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ESSENTIALS Protein-dependent inborn errors of metabolism are caused by in- herited enzyme defects of catabolic pathways or intracellular trans- port of amino acids. Most result in an accumulation of metabolites upstream of the defective enzyme (amino acids and/or ammonia), causing intoxication. Protein-dependent metabolic diseases usually have a low preva- lence except for some high-risk communities with high consan- guinity rates. However, the cumulative prevalence of these disorders is considerable (i.e. at least >1:2000 newborns) and represents an important challenge for all public health systems. Types of protein-dependent inborn errors of metabolism Amino acid disorders—enzyme deficiencies in the proximal part of amino acid catabolism result in accumulation of precursor amino acids which are detectable by ninhydrin (a chemical used to detect ammonia or primary and secondary amines) and thus are called amino acid disorders. Phenylketonuria (PKU) is the most frequent such condition in white people. Organic acid disorders—distal enzyme defects of amino acid deg- radation result in pathological accumulation of organic acids but not the precursor amino acid. These disorders became detectable after the introduction of gas chromatography–mass spectrometry and are called organic acid disorders. Urea cycle defects—breakdown of amino acids results in the re- lease of ammonia that is detoxified by the urea cycle, which is com- posed of five catalytic enzymes, a cofactor producer, and at least two transport proteins. The biochemical hallmark of urea cycle defects is hyperammonaemia. Understanding of the protein-dependent inborn errors is based on the observation that some pathological metabolites impair key intracellular functions, such as energy metabolism, and thus when elevated may become toxic. These metabolites are excreted by urine or following conjugation to L-carnitine or L-glycine. However, in some diseases, such as disorders of tetrahydrobiopterin metabolism, clinical symptoms result from inadequate production of essential metabolites, such as the monoaminergic neurotransmitters. Clinical presentation Children with inherited disorders of amino acid, organic acid, or the urea cycle are usually born at term after an

uneventful pregnancy and are initially asymptomatic. The onset of the first symptoms is varied, ranging from neonatal metabolic decompensation to onset of symp- toms during adulthood. Irreversible organ damage and/or early death often follow if the diagnosis is delayed or missed. Metabolic decompensations in childhood are triggered by excess intake of pro- tein and—most importantly—secondary to breakdown of body pro- tein during episodes that induce catabolism. Family history—if carefully taken, this may reveal important clues to the diagnosis of protein-dependent inborn metabolic errors. Most disorders are inherited as autosomal recessive traits, which may be suspected if the parents are consanguineous or the family has a confined ethnic or geographic background. Carriers for particular disorders and affected children may be more frequent in certain communities (e.g. Amish), ethnic groups (e.g. Ashkenazi Jews, Arabic tribes), or countries that have seen little immigration over many cen- turies (e.g. Finland). Specialist investigations are often started only after a second affected child is born into a family: older siblings may be found to suffer from a similar disorder as the index patient or have died from an acute unexplained disease. Disease spectrum—this is broad, but follows a distinct pattern in specific disorders, for instance: (1) untreated patients with classical PKU and cerebral organic acid disorders characteristically present with neurological symptoms. (2) Acute life-threatening decompen- sation is common in classical organic acid, urea cycle defects, and maple syrup urine disorder; the young infant vomits or refuses to feed and then deteriorates rapidly.
(3) Asymptomatic protein-dependent inborn metabolic errors are rare, but there are a few known enzyme defects, such as histidinaemia, which do not produce disease. Investigation and management Every infant presenting with symptoms of unexplained metabolic crisis, intoxication, or encephalopathy requires urgent evaluation of metabolic parameters, including analyses of arterial blood gases, serum glucose and lactate, plasma ammonia and amino acids, acylcarnitine profiling in dried blood spots, and organic acid analysis in urine. Acute emergency therapy—basic principles are to (1) suppress muscle and liver protein catabolism and ensure a glucose supply above the basal metabolic demand; (2) treat any precipitating illness; (3) reduce increased production of toxic metabolites by reduction or omission of natural protein; (4) enhance detoxifying mechanisms 12.2 Protein-dependent inborn errors of metabolism Georg F. Hoffmann and Stefan Kölker

 and urinary excretion of pathological metabolites; (5) aggressively treat dehydration and acidosis; (6) prevent secondary carnitine de- pletion; and (7) provide alternative routes of ammonia disposal in hyperammonaemia. Long-term treatment—this aims principally to mitigate the meta- bolic consequences of enzyme deficiencies by compensating for them, including: (1) reduction of toxic metabolites by dietary restric- tion of precursor amino acids, prevention of catabolism, stimulation of residual enzyme activity (e.g. with cofactors), and detoxification strategies; and (2) substitution with depleted substrates, such as biotin, cobalamin, or L-dopa. However, efficacy is often low in pa- tients in whom diagnosis is made after the onset of symptoms, hence newborn screening programmes have been introduced in many countries, the criteria for implementation of which include: (1) reli- able presymptomatic disease detection, (2) treatability of the disease, and (3) starting of treatment in presymptomatic children. Successful treatment of affected individuals is often difficult to achieve. Careful supervision in metabolic centres involving an ex- perienced multidisciplinary team is invaluable for the best outcome. Treatment is time- and cost-intensive, often lifelong, and mostly per- formed at home, hence regular training and support of patients and their families is essential to prevent irreversible complications. All patients should carry an emergency card that gives details of their condition and

relevant contact numbers. Parent and patient organ- izations can offer useful support. Detailed description of individual disorders is to be found in the text of this chapter, and further information on diagnosis, genetic testing, treatment and follow-up is available from several online databases (see ‘Further reading’). Introduction Humans depend on dietary protein as a source of amino acids; they are the metabolic basis of all functional and structural proteins in the body. Some amino acids—termed essential—cannot be synthesized by the human body, such l-isoleucine and l-phenylalanine. Renal conservation of amino acids is extremely effective, with clearance values mostly less than 1%. Stool nitrogen losses are about 1 g/day and are mostly of bacterial origin. In contrast to glucose and fatty acids, amino acids taken in excess of requirement cannot be stored but are used for energy. The initial step of degradation is the removal of the amino group. Ammonia enters the urea cycle for conversion to urea. The remaining carbon skeletons are degraded via multistep individual pathways to central metabolic intermediates such as acetyl coenzyme A (CoA) or tricarb- oxylic acid cycle intermediates. Some enzymes require coenzymes, and inherited disease may be due to defects of the apoenzymes or their vitamin coenzymes, for example, biotin, pyridoxine (vitamin B6), or cobalamin (vitamin B12). Amino acids can be specifically detected by the ninhydrin re- action, which became available in the late 1940s, resulting in the identification of disorders such as phenylketonuria (PKU) or maple syrup urine disease. Breakdown of many amino acids occurs mostly intramitochondrially through degradation of CoA-activated car- bonic acids, the so-called acyl-CoA compounds. These nonamino organic acids are not detectable by amino acid analysis. Since defects of the latter phases of amino acid degradation induce accumulation of organic acids but not amino acid precursors, these disorders be- came detectable after the introduction of gas chromatography, es- pecially gas chromatography–mass spectrometry (GC/MS) in the 1960s and 1970s and have been termed organic acid disorders. Thus the terminology amino acid and organic acid disorders is not based on pathophysiological differences but simply on the different analyt- ical approaches. In this chapter, amino acid disorders, urea cycle defects, and or- ganic acid disorders are described; defects in mitochondrial me- tabolism and amino acid transport in the kidney tubule and small intestine are not considered. Historical perspective In 1902, Archibald Garrod introduced the term ‘inborn errors of metabolism’. An extraordinary scientist and paediatrician, he used consanguinity and distribution of cases in families to introduce the hypothesis that autosomal recessive inheritance according to Mendel’s rediscovered laws would explain the occurrence of the alkaptonuria phenotype, a defect in tyrosine degradation. Soon afterwards he also recognized albinism, cystinuria, and pentosuria as inborn errors. Metabolic medicine is closely linked with advances in laboratory techniques. The use of paper chromatography by Bickel and Dent and of automated column chromatography by Moore and Stein opened the field of amino acid disorders. In the late 1960s, Tanaka discovered isovaleric aciduria by GC/MS, and this was followed by the identification of numerous organic acid disorders. More re- cently, the rise of molecular biology has revolutionized the field, and now tandem mass spectroscopy and next-generation sequencing are proving powerful tools in screening and diagnosis. Monogenic defects have been identified for almost every known enzymatic step of protein metabolism. Often it was the discovery of patients with enzyme defects which unravelled individual steps in human metabolism. Until the early 1950s, no treatment of any genetic disorder ex- isted; destiny would take its course, and genetic counselling about recurrence risks was all that could be offered. That changed when, in 1953, Bickel showed that PKU can be successfully treated and that early diagnosis and dietary treatment change the outcome from severe learning difficulties to normal psychosocial develop- ment. Subsequently, many other metabolic diseases became man- ageable in a similar way using the

