Genetic Metabolic Disease

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Abstract

Inborn errors of metabolism (IEM) constitute inherited enzyme or transport protein defects, potentially leading to accumulation of toxic substrates or deficiency of essential products of any given process. There are often further consequences of the primary perturbation, leading to compensatory physiology or further interference in cellular processes. These biochemical effects can, in some instances, lead to fetal developmental abnormalities and in others to dramatic postnatal compromise with significant mortality and morbidity. This chapter discusses the following presentations: neonatal acute metabolic encephalopathy, neonatal epileptic epilepsy, liver disease, cardiomyopathy, nonimmune hydrops fetalis, and dysmorphic IEM (including some lysosomal and peroxisomal disorders and congenital disorders of glycosylation). Systematic investigation of these clinical presentations can often lead to a definitive diagnosis. Most of these disorders are rare, but recent medical advances have made many treatable. It is therefore imperative that these disorders are considered early in differential diagnoses, investigated rigorously and expeditiously, and managed appropriately. Newborn bloodspot screening protocols can also identify many of these conditions in the presymptomatic stage, and hence, knowledge of local screening strategies is imperative for those investigating neonates. Screening tests require definitive testing algorithms in order to correctly identify an affected infant.

Keywords

Inborn errors of metabolism (IEM) • Neonatal acute metabolic encephalopathy • Neonatal epileptic epilepsy • Liver disease • Cardiomyopathy • Nonimmune hydrops fetalis • Dysmorphic inborn errors of metabolism • Lysosomal disorders • Peroxisomal disorders • Congenital disorders of glycosylation

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The term "inborn errors of metabolism" was coined by Sir Archibald Garrod in a series of lectures and papers over a century ago [1]. In this chapter, the terms "genetic metabolic disorders" and "inborn errors of metabolism" are considered to be the same entity and will be referred to as IEM. The concept of IEM implies a major genetic component of inheritance, through all known genetic mechanisms, as well as a disturbance in metabolism. These disturbances can be due to an enzyme deficiency causing toxic elevation of substrate, deficiency of an essential product, or failure to transport the metabolite to the appropriate site of function.

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Historical Perspective

In the early twentieth century, concepts such as Mendelian genetics and natural selection incorporating genetic variability were novel concepts, and it was remarkable on this background that Garrod noted that individuals were also susceptible to chemical variability, which sometimes resulted in disease [1]. In particular, in newly diagnosed individuals with a condition called alkaptonuria, he observed that the alkaptone (blackening) urine was seen in several family members prior to the onset of the clinical condition. The chemistry was clearly hereditary and was noted to predispose to a clinical condition. In subsequent works, he noted some important factors that remain critical when considering the clinical investigation of IEM. Firstly, there is variation in the individuals who excrete the chemical, i.e., there is a continuum of excreted metabolites. Secondly, excretion of such metabolites may not necessarily lead to clinical disease. In his words, "[T]here are many of us who possess latent defects of structure and function, inborn and for the most part inherited, which are apt to be revealed sooner or later, by the effects produced by external influences which are innocuous to the average man…For some individuals, these trifling traumata which go to make up the wear and tear of life, are apparently provoking causes of grave disorders" [2]. None of the conditions originally considered by Garrod would be considered grave in early childhood, but the concept has held true for disorders that have subsequently been described.

Garrod's approach of intellectual inquiry of biochemical physiology, with subsequent link to clinical conditions, has been the hallmark of identification of IEM. In 1934, Følling used the ferric chloride test to identify phenylalanine metabolites in the urine of some intellectually impaired individuals, which subsequently came to be known as phenylketonuria (PKU) [3]. Shortly thereafter, Hans Krebs had described the process of oxidation of citric acid; Gerti and Cori identified the processes of glycogen degradation, subsequently describing the biochemical bases of related disorders [4, 5]. In Christian de Duve's investigation of glucose metabolism, he fortuitously identified an intracellular organelle called the lysosome when trying to separate intracellular fractions [6]. His colleague Henri-Geri Hers went on to identify the enzymatic basis of the first lysosomal storage disorder, acid maltase, in Pompe disease [7]. Again, his comments in 1963 were telling: "[I]t can be expected that other deposition diseases might be explained on the basis of the absence of other lysosomal enzymes." There are currently approximately 50 of such disorders. In the 1980s, Jaak Jaeken from the same unit in Leuven that had discovered the lysosome and related storage disorders went on to describe a new group of conditions related to dysfunction within the endoplasmic reticulum and Golgi apparatus called congenital disorders of glycosylation [8]. Again, a huge range of these conditions is now recognized.

Over the course of the last 50 years, there have been more trained dedicated specialists managing inborn errors of metabolism. This has arisen because a large number of conditions previously thought of as fatal and untreatable can now be treated. This chapter has an emphasis on identifying treatable causes rapidly with first-tier tests. More complex definitive investigations are considered as second- or third-tier tests. Of course, in this day and age, discussion of investigations would not be complete without considering the role of genetic investigation for these disorders. As sequencing methodologies become more high-throughput, massively parallel sequencing is becoming more of a first-line investigation in some scenarios.

When to Think of an IEM

There are sometimes some clues about an IEM from the medical history such as unexpected death in previous siblings. The majority of conditions are autosomal recessive and rare, so there may not be clues from the extended pedigree unless the family is consanguineous (which is usually not spontaneously volunteered) or originates from a geographically or ethnically isolated community. X-linked disorders may have expressed in previous generations, particularly but not exclusively in males, such as in ornithine transcarbamylase deficiency. Conditions caused by primary mutations of mitochondrial DNA (mtDNA) may show so-called maternal inheritance as these conditions are transmitted through the generations almost exclusively by mitochondria in the ovum down the maternal lineage and not the sperm [9]. From the pathologists' perspective, understanding the organ and the organelle from which an enzyme deficiency arises helps direct biochemical testing of that particular tissue organelle. However, there is a huge variation in the clinical manifestations of specific deficits within each organelle, meaning that the clinician often has to investigate several intracellular compartments and organelles in order to reach a specific diagnosis.

This chapter is divided into several sections, firstly, the diagnostic methods used to identify these disorders and then individual sections based on the mode of clinical and/or biochemical presentation.

Laboratory Methods of Diagnosing Inborn Errors of Metabolism

The relationship between the clinicians and laboratory staff cannot be underplayed in the diagnosis of inherited metabolic disorders. From the moment such a disorder is suspected, constant dialogue with the laboratory staff will ensure an efficient processing of samples to obtain the correct result in a timely manner. Furthermore, the correct timing of

samples may be critical for obtaining a diagnosis, as metabolites indicative of a disorder may not be increased unless the samples are collected at a time when the patient is stressed.

Routine biochemical tests such as arterial blood gasses, plasma electrolytes, glucose, urea, creatinine, liver function tests, routine hematological tests, and various endocrinological tests, which may provide helpful pointers to a specific diagnosis, are supplemented by specialist biochemical genetics tests, including urine organic acids, amino acids and acylglycines, plasma acylcarnitines, and other specialized plasma assays.

Routine Biochemistry Tests

Plasma Ammonium

In most laboratories, the measurement of plasma ammonium is performed enzymatically using autoanalyzers. However, as robust as the assay is, errors arise mostly from sample collection and storage. If the blood sample is lysed or sits at room temperature for any length of time, amino acids can be spontaneously deaminated and ammonium can be released from red blood cells, and so results from capillary blood samples should be treated with suspicion [10]. Ideally, the sample should be taken from a free-flowing vein and placed immediately on ice. The sample should be separated within 20 min of collection.

Plasma Lactate and Pyruvate

A decreased lactate-to-pyruvate ratio can be observed in disorders of pyruvate metabolism including pyruvate dehydrogenase deficiency, whereas an increased ratio is observed in some but not all mitochondrial disorders. Correct specimen collection and handling is crucial in achieving reliable results. The patient should be rested, and there should be minimal use of a tourniquet. The sample should be taken into a tube containing an acid precipitant on ice and thoroughly mixed before being centrifuged to remove the supernatant. Lactate and pyruvate are measured in one sample, and the acid precipitant retards the interconversion of pyruvate to lactate.

Specialist Biochemical Genetics Tests

Organic Acids

Organic acid and acylglycine analysis, usually by gas chromatography–mass spectrometry (GC-MS), is available primarily in specialist biochemical genetics laboratories. Although they can be measured in a variety of fluids, urine is the most easily obtained and is the most commonly used for the analyses. These relatively low-molecular-weight molecules are intermediates or end products of amino acid, carbohydrate, lipid, or biogenic amine metabolism. In urine these compounds are stable at −20 °C, so it is important that the sample is frozen immediately after collection if there is likely to be any delay in transport to the laboratory. The timing of the sample collection is also important, with the first available urine at the time of decompensation being the most informative.

Following initial quantitation of urinary creatinine, a volume of urine equivalent to 1 mmol/L of creatinine is acidified after the addition of internal standards and extracted into ethyl acetate. The choice of internal standard varies between laboratories but may include both unlabeled nonphysiological compounds and/or isotopically labeled variants of "important" organic acids (e.g., hexanoylglycine or orotate). After the ethyl acetate extracts have been dried, they are derivatized to trimethylsilyl derivatives. This makes the compounds volatile and protects the organic acids from decomposition throughout the separation in the gas chromatograph. The mass spectrometer is operated in scan mode, which allows for identification based on both retention times and mass spectra. This method can allow detection of more than 200 compounds, not all of them technically organic acids, e.g., glycerol, glycine conjugates, and drug metabolites.

While the method is robust, there are factors that may affect the ability of the laboratory to interpret the results. As mentioned previously, the timing of the sample collection is most important, and the best sample for analysis is the one taken before any clinical interventions. This is particularly so in long-chain fatty acid oxidation defects (Fig. 11.1) or

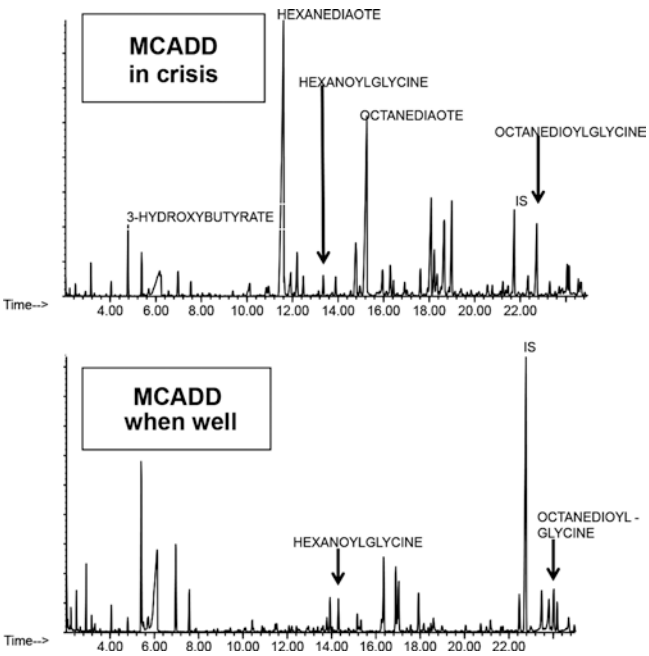

Fig. 11.1 Gas chromatograms (*GC*) of urine from a child with medium-chain acyl-CoA dehydrogenase (*MCAD*) deficiency. The upper panel shows the presence of the signature metabolites during an episode of metabolic decompensation. The lower panel shows a GC tracing of urine from the same child 48 h after the acute presentation. *IS* internal standard

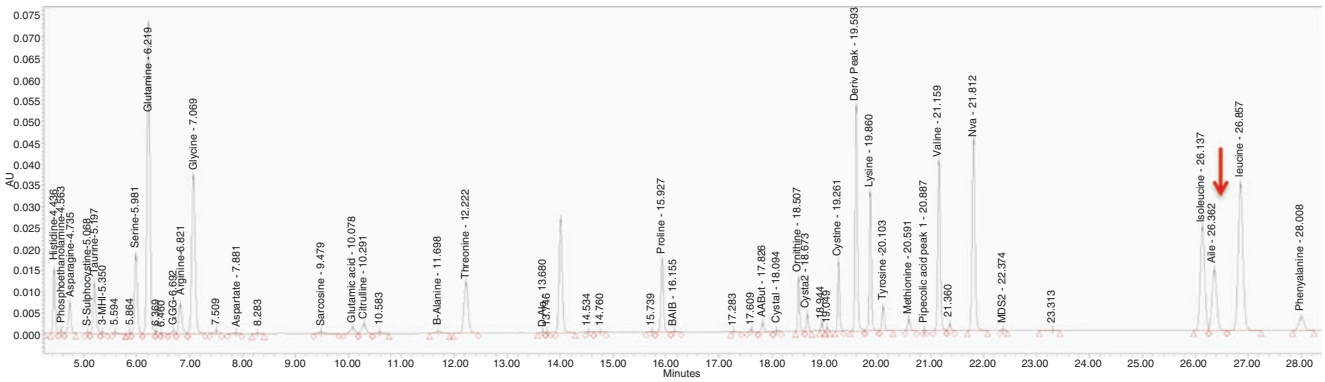

Fig. 11.2 Characteristic plasma amino acid profile for maple syrup urine disease (*MSUD*). Note the increased alloisoleucine (26.36 min), which is pathognomonic for the disorder

disorders associated with intermittent orotic acidurias (e.g., the urea cycle disorder ornithine transcarbamylase deficiency), where the organic acid analysis will not necessarily show the diagnostic metabolites when the patient is well. The storage of the sample will also affect the results. Labile metabolites such as succinylacetone can be degraded when stored at room temperature for extended periods, potentially leading to a false-negative result.

