrenoleukodystrophies and infantile Refsum disease, nerve biopsies may be replaced by biopsies of the skin and conjunctiva.

43.7 Brain

In metabolic diseases, premortem morphological studies of brain tissue for diagnostic purposes have become rather obsolete. Among the metabolic disorders, there are hardly any which display disease-specific morphological findings confined to the brain and not encountered in extracerebral tissues. Conversely, pathology of those metabolic disorders clinically predominant in the brain does not appear disease specific, such as in amino acid disorders or neurotransmitter defects. Moreover, brain biopsy is largely confined to the cortex and subcortical white matter, and this would fail to identify subcortical pathology and respective diseases as seen in Wilson disease or those mitochondrial encephalomyopathies which do not show mitochondrial pathology in biopsied muscle, such as Leigh syndrome.

In addition, the vast achievements in biochemical and molecular data affecting the brain have deplorably been insufficiently correlated with postmortem brain pathology.

Remember

Brain biopsy can now be considered an obsolete diagnostic procedure to procure pathomorphology of metabolic disorders, but autopsies of the brain are still important to confirm in vivo diagnosis and secure morphological information in newly defined metabolic conditions as well as show topography of the pathology.

43.8 Liver

For morphological studies (Table 43.7), a liver biopsy may be a very rare procedure because its pathology in numerous neurometabolic diseases is equally well expressed in other tissues more easily accessible, such as the skin or skeletal muscle. Only in peroxisomal disorders (Powers 2004), which may be divided into those with abnormal or absent peroxisomes and those with regular peroxisomes, the liver as biopsy target may be the best choice, before or after respective biochemical and molecular investigations. Peroxisomes are absent in Zellweger syndrome, neonatal adrenoleukodystrophy, and infantile Refsum disease. At the light microscopic level, the absence of the marker enzyme for peroxisomes, catalase, may suggest absence of peroxisomes which may then be confirmed by electron microscopic examination. In other peroxisomal conditions, peroxisomes may be present, but enlarged or abnormally structured, and "angulate lysosomes" may be encountered.

Among lysosomal disorders, Gaucher disease may be recognized morphologically in the liver but not in the skin or skeletal muscle. It is of course recognized in the bone marrow. Many other lysosomal diseases are morphologically evident in the liver (Fig. 43.5). Although the liver

Table 43.7 Morphological expression of neurometabolic diseases in liver

Peroxisomal diseases
Infantile Refsum disease
Neonatal adrenoleukodystrophy
Zellweger disease
Lysosomal diseases
Vacuolar forms
MPS, ML, oligosaccharidoses
Non-vacuolar forms
Fabry disease
Gaucher disease
Type II glycogenosis
GM1 gangliosidosis
NCL
Niemann-Pick disease
Polyglucosan diseases
Lafora disease
Type IV glycogenosis

may be affected in mitochondrial diseases and even contain abnormally structured mitochondria, an advantage of liver biopsy over skeletal muscle biopsy exists among the mitochondrial disorders only in those affecting the liver but not the skeletal muscle, such as the mitochondrial DNA depletion syndromes.

Remember

The liver is the most important biopsy target in peroxisomal disorders to distinguish between those with defective biogenesis of peroxisomes resulting in absence or smallness of peroxisomes and those defined by individual enzyme deficiencies when peroxisomes are present in hepatocytes. Although lysosomal diseases widely affect the liver, there are more easily accessible tissues, such as the lymphocytes and skin. In non-neuronopathic Gaucher and Niemann-Pick diseases, the liver is a warranted biopsy target.

43.9 Skeletal Muscle

The main cytological components of the skeletal muscle are the multinucleated striated muscle fibers (Table 43.8). They may show a remarkable amount of abnormal mitochondria, i.e., increase in number, increase in size, abnormal shape, and pathological solid inclusions. Abnormal mitochondria are seen in a large number, but not all mitochondrial diseases. However, individual mitochondrial myopathies do not show different ultrastructural patterns of mitochondria. In light microscopic specimens, accumulation of abnormal mitochondria in muscle fibers may give rise to "ragged red fibers" when employing the modified Gomori trichrome stain, to "ragged blue

Table 43.8 Morphological manifestation of neurometabolic diseases in skeletal muscle

Mitochondrial diseases
Lysosomal diseases
Vacuolar forms
MPS, ML, oligosaccharidoses
Non-vacuolar forms
Fabry disease
Type II glycogenosis
GM_1 gangliosidosis
Mucolipidosis IV
Neuronal ceroid lipofuscinoses (NCL)
Niemann-Pick disease
Vitamin E deficiency
Polyglucosan diseases
Lafora disease
Type IV glycogenosis
Polyglucosan body myopathy

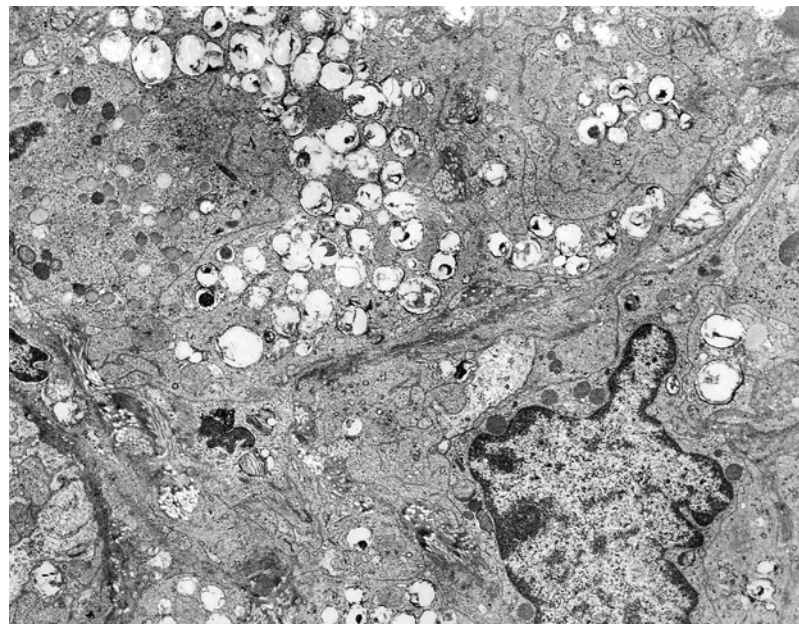

Fig. 43.5 Electron microscopic membrane-bound lysosomal vacuoles with some electron-dense dark amorphous material in hepatocytes of the liver in Niemann-Pick disease type B

fibers" in oxidative enzyme histochemical preparations, such as NADH nitroblue tetrazolium (NBT) reductase and succinic dehydrogenase (SDH), or to "ragged brown fibers" in cytochrome-C oxidase preparations. Many times, however, ragged red fibers may lack enzyme histochemical activity of cytochrome-C oxidase, being so-called COX-negative muscle fibers, while SDH in ragged blue fibers is well expressed, an observation which is quite conspicuous in a combined COX-SDH enzyme histochemical preparation (Vogel 2001).

Among the lysosomal diseases, type II glycogenosis, in both the infantile and non-infantile types, is best studied in the skeletal muscle (Fig. 43.6). The formation of lysosomal glycogen in type II glycogenosis is ubiquitous. Lysosomal glycogen storage is not confined to striated muscle fibers but is apparent in other tissue components of skeletal muscle, such as satellite cells, mural cells of vessels, and fibroblasts. In postinfantile type II glycogenosis, peculiar globular inclusions, giving a reducing appearance with menadione-linked NBT, may be encountered in muscle fibers (Sharma et al. 2005). Mucolipidosis IV may show lysosomal lamellar bodies in muscle fibers and lysosomal vacuoles in interstitial cells. Niemann-Pick disease, especially type C, may affect compartments of both striated and nonstriated cells with the formation of typical lysosomal inclusions. On the other hand, vacuolar lysosomal disorders, such as mucopolysaccharidoses, may readily be recognized in interstitial cells within skeletal muscle but not in striated muscle fibers. The presence of nerve fascicles may provide evidence of lysosomal leukodystrophies, such as metachromatic and globoid cell forms. In Lafora disease, membrane-bound glycogen and debris displaying increased activity of acid phosphatase suggest lysosomal involvement, together with fibrillar glycogen which may be more apparent as polyglucosan bodies in type IV glycogenosis and its non-infantile variants. Type-specific lipopigments of different genetic (CLN) forms of NCL are encountered in striated muscle fibers and interstitial cells, largely granular lipopigments (so-called granular osmiophilic deposits (GROD) in the genetic CLN1 form). Curvilinear/rectilinear, but not fingerprint, profiles are encountered in the genetic forms CLN2, CLN3, CLN5, CLN6, CLN7, and CLN8 (Table 43.9). Finely granular lipopigments also accrue in the skeletal muscle and peripheral nerve in vitamin E deficiency, both the hereditary and acquired forms. This group includes abetalipoproteinemia or Bassen-Kornzweig syndrome.

Biopsied unfixed frozen muscle may also be a suitable organ for biochemical studies, such as the muscle-specific biochemical abnormalities seen in mitochondrial DNA-related, organ-defined

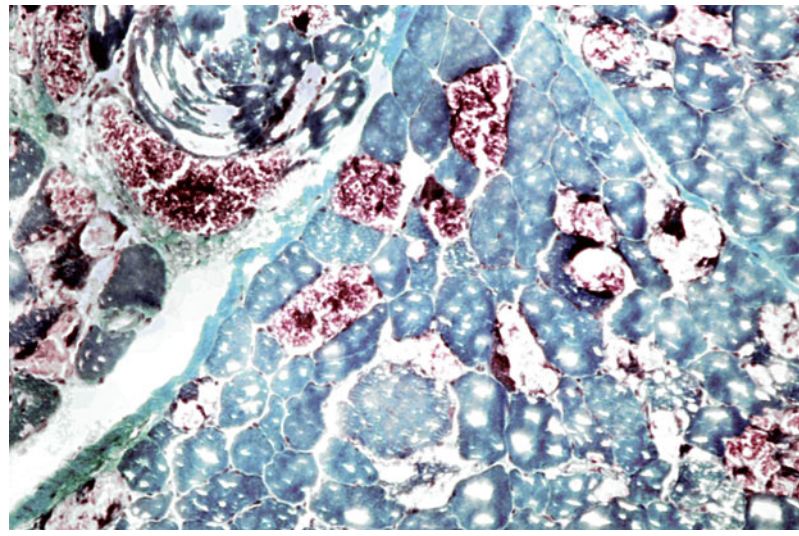

Fig. 43.6 Light microscopic deposition of lysosomal glycogen in and destruction of muscle fibers of skeletal muscle, type II glycogenosis, modified Gomori trichrome stain

mitochondrial disorders and in non-infantile type II glycogenosis. The latter condition may show normal acid maltase levels in circulating blood cells, often the source for biochemical investigation of type II glycogenosis, but absent or reduced activities in the skeletal muscle (Sharma et al. 2005).

Other glycogenoses, such as types III, V, and VII, displaying increased storage of sarcoplasmic nonmembrane-bound glycogen in skeletal muscle fibers, are associated with enzyme histochemical deficiency of the respective two enzymes myophosphorylase and phosphofructokinase in glycogenoses V and VII. The sarcoplasmic glycogen is strongly PAS positive but liable to digestion by diastase; it may fade during prolonged postsurgical tissue handling rendering the myopathology that of vacuolar myopathies. In glycogenosis type IV, fibrillar diastase-resistant polyglucosan bodies are encountered in myofibers. Increased amounts of sarcoplasmic lipid droplets, often confluent and then appearing as larger droplets at the light microscopic level, suggest a lipid myopathy, so-called neutral lipid storage disease with or without associated mitochondrial defects, while lysosomal lipid accumulation is evidence of a rare lysosomal disorder, the lipase-deficient Wolman disease.

Since cardiomyocytes, though uninuclear cells, show a similar constitution as skeletal muscle fibers, they may also be affected in metabolic disorders; thus, skeletal muscle biopsy may serve as a substitute for cardiac biopsy.

Remember

The skeletal muscle is the primary and most important target for biopsy in mitochondrial diseases, in muscle-affecting glycogenoses, and in neutral lipid storage diseases. For proper diagnosis in skeletal muscle tissue, it is essential not only to perform a light and electron microscopic study but also to provide tissue for important biochemical and mitochondrial genomic investigations (mitochondrial diseases, glycogenoses, and lipid disorders). Preservation of unfixed frozen muscle tissue and archival storage is a diagnostic prerequisite.

Table 43.9 Up-to-date nosographic classification of childhood neuronal ceroid lipofuscinoses (NCL)

NCL, infantile (INCL) Santavuori-Haltia	*CLN1/PPT1*	1p32	Lysosomal palmitoyl protein thioesterase 1 (PPT 1)	GROD
	CLN14/KCTD7	7q11.21	KCTD7 protein	GROD, FP
NCL, late-infantile (LINCL) Jansky-Bielschowsky	*CLN2/TPP1*	CLN2: 11p15	Lysosomal tripeptidyl peptidase 1 (TPP 1)	CP
	CLN1			GROD
	CLN10			GROD
NCL, juvenile (JNCL) Spielmeyer-Sjögren-Vogt	*CLN3, CLN5, CLN9, CLN10,*	CLN3: 16p12	Transmembranous CLN3 protein (battenin)	FP
	CLN12/ATP13A2	1p36	ATP13A2 protein	Lamellar
NCL, Finnish variant (vLINCL)	*CLN5*	13q31–32	Soluble CLN5 protein	CP, FP
NCL, early-juvenile Indian/ Czech-Roma variant (eJNCL/ aLINCL)/partly lake-Cavanagh	*CLN6*	15q21–23	Transmembranous CLN6 protein	CP, FP
NCL, late-infantile Turkish variant (vLINCL)	*CLN7/MFSD8*	4q28.1–2	Transmembranous CLN7 protein	CP, FP
"Northern epilepsy"	*CLN8*	8p23	Transmembranous CLN8 protein	CP, FP
NCL, juvenile (JNCL)	*CLN9*	Not known	Unknown	GROD, CP, FP
NCL, congenital (CNCL), juvenile, late infantile	*CLN10/CSTD*	11p15	Cathepsin D	GROD

GROD granular osmiophilic deposits, *FP* fingerprint profiles, *CP* curvilinear profiles

43.10 Other Tissues

Morphologically nonsystematic observations concern biopsies of other tissues such as the kidney, lymph nodes, spleen, appendix, or even urinary sediment. The latter contains lysosomal residual bodies derived from degenerated sloughed-off renal tubular cells. The bone marrow is particularly useful in Gaucher and Niemann-Pick diseases.

Take-Home Messages

- Certain neurometabolic disorders are marked by cerebral and extracerebral morphological manifestations of an individual, i.e., lysosomal, peroxisomal, mitochondrial, and polyglucosan diseases, these being single-organelle/multi-organ disorders. The extracerebral biopsy facilitates and corroborates the diagnostic armamentarium in these conditions, according to selective tissue manifestation and accessibility by biopsy. The preferential sites for biopsy are the blood lymphocytes, skin, and rectum in lysosomal diseases, the skeletal muscle in mitochondrial diseases, the liver in peroxisomal disorders, and the skin and skeletal muscle in polyglucosan diseases. Electron microscopy is the most rewarding diagnostic technique requiring immediate post-bioptic fixation of the tissues, especially in lysosomal and peroxisomal diseases. Many mitochondrial diseases are also recognized in skeletal muscle by light microscopy including mitochondria-related enzyme histochemical preparations.