substrate deprivation strategy. Pharmacological doses of vitamins proved useful in defects of cobalamin and biotin metabolism, homocystinuria, and others. Simultaneously with the perception that identification of chil- dren before the onset of clinical symptoms is indispensable to im- prove the outcome, reliable and cheap screening methods have been developed. In the United States of America, Guthrie set the cornerstone for newborn screening by developing a bacterial in- hibition assay to detect PKU. Despite early disagreement and re- sistance by the medical profession, newborn screening has proven its worth over the years and the test is still called the ‘Guthrie test’ worldwide. In 1999, the World Health Organization announced orphan dis- eases as a major future health challenge. Among these diseases,

SECTION 12 Metabolic disorders 1944 disorders of amino acid and organic acid metabolism are especially important because of their cumulative prevalence (>1:2000 new- borns) and because successful therapy is available for most of them. Inborn metabolic diseases have become a significant challenge for healthcare systems, particularly in countries where infectious dis- eases and other perinatal problems are receding in importance. Aetiology, genetics, pathogenesis, and pathology The clinical manifestations of most protein-dependent inborn errors are thought to result from toxicity of the accumulating key metabolites to specific organs inducing selective or multiple organ failure. This ‘toxic metabolite hypothesis’ has influenced research and allowed the development of effective treatment. Despite increasing knowledge of pathophysiology, the most rele- vant concepts are derived from clinical research. For example, de- fects of all six enzymes in the degradation pathway of phenylalanine and tyrosine are known by now (see also ‘Defects of phenylalanine and tyrosine metabolism’). Defects in the first enzyme, phenyl- alanine hydroxylase, cause PKU (learning difficulties, seizures, ataxia, paresis, behavioural problems) and deficiency of tyrosine aminotransferase, the next enzyme, induces tyrosinaemia type II (corneal erosions, painful hyperkeratotic lesions, behavioural prob- lems). A defect of 4-hydroxyphenylpyruvate dioxygenase is the cause of tyrosinaemia type III which is possibly a nondisease, only a few patients develop neurological manifestations. A block in the next step of the pathway, 4-hydroxyphenylpyruvate dioxygenase, results in alkaptonuria (ochronosis, arthritis, heart disease), whereas deficiency of the last enzyme, fumarylacetoacetase, pro- duces a disease deadly in early childhood, tyrosinaemia type I, presenting with failure to thrive, liver failure, hepatosplenomegaly, hepatocarcinoma, and porphyria-like crises. The distinct syndromes resulting from defective breakdown of aromatic amino acids could never have been inferred simply by biochemical exploration of the metabolic pathway. Epidemiology As a group, protein-dependent disorders are by far the most common, acutely life-threatening inborn errors of metabolism (esti- mated prevalence >1:2000 newborns). However, reliable epidemio- logical data are scarce as all reports suggest a significant portion of patients who evade diagnosis and are considered to have neonatal sepsis or sudden infant death syndrome. All disorders cannot be re- liably ascertained clinically, and until recently population neonatal screening has only been implemented for PKU. Most epidemio- logical data are available from European countries, Japan, and the United States of America, highlighting variations based on ethnic background, migrations, and/or genetic isolation. In a few com- munities, the prevalence of individual disorders may increase up to five times the cumulative prevalence of amino acid and organic acid disorders in European countries, Japan, and the United States of America. For example, glutaric aciduria type I is found in up to 1 in 300 newborns in the Amish Community (United States of America) and the Oji-Cree First Nations (Canada), and in Qatar the preva- lence of classic homocystinuria is 1 in 600 newborns. Prevention With the first successful treatment of a young girl with PKU, the need for timely diagnosis and implementation of treatment became

imperative. In most inborn errors, affected neonates are completely asymptomatic and onset of irreversible symptoms during infancy and childhood can often be prevented if treatment is started while the child is asymptomatic. Since inborn errors of metabolism are rare, only neonatal mass screening can guarantee timely detection. However, which diseases are the most appropriate for screening re- mains debatable. The criteria of Wilson and Jungner (1968) for an implementation to newborn screening include: (1) reliable disease detection in a presymptomatic state of the disease, (2) treatability of the disease, and (3) the start of treatment in the presymptomatic chil- dren. In the 1960s, these criteria were achieved for PKU screening, which developed into one of the most important programmes of preventive medicine. Additional inborn errors such as maple syrup urine disease, galactosaemia, congenital hypothyroidism, and biotinidase deficiency were incorporated into newborn screening programmes of some countries. In the 1990s, a revolutionary technology, tandem mass spectros- copy (MS/MS), was adopted for newborn screening. The possi- bilities of multianalyte detection by MS/MS led to a change in the screening paradigm, that is, one test for many diseases (instead of one test for one disease). MS/MS improved screening for diseases from the conventional screening panels and opened the chance for inclusion of many other inborn errors of metabolism. However, each novel candidate disease has to be evaluated with respect to whether this disease fulfils the criteria for a disease to be screened (see Chapters 2.12 and 12.1), taking into consideration differences in national healthcare systems. As a consequence, the number of screened inborn errors of metabolism varies considerably ranging from two disorders (United Kingdom, Switzerland) up to more than 50 disorders (some parts of the United States of America). Notably, the United States screening panel also includes conditions that can be regarded as nondiseases or have at least a doubtful pathological meaning, such as the 3-methylcrotonyl CoA carboxylase deficiency. It should be appreciated that a liberal expansion of the screening panel burdens the healthcare system, the affected individuals, and the increasing number of false-positive individuals and their fam- ilies. Given these difficulties, it is to be hoped that screening politics will become harmonized in a joint international effort. Clinical considerations and diagnostic work-up History A careful family history may reveal important clues to the diagnosis of protein-dependent inborn metabolic diseases. Most disorders are inherited as autosomal recessive traits which may be suspected if the parents are consanguineous or the family has a confined ethnic or geographic background. Carriers for particular disorders and affected children may be more frequent in certain communities (e.g. Amish), ethnic groups (e.g. Ashkenazi Jews, Arabic tribes), or countries that have seen little immigration over many centuries (e.g. Finland). Often specialist investigations are started only after a second affected child is born into a family. Older siblings may be