Sample contaminants make interpretations particularly difficult. Fecal matter, skin care products, and plasticizers confuse the profile and may obscure small increases in diagnostic metabolites.

It is important to note that the organic acid profile may sometimes need to be interpreted in conjunction with the plasma acylcarnitine and amino acid profiles in order to be able to make a precise diagnosis. Kumps et al. provide a comprehensive list of organic acids and acylglycines, the disorders associated with them, and artifacts that may confuse the diagnosis [11].

Amino Acid Analysis

The ability to measure amino acids in a variety of physiological fluids remains central to the diagnosis of many inherited metabolic disorders. There are a number of analytical techniques that can be used to detect and quantitate amino acids, but high-performance liquid chromatography (HPLC) remains the most popular method and can be applied to urine, plasma, and cerebrospinal fluid (CSF) samples. The method requires derivatization, as the amino acids lack a strong chromophore and are otherwise difficult to measure with sufficient sensitivity and selectivity. Precolumn derivatization, where the amino acids are derivatized before separation and detection, has many advantages as it reduces the amount of reagent required, and any excess reagent can be separated from the individual amino acids. This derivatization also allows for separation on reverse phase columns, which provide excellent separation and run times. Postcolumn derivatization methods, where the amino acids are derivatized after separation on the column but before the detector, are also widely used. The advantage of this technique is that the sample matrix has been removed before derivation, making it useful for a wide range of samples. The disadvantage is that the derivatizing reagent is constantly flowing, making it difficult to increase sensitivity.

The interpretation of amino acid profiles may be complicated by secondary abnormalities in plasma and urine as a consequence of hepatic dysfunction, renal tubular disorders, and the catabolic state of the individual. Clinical information will often aid in the interpretation of atypical amino acid profiles. For inherited metabolic disorders, the abnormalities seen in the amino acid profile are usually fairly obvious, for example, the amino acid profile diagnostic of maple syrup urine disease (Fig. 11.2). Table 11.1 shows the range of amino acid abnormalities in plasma and their associated diagnoses.

Glycosaminoglycan Screening

Glycosaminoglycans (GAGs) are typically increased and/or show a typical qualitative profile in mucopolysaccharide (MPS) disorders. There are many ways to screen for increased excretion of GAGs in urine. Most of them are based on a binding assay, with the dimethylmethylene blue (DMB) spectrophotometric assay being the most common. The DMB binding assay is a direct binding assay that can be read immediately in a spectrophotometer at 520 nm. It is a highly sensitive and specific assay but is subject to interference, especially from drugs excreted as sulfate conjugates [12].

While the assay is relatively simple, there is a requirement that individual laboratories generate their own reference ranges, as the amount of GAGs excreted falls exponentially with age. There is also some overlap between the milder variants of the disorders and the normal population. Even when there are slight increases in GAG excretion or where there is a strong clinical suspicion, qualitative GAG analysis by high-voltage electrophoresis (Fig. 11.3) or specific quantitative enzyme analysis should be conducted.

Table 11.1 Amino acid abnormalities associated with specific inborn errors of metabolism

Increased *S*-sulfocysteine	Isolated sulfite oxidase deficiency and molybdenum cofactor deficiency
Increased GLN Decreased CIT (<3)	Ornithine carbamoyl transferase, carbamoyl phosphate synthetase, or N-acetylglutamate synthase deficiencies
Increased GLN, CIT (>300)	Citrullinemia
CIT (60–300)	Pyruvate carboxylase deficiency
Increased GLN, ASA, ASAA	Argininosuccinic aciduria
Increased ARG	Hyperargininemia
Moderate increase CIT, ARG, THR	Citrullinemia type II
Increased ALA	Primary lactic acidosis
Increased GLY	Nonketotic hyperglycinemia
Decreased SER	Serine deficiency disorders (confirm in CSF)
Increased PEA	Hypophosphatasia
Increased VAL, LEU, ILE, AILE	Maple syrup urine disease (AILE is pathognomonic)
Increased MET	Methionine adenosyltransferase deficiency or glycine N-methyltransferase deficiency or homocystinuria
Increased PIP	May be present in cases of pyridoxine-responsive seizures
Increased TYR	Tyrosine aminotransferase deficiency (oculocutaneous or tyrosinemia type II) or hawkinsinuria
Increased MET	Fumarylacetoacetase deficiency (tyrosinemia type I)
MET	
Increased PHE	Phenylketonuria or a pterin defect
Increased ORN	Hyperornithinemia

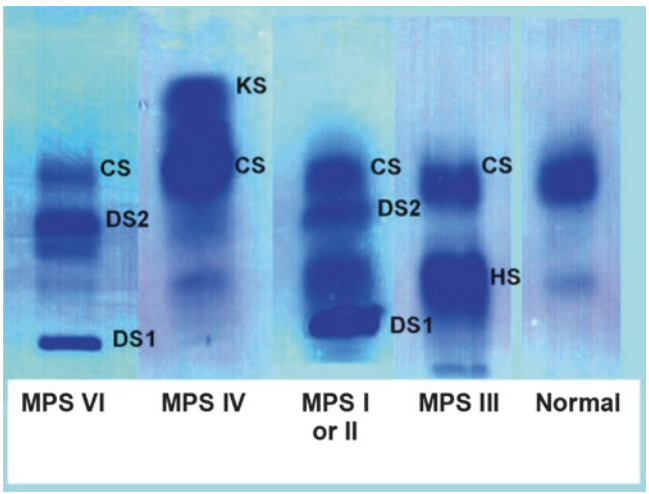

Fig. 11.3 High-voltage electrophoresis of glycosaminoglycans. *CS* chondroitin sulfate; *HS* heparin sulfate; *DS* dermatan sulfate; *KS* keratan sulfate; *MPS1* Hurler, Scheie, or Hurler–Scheie syndrome; *MPS II* Hunter syndrome; *MPS III* Sanfilippo syndrome; *MPS IV* Morquio syndrome; *MPS VI* Maroteaux–Lamy syndrome

Table 11.2 Peroxisomal disorders and their associated biochemical signatures

Disorder	VLCFA	Pristanic acid	Phytanic acid
ZSDs	↑	N -↑	N -↑
RDCP type I	N	↓- N	N -↑
x-ALD	↑	N	N
ACOX1 deficiency	↑	N	N
DBP deficiency	↑	N -↑[a]	N -↑
SCPx deficiency	N	N -↑[a]	N -↑
AMACR deficiency	N	N -↑[a]	N -↑
Refsum disease	N	N	↑↑

ZSD Zellweger spectrum disorder, *RDCP* rhizomelic chondrodysplasia punctata, *x-ALD* X-linked adrenoleukodystrophy, *ACOX1* straight-chain acyl-CoA oxidase, *DBP* D-bifunctional protein, *SCPx* sterol carrier protein X, *AMACR* alpha-methylacyl-CoA racemase
[a]Pristanic acid > phytanic acid

Acylcarnitine Profile

Refinements to tandem mass spectrometry by fast atom bombardment (FAB) ionization [13] led to the use of electrospray ionization (ESI), enabling the automated introduction of samples into a continuously flowing solvent stream. By extracting, butyl esterification, plasma, amniotic fluid, urine, or dried blood spots, acylcarnitine species can be detected and quantified.

Other GC-MS Methods

While organic acids and acylglycines are analyzed by GC-MS in scan mode, by selecting only the ions of interest, we can increase the sensitivity of an assay. The GC-MS apparatus can be operated in selective ion monitoring (SIM) mode for assays including plasma very long-chain fatty acids, including pristinate and phytanate, to detect peroxisomal disorders (Table 11.2), and 7-dehydrocholesterol to detect Smith–Lemli–Opitz syndrome.

Expanded Newborn Screening

Since Guthrie provided evidence that spots on filter paper could diagnose phenylketonuria [14], many countries have used this approach for the diagnosis of a variety of conditions from such samples. The Wilson and Junger criteria for screening tests are often cited to provide a framework for such strategies. These incorporate identifying significant treatable conditions that can be tested using a reliable, sensitive, and specific test to intervene in the presymptomatic phase for a specific condition in a cost-effective manner. These criteria have been applied to identify a range of conditions worldwide including congenital hypothyroidism, hemoglobinopathies, and cystic fibrosis [15–17].

With the advent of tandem mass spectrometry (TMS) with electrospray ionization, the number of disorders that can be identified has expanded from phenylketonuria, galactosemia, congenital hypothyroidism, and cystic fibrosis testing to testing for a number of amino acidemias, fatty acid oxidation (FAO)

Table 11.3 Inborn errors of metabolism detected through expanded newborn screening using electrospray tandem mass spectrometry

Disorder	Diagnostic metabolites
Amino acidemias	
Phenylketonuria	Phenylalanine + tyrosine
Maple syrup urine disease	Leucine + isoleucine
Homocystinuria	Methionine
Citrullinemia	Citrulline
Tyrosinemia type I	Tyrosine + methionine
Organic acidemias	
Propionic acidemia	Increased C3
Methylmalonic acidemias	Increased C3
Isovaleric acidemia	Increased C5
3-Methylcrotonyl-CoA carboxylase deficiency	Increased C5
Glutaric acidemia type I	Increased C5DC
Hydroxymethylglutaric acidemia	Increased C5OH
Fatty acid oxidation disorders	
MCAD deficiency	Increased C8, C10:1
VLCAD deficiency	Increased C14, C14:1
LCHAD and TFP deficiency	Increased C16OH, C18OH
Glutaric aciduria type II	Increased C4, C5, C8, C10
CPT II deficiency	Increased C16, C18:1

disorders, and organic acidurias (Table 11.3) [18]. The implementation of such screening broadened the remit of screening beyond the Wilson and Junger criteria, as TMS provides a profile of variations for different conditions rather than a specific test for a single disorder. TMS has specifically been successful for the screening of MCAD deficiency and MSUD, but its utility in some other conditions has been questioned. As technology has advanced, so has the range of conditions that are being considered or being implemented for screening worldwide. These include severe combined immunodeficiency, congenital adrenal hyperplasia, lysosomal storage disorders, and Duchenne muscular dystrophy [19, 20]. There is considerable variation between screening programs worldwide and debate about screening strategies. Whole exome and genome screening technologies are actively being considered by some programs, and hence, the field could change further in the coming years.

It is important to remember that symptoms may appear before the dried blood spot has been collected or indeed before the sample is received in the laboratory. If one of these disorders is suspected, the newborn screening laboratory should be contacted to ensure results are communicated to the attending clinicians.

It must also be remembered that milder or intermediate forms of inborn errors of metabolism may have normal newborn screening profiles, and so if one of these disorders is suspected because of the clinical presentation, a normal newborn screen should not deter the clinician from performing further investigations through the biochemical genetics reference laboratory [21].

Genetic Testing

A precise metabolic diagnosis can often be reached through biochemical genetic analyses of urine, blood, and/or CSF and/or specific enzymatic assays of cultured cells, white or red blood cells, or tissue samples. In such situations, while it may be possible to apply traditional sequencing techniques to the causative single gene, establishing the precise molecular genetic pathology is unnecessary for diagnosis but may be useful in helping parents in their reproductive decision making. In addition, knowledge of the specific mutations causing the disorder may allow better phenotype–genotype correlations and more accurate prognostication and may guide more targeted therapies, e.g., hydroxocobalamin for methylmalonic aciduria caused by mutations in the cblA complementation group. On the other hand, biochemical genetic analyses may lead to the diagnosis of a specific disorder that is caused by mutations in one of a number of gene products forming a multienzyme complex (e.g., maple syrup urine disease, where the α-ketoacid dehydrogenase enzyme is composed of four distinct subunits, each encoded by a different gene), or biochemical genetic analyses may point to a particular pathway but not the precise enzyme or transporter defect (e.g., a type I serum transferrin isoform profile may be a consequence of mutations in 1 of more than 20 different congenital disorders of glycosylation). In these latter settings, sequential Sanger sequencing of these genes is inefficient, laborious, and costly, and so next-generation sequencing (NGS) is now a very viable option, using an exome-capture gene panel sequencing approach. However, NGS does not supplant the importance of good clinical acumen and focused biochemical genetic testing.

Clinical Presentations of IEM

Acute Metabolic Encephalopathy

There are a number of conditions often classified as "small molecule disorders," whereby the consequence of a primary IEM can lead to disruption of carbohydrate, fat, or protein homeostasis resulting in acid–base instability, hypoglycemia, and/or potentially hyperammonemia. Inborn perturbations of such macronutrient metabolism often lead to acute energy deficit and secondary biochemical consequences [9]. Glycogenolysis, gluconeogenesis, lipolysis, fat oxidation, and protein catabolism support energy delivery in the form of glucose, ketones, and lactate to vital organs. An understanding of this normal energy metabolism is essential, as understanding abnormal physiology associated with an IEM allows medical intervention aimed at reverting to homeostasis normality as quickly as possible. There are often secondary consequences of accumulating metabolites such as the

inhibition of N-acetylglutamate synthase by propionyl coenzyme A leading to secondary hyperammonemia as a result, which can contribute to the severe metabolic derangement in the neonatal presentation of propionic and methylmalonic acidemia [22]. The pathways are intricately linked, as summarized in Fig. 11.5. The neonatal period is a time of catabolism, and those infants with an IEM of macronutrient processing are prone to present at this time. Typically, babies are born in relatively good condition with a symptom-free period that may last hours or days. Subsequent relentless accumulation of substrate or deficiency of product leads to instability often with nonspecific signs such as vomiting leading to encephalopathy with irritability, profound lethargy, coma, and death [23].