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Postmortem Investigations

44

Piero Rinaldo

Key Facts

- All cases of sudden and unexpected death in childhood should be evaluated for a possible underlying metabolic disorder.
- A history of "normal" newborn screening for metabolic disorders is not a sufficient reason to decline postmortem evaluation.
- The most important specimens to collect at autopsy are blood and bile, spotted on filter paper.

The high mortality rate that is associated with acute episodes of metabolic decompensation has led to a perceived association between sudden unexpected death in early life (www.sudc.org) and several inborn errors of amino acid, organic acid, ammonia detoxification, and energy metabolism (Dott et al. 2006). However, in most cases, these conditions do cause acute illness with obvious clinical symptoms that precede death by hours or days. The situation is significantly different when considering the large number of cases affected with a fatty acid oxidation (FAO) disorder who were diagnosed either postmortem or, retrospectively, after the identification of an affected sibling. The latter situation has become a relatively common event since the achievement of greater awareness (Bennett and Rinaldo 2001) and, particularly, because of the broad implementation of expanded newborn screening for these disorders (Watson et al. 2006). Based on numerous observations, it has been postulated that without preventive intervention (i.e., newborn screening) FAO disorders could be responsible for up to 5 % of children who die suddenly and unexpectedly from birth to 5 years of age, particularly among those with evidence of acute infection that routinely would not represent a life-threatening event (Boles et al. 1998). While newborn screening eventually will bring the number of unexpected fatalities in infants and children close to none (Rosenthal et al. 2015), it will take several decades to have the same impact on adults who were not screened at birth. Therefore, it would be prudent for medical examiners to keep FAO disorders in the differential diagnosis of sudden adolescent and adult death with diverse clinical history (Raymond et al. 1999; Randall et al. 2015).

Figure 44.1 shows a flow chart for the evaluation of sudden and unexpected death that is

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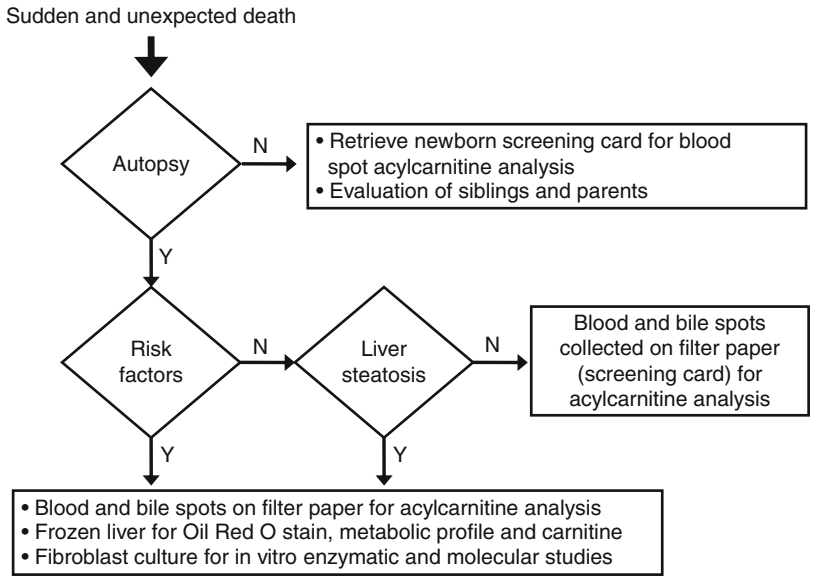

Fig. 44.1 Protocol for the postmortem screening of FAO disorders (From Rosenthal et al. (2015))

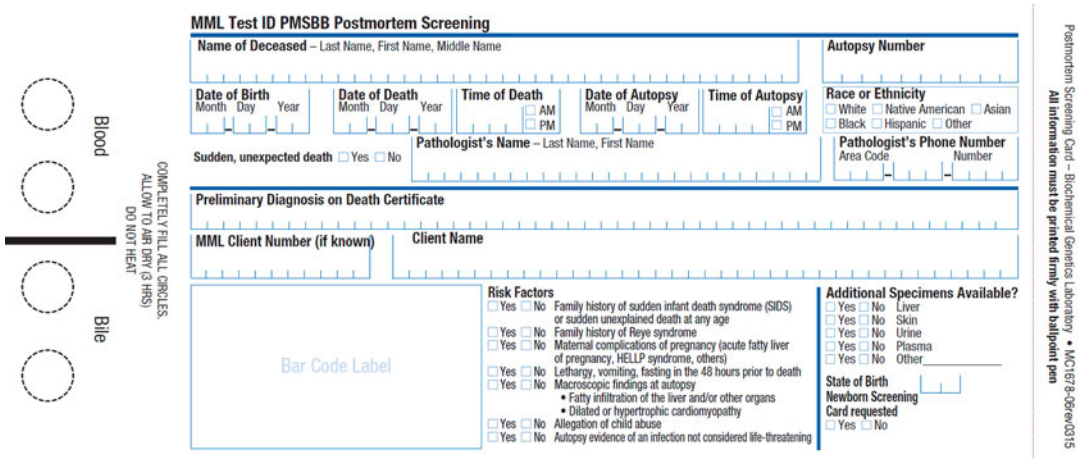

Fig. 44.2 Example of filter paper card for collection of postmortem blood and bile specimen

centered on the analysis of acylcarnitines in blood and bile spots (Rinaldo et al. 2002). Postmortem blood (Chace et al. 2001) and bile (Rashed et al. 1995) could be conveniently collected on a single filter paper card (Fig. 44.2), very similar to those used for newborn screening, which can be shipped at room temperature once properly dried. It is important to underscore the fact that both specimens should be collected in order to detect patients who may show mild or no apparent abnormalities in blood alone (Rinaldo et al. 2005). In cases with a higher level of suspicion, an effort should be made to collect a skin biopsy (Gray et al. 1995). It has been possible to grow a viable line of cultured fibroblasts from a biopsy of the Achille's tendon collected as long as 72 h after death.

Although fatty infiltration of the liver and/or other organs (heart, kidneys) is a common observation in FAO disorders, the finding of macroscopic steatosis cannot be used as the only criterion in deciding whether to investigate a possible underlying FAO disorder during the postmortem evaluation of a case of sudden death (Dott et al. 2006; Boles et al. 1998). Special attention should be paid to the risk factors listed in Table 44.1, and allegations of child abuse should also be fully investigated, with the exception of obvious cases of trauma/physical harm. The frozen skin biopsy could be discarded at a later time without further testing when a credible cause of death has been established, but could otherwise be crucial to reach a proper diagnosis and conclusive confirmation in vitro. If parental permission to perform an autopsy is not granted, it might be possible to retrieve leftover specimens collected during resuscitation efforts which may still be available in the clinical laboratory. In cases when no autopsy was performed, retrieval of any unused portion of the blood spots collected for newborn screening could be arranged via a request submitted in writing to the local laboratory (for a template, see The metabolic autopsy: postmortem screening in cases of sudden, unexpected death), as long as the period of storage, ranging from only a few weeks to indefinitely, had not expired already in the state where the patient was born (Lewis et al. 2011).

Table 44.1 Factors which increase the risk of an undiagnosed FAO disorder

Birth at a location not yet providing expanded newborn screening by MS/MS
Family history of sudden infant death syndrome (SIDS) or other sudden, unexplained deaths at any age
Family history of Reye syndrome
Maternal complications of pregnancy (acute fatty liver of pregnancy, HELLP syndrome, others)
Lethargy, vomiting, fasting in the 48 h prior to death
Macroscopic findings at autopsy
Fatty infiltration of the liver and/or other organs
Dilated or hypertrophic cardiomyopathy
Allegation of child abuse (excluding obvious cases of trauma, physical harm)
Autopsy evidence of infection that routinely would not represent a life-threatening event

44.1 Specimen Requirements

44.1.1 Blood and Bile

Blood specimen collection is usually drawn into heparin-containing tubes from the proximal aorta or by intracardiac puncture. Bile collection is obtained by direct puncture of the gallbladder. These specimens are well preserved when spotted on a filter paper card. Two circles of the card are used for blood, two circles for the bile (each 25 µl of volume). Blood and bile have to be dried before sending the filter paper card to the laboratory which performs the analysis. Relevant demographic patient information should be provided, together with a summary of relevant autopsy findings.

44.1.2 Skin/Tendon for Fibroblast Culturing

In cases with any risk factors, a 5 × 5-mm skin specimen should be collected and placed in culture media. If culture media is unavailable, the specimen can be placed in sterile saline. Although saline is not an optimal media for skin, it will be sufficient in most cases if the specimen is forwarded immediately for cell culturing. The skin specimen should be shipped at room temperature via overnight delivery.

44.1.3 Urine

If urine is present, it should be collected and stored for all cases of sudden, unexpected death. Urine may be collected on a second filter paper card by swabbing the bladder. The

specimen should be allowed to dry for 2–3 h. The urine specimen should be stored at room temperature until the results of the postmortem screen on blood and bile and the results from the original newborn screening card are available.

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Neuroradiology

45

Inga Harting and Nicole I. Wolf

As many children and adults with inherited metabolic disorders present with neurologic symptoms or develop these during the course of their disease, imaging of the brain is one of the most important diagnostic tools in this field. Magnetic resonance imaging (MRI) is the method of choice to assess changes of morphology and maturation as well as potential signal alterations. The indication for cranial computed tomography (CCT) should be limited to emergency situations in an unstable patient, trauma investigation with focus on fractures or cases with suspected calcification and indeterminate MRI findings. Otherwise MRI is preferable, in particular in patients with acute encephalopathy, due to the much greater sensitivity of MRI for alterations of brain parenchyma.

Interpretation of cerebral imaging studies is not easy, and care should be taken that in patients with the diagnosis or suspicion of an IEM, images should be reviewed personally together with a neuroradiologist experienced in this field. Even "normal" MRIs should be re-evaluated as some abnormalities escape detection by (neuro)radiologists unfamiliar with metabolic disorders. In infancy, normal brain maturation – the process of myelination, which is more or less completed at the age of 24 months at least in the MRI – has to be taken into account. Not only may changes be subtle, they may be all together lost if MR sequences are not adjusted for the higher water content and different relaxation of un- or incompletely myelinated brain. Imaging should be repeated if there are new neurologic symptoms, if there is regression and also if the first study has been done/acquired during the first 2 years of life in order to assess whether myelination has been completed.

45.1 Basic/Routine MRI Sequences

45.1.1 T2-Weighted and FLAIR Images

T2-weighted and FLAIR (fluid attenuated inversion recovery) images are basically water-weighted images and characterised by the dark signal of mature, myelinated white matter compared to cortex. As most pathological processes in the CNS are associated with increased water content, they are used to search for any abnormality. T2-hypointense signal is comparatively rare and

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due to either air, flow, blood degradation products and iron accumulation, calcifications or melanin. T2-weighted and FLAIR images differ by the signal of CSF which is bright on T2-weighted images and suppressed on FLAIR images, the latter facilitating the detection of lesions near CSF-containing structures, e.g. in periventricular white matter or cortex. One should keep in mind that the inversion pulse in FLAIR sequences is set to suppress the signal of free water, namely, normal CSF. Suppression of CSF will therefore (diagnostically) fail with increased protein content or blood degradation products in CSF or in some cases if the sulci are too thin. One should also remember that FLAIR images have limited sensitivity in neonates and infants.

45.2 T1-Weighted Images

On T1-weighted images, myelinated white matter is hyperintense (brighter) compared to cortex and CSF is strongly T1 hypointense. Signal on T1-weighted images is commonly hypo- or isointense in pathologies, while T1 hyperintensity is uncommon and may be caused by fat, blood degradation products (methaemoglobin), ferritin, calcifications, minerals (e.g. manganese), melanin and re- or hypermyelination.

45.3 Effect of Brain Maturation on T1- and T2-Weighted Images

Myelination results in contrast inversion between cortex and white matter which allows not only monitoring of brain maturation on MRI but also diagnosis of myelination changes. Unmyelinated white matter is hypointense compared to cortex on T1-weighted images and hyperintense on T2-weighted images. Signal changes due to myelination are seen earlier on T1-weighted images, which are sensitive to small amounts of myelin, whereas hypointense signal of myelinated white matter on T2-weighted images is only seen with larger quantities of compacting, more mature myelin. Faster T1 relaxation with increasing signal on T1-weighted images is due to increasing choline and glycolipid content of myelin, the increasing deposition of myelin membrane and the double-membrane structure of myelin with strong interaction between intramyelinic water and myelin lipids. Signal on T2-weighted images on the other hand is related to the density and relaxation properties of water molecules within the extracellular space and axons, reflecting the decreasing content of protons of myelinated white matter (Barkovich 2000; Barkovich et al. 1998).

In infants (and patients with hypomyelination), myelination is assessed best using T1w sequences. With the exception of the frontotemporal subcortical white matter, myelination is complete on T1-weighted images at the age of 9 months and on T2-weighted images around 18 months; myelination should be completed on T2-weighted images by the age of 2 years. Consequently after the age of 9 months, T2-weighted images are most informative regarding progress of myelination as well as involvement of white matter in metabolic and non-metabolic disorders.

Gyration, the increasing sulcal depth in relation to gyral width and ramification of gyri, is another indicator of brain maturation observable on MRI. An "adult" gyral pattern with a frontopolar sulcal depth at least equivalent to gyral width is usually present by the age of 3 months (van der Knaap et al. 1996).

Remember

Myelination is only completed at the age of 2 years. Some white matter changes are not visible (well) in unmyelinated white matter. It may be important to repeat imaging after the age of 2 years if the first MRI was acquired at a much younger age.

45.4 Pattern Recognition Approach in Interpreting MRI

A pattern recognition approach greatly facilitates differential diagnosis. In this approach, affected structures are analysed and compared with known disorders. It is a powerful method not only for

established disorders but also for the differentiation of new disorders, especially leukoencephalopathies, as detailed in the landmark article of van der Knaap and Schiffmann (2009) providing a systematic approach for the interpretation of MRI findings in this disease group. Typical and diagnostic patterns include the classical form of X-linked adrenoleukodystrophy (X-ALD), Canavan disease, infantile neuroaxonal dystrophy (INAD), pantothenate kinase-associated neurodegeneration (PKAN) or L2-hydroxyglutaric aciduria. Table 45.1 gives an overview of cerebral structures affected in important inborn errors of metabolism. The most obvious differentiation is between affected grey or white matter. If grey matter is involved, involvement of cortex, deep grey matter (putamen versus pallidum) and cerebellar grey matter must be differentiated, also increased (hyperintense) or decreased (hypointense) T2 signal.

If primarily white matter is involved, the first differentiation is that between hypomyelination and all other white matter changes. Hypomyelination is a separate category defined by a significant and permanent myelin deficit. It has to be distinguished from delayed myelination, in which myelination progresses, but only at a very slow pace. In children younger than 2 years, two MRIs at least 6 months apart must be made demonstrating unchanged myelination to prove the diagnosis of hypomyelination (Pouwels et al. 2014).

With all other white matter changes, one must assess the location of affected white matter (subcortical/arcuate fibres, central, periventricular, corpus callosum, brainstem), the gradient (symmetry, anterior vs. posterior white matter, central vs. peripheral white matter, rostral vs. caudal white matter) and other additional characteristics of white matter involvement (contrast enhancement, vacuolisation or cysts, calcifications, swelling, small vs. large and isolated vs. confluent lesions).