 found to have a similar disorder to the index patient, or to have died from an acute unexplained disease classified as ‘sepsis with uniden- tified pathogen’, ‘encephalopathy’, or ‘sudden infant death syndrome’. Notably, the disease course of the same disorder may vary consid- erably even within families depending on genotype–phenotype correlation (if any), varying X-inactivation in female carriers (e.g. ornithine transcarbamylase deficiency), and dominant disorders with variable penetration (e.g. Segawa’s disease). As a result of the successful treatment of inborn errors of metab- olism, an increasing number of affected women are reaching repro- ductive age. If they become pregnant, there may be a risk for their fetuses to be harmed by toxic metabolites from the mother. Especially important is maternal PKU, which is likely to become a major health problem. Other maternal conditions may cause ‘metabolic’ disease in the neonate or infant postnatally, for example, methylmalonic aciduria

and hyperhomocystinaemia, in fully breastfed children of mothers who have pernicious anaemia or who are on a vegan diet, which fosters nutritional vitamin B12 deficiency. Clinical spectrum The range of clinical and biochemical manifestations of the protein- dependent metabolic errors is wide. Here we focus on the clinical manifestation and differential diagnosis of disorders presenting with acute metabolic decompensations (Boxes 12.2.1 and 12.2.2). There is only a limited repertoire of pathophysiological sequences in the response to metabolic intoxication and, consequently, a limited number of therapeutic measures. Timely and correct intervention during the initial episode is a critical prognostic factor. Many protein-dependent metabolic errors already manifest in the first days of life with progressive irritability or drowsiness. Most typically, a young infant may vomit or refuse to feed and then rap- idly deteriorates. The initial erroneous diagnoses are usually neo- natal sepsis or intracranial haemorrhage: a presumptive diagnosis of a protein-dependent inborn error should be considered with equal priority. Children with milder forms may be repeatedly admitted, for example, with unusual metabolic acidosis, hypoglycaemia, or neutropenia in the course of common infections especially gastro- enteritis, before an inborn disorder of metabolism is considered, and routine clinical chemistry may be normal in between crises. A substantial number of patients with protein-dependent inborn errors of metabolism may present differently with acute encephalop- athy or chronic and fluctuating progressive neurological disease. The so-called cerebral organic acidaemias (e.g. glutaric aciduria type I) characteristically present with (progressive) neurological symptoms such as ataxia, myoclonus, extrapyramidal symptoms, and metabolic stroke. Routine clinical chemistry is often unrevealing. Important diag- nostic clues such as progressive disturbances of myelination, cere- bellar atrophy, frontotemporal atrophy, signal abnormalities, and/or infarcts of the basal ganglia can be derived from MRI of the brain. Chronic subdural effusions, haematomas, and retinal haemorrhages in infants and toddlers are characteristic findings in glutaric aciduria type I, although they are more commonly due to child abuse. Laboratory investigations The early consideration of metabolic diseases is of the utmost im- portance. Basic evaluation of metabolic parameters including ana- lyses of blood gases, serum glucose and lactate, plasma ammonia and amino acids, acylcarnitine profiling in dried blood spots (MS/MS), and organic acid analysis in urine (GC/MS) should be performed on an emergency basis in every patient presenting with symptoms of unexplained metabolic crisis, intoxication, or encephalopathy. Routine laboratory parameters Diagnostic clues can be obtained from routine laboratory inves- tigations such as electrolytes (also required for the calculation of the anion gap), urinary ketones, serum transaminases, and cre- atine kinase. Any child admitted to an intensive care unit with life- threatening nonsurgical illness should be tested for these parameters. Box 12.2.1 Presentation of organic acidurias Intoxication • Kussmaul tachypnoea/acidotic breathing • Peculiar smell • Refusal of/adverse reaction to feeding • Protracted episodic vomiting • Erroneous diagnosis of pyloric stenosis (with acidosis) • Reye’s syndrome presentation • Hepatomegaly/liver failure • Rhabdomyolysis • Sudden infant death syndrome (SIDS) or ‘near miss’ SIDS
Acute encephalopathy • Coma • Seizures (myoclonic, intractable) • Acute profound dyskinesia • Pseudotumour cerebri • Cerebral/intraventricular haemorrhage in full-term babies • Stroke-like episodes Chronic encephalo(myelo)pathy • Progressive psychomotor deterioration • Macrocephaly • Ataxia (progressive) • Hypotonia • Dystonia, athetosis • Myoclonus • Seizures (myoclonic, intractable) • Peripheral neuropathy • Pyramidal signs—‘cerebral palsy’ • Pronounced deficiency of speech • Congenital cerebral malformations Box 12.2.2 Clinical chemical indices of organic acidurias • Metabolic acidosis • Increased anion gap • Hyperglycaemia • Ketosis and ketonuria (especially suggestive in newborns) • Lactic acidosis • Hyperammonaemia • Hyperuricaemia • Hypertriglyceridaemia • Increase of transaminases • Granulocytopenia,

thrombocytopenia, and anaemia • Hypoketotic hypoglycaemia (fatty acid oxidation defects) • Increased creatine kinase (fatty acid oxidation defects) • Myoglobinuria (fatty acid oxidation defects)

SECTION 12 Metabolic disorders 1946 Amino acid analysis Many metabolic parameters show considerable diurnal fluctu- ations. For example, plasma amino acid concentrations are highly dependent on the metabolic status, and standard samples should be obtained at least 4 h postprandially. Many amino acids can be reliably quantified in dried blood spots by MS/MS (e.g. for PKU). Homocysteine and tryptophan require specific methods (usu- ally high-performance liquid chromatography (HPLC)) for exact quantification. Regular amino acid analyses are required in patients on specific dietary treatments to adjust intake of amino acids and to recognize a deficiency of essential amino acids and micronutrients. For optimal results, it is important to separate plasma as soon as possible and to ship samples frozen on dry ice. Haemolysis or shipment of whole blood results in useless values for some amino acids. Some potential problems are summarized in Box 12.2.3. Quantitative urinary amino acid analysis is indicated only if a renal tubular reabsorption defect such as cystinuria is sus- pected, and (in addition to plasma analysis) in hyperammonaemia when increased urinary excretion of specific metabolites (e.g. argininosuccinate) may be diagnostic. Organic acid analysis Organic acid analysis is best performed on early morning urine specimens. Complete information of the clinical status and recent management of the patient is indispensable for correct interpret- ation, which is based on key diagnostic metabolites or character- istic biochemical patterns. Repeated analyses may be necessary, preferably during exacerbation of metabolic decompensation, since analyses may be intermittently normal. Characteristic me- tabolites may, however, also become masked in severe metabolic decompensation and ketosis. Some patients with organic acid dis- orders may exhibit only slight elevations of diagnostic metabol- ites that may be underestimated by conventional analysis, such as in 4-hydroxybutyric aciduria, glutaric aciduria type I, and N-acetylaspartic aciduria. In these disorders, quantification by stable isotope dilution assays is preferred. This is also the method of choice for biochemical prenatal diagnosis of organic acid dis- orders in amniotic fluid, if the causative mutations are not avail- able, providing more rapid diagnosis than enzyme analysis of cultured amniocytes. Acylcarnitine analysis A complementary and rapid diagnostic technique for some or- ganic acid disorders is the analysis of acylcarnitine by MS/ MS—by analogy to newborn mass screening—since accumu- lating acyl-CoA esters are in equilibrium with corresponding acylcarnitines. Principles of treatment General aspects Protein-dependent metabolic disorders are chronic conditions that involve various organ systems and thus require a multidisciplinary approach to care and treatment. Patients with genetic diseases that are prone to acute decompensations should carry an emergency card. Vaccinations should be carried out as recommended and should also include vaccinations against varicella, hepatitis A, pneumococcus, and influenza. Special precautions must be taken before, during, and after surgery/anaesthesia. Dietary treatment In many protein-dependent errors of metabolism, therapy is based on reduced intake of precursors in deficient pathways, prevention of catabolism, and an intensification of therapy during intercurrent illnesses. This aims to diminish the supply of toxic metabolites and restore energy supply. Dietary treatment must meet the general, age- dependent, and individual requirements for energy and essential nutrients to ensure normal growth and development (Table 12.2.1). Protein deficiency induces catabolism, failure to thrive, and growth retardation, and secondary depletion of essential amino acids and micronutrients may induce life-threatening complications such as lactic acidosis (thiamine or biotin depletion) or pellagra (niacin depletion). Supplementation of precursor-free