The small molecule disorders resulting in acute neonatal metabolic decompensation are considered below as amino acidopathies, organic acidopathies, urea cycle, fat oxidation, and glucose-related disorders.

Amino Acidopathies

Maple syrup urine disease (MSUD) is the most common acute amino acidopathy presenting in the neonatal period, but it is still rare with an approximate incidence of 1 in 200,000 births [18]. However, there are some populations such as the Pennsylvania Mennonites and in the Philippines where incidence is much higher [24, 25]. Deficiency of the enzyme complex branched-chain ketoacid dehydrogenase (BCKD) results in the accumulation of leucine, isoleucine, and valine. The accumulation of these branched-chain amino acids does not typically lead to hypoglycemia or severe acidosis, and so, there is often little to find in simple blood gas analyses. The finding of gross accumulation of valine, isoleucine, and leucine (Fig. 11.2) and their respective ketoacids in either the urine or blood is typical. Significant elevation of plasma or whole blood alloisoleucine is pathognomonic for this disorder [24]. The distinctive smell of caramelized sugar or maple syrup is thought to be due to the accumulation of sotolone in the urine or sweat. BCKD is similar to pyruvate dehydrogenase complex (PDHC) and the glycine cleavage system (GCS) in that all are multienzyme complexes and so mutations in different genes can have the same biochemical consequences. Hence, mutations predicting null activity of the enzyme complex are most likely to present in the neonatal period, and those with residual enzyme activity may not have their initial presentation until later in life, often provoked by the first illness leading to catabolism, or with weaning from breastfeeding to cows' milk and solids, resulting in an increase in protein intake beyond the metabolic capability of affected metabolic pathway. Mutations of the E1-alpha subunit have been classified as MSUD type I and of the E2-beta subunit as type II [26]. MSUD type III is due to dihydrolipoamide dehydrogenase deficiency, but this is a deficiency of a moiety integral to other complexes including pyruvate dehydrogenase complex (PDHC) and the alpha-ketoglutarate-dehydrogenase complex (KGDC) [27]. Hence, this is a more complex disorder of three different metabolic pathways, and is not MSUD alone, and so is considered to be a different condition. For MSUD, it has often been suggested that thiamine, an essential cofactor for the BCKD enzyme, can be an effective therapy as a cofactor for BCKD deficiency, but in fact this is rarely the case. It is also not pragmatic to embark in an experimental therapeutic trial of thiamine in an acutely encephalopathic child. The pathogenesis of the disorder appears to be related to leucine encephalopathy, which exerts cytotoxic damage to astrocytes with associated cerebral edema. Elevations of valine and isoleucine have no apparent clinical consequences. The natural history of classical MSUD is for babies to progress through lethargy to neurological abnormalities such as cycling dystonic movements and seizures, often with death due to herniation of the cerebellar tonsils into the foramen magnum [23]. Early therapeutic intervention, with life-long rigorously monitored treatment, can lead to normal intellectual outcome [24].

Organic Acidopathies

Catabolism of amino acids can lead to the production of more volatile organic acids that interfere with biochemical processes including acid–base and nitrogen balance. Methylmalonic acidemia (MMA), propionic acidemia (PA), and isovaleric acidemia (IVA) are all formed in the catabolism of branched-chain acids and can lead to catastrophic neonatal presentation. Hypoglycemia and severe increased anion gap metabolic acidosis with elevated lactate and ketones are features, but there can be secondary manifestations including hyperammonemia (as mentioned previously), hypocalcemia, and pancytopenia [9]. PA and MMA were originally described as ketotic hyperglycinemia, with gross elevation of glycine being typical during episodes of acute metabolic decompensation [28]. Severe classical forms of each of these disorders can rapidly progress to death with profound metabolic acidosis and multiorgan failure [29, 30].

Classical MMA occurs as a result of a deficiency of methylmalonyl-CoA mutase, with null activity referred to as mut^0. Those with residual mutase activity are classified mut^-. The cofactor for MMA mutase is adenosylcobalamin (AdoCbl), and neonates may present with metabolic acidosis due to maternal vitamin B12 deficiency (in fact, babies may be detected through newborn screening with congenital vitamin B12 deficiency where the mother was a vegan) or with disorders of the biogenesis of AdoCbl. In vitro complementation studies have been used to assign the genetic locus of the deficit of AdoCbl biogenesis, which are time-consuming and available only in a few specialist laboratories worldwide. Complementation groups A and B have defects in the biosynthesis of AdoCbl alone, while

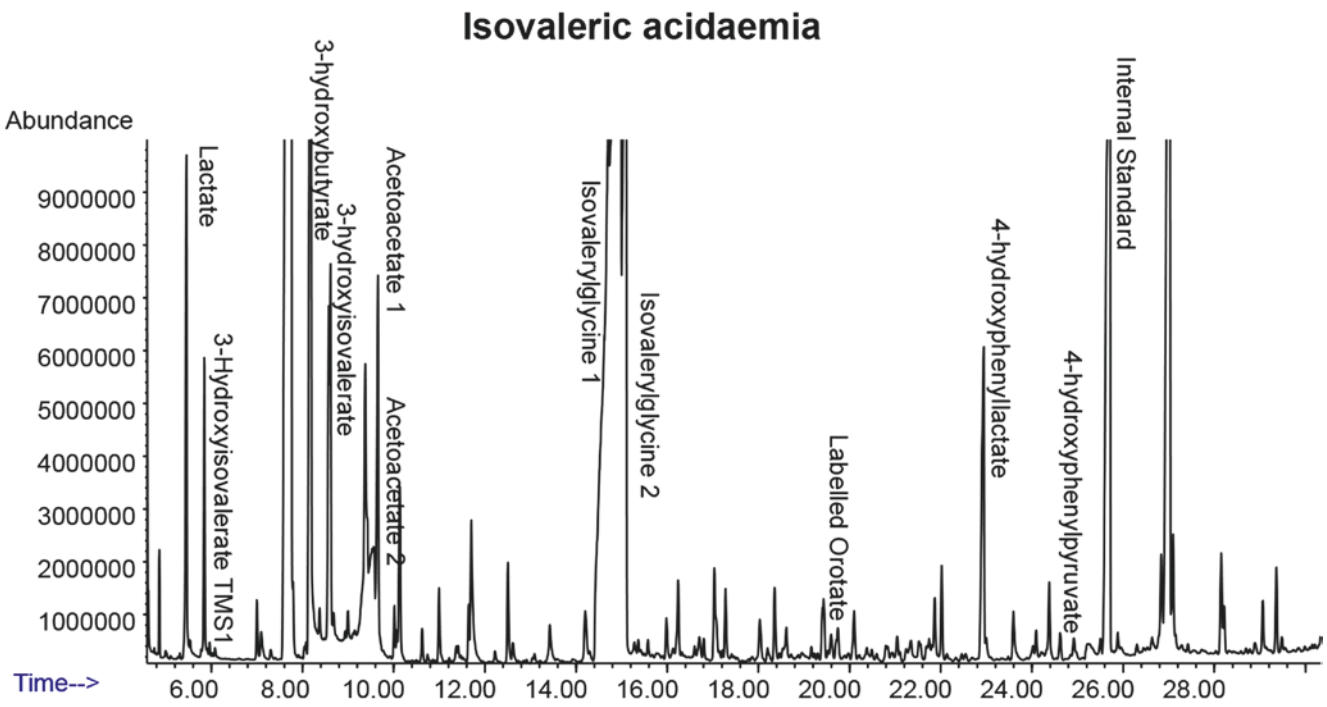

Fig. 11.4 Urine organic acid and acylglycine profile seen in isovaleric acidemia

complementation groups C, D, and F result in defects in both methyl- and adenosylcobalamin, with the latter three complementation groups having elevations of both homocysteine and MMA as a consequence. Disorders of the biogenesis of AdoCbl usually respond well to pharmacological doses of hydroxycobalamin, but it is important to consider what is being treated before vitamin B12 is administered [31]. In short, when MMA is identified in a sick neonate, it needs to be clear whether this is nutritional B12 deficiency, a sole defect of AdoCbl biosynthesis, a combined defect of methyl- and AdoCbl biosynthesis, or a deficiency of MMA mutase. Each has a different treatment course and prognosis. In particular, patients, with mut^0 and to a lesser extent mut^-, can have frequent episodes of metabolic decompensation, require rigorous dietary management, and can develop severe cardiomyopathy or progressive renal disease. Long-term complications may include the development of diabetes mellitus or recurrent episodes of pancreatitis. Patients with Cbl C deficiency also have many visual and neurocognitive long-term problems that require meticulous surveillance [30, 32]. Therefore, when MMA is significantly elevated in the urine or plasma of a neonate, the following contemporary samples should be collected and analyzed as a matter of urgency prior to treatment: serum B12 from both the child and the mother, total homocysteine and plasma amino acids, and blood ammonium. If serum B12 levels are well within the normal range, then further genetic studies or fibroblast complementation studies are indicated.

PA can be elevated in propionyl CoA carboxylase deficiency (PCC), when methylcitrate is also elevated, and in holocarboxylase synthetase and biotinidase deficiencies. PCC has two subunits, PCCA and PCCB, the isolated deficiency of either of which causes isolated propionic acidemia [29]. The cofactor for this enzyme is biotin, which can rarely be deficient in the diet. Biotinidase is an enzyme responsible for recycling biotin, which when deficient can lead to biotin dependency. However, this disorder rarely manifests clinically in the neonatal period. Holocarboxylase synthetase has a role in incorporating biotin into the functional unit of several carboxylase enzymes including PCC, pyruvate carboxylase, 3-methylcrotonyl-CoA carboxylase, and acetyl-CoA carboxylase, giving a very distinctive biochemical signature including PA, methylcitrate, lactic acid, and 3-methylcrotonyl-CoA and its esters. This condition by contrast can have a severe neonatal presentation, which is sometimes unresponsive to biotin [33].

IVA can present with neonatal decompensation due to isovaleryl-CoA dehydrogenase (IVD) deficiency. Gross elevations of IVA can lead to an odor resembling "sweaty feet." The urine metabolic screen will reveal elevated levels of 3-hydroxyisovalerate and isovalerylglycine (Fig. 11.4). IVD utilizes flavin adenine dinucleotide (FAD) as a hydrogen acceptor. Secondary elevation of IVA can occur due to defective FAD processing in a condition called multiple acyl-CoA dehydrogenase deficiency (MADD), which, as the name implies, results in functional defects of a number of key enzymes, again resulting in a complex signature profile of biochemical abnormalities in urine and blood [34].

Urea Cycle Disorders

Accumulation of surplus nitrogen from deamination of amino acids can lead to ammonium production. The urea cycle is a process of removing ammonium from the body in the more inert form of urea.

The process generates arginine, which itself has a vital role in the production of nitric oxide. Inborn errors of all enzymatic stages of the urea cycle have been identified. All are autosomal recessive apart from ornithine transcarbamylase (OTC) deficiency, which is an X-linked disorder. Classical forms of the proximal disorders, carbamoyl phosphate synthetase (CPS), N-acetylglutamate synthase (NAGS), OTC, argininosuccinate synthase (ASS), and argininosuccinate lyase (ASL) deficiencies all can present in the neonatal period with features of ammonium intoxication. These include hyperventilation with respiratory alkalosis in the early stages, rapidly progressing in hours with neurological features of vomiting, irritability, seizures, lethargy, coma, and then death. As circulatory compromise ensues, the respiratory alkalosis changes quickly to metabolic acidosis. All forms of these disorders are treatable with good long-term outcomes, and hence, rapid identification and treatment is imperative [35]. Patients with tachypnea may be erroneously thought to have sepsis or respiratory distress syndrome, but a respiratory alkalosis is not consistent with those causes. In all five of the aforementioned disorders, plasma glutamine is typically elevated when ammonium is elevated, and plasma arginine is low. The plasma citrulline and argininosuccinate may be elevated or low depending on whether the enzyme deficiency is proximal or distal to these metabolites (see Fig. 11.5). The most common of these disorders is OTC deficiency. These patients typically have a low citrulline and excrete orotic acid in the urine (formed from surplus carbamoyl phosphate). OTC deficiency is a heterogeneous disorder with later onset forms being well recognized. It is important to be aware that though X-linked, females can be just as severely affected as boys due to skewed X-inactivation, meaning that female neonatal presentation of OTC deficiency can occur. Females and males with some residual enzyme activity can present later in life after excessive protein intake or after a metabolic stress such as a severe catabolic disorder, after running a marathon, in the postoperative period, or postpartum. In addition, exposure to sodium valproate has caused fatal hyperammonemia in a number of males with milder OTC deficiency.