Remember

Pattern recognition is a powerful tool and allows the diagnosis of many disorders based on their typical MRI findings. This approach

Table 45.1 MRI Findings in inherited metabolic disorders

Grey matter	
Cerebellar atrophy	Nonspecific; present in many metabolic and degenerative conditions
High T2w signal of cerebellar cortex	INAD, Marinesco-Sjögren syndrome
Basal ganglia and thalamic involvement (symmetrical)	
T2 hyperintensity	Mitochondrial disorders, PDHc deficiency, Wilson's disease, organic acidurias, Alpers disease (often unilateral), if medial think of *SLC19A3*
T2 hypointensity (pallidum)	NBIA (including PKAN and INAD)
T2 hypointensity (thalamus)	INCL, Tay-Sachs disease, Krabbe disease, Salla disease
Dentate involvement	L2-hydroxyglutaric aciduria, cerebrotendinous xanthomatosis
Cerebral atrophy	Nonspecific, present in many neurodegenerative disorders
Stroke-like lesions	MELAS, Alpers disease, urea cycle disorders
Polymicrogyria	Zellweger disease and other peroxisomal disorders, fumarase deficiency
Cobblestone lissencephaly	Walker-Warburg syndrome and other O-glycosylation defects
White matter	
Delayed myelination	Nonspecific, present in many inherited and acquired conditions
Hypomyelination	Fucosidosis, Salla disease, galactosemia (mild), folate transporter (FOLR1) deficiency (inconstant)
Early involvement of subcortical white matter	Galactosemia, L2-hydroxyglutaric aciduria
Central white matter/ centrum semiovale	Krabbe disease, metachromatic leukodystrophy, X-ALD, phenylketonuria, Lowe syndrome, disorders of cytosolic methyl group transfer, various other disorders

(continued)

Table 45.1 (continued)

Miscellaneous	
Subdural effusions	Glutaric aciduria type I, Menkes disease
Elongated and tortuous arteries	Menkes disease
Reduced opercularisation	Glutaric aciduria type I (not specific)
Agenesis of the corpus callosum	PDHc deficiency, mitochondrial disorders, fumarase deficiency, SLO
Caudothalamic cysts	Zellweger syndrome
Calcifications	Mitochondrial disorders (especially Kearns-Sayre syndrome), folate deficiency. NBIA

INAD infantile neuroaxonal dystrophy, *MELAS* mitochondrial encephalopathy, lactic acidosis and stroke-like episodes, *NBIA* neurodegeneration with brain iron accumulation, *PDHc* pyruvate dehydrogenase complex, *PKAN* pantothenate-kinase-associated neurodegeneration, *SLO* Smith-Lemli-Opitz syndrome, *X-ALD* X-linked adrenoleukodystrophy

can avoid unnecessary and often expensive or invasive diagnostic tests. Abnormal findings on brain imaging should therefore always be revised by experts in this field.

45.5 Diffusion-Weighted Imaging

Diffusion-weighted imaging measures random movement of water molecules. Diffusion of water molecules results in increased dephasing of protons, which in turn results in signal loss. Unrestricted diffusion of water, e.g. in the CSF-filled ventricles, correspondingly results in a greater signal loss than restricted diffusion in normal parenchyma (Fig. 45.1b, e). Diffusion properties of brain parenchyma are predominantly determined by the extracellular space, while the intracellular space – packed with organelles and other obstacles to free water movement – contributes only little. In order to allow visualisation of these minuscule movements, ultrafast sequences used to freeze motion are combined with strong, opposed gradients applied to cancel coherent motion. As the sequences used are basically T2-weighted sequences, diffusion-weighted images simultaneously depict changes of T2 relaxation and of diffusion changes. It is consequently impossible to differentiate areas of T2 hyperintensity (Fig. 45.1a, d) and restricted diffusion – less movement resulting in lower signal loss with consequent hyperintensity – on diffusion-weighted images. Calculation of apparent diffusion coefficient (ADC) eliminates the T2 effect, allows identification of diffusion changes and is part of standard diffusion package provided with most MR scanners. ADC describes the overall gross mobility of water molecules and is usually averaged for the three directions in space. It is also possibly to quantify the magnitude of direction-dependent diffusion properties, e.g. fractional anisotropy, which however requires availability of diffusion tensor imaging which is not standard and more time consuming and will not be further covered here.

On ADC maps freely moving water, e.g. normal CSF, is shown as bright compared to the more restricted water diffusion within brain parenchyma. Areas of restricted diffusion in the brain are consequently darker than normal parenchyma (Fig. 45.1c), whereas areas in which water molecules can move more easily are hyperintense compared to normal parenchyma (Fig. 45.1e) and cystic areas have a signal equivalent to CSF. Although it is of course easier to look for bright signal on diffusion-weighted images than to search for lower signal on ADC maps, hyperintensity on diffusion-weighted images should always be counterchecked on ADC maps for corresponding lower signal in order not to misdiagnose an increased T2 signal as restricted diffusion (so-called T2 shine through, Fig. 45.1d–f). Moreover, one should keep in mind that diffusion-weighted imaging is sensitive to susceptibility artefacts, e.g. that calcification and haemorrhage alter the signal on diffusion-weighted images and ADC maps and that an area with subacute haemorrhage will apparently be diffusion restricted. This necessitates cross referencing with T1-, T2- and if available T2*-weighted gradient echo or susceptibility-weighted images, the latter two depicting susceptibility artefacts as hypointense areas.

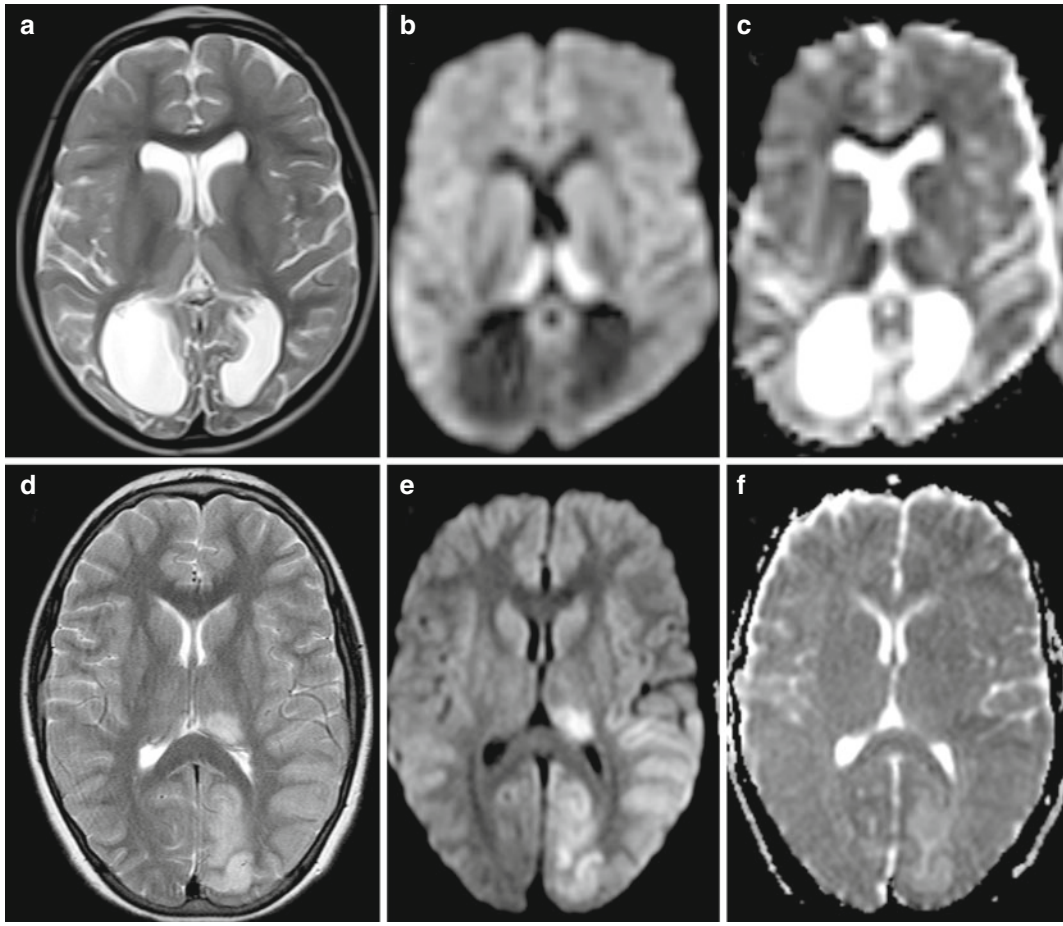

Fig. 45.1 Examples of diffusion-weighted imaging. (**a**–**c**) Symmetrical hyperintensity of the thalamus on T2w (**a**) and diffusion-weighted images (**b**) in a patient with Leigh-like syndrome. Hypointensity on the ADC map (**c**) signifies restricted diffusion. (**d**–**f**) Hyperintensity of the left thalamus, temporal and occipital lobes on T2w (**d**) and diffusion-weighted images (**e**) in a patient with Alpers disease, which is predominantly due to the so-called T2 shine-through effect as revealed by corresponding iso- and hyperintensity on the ADC map (**f**)

Increased diffusion is a common finding and associated with processes that increase the extracellular space, e.g. vasogenic oedema, gliosis, demyelination and hypomyelination. Vice versa restricted diffusion is found in circumstances associated with a decrease of the extracellular space, namely, cytotoxic oedema, intramyelinic oedema and highly cellular tumours; moreover, diffusion is restricted in abscess and empyema. Of note, restricted diffusion may be a transient phenomenon, e.g. with transient ischaemic attacks or epileptic seizures.

45.6 ^{1}H-MR Spectroscopy

^{1}H-MRS allows non-invasive in vivo detection of metabolites. Its basis is the so-called chemical shift phenomenon, namely, that the difference (shift) between a proton's resonance frequency and that of the externally applied magnetic field depends on the shielding effect of the proton's surrounding electron cloud which in turn is determined by its chemical bonds and neighbourhood. This difference of frequency allows identification of metabolites; it determines peak position on the frequency axis (x axis) and is usually expressed

as parts per million (ppm) relative to the scanner's frequency (e.g. approximately 63 MHz at 1.5 T). The chemical neighbourhood also determines a proton's relaxation, which is the process during which the proton releases the energy absorbed during excitation. Protons with fast relaxation, e.g. in lipids, can only be detected during a short span of time, whereas metabolites with longer relaxation times, e.g. lactate, emit their energy for some hundred milliseconds. The time between excitation and read-out of signal, the echo time (TE), therefore determines which metabolites will be visible in the spectrum. Long TE spectra (135 or 270 ms) only detect peaks from the four major compounds N-acetylaspartate (NAA), cholines, creatine and phosphocreatine (Cr) and lactate. Short TE spectra (≤30 ms) detect many more metabolites at the price of more complex spectra with overlapping resonances and more challenging quantification (Fig. 45.2a, b).

Sensitivity of MRS is limited by the low concentration of metabolites with resultant relatively large volumes of interest (≥3–4 ml at 1.5 T). Magnetic field inhomogeneities induced by interfaces with bone or air, large vessels, blood degradation products or calcifications will seriously hamper MSR. Under ideal conditions the baseline of an MR spectrum will be flat and the area under a peak is proportional to the number of spins creating the signal. Standard post-processing usually determines metabolite peak areas by integrating the area under the curve over a predetermined frequency interval, which works quite well for the major metabolites, NAA, Cr and Cho, though less reliably for myo-inositol (mIns) and small lactate resonances. For evaluation of additional metabolites detected by short TE spectra, phase and eddy current corrections as well as usage of a complex model function for peak identification and quantification are advisable. Metabolite concentrations are commonly expressed as concentration ratios with respect to Cr. While this approach might miss changes due to tissue rarefaction and/or increased water signal, absolute quantification is more complex due to influences of software and hardware parameters including water suppression, alterations of relaxation times with pathology, partial-volume effects, etc. Irrespective of the method of quantification, patient values should be compared to an age-matched control group examined at the same MR scanner with identical sequence parameters and head coil (Oz et al. 2014).

45.7 Major Metabolites

N-acetylaspartate (*NAA*) is a marker of neuroaxonal function and density as it is almost exclusively confined to neurons and axons in the mature brain. NAA is synthesised in neuronal mitochondria from aspartate and acetyl-CoA and hydrolysed in astrocytes. Its function remains unclear; among other functions it has been implicated as a precursor for the putative neurotransmitter NAAG, a storage form for the neurotransmitter aspartate, a donor of acetyl-CoA in brain lipid synthesis and an osmolyte. Its singlet peak from the N-acetyl-methyl resonance at 2.02 ppm is the most prominent peak in normal spectra. The much smaller resonance of N-acetyl-aspartyl glutamate (NAAG) at 2.05 ppm overlaps with NAA resulting in a shoulder of the peak. It cannot be reliably resolved from NAA and the N-acetyl resonances are usually evaluated together as tNAA.

The singlet peak at 3.02 ppm is due to the methyl groups of *creatine and phosphocreatine* (*tCr*) with minor contributions from GABA, lysine and glutathione. A smaller peak from the methylene resonances of tCr is found at 3.93 ppm. tCr is located in the intracellular compartment, and, at least in rats, its concentration in glial cell exceeds that of neurons and has been considered a marker for cellular density. It is often used as an internal reference for quantification as it is considered to remain stable in most situations; however changes have been observed in childhood white matter disorders where tCr was the most important discriminative MRS parameter.

Cholines (*Cho*) are compounds of membrane phospholipids and practically invisible while membrane bound due to their very fast relaxation. The major contribution to the singlet peak at 3.22 ppm comes from water-soluble choline, glycerophosphocholine and phosphocholine. The

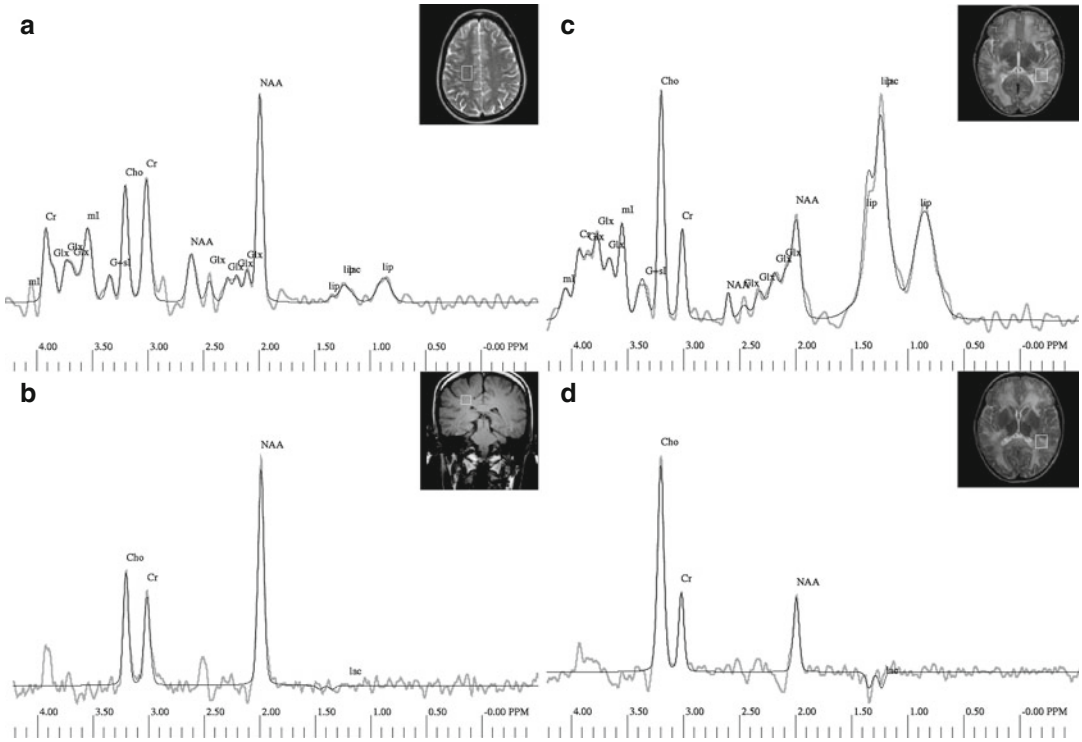

Fig. 45.2 Examples of ^{1}H-MR spectroscopy. (**a**, **b**) Control white matter spectra with short (**a**) and long (**b**) echo time. NB software on most scanners will fit all potential metabolites as in B where lactate is fitted to noise. (**c**, **d**) Short (**c**) and long (**d**) echo time spectra of demyelination in a patient with X-ALD with elevated Cho and Lip, mildly elevated mIns. The small lactate peak seen at long echo time (**d**) is hidden by the overlapping Lip resonances at short echo time (**c**)

Cho resonances are indicators for the rate of turnover and density of membranes; they are elevated with myelination, demyelination, inflammation and tumour proliferation.