mixtures of amino acids and semisynthetic supplements of minerals and trace elements minimizes the risk for malnutrition. Pharmacotherapy Carnitine at daily doses of 50–200 mg/kg body weight is essential for the elimination of accumulating toxic acyl-CoA compounds and for the restoration of intramitochondrial free CoA-SH in most organic acid disorders. In cofactor-responsive disorders, enzyme activity may be restored by specific vitamins, for example, in biotinidase deficiency, cobalamin-responsive methylmalonic acidurias, and riboflavin-responsive multiple acyl-CoA dehydrogenase deficiency. Box 12.2.3 Some pitfalls of amino acid analysis • Shipping/storage without adequate cooling: ↓ glutamine, asparagine, cysteine, homocysteine; ↑ glutamate, aspartate • Haemolysis: ↓ arginine, glutamine; ↑ aspartate, glutamate, glycine, ornithine • Postprandial changes: all amino acids Table 12.2.1 Protein requirements Age Revised safe values (g/kg per day) 0–1 months 2.69 1–2 months 2.04 2–3 months 1.53 3–4 months 1.37 4–5 months 1.25 5–6 months 1.19 6–9 months 1.09 9–12 months 1.02 1–3 years 1.0–0.92 4–10 years 0.88–0.86 11–18 years 0.86–0.77 Source data from Dewey KG, et al. (1996). Protein requirements of infants and children. Eur J Clin Nutr, 50 Suppl 1, S119–47.

12.2 Protein-dependent inborn errors of metabolism 1947 The accumulation of toxic metabolites derived from gut bacteria, such as propionic acid, can be reduced by intestinal antibiotics (e.g. metronidazole). Emergency treatment Treatment of intercurrent illness at home Protein-dependent inborn errors of metabolism often present with acute life-threatening decompensation requiring prompt decisions and measures. A limited number of therapeutic measures have to be taken immediately (Box 12.2.4, Table 12.2.2). It is imperative to decrease catabolism at an early stage of de- compensation. As this usually happens at home, it is essential to educate the family adequately. Home treatment should in- clude adequate control of fever and vomiting, moderate protein restriction, and ample calories, glucose, and fluid (Box 12.2.4). Intake of natural protein can be completely eliminated for the first 24 h of illness, especially if the patient receives precursor-free supplements of amino acids. After 24 h, stepwise reintroduc- tion of natural protein is necessary to prevent protein catabolism. Immediate hospital admission and intravenous treatment is in- dicated when vomiting persists, fluid and dextrose intake remain poor, the clinical condition deteriorates, or the disease course is prolonged. On admission to hospital, these patients must be as- sessed and treated without delay. If emergency management is carried out in peripheral hospitals, this should ideally be super- vised in consultation with a knowledgeable and experienced physician or paediatrician. Emergency treatment in hospital Provision of ample quantities and control of fluid and electrolytes is indispensable and must be continued before any laboratory results are available. Glucose infusions must be adapted to age to provide an adequate energy supply. For example, in neonates glucose infusion is usually started at 10 mg/kg per min (i.e. 14.4 g glucose/kg body weight per day). An insulin drip may be necessary to prevent hyper- glycaemia and to induce an anabolic state. Overhydration is rarely a problem in metabolic crises as they are mostly accompanied by dehydration. Electrolytes, glucose, lactate, and acid–base balance should be checked at least every 6 h and serum sodium should be maintained at no less than 138 mmol/litre. If lactate is constantly increasing while the glucose supply is increasing, one should con- sider a primary defect or secondary inhibition or energy metab- olism, such as in classic organic acid disorders. Antibiotics should be started if there is evidence for an infectious cause. Antipyretics should be administered liberally since they help to reduce the add- itional bioenergetic costs of fever. Carnitine is essential for the elimination of toxic acyl-CoA es- ters in organic acidaemias, to prevent secondary carnitine deple- tion, and to replenish the intracellular CoA pool. Carnitine should be administered intravenously at 100 to 200 mg/kg per day. In

hyperammonaemia, nitrogen-disposing drugs are used: • Sodium benzoate, 250 mg/kg as bolus initially over 1 to 2 h, then 250 (to 500) mg/kg per 24 h. Box 12.2.4 Basic principles for acute emergency therapy 1 Suppress muscle and liver protein catabolism and ensure a glucose supply above the basal metabolic demand 2 Treat the precipitating illness 3 Reduce increased production of toxic metabolites by reduction or omission of natural protein 4 Enhance detoxifying mechanisms and urinary excretion of patho- logical metabolites 5 Aggressively treat dehydration and acidosis 6 Prevent secondary carnitine depletion 7 Provide alternative routes of ammonia disposal in hyperammonaemia Table 12.2.2 Home and outpatient emergency treatment Age (years) % kcal/100 ml Daily amount A. Glucose polymer/maltodextrin solutiona 0–1 10 40 150–200 ml/kg 1–2 15 60 95 ml/kg 2–10 20 80 1200–2000 ml/day

> 10 25 100 2000 ml/day B. Protein intake Natural protein Stop (if amino acid supplements are administered) or reduce to 50% of maintenance therapy (if no amino acid supplements are administered). Reintroduce and increase within 1–2 days Amino acid mixtures If tolerated, amino acid supplements should be administered according to maintenance therapy, e.g. 0.8–1.0 g/kg body weight/dayb C. Pharmacotherapy l-Carnitine Double carnitine intake: 200 mg/kg body weight/day orally (if tolerated) Antipyreticsc If temperature >38.5°C, e.g. ibuprofen (10–15 mg/kg body weight per dose, 3–4 doses daily) a Maltodextran/dextrose solutions should be administered every 2 h day and night. If neonates and infants already receive a specific dietary treatment, protein-free food can be continued but should be fortified by maltodextran. Patients should be reassessed every 2 h. b All calculations should be based on the expected weight, not the actual weight. c Paracetamol administration may be dangerous in acute metabolic decompensation (risk for glutathione depletion).