Disorders of Fat Catabolism

Triglycerides are composed of a 3-carbon glycerol with each carbon atom forming an ester with a long-chain hydrocarbon molecule called a fatty acid or acyl group. The acyl group is an energy dense molecule that is mobilized from triglycerides in response to humoral triggers such as glucagon. Long-chain fatty acids released into the plasma can pass across the hepatocyte's plasma membrane but are re-esterified with carnitine to cross the mitochondrial membrane (by a three-enzyme process collectively known as the carnitine cycle) to undergo fatty acid oxidation in a four-step process in the mitochondrial matrix. Each cycle of the fatty acid oxidation complex results in terminal two-carbon atoms of the fatty acid being removed from the hydrocarbon chain as acetyl-CoA, which can then selectively be utilized for ketone body synthesis as indicated in Fig. 11.5. There are inborn errors of carnitine transport, esterification with fatty acids, carnitine recycling, fatty acid oxidation, and ketone body production that occur in these processes. These lead fundamentally to tissue energy deficit, as well as, in some cases, intoxication with fatty acids. The features of these disorders in the neonatal period are typically (1) hypoketotic hypoglycemia, (2) hepatic dysfunction, or (3) cardiomyopathy and cardiac dysfunction [36, 37].

There is huge variation in the presentation of fat oxidation disorders. The most common of these, medium-chain acyl-CoA dehydrogenase (MCAD) deficiency, can present in the neonatal period, rapidly leading to death, but more typically presents in infancy [38]. Severe forms of the longer-chain disorders such as long-chain hydroxyacyl-CoA dehydrogenase (LCHAD) and very long-chain acyl-CoA dehydrogenase (VLCAD) deficiencies tend to have more severe multisystem dysfunction [37]. Biochemical findings include hypoglycemia with hypoketosis and a high free fatty acid to ketone ratio. Liver transaminase elevation, raised creatine kinase, and high blood ammonium may be seen. Metabolic acidosis may be a feature but is not typically as fulminant as in organic acidurias. An indication of the underlying diagnosis may be possible from identification of specific acylcarnitine species in the blood or low total carnitine. Urine organic acids may demonstrate a profile of elevated dicarboxylic acids with relatively low ketones or even identify specific acylglycine conjugates. As with all acutely presenting IEM, samples should be collected at the time of acute decompensation, as many of the diagnostic metabolites can become normal with treatment.

FAD is the proton acceptor for several dehydrogenase steps in fat oxidation and amino acid and choline catabolism. FAD itself forms part of two complexes, electron transfer flavoprotein (ETF) and ubiquinone oxidoreductase (ETF-QO), which ultimately facilitate transport of electrons in the electron transport chain of the mitochondria. Deficiencies of ETF and ETF-QO result in a heterogeneous condition called multiple acyl-CoA dehydrogenase deficiency (MADD), also known as glutaric aciduria type II (GA II). This may present mainly as a defect of fatty acid catabolism, although it may also have features of an organic aciduria such as isovaleric acidemia [34]. Severe forms have congenital malformations such as cystic dysplastic kidneys and absence of the corpus callosum. Because riboflavin

forms FAD, similar biochemical manifestations can be seen in nutritional riboflavin deficiency or defects of riboflavin transport [39].

Disorders of Carbohydrate Utilization

Glucose forms the major energy source for most individuals, and difficulties can arise if energy cannot be produced from glucose. Glycogen storage diseases (GSD) do not usually present in the neonatal period but can do so [40]. Glycogenolysis is triggered by hypoglycemia, which is meant to be a rapid mechanism of generating glucose from glycogen. GSD type I is a defect in generating glucose from glucose-6-phosphate either from glycogen breakdown or gluconeogenesis. Relatively short fasting between feeds can occur in normal neonates, and, in this condition, glucose cannot be produced. Profound fasting can lead to hypoglycemia, and secondary lactic acidosis can occur. The typical findings seen later in life such as hyperlipidemia, hyperuricemia, and hepatomegaly are acquired problems and may or may not be present in neonates. If glucagon is given, an apparent worsening of lactate and glucose profiles can occur in keeping with the physiology of GSD 1 [40]. Giving adequate glucose normalizes biochemistry. GSD 3 rarely presents in neonates, but this is characterized by severe ketosis and hypoglycemia after short fasts. Observing pre- and postprandial glucose, ketones and lactate profiles can help delineate the different forms of GSD. Glucose requirements above neonatal physiological requirements of 9 mg/kg/min are usually due to humoral dysregulation such as with persistent hyperinsulinemic hypoglycemia of infancy (PHHI).

Glucose is metabolized by various steps in the glycolytic pathway to pyruvate, which is converted by pyruvate dehydrogenase to acetyl coenzyme A by the pyruvate dehydrogenase complex (PDHC) as shown in Fig. 11.5. The complete oxidation of acetyl-CoA in the citric acid cycle is a major mechanism for the generation of energy for cellular activities, with the mitochondrial respiratory chain being responsible for most ATP generation. PDHC is a complex comprising of three main catalytic subunits: pyruvate dehydrogenase (E1), dihydrolipoyl transacetylase (E2), and dihydrolipoamide dehydrogenase (E3) [41]. E3, as discussed earlier, is common to BCKD and KGDC. PDHC deficiency has a broad phenotypic spectrum but can present with neonatal lactic acidosis after feeds are introduced. Lactate can increase if an excessive amount of glucose is given but improves with lower glucose delivery. A high-fat/low-carbohydrate (ketogenic) diet can improve the outcome because critical organs like the brain and cardiac and skeletal muscles can use ketone bodies as an alternate energy source. When lactate is elevated, the lactate/pyruvate ratio is often <20, indicative of pyruvate accumulation, but this is an inconsistent finding. PDHC deficiency can also present as Leigh disease or with congenital cerebral abnormalities such as the absence of the corpus callosum and cystic necrotic changes of the cerebral cortex.

Table 11.4 Differential diagnosis of neonatal lactic acidosis

Cause	Comments
Spurious	Tourniquet applied for too long, difficult blood collection, or poor sample handling – whole blood lactate needs to be processed quickly and meticulously
Shock	Sepsis, necrotizing enterocolitis, cardiac failure, patent ductus arteriosus, cardiopulmonary arrest, multiorgan failure
Administered fluids	Lactate in intravenous fluids and peritoneal dialysate
Hypoxic ischemic encephalopathy	Lactate can remain elevated for several days, but some of these babies could have an MRCD, particularly if the lactic academia is persistent
Neonatal epileptic encephalopathy	Intense seizure disorder could cause secondary lactate elevation, but some may have an MRCD
Glycogen storage disease type I	Lactate typically elevated after fasts of more than 4 h
Organic acidemia	Can usually be discriminated from urine organic acid assessment and an increased anion gap
Pyruvate dehydrogenase complex deficiency	Lactate/pyruvate (L/P) ratio <20. Deteriorates with more glucose and improves with ketogenic diet; L/P ratio may be >25 if there is a primary MRCD

Neonatal Lactic Acidosis

The term "congenital lactic acidosis" is often used to describe a neonate with a mitochondrial respiratory chain disorder (MRCD), but this is unhelpful. There is a differential diagnosis of neonatal lactic acidosis as indicated in Table 11.4 that should be considered before arriving at a potential diagnosis of MRCD.

There is a huge range of presentations of MRCD, and diagnostic testing can be fraught [42]. Neonatal encephalopathy with lactic acidosis is one form of presentation of MRCD. Diagnostic testing will be discussed further in the section on perimortem investigations.

Approach to Testing Neonatal Metabolic Encephalopathy

Newborns have a limited repertoire of behaviors in response to acute pathology, and findings can be nonspecific. Often babies may be thought of as having sepsis [23]. In this situation, basic chemistry including assessment of electrolytes, liver function, glucose, blood gas including anion gap, lactate, ketones, and ammonia should be able to screen for most disorders [9]. Second-tier testing would include plasma amino acids, plasma carnitine profile, urine organic acids, and potentially urine amino acids. A biochemical

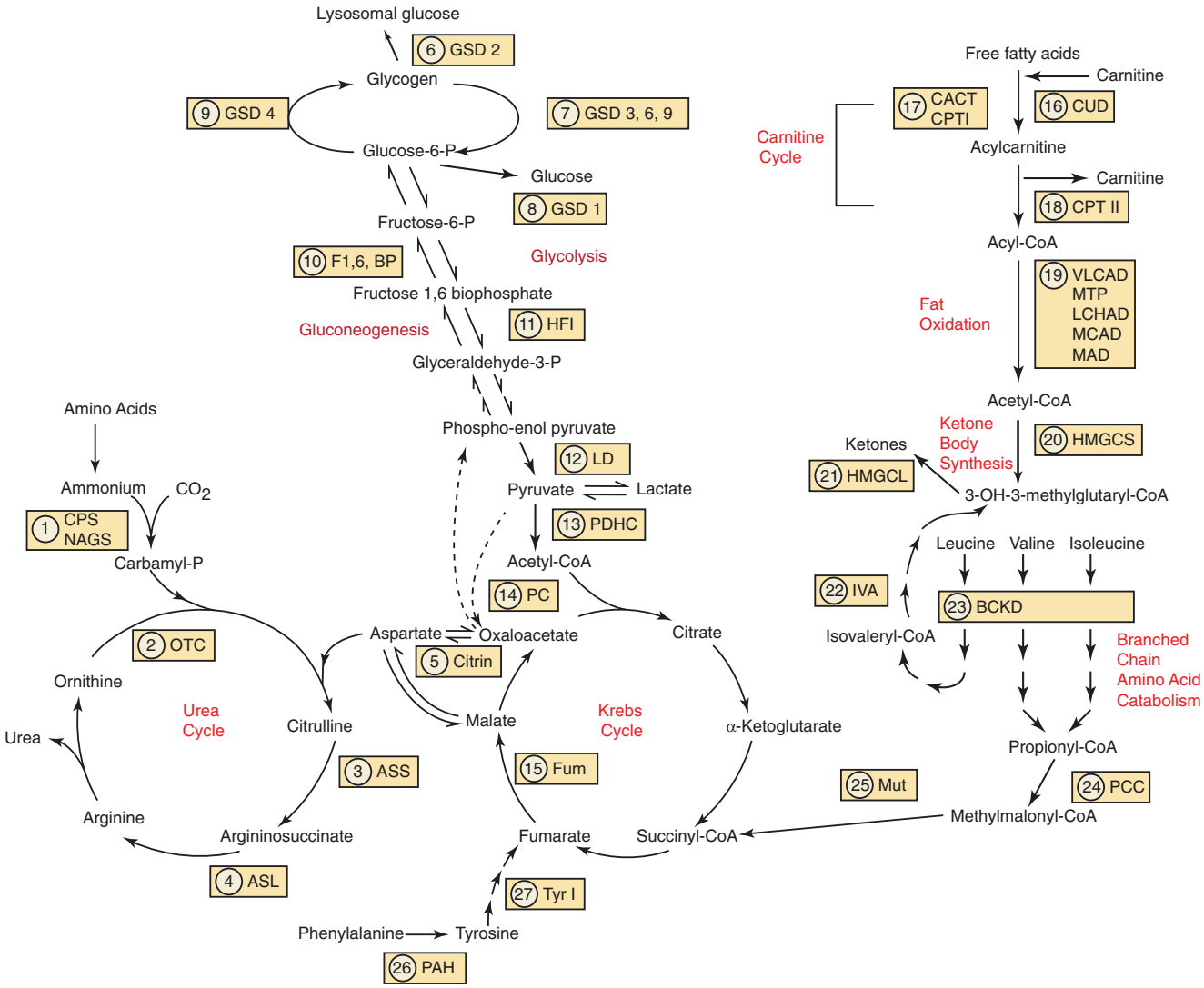

Fig. 11.5 Key metabolic pathways associated with inborn errors of metabolism. Small molecule disorders causing predominantly acute metabolic encephalopathy. Disorders or enzymes that are deficient are referred to in the text. (1) *CPS* carbamoyl phosphate synthase, *NAGS* N-acetylglutamate synthetase. (2) *OTC* ornithine transcarbamylase. (3) *ASS* argininosuccinate synthase (type I citrullinemia). (4) *ASL* argininosuccinate lyase (argininosuccinic aciduria). (5) *Citrin* (type II citrullinemia, neonatal intrahepatic cholestasis of infancy due to SLC25A13 deficiency). (6) *GSD 2* glycogen storage disease type II (also known as lysosomal acid glucosidase, acid maltase deficiency, and Pompe disease). (7) *GSD 3,6,9* glycogen storage disease types 3, 6, and 9 (glycogen brancher enzyme, glycogen phosphorylase, glycogen phosphorylase kinase). (8) *GSD 1* glycogen storage – type I (glucose-6-phosphatase deficiency – von Gierke's disease). (9) *GSD 4* glycogen storage disease type IV (glycogen brancher enzyme deficiency). (10) *F1,6 BP* fructose 1, 6-bisphosphatase deficiency. (11) *HFI* hereditary fructose intolerance (aldolase B deficiency). (12) *LD* lactate dehydrogenase. (13) *PDHC* pyruvate dehydrogenase complex. (14) *PC* pyruvate carboxylase. (15) *Fum* fumarase. (16) *CUD* carnitine uptake disorder (SLC22A5 deficiency). (17) *CACT* carnitine–acylcarnitine translocase (SLC25A20 deficiency); *CPT I* carnitine palmitoyltransferase I. (18) *CPT II* carnitine palmitoyltransferase II. (19) Disorders of mitochondrial fatty acid oxidation spiral: *VLCAD* very long-chain acyl-CoA dehydrogenase, *MTP* mitochondrial trifunctional protein, *LCHAD* long-chain hydroxyacyl-CoA dehydrogenase, *MCAD* medium-chain acyl-CoA dehydrogenase, *MAD* multiple acyl-CoA dehydrogenase. (20) *HMGCS* 3 hydroxy-3-methylglutaryl-CoA synthase. (21) *HMGCL* 3 hydroxy-3-methylglutaryl-CoA lyase. (22) *IVA* isovaleric acidemia (isovaleryl-CoA dehydrogenase deficiency). (23) *BCKD* branched-chain alpha-ketoacid dehydrogenase. (24) *PCC* propionyl-CoA carboxylase. (25) *Mut* (methylmalonyl-CoA mutase). (26) *PAH* phenylalanine hydroxylase. (27) *Tyr I* tyrosinemia (due to fumarylacetoacetate hydrolase deficiency)

testing algorithm is outlined in Fig. 11.6. A lactate/pyruvate ratio can be measured if lactate is elevated and PDHC deficiency or MRCD is suspected. It should be ensured that a newborn screening blood spot test has been collected, and if death seems imminent, consider perimortem investigations (see later).