J-coupling between its methyl groups results in the characteristic *lactate* (*Lac*) doublet at 1.33 ppm, which is upright at short TE and 270 ms but inverted with a TE of 135 ms. Trace amounts may just be detectable in the visual cortex with a concentration of 0.5–1.0 mM but is hardly detected anywhere else in the normal brain. Lac accumulates if the glycolytic rate exceeds the capacity for catabolism or transportation via blood flow. This occurs with increased anaerobic glycolysis, e.g. energy failure and defects of oxidative phosphorylation, or elevated numbers of cells dependent on anaerobic glycolysis, namely, macrophages and neoplastic cells.

With short TE spectra, the most obvious additional peak is that of the collapsed multiplet resonance of *myo-inositol* (*mIns*) at 3.56 ppm. Additional, minor contributors to this peak are inositol monophosphate, phosphatidyl inositol and inositol diphosphate. Being only present in glia, mIns is a glial marker and increases with astrogliosis. As a compound of myelin phospholipids, it is linked with growth and destruction of myelin membranes. It is moreover an osmoregulator and precursor for the inositol-dependent calcium release, for which it might provide a storage form.

With short TE, an additional complex pattern of coupled, small resonances becomes visible between 2.1 and 2.5 ppm and around 3.8 ppm, which are assigned to *glutamate* (*Glu*) and *glutamine* (*Gln*). Glu, the most important excitatory neurotransmitter, is released from Gln in the

presynaptic neuron and then either converted to *GABA*, the most important inhibitory neurotransmitter with resonances at 1.90, 2.30 and 3.03 ppm, or released into the synaptic cleft. Released Glu is taken up by the astrocyte and processed to Gln which then is transported back to the presynaptic neuron. Considering their localisation, Gln may be considered a glial marker and Glu a neuronal marker; reliable detection of all three overlapping metabolites, in particular of Gln and GABA, is difficult.

Broad macromolecular and free lipid (Lip) resonances around 0.9 and 1.3 ppm are also seen with short TE. Elevated free lipids are best seen at short TE. They occur with catabolic processes, e.g. acutely demyelinating lesions. If overlap of Lip resonances precludes identification of Lac, the diminished or absent Lip signal and inverted Lac doublet at 135 ms will generally allow differentiation.

45.8 Patterns of Metabolite Changes

Three main groups of metabolite changes can be observed in neurometabolic disorders, namely, (1) changes related to structural alterations, e.g. changes of myelin, glial and neuronal cells, (2) elevated lactate indicating impaired oxidative phosphorylation and (3) disease-specific changes due to the enzymatic defect (Oz et al. 2014).

In active *demyelination* (Fig. 45.2c, d), membrane turnover is increased; Cho and in some cases Lip are elevated. Lac may be elevated and is thought to be due to infiltration of macrophages. Neuronal damage and loss are reflected by lower tNAA. mIns increases due to increased membrane turnover and gliosis. In end-stage demyelination Cho, tCr and tNAA are decreased and the spectrum is dominated by high mIns reflecting white matter rarefaction and gliosis.

In hypomyelinating diseases synthesis and turnover of myelin membranes are low and on MRS Cho is often low. Elevation of tCr and mIns is the most characteristic finding when comparing hypomyelination with demyelination, myelin vacuolation and cystic white matter degeneration indicating gliosis. In a study myelin vacuolation was associated with marked decreases of tNAA, tCr and Cho with near-normal mIns consistent with axonal damage or loss and with astrocytic proliferation, whereas patients with cystic white matter degeneration were characterised by the decrease of all normal metabolites and the presence of lactate (van der Voorn et al. 2006).

Disease-specific metabolites result from absence or abnormal accumulation of metabolites due to the underlying enzymatic defect (Table 45.2). One example is the massive reduction of tCr in creatine deficiency syndromes, which may be accompanied by a guanidinoacetate peak at 3.78 ppm in patients with deficiency of guanidinoacetate methyltransferase. The absence of the NAA peak has as yet remained a single finding. Abnormally increased disease-specific metabolites are succinate in deficiency of succinate dehydrogenase, NAA in Canavan disease, branched-chain amino acids in maple syrup urine disease, glycine in non-ketotic hyperglycinaemia, arabitol and ribitol resonances in defects of polyol metabolism, elevated GABA in GABA transaminase deficiency or glutaric acid in glutaric aciduria type 1 (Harting et al. 2015).

45.9 Suggested Standard MRI Protocol

A minimal protocol should include axial T2-, T1-weighted and FLAIR images and sagittal T1-weighted images, preferably acquired as a 3D sequence with reconstruction in coronal and axial planes. In case of cerebellar abnormalities, coronal images are particular informative and additional T2-weighted or FLAIR images in the coronal plane helpful. Diffusion-weighted imaging is fast and if available should be included in the standard protocol and particularly requested with suspected stroke-like episodes or “metabolic stroke”. In addition gradient echo T2-weighted or suscep-

Table 45.2 Typical ^{1}H-MRS abnormalities in inherited metabolic disorders

^{1}H-MRS finding	Disease
Absent/massively reduced creatine peak	Creatine synthesis (GAMT, AGAT) and transport (CRTR) deficiency
Elevated lactate	Mitochondrial disorders, pyruvate dehydrogenase deficiency, other IEMs, non-metabolic conditions (ischaemia, infection, neoplasia)
Elevated polyols	Ribose-5-phosphate isomerase deficiency
Strongly increased NAA	Canavan disease
Absent NAA	Aspartate N-acetyltransferase defect
Increased glycine	Nonketotic hyperglycinaemia
Increased choline	Demyelination (e.g. metachromatic leukodystrophy, X-ALD)
Increased succinate	Complex II deficiency
Increased lipids	Sjögren-Larsson syndrome, demyelination

tibility-weighted images are helpful in detecting blood degradation products and calcification, while T1-weighted inversion recovery sequences increase the contrast between cortical grey and white matter and thereby the detection of associated malformations of cortical development.

^{1}H-MRS is a valuable addition, but one should be aware that the diagnostic yield differs with the available post-processing technique: A long or short TE spectrum with standard post-processing will show a sufficiently large increase or decrease of NAA and/or Cho and a sufficiently large increase of mIns and lactate. It will identify Cr-deficiency syndromes with their massive reduction of Cr and the clearly increased NAA of Canavan disease in conjunction with a typical MR pattern. Anything else will require dedicated post-processing, which usually requires manual archiving as spectroscopy raw data are commonly not saved in the digital radiological viewing system. In case of single-voxel spectroscopy, voxels should be placed in areas of signal abnormality or if absent in basal ganglia and white matter of the centrum semiovale.

45.10 Neuroradiologic Findings in IEM

This overview is by no means complete. We aim at providing a framework for interpreting the most pertinent anomalies in brain imaging. The most important and frequent disorders are covered.

45.10.1 Associated Brain Malformations

Occasionally, IEMs lead to brain malformations (Fig. 45.3). The cortex is often involved, as seen with the cobblestone cortex of Walker-Warburg syndrome (Fig. 45.3a–c), a disorder of O-glycosylation, or with perirolandic and perisylvian polymicrogyria in Zellweger disease (Fig. 45.3d–f) and other peroxisomal disorders. In Walker-Warburg syndrome, the cerebellum and brainstem are also highly abnormal, with a characteristic silhouette of pons and medulla oblongata on sagittal images. Cerebellar cortical cysts can also be present in O-glycosylation defects. Fumarase deficiency can lead to polymicrogyria and agenesis of the corpus callosum. The latter can also be seen in pyruvate dehydrogenase complex deficiency. Cerebellar hypoplasia is a feature of congenital disorders of glycosylation (CDG) type 1, now called PMM2-CDG, and other congenital disorders of glycosylation. Midline abnormalities including holoprosencephaly and malformations of the corpus callosum may be found in Smith-Lemli-Opitz syndrome. Partial corpus callosum agenesis is seen in some patients with *EARS2* mutations.

45.10.2 Grey Matter Disorders

45.10.2.1 Generalised Atrophy

In many IEM primarily affecting grey matter, brain imaging shows supratentorial or generalised atrophy, which is a nonspecific finding.

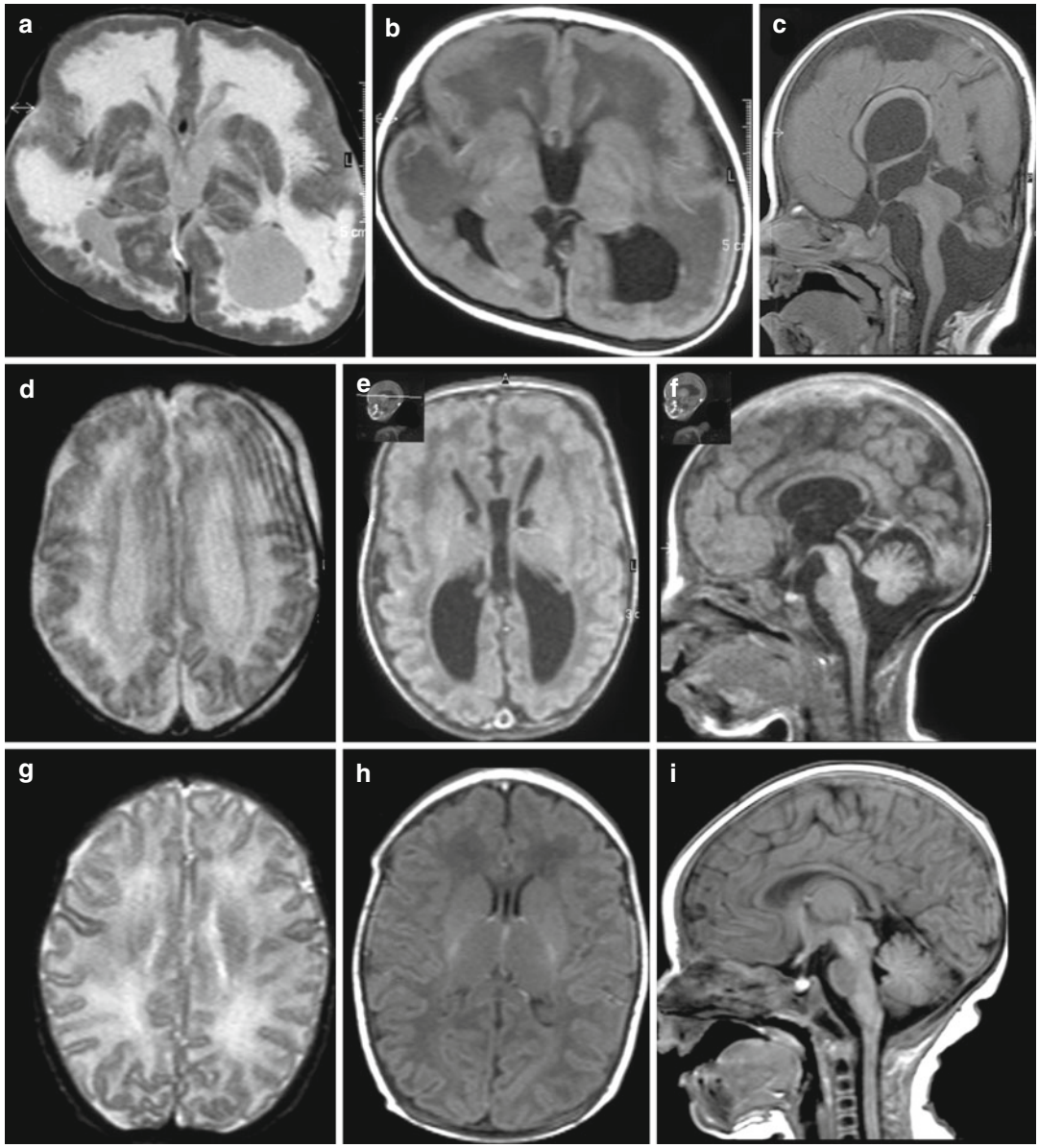

Fig. 45.3 Examples of metabolic disorders with associated brain malformations (**a**, **d**, **g**: T2w; **b**, **c**, **e**, **f**, **h**, **i**: T1w; **g**–**h** normal neonate for comparison). (**a**–**c**) Walker-Warburg syndrome with cobblestone cortex and characteristic brainstem silhouette on sagittal images resulting from hypoplasia of pons, enlarged tectum and a "kink" in the dorsal pons; the cerebellum is dysplastic. (**d**–**f**) Zellweger syndrome with polymicrogyria and germinolytic cysts near the foramina of Monroi; the basis pontis and vermis are mildly hypoplastic

Often, these disorders present with dementia and motor decline, as, e.g. Niemann-Pick disease type C or the neuronal ceroid lipofuscinoses. In primarily neuronal disorders with postinfantile-onset characteristic but unspecific, MR changes comprise a hazy, slightly elevated signal of periventricular white matter, loss of contrast at the border of cortex and white matter and thinned cortical ribbon. Early-onset profound atrophy of the cerebrum, cerebellum and brainstem (also called pontocerebellar hypoplasia type 6) is a feature of recessive mutations in

RARS2. If grey matter disorders start early in life, myelination is, sometimes severely, impaired, which might guide investigations in the wrong direction. Early severe atrophy and grey matter symptoms as prominent epilepsy point to a primary neuronal process and help with the differential diagnosis.