 • Sodium phenylacetate, 250 mg/kg as bolus initially over 1 to 2 h, then 250 (to 600) mg/kg per 24 h; alternatively, sodium phenylbutyrate is administered at the same concentration orally. • Arginine hydrochloride, 420 mg/kg (i.e. 2 mmol/kg) as bolus initially over 1 to 2 h, then 420 mg/kg per 24 h. If the response to emergency treatment is poor, the patient de- teriorates, or the ammonia concentration exceeds 400 to 500 µmol/ litre (neonate, infant), haemofiltration or haemodialysis should be urgently considered. Since intracranial pressure due to cerebral oe- dema appears earlier in older children, adolescents, and adults than in newborns, infants, and younger children, extracorporeal detoxi- fication should be considered if ammonia concentration exceeds 200 µmol/litre or even as first-line treatment. If persisting lactic acid- osis is present, thiamine (100–500 mg/day) and biotin (10–20 mg) should be given empirically. Monitoring of treatment Dietary treatment without adequate monitoring is dangerous since disease-specific complications, therapy-specific adverse events (e.g. malnutrition), and developmental delay might be overlooked. Anthropometric parameters such as weight, height, and head cir- cumference should be recorded at each visit. Psychomotor devel- opment must be regularly assessed with appropriate tests. Weight loss or insufficient weight gain in affected children is often caused by inadequate dietary treatment and may herald impending metabolic decompensation. The major aim of biochemical monitoring is to ensure that nu- trition is not

compromised. Biochemical evaluation includes blood count, serum electrolytes, calcium, phosphate, magnesium, ferritin level, liver and kidney function tests, alkaline phosphatase, total protein, albumin, prealbumin, transferrin, cholesterol, triglycer- ides, zinc, copper, retinol (plasma), carnitine, ammonia, lactate, and plasma amino acids. Although analyses of specific metabolic parameters are required to confirm the diagnosis of an inborn error of metabolism, these parameters are often not informative for bio- chemical follow-up monitoring since the relationship between the metabolic parameters and outcome is unclear for most disorders. However, regular monitoring of some metabolic parameters is ne- cessary since they are directly related to the outcome. For example, plasma phenylalanine is monitored in PKU, plasma leucine in maple sugar urine disease, plasma glutamine and arginine in urea cycle defects, and plasma homocysteine in trans-sulphuration and remethylation defects. Likely future developments The scientific and technological advances described in the previous sections have offered much benefit to patients with inborn errors of metabolism. To implement and utilize them properly, much remains to be done. Initially, metabolic physicians and scientists need to combine their efforts and concentrate on well-conducted international studies and development of evidence-based guide- lines. Significant differences still exist in the diagnostic procedures, treatment, and monitoring of many diseases, resulting in a wide variation in outcome. Even for PKU, the disease with the greatest and longest experience in successful therapy, current guidelines recommend different cut-offs for the indication of treatment ran- ging from 400 µmol/litre in the United Kingdom to 360 µmol/litre in the United States of America and 600 µmol/litre in France and Germany. The knowledge of the academic community must be combined and structured, transferred to the physicians and other medical staff, and implemented in healthcare systems. Nowadays, this process has become much easier by means of numerous re- commendations, information, and even projects available on the Internet, permanent professional email round tables, Internet editions of book and journals, and open-access databases. In the necessary implementation process, regional differences such as availability of funds, local pathology, and religious and geographic factors must be taken into account. Accordingly, specialized na- tional metabolic centres and appropriate metabolic networks should be established and properly maintained. Unfortunately, novel diagnostic and therapeutic possibilities (Box 12.2.5), such as newborn screening or enzyme replacement therapy, are relatively expensive and are still unrealistic for many countries where there are no screening programmes and perhaps no well-organized healthcare system. Individual disorders A summary of protein-dependent inborn errors of metabolism including the enzyme defect, incidence, gene locus, and Online Mendelian Inheritance in Man (OMIM) number is given in Table 12.2.3. Urea cycle defects Aetiology/pathophysiology The major source of ammonia is catabolism of protein, which is detoxified to urea in the liver (Fig. 12.2.1). The efficiency of hep- atic ammonia detoxification is enhanced through the action of glu- tamine synthase. Hyperammonaemia (plasma ammonia >80 µmol/ litre in newborns; >50 µmol/litre after the newborn period) is caused by increased production (e.g. by intestinal urease-producing bac- teria) or decreased detoxification of ammonia. Decreased detoxifi- cation results from inherited or acquired deficiency of key enzymes and transporters of the urea cycle, or bypassing of the liver (e.g. open hepatic duct). Secondary impairment of ammonia detoxification re- sults from conditions where glutamate or acetyl-CoA are decreased, Box 12.2.5 New treatment strategies in inborn errors of metabolism • Supplementation with end products • Anaplerotic therapy • Enzyme replacement • Chemical chaperones • Specific blockade of biosynthetic pathways • Specific blockade of degradation pathways • Specific blockade of pathophysiological signalling • (Stem) cell therapy • Gene therapy

12.2 Protein-dependent inborn errors of metabolism 1949 Table 12.2.3 Summary of protein-dependent inborn errors of metabolism Disease Enzyme defect Incidencea Gene map locus Gene name OMIM (phenotype number) Defects of the urea cycle Argininaemia Arginase 1 1:100 000 6q23 ARG1 207800 Argininosuccinic aciduria Argininosuccinate lyase 1:50 000 7cen–q11.2 ASS1 207900 Citrullinaemia type I Argininosuccinate synthetase 1 1:50 000 9q34 ASL 215700 Deficiency of Citrin <1:200 000 7q21.3 SLC25A13 605814 (neonatal onset) 603471 (adult onset) Deficiency of N-Acetylglutamate synthase <1:200 000 17q21.3 NAGS 237310 Deficiency of Carbamoylphosphate synthetase 1 1:50 000 2q35 CPS1 237300 Deficiency of Ornithine carbamoyltransferase 1:30 000 Xp21.1 OTC 311250 Dibasic amino aciduria II, lysinuric protein intolerance <1:200 000 14q11.2 SLC7A7 222700 Hyperornithinaemia–hyperammonaemia– homocitrullinuria syndrome Ornithine transporter <1:200 000 13q14 SLC25A15 238970 Carbonic anhydrase VA deficiency Mitochondrial carbonic anhydrase VA Unknown 16q24.2 CA5A 114761 Defects of branched-chain amino acid metabolism Isovaleric aciduria Isovaleryl-CoA dehydrogenase 1:80 000 15q14–q15 IVD 243500 Maple syrup urine disease Branched-chain keto acid dehydrogenase (lipoamide) 1:200 000 248600 Type Ia E1 component α-chain 19q13.1–q13.2 BCKDHA Type Ib component β-chain 6p21–p22 BCKDHB Type II dihydrolipoamide branched-chain
transacylase (E2 component) 1p31 DBT 3-Methylcrotonylglycinuria 3-Methylcrotonyl-CoA carboxylase 1:60 000 210200 α-subunit 3q25–q27 MCCC1 β-subunit 5q12–q13 MCCC2 3-Methylglutaconyl-CoA hydratase deficiency
(3-methylcrotonyl aciduria type I) 3-Methylglutaconyl-CoA hydratase <1:200 000 9q22.31 AUH 250950 TAZ defect or Barth syndrome (3-methylglutaconic aciduria type II) Tafazzin <1:200 000 Xq28 TAZ 302060 OPA3 defect or Costeff's syndrome
(3-methylglutaconic aciduria type III) OPA3A and OPAB protein <1:200 000 19q13.2–q13.3 OPA3 258501 3-Methylglutaconic aciduria type IV (i.e. MEGDEL syndrome, TMEM70 defect, or not otherwise specified) E.g. polymerase-γ, transmembrane protein 70, succinate-CoA ligase, serine active site- containing protein 1 or not yet classified <1:200 000 e.g. 15q26.1, 8q21.11, 13q14.2, 6q25.3, or ? e.g. POLG1, TMEM70, SUCLA2, SERAC1, or? 250951 (if not otherwise specified) DNAJ19 defect or DCMA syndrome
(3-methylglutaconic aciduria type V) Translocase of the inner mitochondrial membrane 14 Unknown 3q26.33 DNAJC19 610198 (continued)