Treatment of Neonatal Metabolic Encephalopathy

As mentioned previously, the course of many of these disorders is rapidly fatal and yet most can be treated [38, 43]. Therefore, the possibility of an IEM must be considered

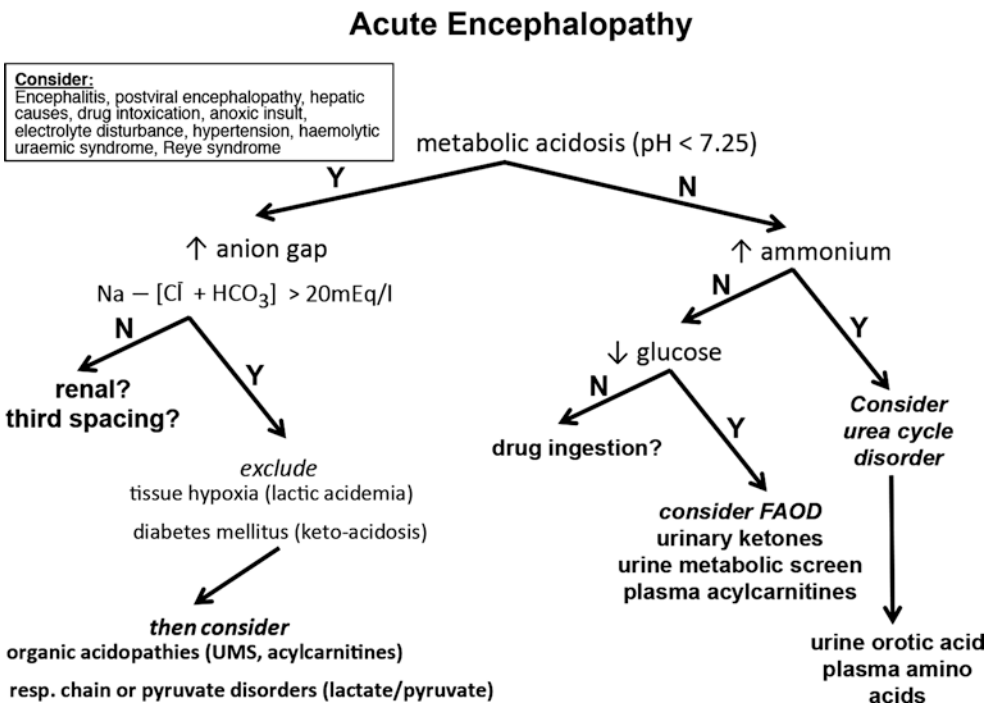

Fig. 11.6 Diagnostic algorithm for inborn errors of metabolism presenting as an acute encephalopathy

early in the differential diagnoses (Fig. 11.6), and investigation must occur in parallel with consideration of treatment [23]. In generic terms, these are disorders of energy metabolism, and treatments are geared toward circumventing the enzyme block and restoring energy provision to the infant by other means. Temporary elevation of toxic metabolites such as ammonium may need to be removed as part of the initial resuscitative phase of treatment by dialysis/hemofiltration and/or ammonia-lowering medications. The provision of substrates upstream of an enzyme block needs to be withheld initially and restricted long term; for example, leucine in MSUD or long-chain fat in long-chain fat oxidation disorders [26]. Products downstream of an obstruction may need to be replenished, such as arginine in urea cycle disorders [35]. The restriction of whole protein in disorders of specific amino acid catabolism necessitates whole protein restriction with replenishment of the amino acids not directly involved in the disorder. The micronutrients associated with whole protein such as calcium in milk need also to be replenished. Restoration of energy supply is possible, usually by using an alternative macronutrient such as fat in the ketogenic diet used for PDHC deficiency [41].

Neonatal Epileptic Encephalopathy (NEE)

Severe seizure disorders are seen in children with hypoxic ischemic encephalopathy. Some neonates develop intractable seizures in the neonatal period with no apparent cause and are refractory to treatment with standard doses of anticonvulsant therapy. There is a range of quite different IEM that can cause seizures. It is important to note that not only can a diagnosis be made but some disorders can be effectively treated. Failure to implement therapy in a timely manner can lead to severe irreversible neurotoxicity and death. Most notable among these causes are pyridoxine-responsive seizures and pyridoxal phosphate-responsive seizures, which can reverse seizure activity in minutes [44, 45]. There is also a novel treatment for molybdenum cofactor deficiency type A [46]. Evaluation would incorporate consideration of disorders that cause metabolic encephalopathy as above, but there is usually some indication from baseline biochemical evaluation. Some disorders may be associated with dysmorphic syndromes such as sulfite oxidase deficiency or Zellweger syndrome [47]. Baseline biochemistry should include assay electrolytes, liver function tests, full blood count, uric acid, creatine kinase, and vitamin and mineral assessment including vitamins B1, B2, B6, B12, and folic acid as well as calcium, magnesium, copper, and zinc. Investigations looking at the contemporary concentration and ratios of metabolites such as glucose, lactate, glycine, and serine in the plasma and cerebrospinal fluid can screen for specific conditions. If possible, assay of CSF neurotransmitters and pterins should be performed, but this is only assayed in selected laboratories worldwide and has meticulous collection conditions [48]. Table 11.5 indicates causes that should be considered [44, 45, 48–58] (Figs. 11.7 and 11.8).

In addition to the IEM listed causing NEE, there are many genetic types of epilepsy classified as different forms of

Table 11.5 Genetic metabolic causes of neonatal epileptic encephalopathy [44, 45, 48–58]

Disorder	Test	Comment
Nonketotic hyperglycinemia	Paired CSF/plasma glycine (amino acids) ratio typically >0.04 Urine amino acids may show glycine	Classical NKH is a defect in one of the 3 genes (*GLDC*, *AMT*, *GCSH*) forming the glycine cleavage system (GCS). Fulminant seizure disorder with periods of early apnea. However, apnea subsides leaving a refractory seizure disorder. Assay of GCS activity from hepatic tissue is now rarely performed. Atypical forms of NKH may have elevated CSF glycine with a normal ratio. Defects of iron–sulfur cluster biogenesis can cause variable defects of GCS, PDHC, and MRCD
Sulfite oxidase (SUOX)/molybdenum cofactor deficiency (MoCo)	Urine amino acids – S-sulfocysteine identified in both disorders. Low cystine and uric acid in MoCo deficiency. Urine purines and pyrimidines delineate disorders Bedside urine sulfite measurements may be unreliable	Classical sulfite oxidase deficiency leads to aggressive often terminal seizure disorder. Congenital malformations include, prominent forehead, small nose, long philtrum, and thick lips. Patients acquire dislocated lenses, cerebral atrophy and cystic encephalomalacia (Fig. 11.7). Defects identified in SUOX gene. MoCo deficiency leads to combined deficiencies in sulfite oxidase, xanthine dehydrogenase, mitochondrial amidoxime reducing component (mARC), and aldehyde oxidase. MoCo complex is composed of 3 subunits – MoCo A, B, and C. Cyclic pyranopterin monophosphate can be used to treat type A
Serine biosynthesis disorders	CSF amino acids (PLAA not definitive) *PSAT1* and *PHGDH* gene testing	Seizures typical later than neonatal period but could present in neonatal period with recurrent apnea
Hyperinsulinism–hyperammonemia (HI–HA) syndrome	Hypoglycemia, hyperinsulinism Hyperammonemia Glutamate dehydrogenase (GDH) gain of function mutations	Leucine-sensitive hyperinsulinism with hyperammonemia refractory to treatment with low protein diet or ammonia scavengers. Seizures often related to profound hypoglycemia but can occur persistently. Hyperammonemia thought to be benign
D2-hydroxyglutaric aciduria	Urine organic acids	D2HGDH and IDH2 genetic mutations
Biotinidase deficiency	Dried blood spot biotinidase Urine organic acids Plasma acylcarnitines	Seizures typical later than neonatal period and patients have dermatological features. Holocarboxylase deficiency more likely to cause early onset seizure disorder
Pyridoxine-responsive seizures (antiquitin)	Urinary α-aminoadipic semi-aldehyde; *ALDH7A1* gene testing	Clinical trial indicated in refractory NEE of treatment for all children under 1 year Nonspecific elevation of urine pipecolic acid (organic acids)
Pyridoxal phosphate-responsive seizures (PNPO)	Abnormal CSF amino acid profile Abnormal CSF neurotransmitter profile High urine vanillactate (organic acids) Low CSF pyridoxal phosphate and folate *PNPO* gene testing	Clinical trial indicated in refractory NEE Folinic acid can also be used as an adjunct
Hereditary folate transport disorder	CSF methionine and folate Increased urinary formiminoglutamic acid Mutations of *SLC46A1* gene	Presents with hypotonia, developmental delay, seizures, and athetosis
Mitochondrial respiratory chain disorders	CSF and blood lactate Fresh/frozen liver and muscle assays	Leigh or Alpers phenotype. Could present with isolated seizures with or without lactic acidosis. May have liver dysfunction
Pyruvate dehydrogenase complex deficiency	Lactate/pyruvate ratio, fibroblast enzyme assay	Could present with acute metabolic decompensation with lactic acidosis, seizures, or developmental delay. Cystic encephalomalacia seen in those with a neonatal onset
Fumarase deficiency	Urine organic acids (fumarate) *FH* gene testing	Associated with intractable seizures, acquired microcephaly, and developmental delay
Peroxisomal disorders	Plasma VLCFA, red-cell plasmalogens, and serum bile acids (see Table 11.3) Targeted exome sequencing	Often some dysmorphic features. May have liver dysfunction. Could present as neonatal adrenoleukodystrophy. Heterogeneous presentations

(continued)

Table 11.5 (continued)

Disorder	Test	Comment
Tay–Sachs Sandhoff Krabbe	White-cell enzymes Genetic testing of relevant gene	Seizures not typical in the neonatal period but neurological signs such as excessive startle reaction and hyperreflexia are acquired. Cherry red spot may be present
GM3 synthase deficiency	*ST3GALV* gene testing	Very rare and more prevalent in Amish community
Neuronal ceroid lipofuscinosis	Assay of enzymes PPT1 and TPP1 Targeted exome sequencing	More than 15 genetic forms of NCL (Batten disease) are known – 2 of the lysosomal enzymes are readily available to measure but others are not. Overlapping phenotypes between different genotypes with progressive retinopathy, developmental delay, and seizures typical
Congenital disorders of glycosylation (CDG)	Transferrin isoforms (N-glycosylation) PMM enzyme assay Apolipoprotein CIII isoforms (O-glycosylation) Targeted exome sequencing	Wide range of different presentations. Can present with seizures, stroke, or stroke-like episodes. May have coagulopathy of prothrombotic tendency. O-glycosylation defects include Walker–Warburg syndrome and Fukuyama muscular dystrophy
GLUT 1	Paired CSF and plasma glucose *SLC2A1* mutation testing	Neonatal epileptic encephalopathy unusual but occurs in infancy. Acquired microcephaly and developmental delay. Hypoglycorrhachia (CSF glucose, less 2.6 mmol/L with ratio to plasma <0.5, but this is variable). Low CSF lactate. Autosomal dominant condition
Menkes	Ceruloplasmin, copper low but can be unreliable in neonates *ATP7A* mutation testing	Seizures unlikely in neonatal period but can present with hypothermia, skin laxity, pudgy cheeks, and sparse hair Kinky "steel wool" hair (Fig. 11.8) evolves in infancy. Seizures typical from 2 months of age. Wormian bones in skull X-ray
Familial hypomagnesemia	Serum magnesium and calcium *CLDN16* gene sequencing	Generalized hypocalcemic–hypomagnesemic seizures occur due to a defect in intestinal and renal reabsorption of magnesium

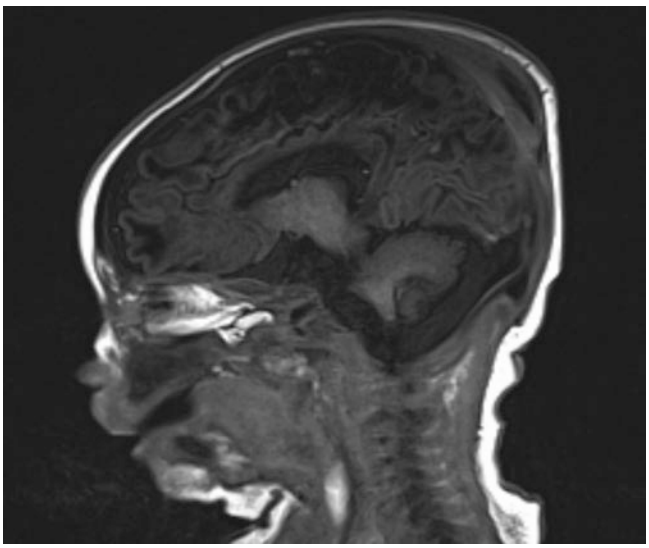

Fig. 11.7 Brain MRI of a 5-day-old infant with molybdenum cofactor deficiency type A. The features are those of cystic encephalomalacia with multiple cysts in the subcortical white matter. An occipital subdural collection can also be seen