45.10.2.2 Involvement of Deep Grey Matter Structures

Basal ganglia involvement is found in many different IEMs. Mitochondrial disorders, especially Leigh syndrome, have a predilection for the basal ganglia. In the acute phase, these structures show swelling with elevated T2 signal. In the chronic phase, T2 signal may stay increased, but volume usually decreases. Often, these abnormalities are symmetric, but they can be unilateral. Other disorders with energetic failure also involve the basal ganglia. T2 signal elevation of basal ganglia, albeit usually milder, can also be found in Wilson's disease (and in some patients, there may also be T2 hypointensity), sometimes in combination with the face of the giant panda sign in the mesencephalon (Bandmann et al. 2015). Recently, a distinctive pattern of basal ganglia involvement has been described in MEGDEL (3-methylglutaconic aciduria with deafness, encephalopathy and Leigh-like syndrome) with a partially spared putamen (Wortmann et al. 2015) (Fig. 45.4c). Involvement of the medial thalamus should prompt investigation for *SLC19A3* deficiency, a treatable IEM, which, in its most severe form, leads to early-infantile onset with involvement of virtually all brain structures including basal ganglia and thalami. Symmetric T2 hyperintensity of the pallidum can be seen in pyruvate dehydrogenase complex deficiency. The characteristic pattern of symmetric striatal (and concomitant pallidal) changes together with wide anterior temporal, and Sylvian CSF spaces have become rare with presymptomatic diagnosis and treatment of glutaric aciduria type I (Fig. 45.4a, b). T2 hyperintensity of the pallidum is not infrequently seen in asymptomatic patients possibly related to delayed myelination. Widened CSF spaces may normalise with treatment and white matter changes, increasing with age, and predominantly periventricular may be the only finding (Harting et al. 2009).

T2-hypointense signal of the pallidum indicates abnormal iron accumulation within the spectrum of neurodegeneration with brain iron accumulation (NBIA; Fig. 45.4f, g). A spot of hyperintensity, interpreted as gliosis, within the hypointense pallidum is called eye of the tiger and suggests PKAN, but has recently also been associated with mutations in *COASY*, causing NBIA6. In combination with cerebellar atrophy and signal elevation of the cerebellar cortex, best visualised on FLAIR images, it points to INAD (Fig. 45.4h, i). Other structures, which may be involved in abnormal iron accumulation with a resulting T2-hypointense signal, are nucleus ruber and substantia nigra and the dentate nucleus (Ward et al. 2014).

T1-hyperintense signal of the pallidum in the context of IEM can be found in liver disease including Wilson's disease and in manganese accumulation, e.g. from parenteral nutrition, but has recently also been described as secondary to mutations in *SLC30A10* coding for a manganese transporter.

45.10.2.3 Cortical Involvement

Cortical involvement is seen in acute presentations of certain mitochondrial disorders, especially MELAS and Alpers disease (Fig. 45.4d, e). Cortical signal is increased on T2-weighted images, and the cortex is swollen. Subcortical white matter involvement is possible. These abnormalities do not correspond to occlusion of one of the large arteries and are therefore also called stroke-like episodes. Clinically, this is usually accompanied by frequent epileptic seizures or even refractory status epilepticus, and it is difficult to decide whether seizures lead to these cortical abnormalities or vice versa. The parietooccipital cortex is most frequently involved. In Alpers disease, this is usually accompanied by abnormalities of the ipsilateral dorsal thalamus. In the chronic phase, local cortical and subcortical atrophy may ensue. *SLC19A3* deficiency can present with multifocal cortical signal abnormalities; a diagnostic clue is the involve-

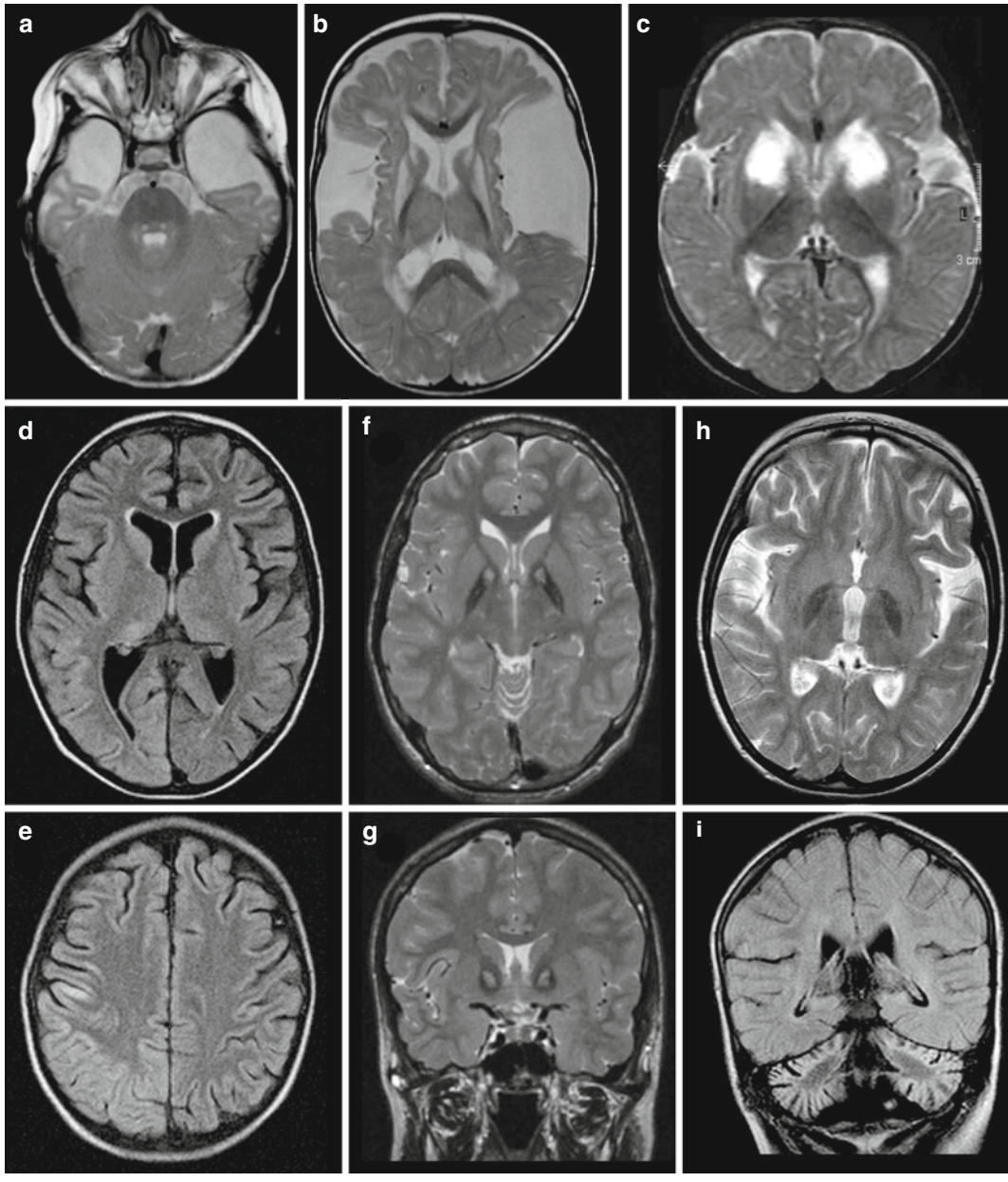

Fig. 45.4 Examples of disorders predominantly affecting grey matter (**a**–**c**, **f**–**h**: T2w, **d**, **e**, **i**: FLAIR). (**a**, **b**) Glutaric aciduria type 1 after an acute encephalopathic crisis with basal ganglia lesions and massive widening of anterior temporal and Sylvian CSF spaces; additional supratentorial white matter changes and central tegmental tract hyperintensity. (**c**) MEGDEL with typical partial sparing of dorsal putamen. (**d**, **e**) Hyperintensity of the right dorsal thalamus in combination with predominantly right parieto-occipital cortical swelling and hyperintensity in Alpers disease. (**f**, **g**) The so-called eye of the tiger in PKAN resulting from central T2-hyperintensity within the hypointense pallidum. (**h**, **i**) Hyperintensity of the cerebellar cortex in combination with a T2-hypointense pallidum in INAD

ment of the medial thalami. Menkes disease shows signal abnormalities of cortex, basal ganglia and white matter, with often severe and early atrophy, which may even lead to subdural

effusions. Tortuous vessels are a characteristic finding. Wormian bones are present on skull X-rays. Rib fractures are possible, due to the connective tissue involvement in this disorder, and can prompt suspicion of nonaccidental injury, especially when found in combination with subdural hygroma.

45.10.2.4 Cerebellar Atrophy

Cerebellar atrophy is a nonspecific finding and has a broad differential diagnosis including IEM (Poretti et al. 2008). It can accompany other abnormalities as supratentorial atrophy, signal abnormalities of the deep grey matter structures or signal abnormalities of the cerebellar cortex itself.

45.10.3 White Matter Disorders

Genetic white matter disorders, or leukodystrophies, mostly come with characteristic MRI abnormalities. Pattern recognition often allows making a diagnosis, confirmed by the appropriate metabolic or genetic test. It should be kept in mind that many of the inherited white matter disorders do not, strictly speaking, belong to the large group of IEM. Metabolic investigations are therefore not always necessary when confronted with the MRI diagnosis of leukodystrophy.

45.10.3.1 Lysosomal Disorders

The most frequent disorder in this group is metachromatic leukodystrophy (MLD). In late-infantile cases, T2-hyperintense white matter abnormalities start in the splenium and the parieto-occipital white matter, sparing the subcortical fibres. In juvenile cases, there is no posterior-anterior gradient, and in adult-onset cases, the frontal white matter is more affected. Especially in late infantile and juvenile cases, there are perivascular stripes of lower signal intensity, due to sulfatide accumulation, leading to the so-called tigroid pattern which is a characteristic of MLD (Voorn et al. 2005; Fig. 45.5c, d). Projection fibres in the internal capsule and later also mesencephalon and brainstem are also affected. Involvement of cerebellar white matter occurs only late. Atrophy especially of the thalami is frequent, and in later stages, global atrophy ensues. Contrast studies may show enhancement of cranial nerves and lumbosacral nerve roots.

Krabbe disease or globoid cell leukodystrophy (GLD) can also lead to a stripe-like pattern in the hemispheric white matter. Characteristically, projection fibres are involved (the pyramidal tracts in the crus posterius of the internal capsule, mesencephalon and brainstem) as well as the cerebellar white matter and the hilus of the dentate nucleus (Fig. 45.5e–g). These structures are abnormally hyperdense in CT studies. In the rare later-onset forms, selective involvement of the corticospinal tracts is typical.

Tay-Sachs disease is strictly speaking a grey matter disorder, but in its infantile form, white matter involvement is typical, sometimes mistaken for hypomyelination. The signal of the basal ganglia and thalami, which appear swollen, is hyperintense on T2-weighted images.

Fucosidosis is one of the few IEMs leading to hypomyelination. A prominent T2-hypointense signal of the pallidum and substantia nigra, less of the thalami, points to this diagnosis.

Fabry disease leads to a vasculopathy, and consequently, brain imaging shows multiple lacunar infarcts and multifocal T2-hyperintense white matter lesions. Larger infarcts and haemorrhages are possible. The pulvinar may show calcifications.

Mucopolysaccharidoses do not have typical white matter abnormalities. Prominent perivascular spaces can be a clue to this diagnosis, but are by no means obligate.

45.10.3.2 Organic Acidurias and Mitochondrial Disorders

L2-hydroxyglutaric aciduria leads to multifocal T2-hyperintense signal abnormalities predominantly in the subcortical white matter in combination with involvement of the pallidum and

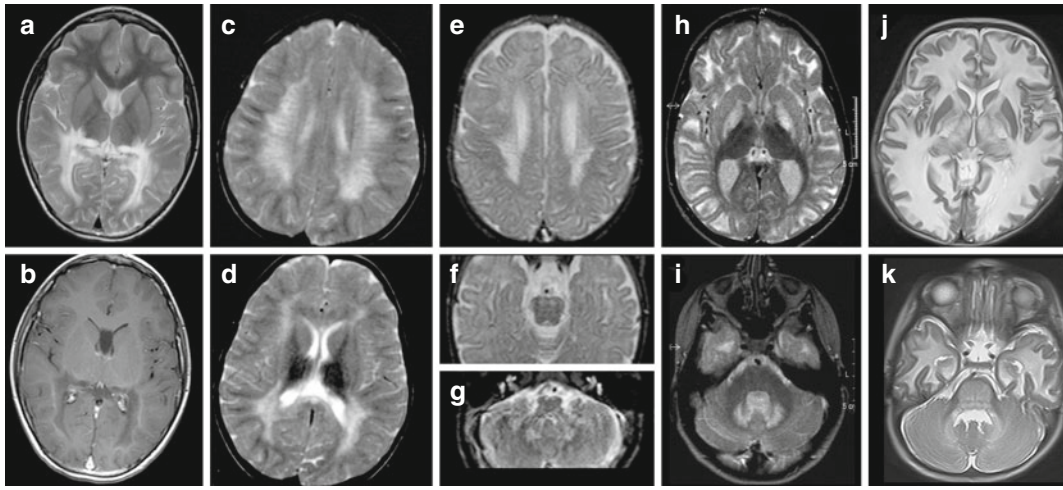

Fig. 45.5 Examples of disorders predominantly affecting white matter (**a**, **c**–**k**: T2w, **b**: T1w+GAD). (**a**, **b**) Characteristic parieto-occipital white matter changes in X-ALD the outermost zone of active demyelination extending anteriorly into internal and external capsules (**a**), enhancement of the middle zone with inflammatory activity and T1 hypointensity, T2 hyperintensity of the innermost, burnt-out zone. (**c**, **d**) MLD with a tigroid pattern of periventricular and lobar white matter changes and involvement of the splenium. (**e**–**g**) A tigroid pattern can also be observed in GLD; note the early involvement of the hilus of the dentate nuclei and brainstem pyramidal tracts. (**h**, **i**) L2-hydroxyglutaric aciduria with characteristic, predominantly subcortical white matter changes and T2 hyperintensity of pallidum and dentate nucleus. (**j**, **k**) Canavan disease with diffuse T2 hyperintensity of supratentorial *white* matter, involvement of brainstem and cerebellum and T2-hyperintensity of the pallidum and thalamus

dentate nucleus (Fig. 45.5h, i). Canavan disease causes diffuse T2 signal elevation of the entire supratentorial white matter with swelling; also globus pallidus and thalamus are abnormal as well as brainstem tracts and cerebellar white matter (Fig. 45.5j, k). Propionic acidemia can cause diffuse involvement of the subcortical white matter in addition to signal abnormalities of deep grey matter structures and cerebellar cortex.

Mitochondrial disorders often have predominant grey matter abnormalities, but some involve white matter. Kearns-Sayre syndrome leads to abnormalities of subcortical and deep white matter, with relative sparing of the periventricular white matter, of the deep grey matter structures (especially globus pallidus and thalamus), midbrain, brainstem and cerebellar white matter. A pathognomonic pattern of widespread supratentorial white matter and cerebellar cortical involvement is seen in complex I deficiency due to mutations in *NUBPL* (Kevelam et al. 2013).

45.10.3.3 Disorders of Amino Acid Metabolism

Patients with phenylketonuria (PKU) can develop mild, multifocal, partly confluent periventricular white matter signal abnormalities. Neonates with non-ketotic hyperglycinaemia show elevated T2 signal of the slightly swollen supratentorial white matter and impaired diffusion of the corticospinal tracts, middle cerebellar peduncles and cerebellar white matter. Glycine is elevated on ^{1}H-MRS. In the neonatal form of maple syrup urine disease (MSUD), the corticospinal tracts show elevated signal and swelling, accompanied by impaired diffusion and swelling of the midbrain and also dorsal part of the pons.