 Disease Enzyme defect Incidencea Gene map locus Gene name OMIM (phenotype number) 2-Methyl-3-hydroxybutyryl-CoA deficiency 2-Methyl-3-hydroxybutyryl-CoA dehydrogenase <1:200 000 Xp11.2 HSD17B10 300438 Methylmalonic aciduria (mut0/mut− defects) Methylmalonyl-CoA mutase 1:100 000 6p12.3 MUT 251000 Propionic aciduria Propionyl-CoA carboxylase 1:200 000 α-chain 13q32 PCCA 232000 β-chain 3q21–q22 PCCB 232050 3-Hydroxyisobutyryl-CoA hydrolase deficiency 3-Hydroxyisobutyryl-CoA hydrolase Unknown 2q32.2 HIBCH 250620 Short-chain enoyl-CoA hydratase deficiency Mitochondrial short-chain enoyl-CoA hydratase 1 Unknown 10q26.3 ECHS1 616277 Defects of lysine, hydroxylysine, and tryptophan metabolism 2-Aminoadipic and oxoadipic aciduria Dehydrogenase E1 and transketolase domains-containing protein 1 <1:200 000 10p14 DHTKD1 204750 2-Oxoadipic aciduria Dehydrogenase E1 and transketolase domains- containing protein 1 <1:200 000 10p14 DHTKD1 245130 Glutaric aciduria type I Glutaryl-CoA dehydrogenase 1:100 000 19p13.2 GCDH 231670 Gyrate atrophy of choroid and retina Ornithine-oxoacid/ ornithine aminotransferase <1:200 000 10q26 OAT 258870 Hyperlysinaemia Saccharopine dehydrogenase/lysine:α- ketoglutarate reductase <1:200 000 7q31.32 AASS 238700 Saccharopinuria Saccharopine dehydrogenase/lysine:α- ketoglutarate

reductase <1:200 000 7q31.32 AASS 268700 Multiple carboxylase deficiency Biotinidase deficiency Biotinidase 1:80 000 3p25 BTD 253260 Holocarboxylase synthetase deficiency Holocarboxylase synthetase <1:200 000 21q22.1 HLCS 253270 Other organic acidurias N-Acetylaspartic aciduria (Canavan's disease) Aspartoacylase; aminoacylase 2 <1:200 000 17pter–p13 ASPA 271900 Ethylmalonic encephalopathy Mitochondrial matrix protein <1:200 000 19q13.2 ETHE1 602473 D-2-Hydroxyglutaric aciduria Type I: D-2-hydroxyglutaric acid dehydrogenase <1:200 000 2p25.3 D2HGDH 600721 Type II: isocitrate dehydrogenase 2 (mitochondrial) <1:200 000 15q26.1 IDH2 613657 L-2-Hydroxyglutaric aciduria FAD-dependent L-2-hydroxyglutarate dehydrogenase <1:200 000 14q22.1 L2HGDH 236792 Combined D-2- and L-2-hydroxyglutaric aciduria Mitochondrial citrate transporter <1:200 000 22q11.21 SLC25A1 615182 Defects of phenylalanine and tyrosine metabolism Alkaptonuria Homogentisate 1,2-dioxygenase <1:200 000 3q21–q23 HGD 203500 BH4 deficiency, dopa-responsive dystonia (dominant) Guanosine-5-triphosphate cyclohydrolase 1:100 000 14q22.1–q22.2 GCH1 128230 Table 12.2.3 Continued

 BH4 deficiency Deficiency of Dihydropteridine reductase <1:200 000 4p15.32 QHPR 261630 Deficiency of Guanosine-5-triphosphate cyclohydrolase <1:200 000 14q22.1–q22.2 GCH 233910 Deficiency of 6-Pyruvoyltetrahydropterin synthase <1:200 000 11q22.3–q23.3 PTS 261640 Deficiency of Sepiapterin reductase <1:200 000 2p13.2 SPR 612716 Deficiency of Pterin-4α-carbaminoline dehydratase Unknown 10q22.1 PCBD1 264070 Phenylketonuria (PKU) Phenylalanine hydroxylase 1:10 000 12q24.1 PAH 261600 Type I (Classical PKU = Phe >1200 µmol/litre) c.50% Type II (Mild PKU = 360–600 µmol/litre ≤ Phe ≤ 1200 µmol/litre) c.30% Type III (Non-PKU HPA/MHP = Phe <360–600 µmol/ litre) c.20% Types II+III (BH4-PAH = Phe <1200 µmol/litre + BH4- responsive) c.35% Hyperphenylalaninaemia with primapterinuria Pterin-4α-carbinolamine <1:200 000 10q22.1 PCBD 264070 Tyrosinaemia type I Fumarylacetoacetase 1:100 000 15q23.1 FAH 276700 Tyrosinaemia type II Tyrosine aminotransferase <1:200 000 16q22.2 TAT 276600 Tyrosinaemia type III 4-Hydroxyphenylpyruvate dioxygenase <1:200 000 12q24.31 HPD 276710 Neurotransmitter diseases and related disorders Deficiency of Aromatic L-amino acid decarboxylase <1:200 000 7p12.1 DDC 608643 Deficiency of Dopamine β-hydroxylase <1:200 000 9q34.2 DBH 223360 Deficiency of GABA transaminase <1:200 000 16p13.2 ABAT 613163 Deficiency of 3-Phosphoglycerate dehydrogenase <1:200 000 1p12 PHGDH 601815 Deficiency of Tyrosine hydroxylase <1:200 000 11p15.5 TH 605407 Folinic acid-responsive epilepsy (see 'Pyridoxine- dependent epilepsy') α-Aminoadipic semialdehyde dehydrogenase (antiquitin) <1:200 000 5q23.2 ALDH7A1 266100 4-Hydroxybutyric aciduria Succinic semialdehyde dehydrogenase <1:200 000 6p22.3 ALDH5A1 271980 Hyperprolinaemia type II L-Δ1-pyrroline-5-carboxylate dehydrogenase <1:200 000 1p36 ALDH4A1 239510 Nonketotic hyperglycinaemia (glycine encephalopathy) 1:60 000 605899 H-protein deficiency 16q22.24 GCSH P-protein deficiency 9p24.1 GLDC T-protein deficiency 3p21.31 AMT Other: transient 605899 Pyridoxal phosphate-dependent epilepsy Pyridox(am)ine 5′-phosphate oxidase <1:200 000 17q21.32 PNPO 610090 Pyridoxine-dependent epilepsy α-Aminoadipic semialdehyde dehydrogenase (antiquitin) <1:200 000 5q23.2 ALDH7A1 266100 (continued)