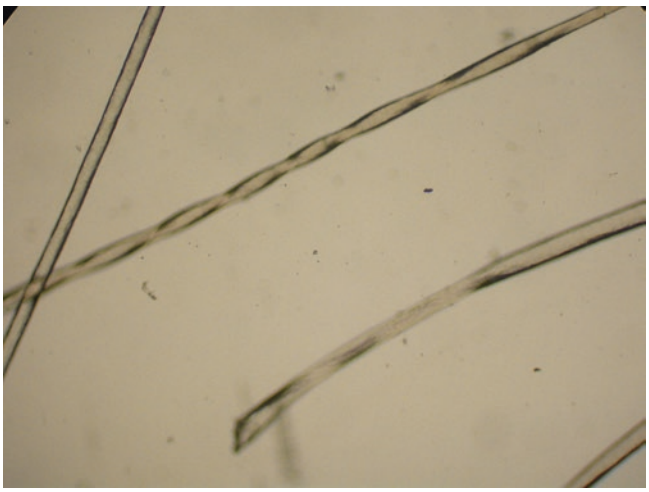

Fig. 11.8 Microscopy of hair from a boy with Menkes disease. These hairs appear flattened and twisted and are brittle

early infantile epileptic encephalopathy (EIEE). There are more than 100 genes listed on the Online Mendelian Inheritance in Man (OMIM) Website as causing this presentation [59]. These include defects of voltage-gated sodium and potassium channels due to mutations of *SCN1A*, *SCN2A*, *KCNQ*, and *KCNQ3*; atypical Rett syndrome in some patients with *MECP2*, *CDKL5*, or *FOXG1* abnormalities; or defects of postsynaptic vesicular docking mechanisms in STXPB1 deficiency [60, 61].

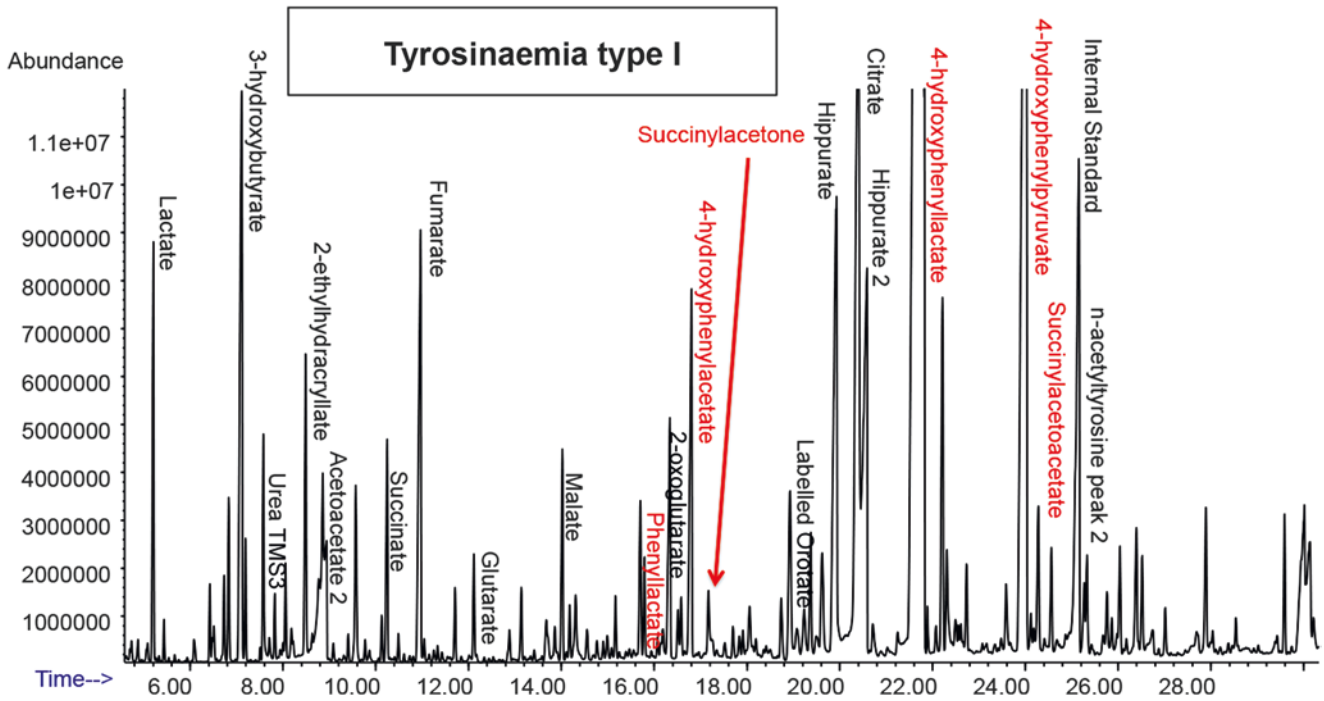

Fig. 11.9 Urine gas chromatography trace of tyrosinemia type I. The diagnostic marker is succinylacetone, while other abnormal metabolites (e.g., phenyllactate, 4-hydroxyphenylacetate, and 4-hydroxyphenylpyruvate) are nonspecific markers of liver dysfunction

Neonatal Hepatic Disease and Nonimmune Jaundice

Hepatic Intoxication

IEM can cause acute hepatic intoxication, progressive cholestasis, or progressive cirrhosis. In addition to a wide range of IEM causing hepatic dysfunction, there is a large range of infective, toxic, and obstructive causes. For progressive liver dysfunction, detailed assessment of transaminases and unconjugated and conjugated bilirubin fractions should be measured. Hemolysis should be considered with significant unconjugated hyperbilirubinemia. Liver synthetic function should be assessed with deteriorating liver function. Liver ultrasound and dynamic assessment of bile acid excretion should be considered [62]. The treatable IEMs that typically cause acute liver failure are classical galactosemia and tyrosinemia type I (see Fig. 11.9 for characteristic urine GC tracing). Children seem to be well in the first few days of life, establishing enteral feeds normally, but the respective toxic agents galactose-1-phosphate and succinylacetone impair hepatic and renal tubular functions, leading quite rapidly over days to a child with synthetic liver failure potentially with jaundice. *Escherichia coli* septicemia is a well-documented association with classical galactosemia. It is imperative to have a high index of suspicion of these two conditions in particular as timely intervention is lifesaving and prevents morbidity [63, 64]. This means stopping feeds, managing with intravenous dextrose/saline, and performing diagnostic tests such that definitive management can be instituted. Several of the organic acidemias and fat oxidation defects can also cause severe liver dysfunction, but other biochemical manifestations are seen. It must be remembered, however, that in liver failure, hypoglycemia, metabolic acidosis, and hyperammonemia may be secondary features. Causes of hepatic liver dysfunction and hyperbilirubinemia are considered in Table 11.6 [55, 63–75].

Neonatal Cholestasis

Neonatal cholestasis is another relatively common presentation of a range of non-metabolic disorders and IEM. Diagnostic testing of a broad range of rare conditions can take time. Some tests are particularly time critical, particularly identifying biliary atresia, which ideally requires surgical correction within the first weeks of life. If impaired biliary flow is demonstrated, a liver biopsy is often indicated. At the time of liver biopsy collection, skin biopsy collection (for Niemann–Pick disease type C) and muscle biopsy (for MRCD) should be considered [67, 76]. Specific features of IEM are not often seen in biopsy reports, with nonspecific hepatitis and giant-cell arteritis being common findings. Alpha-fetoprotein is often grossly elevated but does not correlate with an increased carcinogenic risk. Some causes of cholestasis may have other features such as mitochondrial DNA depletion having raised lactate and neurological signs

Table 11.6 IEM with a predominant hepatic pathology [55, 63–75]

Disorders of hepatic intoxication		
Disorder	**Tests**	**Comments**
Neonatal hemochromatosis	Ferritin, MRI studies, Biopsy	Majority of cases are not hereditary. Rapidly progressive disorder
Tyrosinemia type I	Urine organic acids (succinylacetone) (Fig. 11.9). Plasma amino acids. *FAH* gene testing	Fumarylacetoacetate deficiency. Responds well to treatment Long-term risk of hepatocellular carcinoma without adequate metabolic treatment
Galactosemia	Galactose-1-phosphate assay Gal-1-P-UT assay *GALT* gene testing	Responds quickly to galactose-restricted diet. Oil-drop cataracts typically not seen in the first few days of life but may appear later if untreated. Different forms with variable requirement for treatment. Long-term neurological outcome variable. Galactokinase deficiency develops cataracts without liver dysfunction Bedside urine-reducing sugars are unreliable
Hereditary fructose intolerance	*ALDOB* gene testing (3 common mutations)	Unlikely in neonates but could present with liver dysfunction and lactic acidosis if copious sucrose given
Fructose-1-6-bisphosphate deficiency	*FBP1* gene testing	Unlikely in neonates but could present with liver dysfunction and lactic acidosis with fasting
Mitochondrial DNA depletion	Biopsy and mtDNA quantitation Specific genetic testing	Usually with neurological signs and lactic acidosis
Other mitochondrial disorders	Biopsy and Respiratory chain assay Specific genetic testing	Usually has multisystem involvement and lactic acidosis if it presents in the neonatal period
Fat oxidation disorder	Plasma acylcarnitine profile Fibroblast fat oxidation flux studies DNA testing	Usually associated with hypoketotic hypoglycemia and may have hyperammonemia or cardiomyopathy Dietary long-chain fat and fasting compound abnormalities
Crigler–Najjar syndrome	Plasma bile assays *UGT1A1* gene testing	Deficiency of UDP-glucuronosyltransferase. Null activity leads to persistent neonatal unconjugated hyperbilirubinemia. Partial activity leads to Gilbert syndrome
Disorders associated with cholestasis		
Alpha1-antitrypsin deficiency	Serum alpha1-antitrypsin *SERPINA1* gene testing	PiZ phenotype associated with more severe manifestations. Deficiency of a protease inhibitor that can lead to intracellular inclusions being deposited at times of physiological stress. This initiates chronic inflammation
Disorders of bile acid synthesis	Plasma and urine bile acids	Large range of conditions associated with synthetic defects of bile acids. Normal gamma-glutamyl transferase typical in the context of raised conjugated bilirubin. Acholic stools. Chondrodysplasia seen in some forms
Niemann–Pick disease type C	Fibroblast filipin staining *NPC1* and *NPC2* gene testing	Heterogeneous disorder. Splenomegaly may occur early or be acquired. Neurological findings (including upward gaze palsy) usually acquired in infancy or childhood
Citrin deficiency	Plasma amino acid profile *SLC25A13* gene testing	Heterogeneous disorder prevalent in Southeast Asia. Neonatal intrahepatic cholestasis is one presentation that usually resolves spontaneously. Disorder of mitochondrial malate–aspartate shunt
Progressive familial intrahepatic cholestasis	Serum bile acids Serum cholesterol *ATP8B1*, *ABCB11*, *ABCB4* gene testing	PFIC classified into types 1–3, with defects of intrahepatic cholestasis being primary features and with type I having pancreatic exocrine insufficiency. All progress to cirrhosis
Congenital disorders of glycosylation	Transferrin isoforms (N-glycosylation) PMM and MPI enzyme assay Targeted exome sequencing	CDG Ib – mannose phosphate isomerase (MPI) is treatable causing liver dysfunction and diarrhea. CDG Ia associated with a range of problems including liver hepatocellular and synthetic dysfunction, ascites, and pericardial effusions. Both type Ia and Ib are associated with persistent hyperinsulinemic hypoglycemia of infancy. Other forms of CDG also cause liver dysfunction
Peroxisomal biogenesis disorders	Plasma VLCFA Targeted exome sequencing	Usually some dysmorphic features. May have atypical neurology. Heterogeneous presentations
Disorders causing jaundice due to hemolytic anemia		

Table 11.6 (continued)

Glucose-6-phosphate dehydrogenase deficiency	RBC enzyme assay	The most common X-linked inherited cause of hemolytic anemia. Many patients are asymptomatic but provoked by oxidative stress
Pyruvate kinase deficiency	RBC enzyme assay	The most common form of nonimmune, non-red-cell morphology-related neonatal hemolysis
Gamma-glutamylcysteine synthase deficiency	RBC enzyme assay	Causes glutathione deficiency
Glutathione synthetase deficiency	RBC enzyme assay Urine organic acids (5-oxoproline)	Lactic acidosis and developmental delay can occur
Congenital erythropoietic porphyria	Urine and stool porphyrins Mutation testing	Develop photosensitive rash

or peroxisomal biogenesis defects with dysmorphic features, so detailed multisystem evaluation needs to be performed in such circumstances [47, 77].