In disorders with hyperhomocysteinaemia, both diffuse and multifocal white matter abnormalities are possible as well as sequelae of vascular events (lacunar infarctions and larger strokes). In the spinal cord, dorsal and lateral columns may be affected. In severe cases with early onset, progressive hydrocephalus can be present.

45.10.3.4 Peroxisomal Disorders

X-linked adrenoleukodystrophy (X-ALD) is the most frequent inherited white matter disorder. The childhood cerebral form shows pathognomonic MRI abnormalities (Fig. 45.5a, b). White matter abnormalities typically commence in the splenium and symmetrically spread to the parietooccipital white matter. The white matter lesion consists of three zones: the innermost zone has the highest signal on T2-weighted images, followed by a zone with lower signal (with contrast enhancement, corresponding to inflammatory activity) and then again by a zone with high signal, the actively demyelinating zone. Calcifications can be present. Less frequently, the disease starts in the frontal white matter or in the corticospinal tracts or presents with asymmetric lesions. In adrenomyeloneuropathy (AMN), corticospinal tracts may be prominently involved.

Zellweger syndrome leads to perisylvian polymicrogyria and, in slightly older children, to involvement of the periventricular white matter, corticospinal tracts and cerebellar white matter including the hilus of the dentate nucleus. Peroxisomal D-bifunctional protein deficiency leads to similar abnormalities. It should be kept in mind that in some patients, plasma investigation of peroxisomal abnormalities may remain negative, and diagnosis can only be confirmed by fibroblast studies.

45.10.3.5 Defects of tRNA Synthesis

This growing group of disorders has recently been described and comprises defects of cytosolic and of mitochondrial amino acid tRNA synthetases. In the latter group, elevated lactate in body fluids and the brain is possible; the first group lacks metabolic markers. Some of these disorders have specific MRI patterns. Mutations in *DARS2*, coding for the mitochondrial aspartyl tRNA synthetase, were the first described in this group and cause leukoencephalopathy with brainstem and spinal cord involvement and elevated lactate (LBSL). MRI shows a pathognomonic pattern of multifocal supratentorial white matter involvement in combination with elevated T2 signal of posterior columns and lateral corticospinal tracts in the spinal cord and of inferior and superior cerebellar peduncles, the medial lemniscus, the mesencephalic trigeminal tracts, the intraparenchymal parts of the trigeminal nerves and the corticospinal tracts in the pons. Lactate is often elevated. Recessive mutations in *DARS*, encoding the cytosolic aspartyl tRNA synthetase, lead to hypomyelination with brainstem and spinal cord involvement and severe leg spasticity (HBSL). Some of the affected structures in brainstem and spinal cord are the same as in LBSL (posterior columns and lateral corticospinal tracts in the spinal cord, inferior and superior cerebellar peduncles, the medial lemniscus and the pontine corticospinal tracts). Mutations in *RARS* cause hypomyelination. Mutations in *EARS2* cause leukoencephalopathy with thalamus and brainstem involvement and high lactate (LTBL). Strikingly, partial recovery of these severe abnormalities is possible. *AARS2* mutations lead to a leukoencephalopathy with adolescent or adult onset, rapid decline and, on MRI, involvement of left-right connections and descending tracts.

45.10.3.6 Miscellaneous Disorders

Adult polyglucosan body disease (or glycogen storage disease type IV) shows multifocal T2-hyperintense signal abnormalities, which can be confluent in later stages and also involve the anterior temporal lobe. Cerebellar white matter and brainstem are also involved, the latter being visualised best by sagittal FLAIR images. In galactosemia type 1, myelination is severely delayed, resulting in incomplete myelination, and mild global atrophy may develop. Lowe (oculocerebrorenal) syndrome shows multifocal white matter lesions and small cysts in the periventricular white matter.

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Appendix

Differential Diagnosis of Clinical and Biochemical Phenotypes

Acidosis
Acrodermatitis, enteropathica
Hyperchloremic diarrhea
Infectious
Lactase deficiency
Sucrase deficiency
Renal tubular acidosis (*RTA*)
Cystinosis
Fanconi syndrome
Galactosemia
Glucose, galactose malabsorption
Hepatorenal tyrosinemia
Mitochondrial disorders (especially Pearson and Kearns–Sayre syndromes, *BCS1L* mutations)
Osteopetrosis and RTA
Topiramate

Alopecia
Acrodermatitis enteropathica
Adrenoleukodystrophy/adrenomyeloneuropathy
An (hypo) hidrotic ectodermal dysplasia
Biotin deficiency
Cartilage–hair hypoplasia
Congenital erythropoietic porphyria
CEDNIK syndrome (patchy)
CHILD syndrome (unilateral)
Conradi–Hünermann syndrome
Hemochromatosis
Hutchinson–Gilford progeria
MACS syndrome
Methylmalonic acidemia
Multiple carboxylase deficiency – holocarboxylase Synthetase and biotinidase deficiencies
Porphyria cutanea tarda type II
Propionic acidemia
Rhizomelic chondrodysplasia punctata type I
Trichorrhexis nodosa-argininosuccinic aciduria
Vitamin D-dependent rickets-receptor abnormalities

Angiokeratomas
Aspartylglucosaminuria
Fabry disease
Fucosidosis
Galactosialidosis
GM_1 gangliosidosis
Mannosidosis
Sialidosis
Schindler disease type II

Apparent Acute Encephalitis
Alpers disease
Biotin-responsive basal ganglia disease or thiamine-responsive encephalopathy
Glutaric aciduria I
LARS mutations
Mitochondrial disorders (especially *POLG* mutations, NARP)
Propionic acidemia

Arthritis
Alkaptonuria
Farber disease
Gaucher type I
Gout-HPRT deficiency; PRPP overactivity

G.F. Hoffmann et al. (eds.), *Inherited Metabolic Diseases*, DOI 10.1007/978-3-662-49410-3

Homocystinuria
I-cell disease
Lesch–Nyhan disease
Mucolipidosis III
Mucopolysaccharidosis IS; II

Bleeding Tendency
Abetalipoproteinemia
α-1-Antitrypsin deficiency
Congenital disorders of glycosylation (CDG)
Chediak–Higashi syndrome
Fructose intolerance
Gaucher disease
Glycogenoses types I and IV
Hermansky–Pudlak syndrome
Peroxisomal disorder
Tyrosinemia type 1

Calcification of Basal Ganglia
Albright syndrome
Bilateral striato-pallido-dentate calcinosis
Carbonic anhydrase II deficiency
Cockayne syndrome
Down syndrome
Fahr disease
Familial progressive encephalopathy with calcification of the basal ganglia (Aicardi–Goutières syndrome)
GM_1 gangliosidosis
Hypo-, hyper-parathyreoidism
Krabbe leukodystrophy
Lipoid proteinosis
Microcephaly and intracranial calcification
Mitochondrial disorders (especially Kearns–Sayre syndrome)
Multiple endocrine neoplasia I
Pantothenate kinase-associated neurodegeneration (PKAN, formally Hallervorden–Spatz disease)
Neurofibromatosis
Pterin defects
  Dihydropteridine reductase deficiency
  GTP cyclohydrolase I deficiency
  6-pyruvoyl-tetrahydropterin synthase deficiency
  Sepiapterin reductase deficiency
Spondyloepiphyseal dysplasia

Cardiomyopathy
Congenital muscular dystrophy
Danon disease
Disorders of fatty acid oxidation
Fabry disease
Glycogenosis type III
Hemochromatosis
D-2-Hydroxyglutaric aciduria
3-Methylglutaconic aciduria (Barth syndrome, Sengers syndrome, TMEM70 deficiency, dilated cardiomyopathy with ataxia (DCMA) with mutations in *DNAJC19* gene)
Mitochondrial disorders (other)
Mucopolysaccharidoses
α-Mannosidosis
Pompe disease

Cataracts: Lenticular Opacity
Cerebrotendinous xanthomatosis
Δ1-pyrroline-5-carboxylate synthetase deficiency
Cholesterol biogenesis disorders
Fabry disease
Galactokinase deficiency
Galactosemia
Homocystinuria
Hyperferritinemia-cataract syndrome
Hyperornithinemia (ornithine aminotransferase deficiency)
Lowe syndrome
Lysinuric protein intolerance
Mannosidosis
Mevalonic aciduria
Mitochondrial disorders (e.g., Sengers syndrome)
Multiple sulfatase deficiency
Neonatal carnitine palmitoyl transferase (CPT-II deficiency)
α′-Pyrroline-5-carboxylate synthase deficiency
Peroxisomal disorders
SLC33A1 deficiency

Cerebral Calcification
Abnormalities of folate metabolism
Adrenoleukodystrophy
Aicardi–Goutières syndrome
Biopterin abnormalities
Biotinidase deficiency

Carnitine palmitoyltransferase II deficiency
Cockayne syndrome
Fabry disease (pulvinar sign)
GM_2 gangliosidosis
L-2-hydroxyglutaric aciduria
Hypoparathyroidism
Krabbe disease
Mitochondrial disorders (including Kearns–Sayre and MELAS syndromes)
Osteopetrosis and renal tubular acidosis (carbonic anhydrase II deficiency)

Cerebrovascular Disease
Fabry disease
Familial hypocholesterolemia
Homocystinuria
Menkes disease
5,10-Methylenetetrahydrofolatereductase deficiency
Myocardial infarction

Cerebrospinal Fluid Lymphocytosis
Aicardi–Goutières syndrome

Cerebrospinal Fluid Protein Elevation
Congenital disorders of glycosylation (CDG)
L-2-Hydroxyglutaric aciduria
Krabbe disease
Mitochondrial disorders (Kearns–Sayre, MELAS and MERRF syndromes and *POLG* mutations, Alpers disease)
Metachromatic leukodystrophy
Multiple sulfatase deficiency
Neonatal adrenoleukodystrophy
Refsum disease

Cherry Red Macular Spots
Galactosialidosis
GM_1 gangliosidosis
Mucolipidosis I
Multiple sulfatase deficiency
Niemann–Pick disease
Sandhoff disease
Sialidosis
Tay–Sachs disease

Cholestatic Jaundice
Alagille syndrome
ARC syndrome
α-1-Antitrypsin deficiency
Byler disease
(Progressive familial intrahepatic cholestasis) (PF1C1, BRIC1)
PFIC2 (bile salt excretory pump (BSEP))
PFIC 3 (MDR3)
Citrin deficiency
Cystic fibrosis
Dubin–Johnson syndrome
Fructose bisphosphate aldolase (B) deficiency (HNF-1B)
Hepatic nuclear factor 1β gene mutation
LCHAD deficiency
Mevalonic aciduria
Mitochondrial disorders (GRACILE syndrome caused by *BCS1L* mutations)
Mucolipidosis type II
Niemann–Pick type C disease
Peroxisomal biogenesis disorders
Rotor syndrome
Tyrosinemia, hepatorenal

Chondrodysplasia Phenotypes (Punctate Calcifications (Stipplings))
Conradi–Hünermann syndrome
I-cell disease
Mucolipidosis II, I-cell disease
Peroxisomal disorders
Warfarin embryopathy

Chronic Pancreatitis
Hereditary (dominant) (with or without lysinuria (cystinuria)): with or without pancreatic lithiasis or portal vein thrombosis
With hyperparathyroidism in multiple endocrine adenomatosis syndrome
Mitochondrial disorders (e.g., Pearson, Kearns–Sayre and MELAS syndromes)
Organic acidemias
Regional enteritis (Crohn)
Trauma – pseudocyst

Cirrhosis of the Liver
α-1-Antitrypsin deficiency
Citrin deficiency (citrullinemia)
Cystic fibrosis
Congenital disorders glycosylation
Defects of bile acid synthesis
Galactosemia
Glycogen storage disease type IV
(Neonatal) Hemochromatosis
Hepatorenal tyrosinemia
Mitochondrial disorders
Niemann–Pick type C
Peroxisomal disorders
Pyruvate kinase deficiency
Transaldolase deficiency
Tyrosinemia type I
Wilson disease

Corneal Opacity
Cystinosis
Fabry disease
Fish eye disease (LCAT deficiency)
Galactosialidosis
GM_1 gangliosidosis
Hurler disease (MPS I)
I-cell disease
Mannosidosis
Mitochondrial disorders (including Pearson and Kearns–Sayre syndromes)
Mucolipidosis III
Multiple sulfatase deficiency

Corpus Callosum Agenesis
Adrenocorticotrophic hormone (ACTH) deficiency
Aicardi syndrome
Mitochondrial disorders
Nonketotic hyperglycinemia
Peroxisomal disorders
Pyruvate dehydrogenase complex deficiency
VICI syndrome

Creatine Kinase: Elevated
Aldolase A deficiency
Carnitine palmitoyltransferase II deficiency
COL4A1
Congenital disorders of glycosylation (CDG)
Disorders of fatty acid oxidation
Drugs – toxins, alcohol, statins
Glutaric acidemia II
Glycogenosis III
Glycogenosis V – McArdle
Glycogenosis – posphofructokinase
D-2-Hydroxyglutaric aciduria
Inflammatory myopathy – dermatomyositis, polymyositis infectious myositis
3-Oxothiolase deficiency
Mevalonic aciduria
Mitochondrial disorders (markedly elevated in TK2 deficiency, mild elevation in other disorders)
Myoadenylate deaminase deficiency
Muscular dystrophy – Duchenne, Becker, Walker–Warburg syndrome, and related defects of O-glycosylation
3-Oxothiolase deficiency
Traumatic muscle injury (including intramuscular injections)

Deafness (Sensorineural)
α-Mannosidosis
Biotinidase deficiency
Canavan disease
Fabry disease (ABurlina)
KID syndrome
MEDNIK syndrome
MEGDEL (3-methylglutaconic aciduria type VI, MGCA6)
Mitochondrial disorders (including Pearson, Kearns–Sayre, MELAS, MERRF and MIDD syndromes, succinyl-CoA synthase deficiency, *BCS1L* mutations presenting as complex III deficiency, or Björnstad syndrome)
Peroxisomal disorders
PRPP synthetase abnormality
Refsum disease
SLC33A1 deficiency (MIM 614482)
VICI syndrome

Dermatosis
Acrodermatitis enteropathica

Biotinidase deficiency
Glycogenosis – Ib
Holocarboxylase synthetase deficiency
MTHFR deficiency

Diabetes Mellitus: Erroneous Diagnosis
Congenital disorders of glycosylation
Glycogen storage disease type 0
Isovaleric acidemia
Methylmalonic acidemia
Mitochondrial disorders
3-Oxothiolase deficiency
Propionic acidemia

Diarrhea
Abetalipoproteinemia
Congenital chloride diarrhea
Electron transport disorders
Enterokinase deficiency
Glucose galactose malabsorption
Johansson–Blizzard syndrome
Lactase deficiency
Lysinuric protein intolerance
MEDNIK syndrome
Mitochondrial disorders (including Pearson, Kearns–Sayre, and MELAS syndromes)
Schwachman syndrome
Sucrase deficiency
Sjögren–Larsson syndrome
Wolman disease

Dysostosis Multiplex
β-Mannosidosis
Galactosialidosis
Generalized GM_1 gangliosidosis
Mucolipidosis II, I-cell disease
Mucolipidosis III
Mucopolysaccharidosis I (Hurler, Hurler–Scheie disease)
Mucopolysaccharidosis II (Hunter disease)
Mucopolysaccharidosis III (Sanfilippo disease)
Mucopolysaccharidosis VI (Maroteaux–Lamy disease)
Mucopolysaccharidosis VII (Sly disease)
Multiple sulfatase deficiency