 Disease Enzyme defect Incidencea Gene map locus Gene name OMIM (phenotype number) Defects of trans-sulphuration and remethylation Deficiency of S-adenosyl-homocysteine hydrolase <1:200 000 20q11.22 AHCY 613752 Deficiency of Adenosine kinase <1:200 000 10q22.2 ADK 614300 Deficiency of Cysthationine γ-lyase < 1:70 000 1p31.1

CTH 219500 Deficiency of Glycine N-methyltransferase <1:200 000 6p21.1 GNMT 606664 Deficiency of Methionine adenosyltransferase 1 <1:200 000 10q23.1 MAT1A 250850 Deficiency of Methionine synthase reductase (cobalamin E) <1:200 000 5p15.31 MTRR 236270 Deficiency of Methionine synthase (cobalamin G) <1:200 000 1q43 MTR 250940 Deficiency of 5,10-Methylene-tetrahydrofolatreductase <1:200 000 1p36.22 MTHFR 236250 Homocystinuria Cystathionine-β-synthase 1:100 000 21q22.3 CBS 236200 a Incidences as estimated in the white population; they vary between populations of different ethnic background. <1:200 000 indicates incidence very low but uncertain because not specifically determined. Of some disorders, only two or three families are as yet known worldwide. BH4-PAH, BH4-responsive phenylalanine hydroxylase deficiency; Phe, phenylalanine; PKU, phenylketonuria. Table 12.2.3 Continued

12.2 Protein-dependent inborn errors of metabolism 1953 such as in organic acid defects, mitochondrial β-oxidation de- fects, carnitine depletion, or valproate therapy, or where toxic acyl-CoAs are increased, such as propionyl-CoA in propionic and methylmalonic aciduria or isovaleryl-CoA in isovaleric aciduria. Hyperammonaemia is neurotoxic, resulting in brain oedema, convulsions, and coma. Neuropathological evaluation reveals an alteration of astrocyte morphology including cell swelling (acute hyperammonaemia) and Alzheimer type II astrocytosis (chronic hyperammonaemia). The brain relies on energy-dependent glu- tamine synthesis by astrocytic glutamine synthetase for the removal of excess ammonia. As a consequence, increased brain ammonia is considered to amplify glutamatergic signalling and cause redistri- bution of cerebral blood flow and metabolism, impairment of brain energy metabolism affecting the glutamate/glutamine cycle, and in- creased serotonin secretion. Hyperammonaemia exerts reversible (mostly serotoninergic) and irreversible effects. Peak plasma am- monia concentrations exceeding 500 µmol/litre or a coma lasting more than 2 to 3 days appears to be associated with irreversible de- fects which worsen with the duration of the coma. All inherited urea cycle defects follow an autosomal recessive trait except for ornithine transcarbamylase deficiency which is X-linked. Clinical presentation Urea cycle defects are among the most common inborn errors of metabolism (cumulative incidence is approximately 1 in 40 000 newborns). Six inherited urea cycle defects are well described, that is, deficiencies of N-acetylglutamate synthetase, carbamoyl-phosphate synthase 1, ornithine transcarbamylase, argininosuccinate synthetase and lyase, and arginase 1 (Fig. 12.2.1). Deficiency of glu- tamine synthetase has also been identified but is not described here. Five urea cycle defects share a common but variable clinical presen- tation due to hyperammonaemia. Arginase 1 deficiency and defects of cellular transport including transporter proteins for the dibasic amino acids ornithine (hyperornithinaemia–hyperammonaemia–homocitrullinuria syndrome) and aspartate (citrullinaemia II) result in a more subtle disease with predominantly neurological symptoms. Onset of symptoms may occur at any age; however, it is par- ticularly frequent during the neonatal period, late infancy, and puberty, and is precipitated by excess protein or episodes that in- duce catabolism such as infectious diseases, trauma, or cortisone therapy. In general, symptoms are less severe with increasing age at onset. Neonatal presentation starts after a short asymptomatic interval with poor feeding, vomiting, lethargy, tachypnoea, and/or irritability which cannot be distinguished clinically from neonatal sepsis. Untreated, acute encephalopathy rapidly progresses to death. In infancy, the symptoms are less acute and more variable than in the neonatal period including anorexia, vomiting, developmental delay, and behavioural problems. In X-linked ornithine transcarbamylase deficiency, female carriers may also be affected due to variable in- activation of the X chromosome (the Lyon hypothesis). Clinical presentation ranges from acute liver failure, cognitive disability, and behavioural problems

to psychiatric disease. In arginase 1 defi- ciency, patients usually present with progressive spasticity which is often mistaken for cerebral palsy, seizures, and learning difficulties. Dystonia and ataxia may develop. Acute decompensation occurs rarely. The phenotypic variation of patients with urea cycle disorders as well as evidence-based recommendations for diagnosis, treat- ment, and follow-up have recently been reported by an international consortium of experts. Diagnosis Emergency analysis of ammonia must be part of the basic investiga- tions in all patients at all ages with unclear encephalopathy or acute hepatic failure. Among the inherited hyperammonaemias, two-thirds are due to urea cycle defects and one-third to organic acid and other inborn errors. Blood gas analyses and anion gap determinations may show alkalosis and normal anion gap in urea cycle defects and acidosis and increased anion gap in organic acid disorders. Characteristic biochemical changes (glutamine, alanine, citrulline, ornithine, ar- ginine, argininosuccinic acid, orotic acid, uracil) can be identified by plasma amino acid analysis, GC/MS analysis of urinary organic acids, or HPLC analysis of orotic acids and orotidine. The diagnosis can be confirmed by enzyme analysis in liver tissue (all urea cycle defects except for N-acetylglutamate synthase deficiency), fibro- blasts (argininosuccinate synthase 1 and lyase), or molecular genetic studies. Prenatal diagnosis is possible. Autosomal recessive inherited urea cycle disorders can be identified by molecular genetic studies on chorionic villus biopsy. Enzyme analysis can be performed for deficiencies of argininosuccinate lyase and synthase. Arginase defi- ciency can also be diagnosed biochemically by fetal blood analysis. Therapy and outcome The aim of treatment is to correct the biochemical disorder (glutamine in plasma <800–1000 µmol/litre, ammonia <80 µmol/litre, arginine 80–150 µmol/litre) and to ensure that the patient grows normally and thrives. The major metabolic strategies are (1) reduction of natural protein to decrease ammonia production, (2) supplementation with essential amino acids to prevent malnutrition and to reutilize ni- trogen for the synthesis of nonessential amino acids, (3) replacement of arginine or citrulline which become essential amino acids in all urea cycle disorders except for arginase 1 deficiency, and (4) utiliza- tion of alternative pathways for nitrogen excretion. This last strategy includes application of sodium benzoate (250–500 mg/kg per day) and Fumarate Mitochondrion = Ornithine transporter Cytosol Ammonia $HCO3^-$ CPS1 Carbamyl phosphate Orotic acid Orotidine Uracil Glutamate N-acetylglutamate NAGS Citrulline Aspartate Argininosuccinate Arginine Urea Cycle Ornithine OTC ASS ASL Arginase 1 Urea T T ⊕ Fig. 12.2.1 The urea cycle. ASL, argininosuccinate lyase; ASS, argininosuccinate synthase; CPS1, carbamyl phosphate synthase 1; NAGS, N-acetylglutamate synthase; OTC, ornithine transcarbamylase; T, ornithine transporter. Source data from Zschocke J, Hoffmann GF (2011). Vademecum metabolicum. Manual of metabolic paediatrics, 3rd edition. Schattauer, Stuttgart.