IEM Causing Hydrops Fetalis

IEM account for approximately 1 % cases of hydrops fetalis, with the most important causes summarized in Table 11.7 [40, 50, 68, 69, 78–80]. Most causes of neonatal hemolysis (see earlier) could cause hydrops fetalis. It has been postulated that some IEM may lead to liver synthetic dysfunction causing hypoalbuminemia and consequent hydrops. This potential mechanism is certainly plausible for congenital disorders of glycosylation, as similar physiology is seen postnatally [69]. For several lysosomal storage disorders, liver dysfunction may be a component but so may cardiac dysfunction and tissue dysplasia leading to impaired lymphatic return [55, 68]. The prognosis for hydrops fetalis is often poor, and this remains true for IEM causes. The hematological causes may respond to aggressive treatment, so these must be considered at the forefront of investigations in live fetuses. More often, however, in utero death and stillbirth are typical outcomes. In such circumstances, rigorous investigation needs to occur for an accurate genetic diagnosis that would permit more accurate discussions about recurrence risks and potential prenatal testing in subsequent pregnancies. If nothing else can be done, trying to establish fibroblast growth from the umbilical cord is a priority, particularly in recurrent cases, as this could form the basis of further enzymatic, functional, and genetic testing. If possible, a skeletal survey should be performed and samples collected for VLCFA, transferrin isoforms, leukocyte enzymes for lysosomal storage disorders, as well as whole blood for DNA extraction. If urine is available, total glycosaminoglycans (GAG) may be assessed (although there may be difficulties with interpretation of neonatal samples), specific GAG electrophoresis patterns may be observed, and urine oligosaccharides can be assayed. Careful histological examination of the placenta may also be very instructive, for instance, in glycogen storage disease type IV (Andersen disease; Fig. 11.10). For the hydropic fetus, amniocentesis to

Table 11.7 Nonhemolytic IEM causes of hydrops fetalis [40, 50, 68, 69, 78–80]

Disorder	Test	Gene affected
MPS I (Hurler)	WBC enzymes, GAGS	*IDUA*
MPS IV (Morquio)	WBC enzymes, GAGS	*GALNS*
MPS VI (Maroteaux–Lamy)	WBC enzymes, GAGS	*ARSB*
MPS VII (Sly)	WBC enzymes, GAGS	*GUSB*
Mucolipidosis type II (I-cell disease)	Skeletal survey, WBC enzymes	*GNPTAB*
Type I sialidosis	Fibroblast assay, oligosaccharides	*NEU1*
GM1 gangliosidosis	WBC enzymes	*GLB1*
Farber disease	WBC enzymes	*ASAH1*
Type II Gaucher disease	WBC enzymes	*GBA*
Niemann–Pick A	WBC enzymes	*SMPD1*
Wolman disease	WBC enzymes	*LIPA*
Multiple sulfatase deficiency	WBC enzymes, GAGS	*SUMF1*
Galactosialidosis	WBC enzymes	*CTSA*
Niemann–Pick C	Fibroblast filipin staining	*NPC1*, *NPC2*
Smith–Lemli–Opitz syndrome	7-Dehydrocholesterol assay	*DHCR7*
Congenital disorders of glycosylation	Transferrin isoforms	>20
Peroxisome biogenesis disorders	VLCFA	>20
Pearson syndrome	Bone marrow biopsy	mtDNA deletions
glycogen storage disease type IV (GSD 4)	Postmortem pathology	*GBE1*
Fumarase deficiency	Urine organic acids	*FH*
Greenberg skeletal dysplasia	Fibroblast sterols, skeletal survey	*LBR*

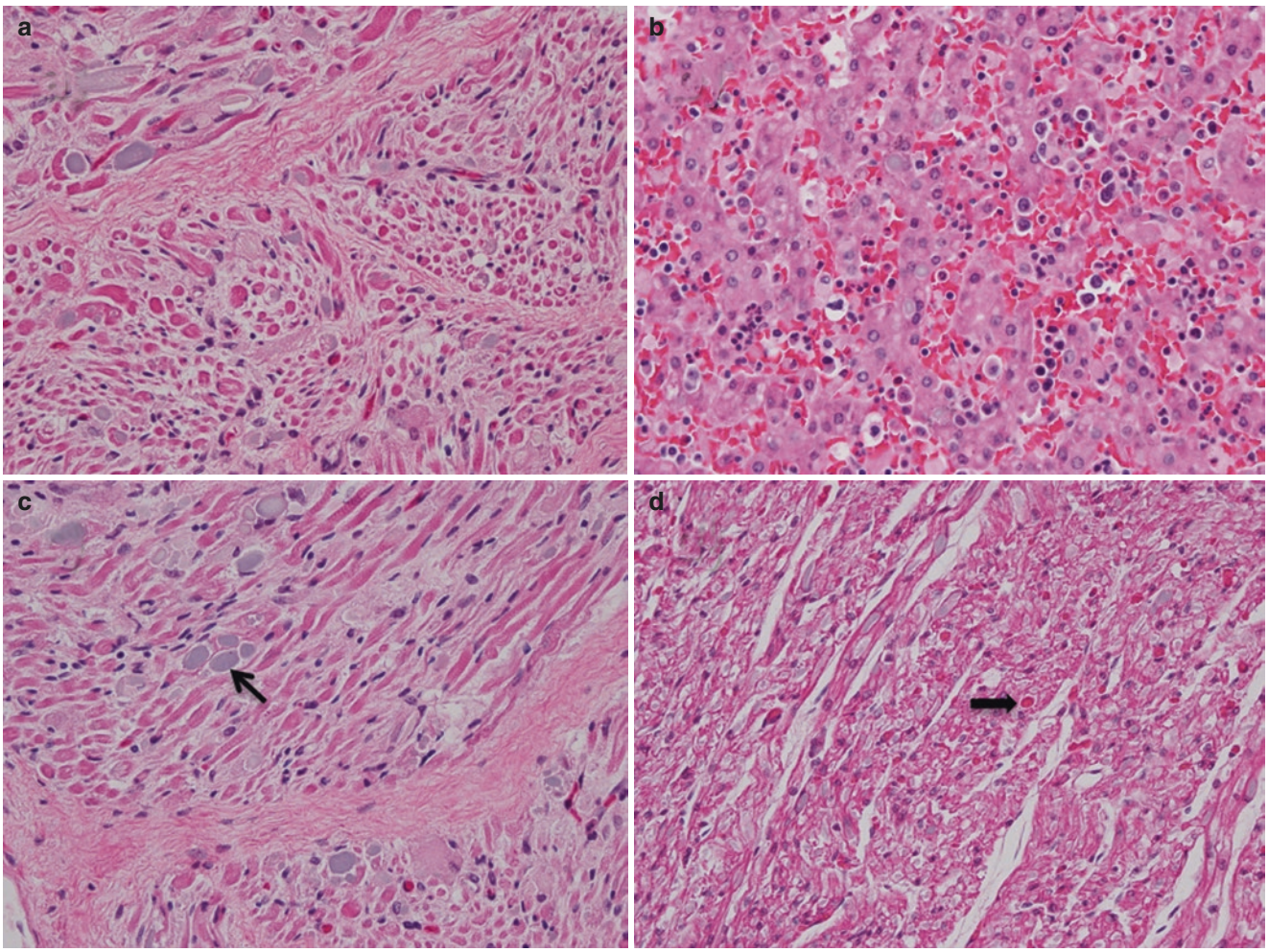

Fig. 11.10 Histopathology diagnostic of glycogen branching enzyme deficiency (glycogen storage disease type IV). Thirty-eight-week gestation pregnancy with 4-week history polyhydramnios. Presentation with abnormal CTG, emergency cesarean section, but death in utero. PASD, colloidal iron-positive storage material in multiple organs including brain and ganglion cells. (**a**) Psoas muscle (×40): varying size globules in skeletal muscle fibers with some fiber atrophy. (**b**) Liver (×40): scattered small globules in hepatocytes. (**c**) Heart (×40): varying size globules in myofibers (*thin arrow*). (**d**) Heart (×40): globules stain with PASD (*thick arrow*), which shows more globules than seen on H&E (Images and descriptions kindly provided by Dr. Susan Arbuckle, Histopathology Department, Children's Hospital at Westmead, Sydney, Australia)

establish a cultured cell line and for amniotic fluid metabolic analysis is often helpful in establishing the diagnosis.

IEM Causing Cardiomyopathy

There are a number of causes of cardiomyopathy in the neonate – some genetic; others not. From the perspective of IEM, the causes can broadly be related to energy deficit with compensatory cardiomyopathy (which may be hypertrophic or dilated), toxicity, or infiltration. In the live child, the cardiology assessment of cardiomyopathy should be comprehensive in order to delineate primary and secondary features such as anatomy, physiology (shunts and oxygenation), integrity of conduction pathways, dimensions, and functional ability of the heart. The metabolic assessment incorporates these physiological parameters in the context of the metabolic milieu. It can sometimes be difficult to distinguish the primary cause with impaired tissue perfusion and lactic acidosis. In the neonatal period, all of the organic acidemias mentioned previously can cause cardiomyopathy. In addition to these, malonic acidemia seems particularly associated with cardiomyopathy. Similarly, several of the severe forms of fat oxidation defects such as VLCAD, carnitine transporter defect, carnitine–acylcarnitine translocase, carnitine palmitoyltransferase II, and multiple acyl-CoA dehydrogenase deficiencies can cause cardiomyopathy. Usually, there are associated features as described in the section on acute metabolic encephalopathies to guide investigation and treatment [9].

For some disorders, skeletal myopathy accompanies cardiomyopathy. Classical Pompe disease (acid alpha-glucosidase or acid maltase deficiency) presents with babies having a normal-appearing heart at birth. However, hypertrophic cardiomyopathy and skeletal myopathy rapidly progress in the first few

weeks of life with a median age of death at 8 months. Other specific clues may include macroglossia and a characteristic electrocardiogram (ECG). Timely intervention can avert this natural history [81]. Barth syndrome is an X-linked disorder of cardiolipin metabolism associated with cardiomyopathy. If neutropenia, urinary 3-methylglutaconic acid, neither of which are invariant features, and cardiomyopathy (usually dilated but may also see left ventricular non-compaction) are observed, particularly in a boy, then sequencing of the *TAZ* gene should be performed [82]. Therefore, first-tier tests for cardiomyopathy would include an arterial blood gas with anion gap calculation, electrolytes, liver function tests, creatine kinase, full blood count (neutropenia for Barth syndrome) and film (vacuolated lymphocytes for lysosomal storage disorders), plasma lactate, glucose, and ketones. A dried blood spot for enzyme assay for Pompe disease should be collected and sent to a reference laboratory. If there is any suspicion of an organic acidemia or fat oxidation defect, both plasma carnitine profile and urine organic acids should be analyzed urgently.

There is a range of other IEM where cardiomyopathy may be observed, but which should also have other systemic features pointing to a specific diagnosis. These include several of the lysosomal storage disorders, congenital disorders of glycosylation, and peroxisomal disorders mentioned previously that cause hydrops fetalis. These, together with mitochondrial respiratory chain disorders, could have an infiltrative element to their presentation, although for the latter, dilated cardiomyopathy may also be seen. Therefore, in selected circumstances, transferrin isoforms, VLCFA, white cell enzymes for lysosomal storage disorders, urine GAGs, and urine oligosaccharides should be determined. If an MRCD seems possible or if the child is moribund, perimortem biopsies should be considered.

IEM with Dysmorphic Features

Disorders causing metabolic encephalopathy in the neonatal period are not typically associated with dysmorphic features. Exceptions include sulfite oxidase deficiency, multiple acyl-CoA dehydrogenase deficiency (MADD), and pyruvate dehydrogenase complex deficiency, which have been described previously. Severe carnitine palmitoyltransferase II deficiency can lead to similar dysmorphic manifestations as MADD. Menkes disease can sometimes be identified in the neonatal period, not typically with the "kinky hair" but with the pudgy cheeks, loose folds of skin, and sparse hair. Seizures are not typically seen in the neonatal period [83].

Peroxisomal Disorders

The peroxisomes are involved in the processing of very long-chain fats greater than 20-carbon length. These fatty acids do not have an important role in acute energy metabolism but are more implicated in structural lipid architecture such as the creation of myelin. Hence, the phenotype of many of these conditions has a neurological component, but there are also dysmorphic features. Traditionally, this group of disorders has been divided into single enzyme disorders (e.g., adrenoleukodystrophy), more complex biogenesis disorders (e.g., Zellweger syndrome), and disorders of specific enzyme/protein import into peroxisomes (e.g., rhizomelic chondrodysplasia punctata). There are at least 20 different genes known to cause peroxisomal disorders with overlapping phenotypes. The neonatal neurological and dysmorphic phenotypes can be broadly separated into Zellweger type and rhizomelic chondrodysplasia punctata (RCDP) type, but there are patients that have overlapping features and others in whom dysmorphism is not a major feature [47].

The Zellweger phenotype is one of a baby with profound hypotonia and several dysmorphic features including a large fontanel, flat occiput and face, macrocephaly with a high forehead, hypertelorism, and single palmar creases. From a neurological perspective, there is usually profound developmental delay, seizures, hypotonia, and decreased deep tendon reflexes. Brain MRI may show polymicrogyria and heterotopias with a hypoplastic corpus callosum. There may be congenital heart disease, pulmonary hypoplasia, liver dysfunction, hydronephrosis, and cryptorchidism. Plasma VLCFA are abnormal, with decreased red blood cell plasmalogens and increased serum phytanic acid. Pipecolic acid is often found in urine organic acids (although it is important to note that small amounts of pipecolic acid may be found in the urine of normal neonates). The RCDP type has a major manifestation of a skeletal dysplasia with proximal limb shortening, joint contractures, and metaphyseal splaying. Widespread calcified punctate stippling of the bones can be seen on skeletal survey in infancy. There may be other dysmorphic features including up-slanting palpebral fissures, frontal bossing, micrognathia, congenital cataracts, as well as sensorineural deafness. There is usually profound neurological compromise with developmental delay, seizures, and cerebral atrophy. While this is classically associated with *PEX7* gene mutations, the manifestations can be caused by mutations in other genes. Plasma VLCFA are typically normal, whereas red blood cell plasmalogens are low and serum phytanic acid is elevated.