Ectopia Lentis (Dislocation of the Lens)
Homocystinuria
Hyperlysinemia
Marfan syndrome
Molybdenum cofactor deficiency
Schwartz-Jampel syndrome
Sulfite oxidase deficiency
Weill–Marchesani syndrome

EEG Burst Suppression Pattern
Anesthesia-deep stages
Anoxia, cerebral hypoperfusion
B_6/pyridoxal-phosphate dependencies
Drug overdose (e.g., phenobarbital)
Mitochondrial disorders (e.g., *SLC25A22* mutations)
Molybdenum cofactor deficiency
Monogenic epilepsy syndromes
Nonketotic hyperglycinemia
Organic acidemias (neonatal encephalopathy-propionic acidemia)
Purine metabolism defects

Exercise Intolerance
Defects of glycogenolysis
Disorders of fatty acid oxidation
3-Oxothiolase deficiency
Mitochondrial disorders
Myoadenylate deaminase deficiency

Fever Syndromes
Familial Mediterranean fever
Hyperimmunoglobulin D syndrome (mevalonic aciduria)
Muckle–Wells syndrome (neonatal onset multisystem inflammatory disease syndrome)
Tumor necrosis factor receptor-associated periodic syndrome

Glycosuria
Cystinosis
Diabetes mellitus
Hepatorenal tyrosinemia
Fanconi–Bickel syndrome – GLUT-2 mutations
Glycogen synthase deficiency
Mitochondrial disorders (Pearson and Kearns–Sayre syndromes)
Renal Fanconi syndrome
Wilson disease

Hair Abnormalities
Adrenoleukodystrophy/adrenomyeloneuropathy (scarce and thin hair)
Argininosuccinic aciduria
Cartilage–hair hypoplasia
Chediak–Higashi syndrome (mild hair hypopigmentation)
Cystinosis (light hair pigmentation)
Griscelli syndrome types 1–3 (silver-gray hair)
Homocystinuria (fine, brittle hair)
Infantile sialic acid storage disease (fair hair)
Kinky hair, photosensitivity, and mental retardation
Menkes disease (pili torti, trichorrhexis nodosa, monilethrix)
Methionine malabsorption syndrome (white hair)
Mitochondrial disorders (complex III deficiency caused by *BCSL1* mutations, including pili torti with deafness or with dental enamel hypoplasia – Björnstad syndrome)
Phenylketonuria (blond hair)
Pili torti: isolated, MIM 261900
Trichothiodystrophy: trichorrhexis nodosa, ichthyosis, and neurological abnormalities (Pollitt syndrome) MIM 27550
VICI syndrome (hypopigmented hair)

HDL (Lipoprotein) Low
Lecithin–cholesterol acyltransferase (LCAT) deficiency (fish eye disease)
Tangier disease
Hypoalphalipoproteinemia

Hemolytic Anemia
Defects of glycolysis
5-Oxoprolinuria
Purine and pyrimidine disorders
Wilson disease

Hemophagocytosis (Erythrophagocytosis)
Carnitine palmitoyltransferase I deficiency
Familial hemophagocytic lymphocytic histocytcosis (perforin deficiency, PRF1)
Gaucher disease
Hemochromatosis
Lysinuric protein intolerance
Niemann–Pick disease
Propionic acidemia
Wolman disease

Hepatic Carcinoma
α-1-Antitrypsin deficiency
Galactosemia
Glycogen storage disease types I and IV
Hemochromatosis
Hepatorenal tyrosinemia
Progressive intrahepatic cholestasis
Thalassemia
Wilson disease
Wolman disease

Hepatic Cirrhosis
α-1-Antitrypsin deficiency
Cholesteryl ester storage disease
Congenital disorder of glycosylation (CDG-X)
Cystic fibrosis
Coeliac disease
Fructose intolerance
Galactosemia
Gaucher disease
Glycogenosis types I and IV
Hematochromatosis
Hepatorenal tyrosinemia
Hypermethioninemia
Mitochondrial disorders (especially mitochondrial DNA depletion syndromes caused by *POLG*, *DGUOK*, and *MPV17* mutations)

Niemann–Pick disease
Progressive intrahepatic cholestasis
Thalassemia
Transaldolase deficiency
Urea cycle disorders
Wilson disease
Wolman disease

Hepatic Failure: Acute
Adenosine kinase deficiency
α-1-Antitrypsin deficiency
Fatty acid oxidation disorders
Galactosemia
Hepatorenal tyrosinemia
Hereditary fructose intolerance
LARS (IFLS1)
MEGDEL (3-methylglutaconic aciduria type VI, MGCA6)
Mitochondrial disorders (especially mitochondrial DNA depletion syndromes caused by *POLG*, *DGUOK*, and *MPV17* mutations; also *TRMU* mutations – NB reversible)
Neonatal hemochromatosis
Niemann–Pick types B and C
Urea cycle disorders
RALF syndrome (IFLS2, mutations in NBAS)
Wilson disease
Wolcott–Rallison syndrome

Hydrops Fetalis
Carnitine transporter deficiency
Congenital disorders of glycosylation
Farber disease (disseminated lipogranulomatosis)
Galactosialidosis
Glycogenosis type IV
GM_1 gangliosidosis
Gaucher disease
Infantile free sialic acid storage disease (ISSD)
Mucolipidosis II (I-cell disease)
Neonatal hemochromatosis
Niemann–Pick disease
Niemann–Pick disease type C
Pearson syndrome (anemia)
Sialidosis
Sly disease-β-glucuronidase deficiency
Wolman disease

3-Hydroxyglutaric Aciduria
Glutaryl-CoA dehydrogenase deficiency
Short-chain hydroxyacyl-CoA dehydrogenase deficiency
Carnitine palmitoyltransferase I deficiency

Hyperammonemia
N-Acetylglutamate synthetase deficiency
α-1-Antitrypsin deficiency
Argininemia
Argininosuccinic aciduria
Carbamoyl phosphate synthetase deficiency
CblC deficiency (rare)
Chemotherapy-induced hyperammonemia
Citrullinemia
Δ1-pyrroline-5-synthase deficiency
Distal renal tubular acidosis
Fatty acid oxidation disorders
HHH syndrome
HMG-CoA lyase deficiency
Hyperinsulinism/hyperammonemia
Isovaleric acidemia
Lysinuric protein intolerance
MCAD deficiency
MEGDEL (3-methylglutaconic aciduria type VI, MGCA6)
Methylmalonic acidemia
Mitochondrial carbonic anhydrase VA deficiency
Mitochondrial disorders (including MEGDEL, TMEM70 deficiency)
Multiple carboxylase deficiency
Ornithine transcarbamylase deficiency
Pyruvate carboxylase deficiency
Pyruvate dehydrogenase complex deficiency
Propionic acidemia
Hyperthermia, malignant carnitine palmitoyl transferase-II deficiency
Transient hyperammonemia of the newborn
Urinary tract infection – urea-splitting bacteria
Valproate
Wilson disease

Hypertyrosinemia
Drug – toxin
Hepatic infection
Hepatorenal tyrosinemia

Hyperthyroidism
Mitochondrial DNA depletion syndromes (especially *DGUOK* and *MPV17* mutations)
Oculocutaneous tyrosinemia
Postprandial
Scurvy
Transient tyrosinemia of the newborn
Treatment with NTBC
Tyrosinemia type II
Tyrosinemia type III (deficiency of 4-hydroxyphenylpyruvate dioxygenase)

Hypoketotic Hypoglycemia
Adenosine kinase deficiency
Carnitine transporter deficiency
CPT I deficiency
3-α-Hydroxyacyl-CoA dehydrogenase deficiency (HADH deficiency, formerly SCHAD)
HMG-CoA lyase deficiency
HMG-CoA synthetase deficiency
LCAD deficiency
LCHAD/MTP deficiency
MCAD
Monocarboxylate transporter 1 deficiency
VLCAD deficiency

Hypophosphatemia
Fanconi syndrome
Hyperparathyroidism
Mitochondrial disorders (including Pearson, Kearns–Sayre, and MELAS syndromes)
X-linked hypophosphatemic rickets

Hypouricemia
Fanconi syndrome, cystinosis, any proximal renal tubular dysfunction
Isolated renal tubular defect (Dalmatian dog model)
Molybdenum cofactor deficiency
Phosphoribosyl pyrophosphate synthetase deficiency
Purine nucleoside phosphorylase deficiency
Wilson disease
Xanthine oxidase deficiency

Ichthyosis
ARC syndrome
CEDNIK syndrome
Chanarin–Dorfman syndrome
Chondrodysplasia punctata type 2
CHILD syndrome (congenital hemidysplasia ichthyosis and limb defects)
COG5-CDG (CDG IIi)
Conradi–Huenermann syndrome
DOLK-CDG (CDG Im)
Gaucher disease
Ichthyosis, split hairs, and aminoaciduria
Krabbe disease
MEDNIK syndrome
MPDU1-CDG (CDG If)
Multiple sulfatase deficiency
PIGL-CDG
Refsum disease
Rhizomelic chondrodysplasia punctata type I
Serine deficiency syndromes
Sjögren–Larsson syndrome
SRD5A3-CDG (CDG Iq)
Sterol oxidase deficiency (SC4MOl deficiency)
X-linked ichthyosis – steroid sulfatase deficiency

Ichthyosis and Retinal Disease
Refsum syndrome
Sjögren–Larsson syndrome

Inverted Nipples
Biopterin synthesis disorders
Citrullinemia
Congenital disorders of glycosylation
Glycogenosis 1b
Hyperphenylalaninemia
Isolated – dominant (MIM163610)
Isovaleric acidemia
Menkes disease
Methylmalonic acidemia
Molybdenum cofactor deficiency
Niemann–Pick type C
SCAD deficiency
Propionic acidemia
Pyruvate carboxylase deficiency
VLCAD deficiency
Weaver syndrome

Isolated Deficiency of Speech as Presentation in Metabolic Disease
D-glyceric aciduria
Histidinemia
3-Methylglutaconyl-CoA hydratase deficiency

Lactic Acidemia
Ethylmalonic encephalopathy
Fatty acid oxidation disorders (long-chain)
Krebs cycle defects
Lues, congenital
MEGDEL (3-methylglutaconic aciduria type VI, MGCA6)
Mitochondrial disorders
Organic acidemia, e.g., propionic acidemia
Pyruvate carboxylase deficiency
Pyruvate dehydrogenase complex deficiency

Leigh Syndrome
Biotinidase deficiency
ECHS1 deficiency
Ethylmalonic Encephalopathy
HIBCH deficiency
MEGDEL (3-methylglutaconic aciduria type VI, MGCA6)
3-Methylglutaconic aciduria
Mitochondrial disorders (complex I, II, III, IV, or V deficiency or multiple respiratory chain defects, fumarase deficiency, succinyl-CoA synthase deficiency, presently more than 80 defined defects)
Pyruvate dehydrogenase complex deficiency

Leukopenia with or without Thrombopenia and Anemia
Abnormalities of folate metabolism
Isovaleric acidemia
Johansson–Blizzard syndrome
Methylmalonic acidemia
3-Oxothiolase deficiency
Pearson syndrome
Propionic acidemia
Shwachman syndrome
Transcobalamin II deficiency
Transaldolase deficiency

Macrocephaly
Alexander disease
Bannayan–Ruvalcaba–Riley syndrome
Canavan disease
Glutaric aciduria type I
Hurler disease
4-Hydroxybutyric aciduria
L-2-hydroxyglutaric aciduria
3-Hydroxy-3-methylglutaric aciduria
Krabbe disease
Mannosidosis
Multiple acyl-CoA dehydrogenase deficiency
Multiple sulfatase deficiency
Neonatal adrenoleukodystrophy
Pyruvate carboxylase deficiency
Tay–Sachs disease

Megaloblastic Anemia
Abnormalities of B_{12} deficiency – vegan or breast-fed infant of vegan mother or mother with pernicious anemia
Abnormalities of folate metabolism, dihydrofolate reductase deficiency
Cobalamin metabolic errors (methylmalonic acidemia, and homocystinuria-Cbl C and D)
CblF cobalamin lysosomal transporter deficiency
Dietary folate deficiency
Folate malabsorption – hereditary – protein coupled folate transport (PCFT) deficiency
Intestinal B_{12} transport deficiency – Imerslund – Grasbeck-cubilin deficiency
Methylmalonic acidemia-homocystinuria-Cbl C and D
Mevalonic aciduria
Mitochondrial disorders
Orotic aciduria
Pernicious anemia – intrinsic factor deficiency
Transcobalamin II deficiency

Metabolic Acidosis and Ketosis
Fabry disease
Familial hypocholesterolemia
Homocystinuria
Isovaleric acidemia
Menkes disease

Methylcrotonyl-CoA carboxylase deficiency
Methylmalonic/propionic acidemia
Multiple carboxylase deficiency
3-Oxothiolase deficiency

Metabolic Stroke/Stroke-Like Episodes
Carbamyl phosphate synthetase deficiency
Chediak–Higashi syndrome
Congenital disorders of glycosylation
Ethylmalonic encephalopathy
Fabry disease
Familial hypercholesterolemia and glutaric aciduria type I
Homocystinuria
Hydroxymethylglutaryl-CoA lyase deficiency
Isovaleric acidemia
Menkes disease
Mitochondrial disorders (including MELAS and MERRF syndromes and defects of coenzyme Q_{10} biosynthesis)
Methylcrotonyl-CoA carboxylase deficiency and methylmalonic acidemia
5,10-Methylenetetrahydrofolatereductase deficiency
Multiple acyl-CoA dehydrogenase deficiency
Ornithine transcarbamylase deficiency
Propionic acidemia
Phosphoglycerate kinase deficiency
Progeria
Pyruvate carboxylase deficiency
Pyruvate dehydrogenase complex deficiency
Purine nucleoside phosphorylase deficiency
Sulfite oxidase deficiency

Methylmalonic Aciduria
B_{12} deficiency, pernicious anemia, including autoimmune
Cobalamin A
Cobalamin C and cobalamin D
Cobalamin X (HCFC1 deficiency)
Imerslund–Gräsbeck – cobalamin enterocyte malabsorption
Mut^o, Mut^-
Succinyl-CoA synthase deficiency
Transcobalamin II deficiency

Mongolian Spot: Extensive
GM_1 gangliosidosis
Hurler syndrome
Niemann–Pick disease

Myocardial Infarction: Cerebral Vascular Disease
Fabry disease
Familial hypercholesterolemia
Homocystinuria
Menkes disease
Oxothiolase deficiency
Propionic acidemia

Neonatal Hepatic Presentations in Metabolic Diseases
Adenosine kinase deficiency
α-1-Antitrypsin deficiency
Cystic fibrosis
Congenital disorder of deglycosylation (NGLY1 deficiency)
Fructose intolerance
Galactosemia
Glycosylation disorders, especially type Ib
Hemochromatosis
Hepatorenal tyrosinemia
Long-chain hydroxyacyl-CoA dehydrogenase deficiency
Mitochondrial disorders (especially mitochondrial DNA depletion syndromes, GRACILE syndrome, *TRMU* mutations – NB reversible)
Infantile Liver failure syndrome type I (*LARS*)
Niemann–Pick type C disease
Mucolipidosis type II
Wilson disease
Wolman disease
Sly disease