Disorders of Cholesterol Biosynthesis

The most common disorder of cholesterol biosynthesis is Smith–Lemli–Opitz syndrome (SLOS), with an estimated incidence between 1 in 20,000 and 1 in 40,000. It is associated with microcephaly and developmental delay, but there are a range of other dysmorphic features including hypertelorism, low-set posteriorly rotated ears, micrognathia,

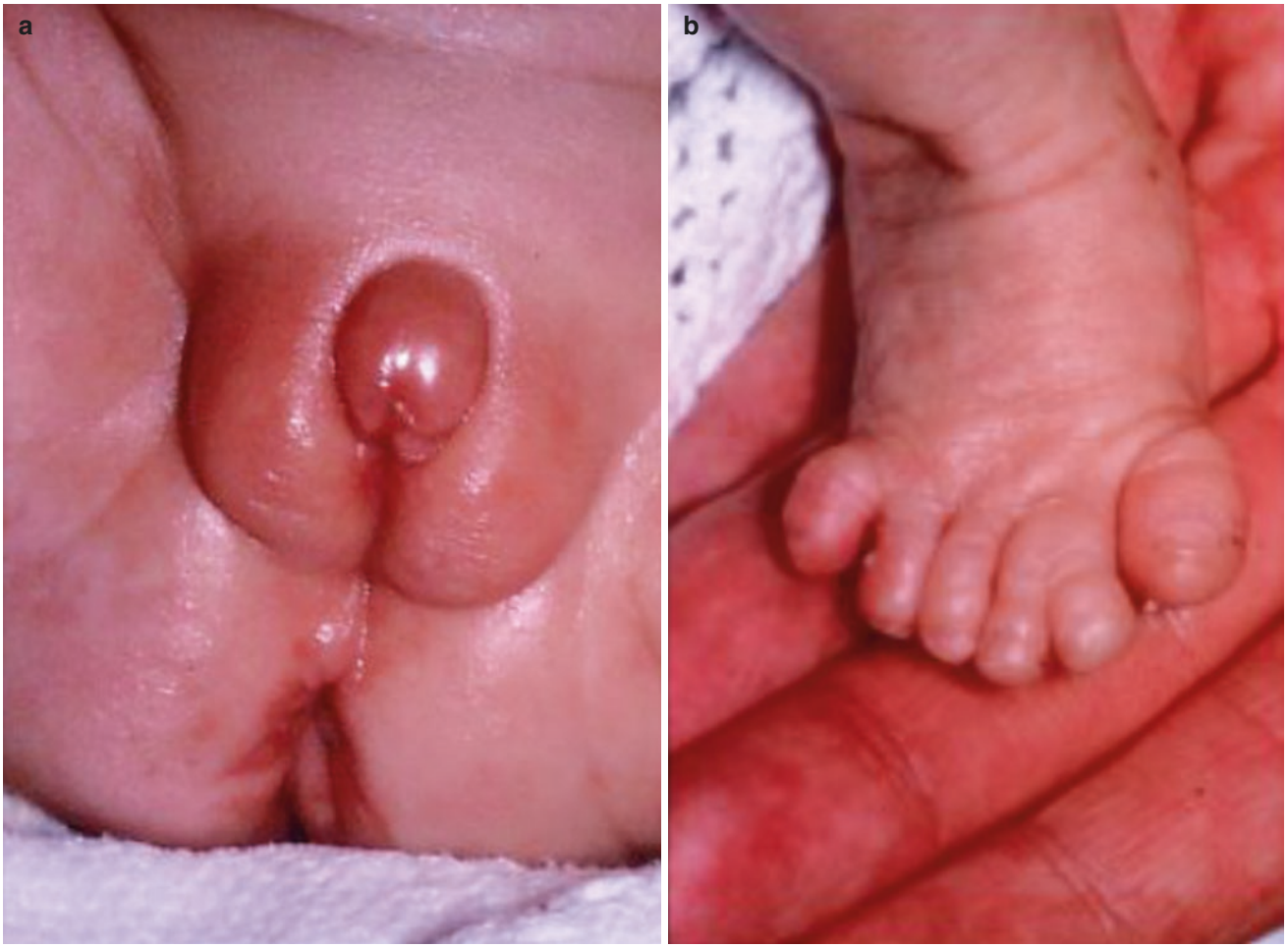

Fig. 11.11 Congenital malformations associated with Smith–Lemli–Opitz syndrome. Male infant with ambiguous genitalia, and two- to three-toe syndactyly as well as postaxial polydactyly

bitemporal narrowing, cataracts, and ptosis with epicanthic folds. There are usually abnormalities of the digits including two- to three-toe syndactyly, postaxial polydactyly, and the toes themselves short and broad. Renal abnormalities are common in both males and females, and males may have hypospadias, ambiguous genitalia, and hypoplastic scrotum (Fig. 11.11). Congenital cardiac abnormalities are also common. From a biochemical perspective, the total cholesterol is low with elevated 7-dehydrocholesterol. SLOS is caused by delta-7-dehydrocholesterol reductase deficiency, encoded for by the *DHCR7* gene. There is quite a broad phenotypic range with some having less obvious dysmorphic features and intellectual deficit [79].

8-Dehydrocholesterol can be elevated in two distinct dysmorphic syndromes: Conradi–Hunermann syndrome, which is an X-linked dominant form of chondrodysplasia punctata (CDPX2), and CHILD syndrome (congenital hemidysplasia with ichthyosiform erythroderma and limb defects), the gene for which (*NSDHL*) is also on the X chromosome. Both can have severe skeletal manifestations and neurocognitive deficits. CDPX2 is associated with a skeletal dysplasia including asymmetric limb shortening, epiphyseal stippling, and hemivertebrae with vertebral calcifications. There may be other connective tissue abnormalities including erythroderma, ichthyosis, and alopecia. CHILD syndrome can be associated with an unusual ipsilateral dysplasia affecting the limb, skin, cranial nerves, thyroid gland, central nervous system, spinal cord, heart, and muscles. In hemizygous males, these disorders are usually lethal, while in females, the phenotypic range is broad due to lyonization. Congenital structural anomalies and abnormalities in sterols are also seen in desmosterolosis, lathosterolosis, and Greenberg skeletal dysplasia.

Congenital Disorders of Glycosylation

The congenital disorders of glycosylation are disorders of application and processing carbohydrate molecules on the surface of proteins. These molecules facilitate identification

of such proteins for targeting function and transporting across tissues. Hence, the disorder of such processing can lead to wide-ranging disturbances in function as well as structural anomalies. To date, there are more than 40 different disorders described, with some having phenotypic overlap but others being distinctly different. The most common is CDG type Ia, caused by phosphomannomutase deficiency encoded by the *PMM2* gene [8, 69]. The broad range of effects that disordered glycosylation on intercellular processes can lead to a variety of clinical features. Dysmorphic features include inverted nipples with peau d'orange skin. Fingers and toes may be elongated, and there can be relatively hypoplastic buttocks associated with abnormal fat pads, which often disappear after infancy. Retinitis pigmentosa can be present, associated with roving eye movements and nystagmus. There can be microcephaly, with MRI scans demonstrating olivopontocerebellar hypoplasia. Patients with other N-linked glycosylation defects may have coloboma of the iris, optic nerve atrophy, and microcephaly. The dysmorphic features of O-glycosylation defects include exostoses, severe skeletal dysplasias, cutis laxa, microphthalmia, lissencephaly, and congenital contractures.

Lysosomal Storage Disorders

Many of the patients with this group of disorders appear normal at birth, but some may have some features in the neonatal period. This could include hepatomegaly in patients with Niemann–Pick disease type C, for instance, or joint contractures and skeletal dysplasia in patients with mucolipidosis type II (I-cell disease) [55, 84]. Patients with Wolman disease may have in utero calcification of the adrenal glands, and congenital adrenal hyperplasia has been reported in sialidosis type II. Features of dysostosis multiplex may be seen incidentally in radiographic images of patients with severe MPS disorders, but this is not typical. Patients with MPS VII can have a depressed nasal bridge and widely spaced eyes in the neonatal period. Babies with galactosialidosis can have absence of the nasal septum, inferior epicanthal creases, micrognathia, and a high arched palate. More subtle progressive dysmorphic features are seen in a wide range of lysosomal disorders.

Maternal Phenylketonuria

Elevations of phenylalanine exert a teratogenic effect on the developing fetus. While pregnant mothers with PKU can tolerate elevated phenylalanine levels without permanent neurocognitive deficit, their babies can develop a series of severe congenital anomalies. These include microcephaly, congenital cardiac defects, and dysmorphic features such as pre- and postnatal growth retardation, a smooth long philtrum, broad nasal bridge, and micrognathia [85]. Rigorous management of maternal phenylketonuria during pregnancy prevents the fetus from acquiring these abnormalities. If the fetus is unaffected by PKU, protein restriction can be liberalized in the latter part of pregnancy as the baby is able to metabolize surplus phenylalanine. However, if the fetus is also affected by PKU, meticulous metabolic control is required throughout all of the pregnancy.

Investigation of the Patient in the Perimortem Period

Many of the conditions listed in this chapter could lead to sudden death in utero or in the neonatal period. In order to elucidate and establish a cause of death to enable more accurate genetic counseling, a number of investigations should be instituted. Evaluation should start with a thorough examination trying to establish what organs are involved. Some conditions are associated with dysmorphic features as discussed. A thorough postmortem examination should be performed and radiological examinations undertaken to look for any evidence of dysostosis multiplex. If possible, samples should be collected before death, as reference intervals are not well validated for postmortem samples. If not possible, samples should be collected as soon as possible after death if metabolic profiling is anticipated. Tissue should be collected from different sites as IEM express themselves differently in different tissues. Table 11.8 indicates investigations that could be considered as well as the samples that should be collected.

Mitochondrial Respiratory Chain Disorders

In the child who dies with severe liver disease, lactic acidosis, cardiomyopathy, and/or intractable seizures, a mitochondrial respiratory chain disorder should be considered. This is a very heterogeneous group of disorders, with some having recognizable genotype–phenotype associations (e.g., POLG deficiency and neonatal hepatic encephalopathy), but there are many disorders that have variable and often nonspecific presentations. The conditions are so variable that at this stage, there is no definitive method for making a diagnosis, but currently, assay of the mitochondrial respiratory chain from the muscle and liver provides the best diagnostic yield. Ideally, both should be collected. Assay needs to be performed on tissue before there has been significant autolysis, as this can cause spurious functional abnormalities. Hence, assays can only be performed on samples collected from a patient during life or within 2 h of death. Samples should be collected (>200 mg) from the muscle, divided in two parts

Table 11.8 Samples that should be collected as part of the "genetic metabolic autopsy"

Sample	Method	Investigations/rationale
Urine: 10 ml	Catheter or suprapubic aspirate – freeze until analyzed	Organic acids, amino acids, GAGS, oligosaccharides
Whole blood (EDTA)	Venipuncture/cardiac aspirate – extract DNA and store	DNA extraction – targeted mutation testing, exome or genome sequencing
Plasma (lithium heparin)	Venipuncture/cardiac aspirate – separate and freeze	Plasma acylcarnitine profile, VLCFA, amino acids, lactate, glucose, ketones, fatty acids, creatine, guanidino acetate
Serum (clotted blood)	Venipuncture/cardiac aspirate – separate and freeze	Serum transferrin isoforms, bile acids
Filter paper blood spots	Capillary blood or venipuncture	Newborn screening tests. Targeted mutation testing. Specific enzyme assay (e.g., Pompe)
Cerebrospinal fluid	Lumbar puncture	Glucose, lactate, amino acids (should be paired with plasma); neurotransmitters, pterins
Muscle and liver biopsy	Open biopsy collected >200 mg tissue (ideally <2 h after death)	Histopathology and mitochondrial respiratory chain assay (see text)
Skin biopsy or knee cartilage	Epithelial tissue stored in fibroblast culture medium (<6 h post death)	Specific enzyme assay (fat oxidation, peroxisomal enzymes, CDG), cultured cell source for future DNA extraction and testing
MRI	Usually brain	Determines scope of brain involvement (e.g., lissencephaly, Leigh disease, olivopontocerebellar hypoplasia)
Skeletal survey	X-ray	Skeletal dysplasia, punctate calcified lesions, exostoses

for histopathology assessment: (1) stored in preservatives such as formalin (for histopathology and immunohistochemistry) and glutaraldehyde (for electron microscopy) and (2) frozen to −80 °C within 20 min of collection. Frozen samples need to be sent to a laboratory with experience in assaying mitochondrial respiratory chain. The laboratory should be able to perform the assays and quantitate mitochondrial DNA relative to nuclear DNA (specifically looking for evidence of mitochondrial DNA depletion). Further genetic testing can be undertaken on affected tissue [42, 77].

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