Odd or Unusual Odor
Dimethylglycinuria
Glutaric aciduria type II
Hepatorenal tyrosinemia
Isovaleric acidemia
Maple syrup urine disease

Phenylketonuria
Treatment of urea cycle disorder with phenylacetate
Trimethylaminuria

Optic Atrophy
ADP-ribosyl protein lyase deficiency
Adrenoleukodystrophy (ALD)
Biotinidase deficiency
Canavan disease
Congenital disorders of glycosylation
GM_1 gangliosidosis
Homocystinuria
Krabbe disease
Menkes disease
Methylmalonic acidemia
Metachromatic leukodystrophy
3-Methylglutaconic aciduria, type III (Costeff)
Mevalonic aciduria
Mitochondrial disorders (including Leber hereditary optic neuropathy (LHON), Leigh syndrome, MERRF, NARP)
Multiple sulfatase deficiency
Neonatal adrenoleukodystrophy
Propionic acidemia
Sandhoff disease
Tay–Sachs disease

Orotic Aciduria
UMP synthase deficiency (hereditary orotic aciduria)
Urea cycle defects – ornithine transcarbamylase deficiency, citrullinemia, argininosuccinic aciduria, arginemia
Purine nucleoside phosphorylase (PNP) deficiency
Phosphoribosylpyrophosphate (PRPP) synthetase deficiency

Osteoporosis and Fractures
Gaucher disease
Glycogenesis I
Homocystinuria
Infantile Refsum disease
Lysinuric protein intolerance
Menkes disease
Methylmalonic acidemia
Mucolipidosis II, I-cell disease
Propionic acidemia

Pain and Elevated Erythrocyte Sedimentation Rate
Fabry disease
Familial hypercholesterolemia
Gaucher disease
Mevalonic aciduria

Pancreatitis
Carnitine palmitoyltransferase II deficiency
Glycogenosis type I
Glycogenosis 1 plus apoE2 type III hypertriglyceridemia
Hereditary dominant, with or without lysinuria; with or without pancreatic lithiasis or portal vein thrombosis
Homocystinuria
Hydroxymethylglutaryl-CoA lyase deficiency
Hyperlipoproteinemia type IV
Isovaleric acidemia
Lipoprotein lipase deficiency, also type IV
Lysinuric protein intolerance
Maple syrup urine disease
Methylmalonic acidemia
Mitochondrial disorders (Pearson, Kearns–Sayre, and MELAS syndromes)
Ornithine transcarbamylase deficiency
Propionic acidemia
Regional enteritis (Crohn)
Trauma – pseudocyst
With hyperparathyroidism in multiple endocrine adenomatosis syndrome

Paralysis of Vertical Gaze
Mitochondrial disorders (including Leigh and Kearns–Sayre syndromes)
Niemann–Pick type C
Peripheral neuropathy

Photophobia
Cystinosis
Oculocutaneous tyrosinemia
Mitochondrial disorders (Pearson and Kearns–Sayre syndromes, NARP)

Polycystic Kidneys
Carnitine palmitoyltransferase II (CPT-II) deficiency
Congenital disorders of glycosylation
Glutaric aciduria type II (multiple acyl-CoA dehydrogenase deficiency) (GA II)
Zellweger syndrome

Psychotic Behavior
Carbamoyl phosphate synthetase deficiency
Cbl disease
Citrin deficiency
Citrullinemia
Hartnup disease
Homocystinuria
Hurler–Scheie, Scheie disease
Krabbe disease
Lysinuric protein
β-Mannosidosis
Maple syrup urine disease
5,10-Methylenetetrahydrofolatereductase deficiency
Metachromatic leukodystrophy
Mitochondrial disorders (especially MELAS)
Neuronal ceroid lipofuscinoses
Niemann–Pick type C disease
Ornithine transcarbamylase deficiency
Porphyria
Pyruvate dehydrogenase deficiency
Sanfilippo disease
Tay–Sachs, Sandhoff – late onset
Wilson disease

Ptosis
Congenital myasthenic syndromes
Dopamine deficiency syndromes
Mitochondrial disorders (including Kearns–Sayre and MNGIE syndromes)

Ragged Red Fibers
Menkes disease
Mitochondrial disorders (affecting mitochondrial DNA directly or mitochondrial DNA maintenance or mitochondrial translation)

Raynaud Syndrome
Fabry disease

Red Urine
Beets
Hematuria
Hemoglobinuria
Myoglobinuria
Drugs: rifampicin, phenolphthalein, nitrofurantoin, ibuprofen, pyridium

Renal Calculi
APRT (adenosine phosphoribosyltransferase) deficiency
Cystinuria
HPRT deficiency – Lesch–Nyhan disease
Mitochondrial disorders (e.g., *CLPB* mutations)
Oxaluria
PRPP synthetase abnormalities
Wilson disease
Xanthine oxidase deficiency

Renal Fanconi Syndrome
Cystinosis
Galactosemia
Glycogenosis I and III
Hepatorenal tyrosinemia
Lowe syndrome
Lysinuric protein intolerance
Mitochondrial disorders (especially Pearson and Kearns–Sayre syndromes, *BCS1L* mutations)
Primary Fanconi syndrome
Wilson disease

Renal Tubular Acidosis (RTA)
Cystinosis
Galactosemia

Heavy metal toxicity
Hepatorenal tyrosinemia
Mitochondrial disorders (especially Pearson and Kearns–Sayre syndromes, *BCS1L* mutations)
Osteopetrosis and RTA
Topamax

Retinitis Pigmentosa
Abetalipoproteinemia
Congenital disorder of glycosylation
Hunter disease
LCHAD deficiency
Mevalonic aciduria
Mitochondrial disorders (including Kearns–Sayre and NARP syndromes)
Neuronal ceroid lipofuscinoses
Peroxisomal biosynthesis disorders
Primary retinitis pigmentosa
Refsum disease
Sjögren–Larsson syndrome (fatty alcohol oxidoreductase deficiency)

Reye Syndrome Presentation
Fatty acid oxidation disorders
Disorders of gluconeogenesis
Urea cycle disorders
Fructose intolerance
Infantile liver failure syndrome type I (IFLS1, *LARS*)
Mitochondrial disorders
Organic academia
RALF syndrome (IFLS2, mutations in *NBAS*)

Rhabdomyolysis
Aldolase A (fructose bisphosphate) deficiency
Disorders of fatty acid oxidation – LCHAD, CPT-II
Drugs – methylenedioxymethamphetamine (MDMA)
Glutaric acidemia I
Glycogenosis V – McArdle – myophosphorylase
VII – Tarui – phosphofructokinase
Glycolysis – phosphoglycerate kinase – phosphoglyceromutase
Infection – myositis
Ischemic injury
Mitochondrial disorders (especially mitochondrial DNA depletion syndromes, complex III deficiency caused by *MTCYB* mutations)
Oxphos defects – complex I, complex II
Quail ingestion – coturnism
Toxin – tetanus, snake venom, alcohol, cocaine, bee venom
Lipin 1 (LPIN1)

Scoliosis
Congenital disorder of glycosylation
Ehlers-Danlos syndromes
Homocystinuria
Marfan syndrome
Pyruvate dehydrogenase complex deficiency

Self-Injurious Behavior
Lesch–Nyhan disease
Neuroacanthocytosis

Spastic Paraparesis
Argininemia
Biotinidase deficiency
HHH syndrome
Metachromatic leukodystrophy
Mitochondrial disorders (including *SPG7*, *IBA57* and *NFU1* mutations)
Pyroglutamic aciduria
Pyruvate dehydrogenase complex deficiency (*PDHX* mutations)
Sjögren–Larsson syndrome
SLC33A1 deficiency (MIM 612539)
Spondyloenchondrodysplasia (acid phosphatase deficiency)

Subdural Effusions
Glutaric aciduria I
D-2-Hydroxyglutaric aciduria
Menkes disease
Pyruvate carboxylase deficiency

Vomiting and Erroneous Diagnosis of Pyloric Stenosis
Ethylmalonic-adipic aciduria
Galactosemia
HMG-CoA lyase deficiency
4-Hydroxybutyric aciduria
D-2-Hydroxyglutaric aciduria
Isovaleric acidemia
Methylmalonic acidemia
3-Oxothiolase deficiency
Phenylketonuria
Propionic acidemia

Xanthomas
Cerebrotendinous xanthomatosis
Familial hypercholesterolemia
Lipoprotein lipase deficiency
Niemann–Pick disease
Sitosterolemia

Reference Books

Blau N, Duran M, Gibson KM, Dionisi Vici C (eds) (2014) Physician's guide to the diagnosis, treatment, and follow-up of inherited metabolic diseases. Springer, Berlin
Detailed collation of clinical and laboratory findings in individual disorders. Very helpful for metabolic specialists and clinicians with experience of metabolic disorders but less well suited to clinicians who do not regularly see patients with inborn errors of metabolism

Blau N, Duran M, Gibson KM (eds) (2008) Laboratory guide to the methods in biochemical genetics. Springer, Berlin
A prime resource for metabolic specialists working in the laboratory that also provides excellent background information for clinicians interested in methodological background information

Blau N, Hoffmann GF, Leonard J, Clarke JTR (eds) (2005) Physician's guide to the treatment and followup of metabolic diseases. Springer, Berlin
The book gives detailed information with regard to the management of metabolic disorders. It is an intermediary between more general textbooks and dedicated original articles. A useful reference book for clinicians who see metabolic patients

Bremer HJ, Duran M, Kamerling JP, Przyrembel H, Wadman SK (1981) Disturbances of amino acid metabolism: clinical chemistry and diagnosis. Urban & Schwarzenberg, Baltimore-Munich
A unique, detailed source of information on amino acids and aminoacidopathies. Contains large tables of normal values in various body fluids. Somewhat outdated and unfortunately out of print, but worth an effort to get second hand

Saudubray JM, van den Berghe G, Walter JH (eds) (2012) Inborn metabolic diseases – diagnosis and treatment, 5th edn. Berlin, Springer
Accessible pathway-based approach to inborn errors of metabolism. Covers all the major groups of metabolic disorders and is particularly helpful for clinicians who seek basic information on diagnosis and treatment of individual defects. Individual chapters written by various authors and of variable quality

Firth HV, Hurst JA (2005) Oxford desk reference clinical genetics. Oxford University Press, Oxford. A well-written, practical text. It is primarily aimed at clinical geneticist and does not have a specific focus on metabolic disorders but is an extremely valuable resource for all clinicians who encounter patient's genetic conditions

Nyhan WL, Barshop BA, Al-Aqeel A (2012) Atlas of inherited metabolic diseases, 3rd edn. Hodder Arnold, London
Detailed clinically oriented monographs of a wide range of individual metabolic disorders with excellent photographs

Rimoin DL, Connor JM, Pyeritz RE, Korf BR (eds) (2006) Emery and Rimoin's principles and practice of medical genetics, 5th edn. Churchill Livingston, New York

An excellent, comprehensive and mostly up-to-date textbook of clinical genetics. Chapters dealing with metabolic disorders are not always convincing. 3 volumes, quite expensive

Sarafoglu K, Hoffmann GF, Roth K (eds) (2017) Pediatric endocrinology and inborn errors of metabolism, 2nd edn. McGraw Hill, New York

Concise, comprehensive, and reasonably prized clinical textbook covering the whole field of metabolic as well as endocrine disorders. A must for every consultant pediatrician, not only endocrinologists and metabolic specialists

Scriver CR, Beaudet AL, Sly WS, Valle D (eds) (2001) The metabolic and molecular bases of inherited disease, 7th edn. McGraw-Hill, New York

The last printed version of the standard textbook on inborn errors of metabolism, extended to four volumes and nonmetabolic conditions. Special emphasis on molecular and pathophysiologal aspects of individual disorders. It has now turned into a huge online knowledgebase ("Online Metabolic and Molecular Bases of Inherited Disease") covering the widest possible range of inherited disorders but unfortunately requires an expensive annual subscription (www.ommbid.com)

Zschocke J, Hoffmann GF, 2011. Vademecum metabolicum – diagnosis and treatment of inborn errors of metabolism, 3 edn. Schattauer, Stuttgart

A concise pocket book containing the essentials of metabolic pediatrics, with short descriptions of most inborn errors of metabolism

Internet Resources

Society for the Study of Inborn Errors of Metabolism, SSIEM (www.ssiem.org) and Society for Inherited Metabolic Disorders (www.simd.org)

The homepages of the SSIEM (international, focus in Europe) and the SIEM (USA) provide excellent links to various Internet resources concerned with inborn errors of metabolism, including learned societies, parent organizations, laboratory directories, etc.

National Center for Biotechnology Information, NCBI (www.ncbi.nlm.nih.gov)

The primary source for molecular biology information on the Internet. Home to Pubmed, OMIM, GenBank, and a vast range of other databases. May be difficult to navigate without some experience.

OMIM (www.omim.org)

This is the online version of "Mendelian Inheritance in Man," the oldest collation of genetic disorders, originally edited by Victor A. McKusick. It is freely available from Johns Hopkins University. The database contains monographs of individual disorders and genes together with references and extensive links to other online resources. OMIM has an emphasis on molecular information, but there are also plenty of clinical data that are useful in daily practice. The number allocated to entries in the OMIM catalog is used in scientific publications worldwide for the exact identification of individual genetic disorders.

GeneTests (www.genetests.org) and Gene Reviews (www.genereviews.org)

A freely available medical genetics information resource, specifically for physicians and other health-care providers, is funded by the National Institute of Health, USA. Its "GeneReviews" are extremely useful monographs with detailed information on clinical presentation, diagnosis, differential diagnosis, management and genetic counseling, molecular genetics, etc., available now for a huge number of individual conditions. There are also directories of clinical centers and diagnostic laboratories particularly in the USA.

Orphanet (www.orpha.net) and EuroGentest (www.eurogentest.org)

Orphanet is a European portal for rare genetic diseases and orphan drugs. It was selected as the central database of EuroGentest, an EU-funded Network of Excellence looking at

various aspects of genetic testing, including quality management, information databases, and education. Together the websites provide a wealth of information on rare diseases and various resources particularly in Europe, including a laboratory directory, clinical centers, patient support groups, etc. The information is available in different languages; access to the site is free of charge.

Human Genome Variation Society Website, HGVS (www.hgvs.org)

The HGVS aims to "foster discovery and characterization of genomic variations." The website provides links to a vast number of locus-specific mutation databases as well as standard recommendations for mutation nomenclature, an important resource for clinical molecular geneticists as well as clinicians and scientists trying to find additional mutation-related information.

Human Gene Mutation Database, HGMD (www.hgmd.cf.ac.uk)

This database on mutations causing human disorders, based in Cardiff, Wales, was originally established for the study of mutational mechanisms in human genes. Mutations were also identified by scanning published articles, a major effort. However, content freely available is not up-to-date, and the full "professional" version provided by a commercial partner is expensive even for academic/nonprofit users.

GeneCards (www.genecards.org)

This is a concise, gene-oriented database that contains information on various functions of human genes including the respective proteins and associated disorders. There are copious links to other Internet databases. GeneCards are particularly useful for geneticists and researchers in the areas of functional genomics and proteomics.

Online Metabolic and Molecular Bases of Inherited Disease (www.ommbid.com)

It is the online successor of the previous standard textbook of inborn errors of metabolism, with detailed information on molecular and pathophysiological aspects of individual disorders. Though vastly expanded to cover the widest possible range of inherited disorders, it unfortunately requires an expensive annual subscription.

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