 sodium phenylbutyrate or phenylacetate (250–600 mg/kg per day) to conjugate glycine or glutamine, resulting in urinary excretion of waste nitrogen in alternative compounds (hippurate, phenyl acetylglutamine). In N-acetylglutamate synthase - deficiency, N- carbamylglutamate can be used as an alternative allosteric activator of carbamoyl phosphate synthase. All patients with urea cycle defects are at risk of acute metabolic decompensation precipitated by metabolic stress such as protein load, infection, anaesthesia, or surgery. To prevent or reverse meta- bolic crises, a stepwise implementation of an intensified emergency treatment is required (see also ‘Emergency treatment’). If diet and pharmacotherapy is insufficient to improve hyperammonaemia sig- nificantly and rapidly, haemofiltration or haemodialysis should be considered. Main factors that determine outcome are duration and severity of hyperammonaemia the specific disease, and age at disease onset are considered as

most important. In general, a beneficial outcome critically relies on rapid diagnosis and immediate start of treatment after the onset of first symptoms. Carbonic anhydrase VA deficiency Aetiology/pathophysiology Bicarbonate cannot enter the mitochondria and thus is generated within the mitochondria by two carbonic anhydrases: VA and VB. Carbonic anhydrase VA provides bicarbonate as a substrate to carbamyl phosphate synthase 1, the first enzymatic step of the urea cycle, as well as to three mitochondrial carboxylases, pyruvate carboxylase, propionyl-CoA carboxylase, and 3-methylcrotonyl- CoA carboxylase which are involved in energy metabolism and the catabolic pathways of branched-chain amino acids, respectively. Combined dysfunction of these four mitochondrial enzymes due to limited availability of their substrate bicarbonate causes a biochem- ical derangement including hyperammonaemia, impaired energy metabolism (affecting gluconeogenesis and tricarboxylic cycle) with lactic acidosis, as well as organic acidurias resembling propionic aciduria and 3-methylcrotonyl-CoA carboxylase deficiency (see ‘3-Methylcrotonylglycinuria’). Presentation Recently, four patients with this disease have been reported. Three of them presented with lethargy, tachypnoea, hypoglycaemia, hyperammonaemia, hyperlactatemia with hyperalaninaemia, and respiratory alkalosis during the first days of life; in one of them, the initial metabolic crisis occurred at age 13 months. During the follow- up, episodes of acute encephalopathy were precipitated by catabolic stress. Motor and mental development was within the normal range in one child, whereas delayed motor development due to ataxia and mild axial hypotonia, psychomotor retardation, or learning difficul- ties was found in the other children. Diagnosis Metabolic tests reveal a unique pattern of elevated lactate and hyperammonaemia with elevated glutamine and alanine, but low citrulline and arginine in plasma in combination with increased urinary excretion of lactate, ketone bodies, propionate metabolites, methylcrotonylglycine, and 3-hydroxyisovaleric acid. The meta- bolic pattern can be identified by analysis of plasma amino acids and organic acids in urine. Notably, newborn screening profiles, specifically C3 and C5OH levels, were unremarkable in all index pa- tients. The diagnosis can be confirmed by molecular genetic testing. Carbamyl phosphate synthase 1 and N-acetylglutamate synthase de- ficiency are the most relevant differential diagnosis. In children with negative molecular genetic test results in CPS1 and NAGS genes, car- bonic anhydrase VA deficiency should be considered. Treatment and outcome Treatment with preventive sick-day management using high-caloric, lipid-rich and low-protein formula to enhance anabolism and to re- duce the formation of toxic metabolites as well as carglumic acid to enhance the activity of carbamyl phosphate synthase 1 can be ad- ministered. Although carbonic anhydrase VA deficiency should be considered as treatable condition, treatment strategies have not yet been studied systematically. The long-term outcome of this disease is unknown. Defects of branched-chain amino acid metabolism Maple syrup urine disease Maple syrup urine disease was first reported in 1954 by Menkes, Hurst, and Craig, who noticed an unusual odour reminiscent of maple syrup in the urines of four infants who died from a rapidly pro- gressive neurological disease. In newborn screening programmes, a prevalence of approximately 1 in 200 000 newborns is encountered but in the Mennonites in Pennsylvania, the prevalence is as high as 1 in 200. Maple syrup urine disease is frequent in other ethnic groups and isolates such as persons of French Canadian origin. In maple syrup urine disease, the branched-chain amino acids leucine, isoleucine, and valine, their corresponding α-keto acids and hydroxy acid derivatives, as well as l-alloisoleucine are increased in physiological fluids. These amino acids and their metabolites accumulate due to inherited deficiency of the thiamine-dependent branched-chain α-keto acid dehydrogenase complex, consisting of subunits E1α, β, E2, and E3 (Fig. 12.2.2). l-Alloisoleucine results from racemization of the 3-carbon of l-isoleucine during transamination. Its elevation is pathogno- monic for maple syrup urine disease. Presentation

Several clinical presentations have been delineated but there is con- siderable overlap. Most frequently the condition comes to light in the first few days of life with lethargy, irritability, poor feeding, and neurological deterioration. Later-onset forms of maple syrup urine disease are slower with failure to thrive, developmental delay, and sometimes seizures; episodic ataxia and stupor sometimes pro- gressing to coma may be precipitated by high protein intake or intercurrent illness. In patients showing a response to thiamine, the condition tends to resemble later-onset maple syrup urine disease. A very rare related disease results from deficiency of lipoamide de- hydrogenase presenting after the neonatal period with lactic acid- osis, hypotonia, developmental delay, abnormal movement, and progressive neurological deterioration. Most patients with maple syrup urine disease have the classic form. If untreated, these neonates quickly deteriorate, developing lethargy, hypotonia alternating with muscular rigidity, opisthotonic posturing, and seizures (Fig. 12.2.3). Despite giving its name to

12.2 Protein-dependent inborn errors of metabolism 1955 the disease, the characteristic odour may be absent. Neuroimaging shows localized or diffuse generalized cerebral oedema. Convulsions appear regularly and electroencephalography reveals abnormalities with comb-like rhythms (5–9 Hz) of spindle-like sharp waves over the central regions and multiple shifting spikes and sharp waves with suppression bursts. Untreated patients succumb within a few days. Prominent neuropathological signs of untreated maple syrup urine disease are cerebral atrophy, including neuron loss in pontine nu- clei and the thalamus and myelin deficiency; spongy degeneration and astrocytic hyperplasia occur. Hypodensities may be present in globus pallidus and thalamus. In a few patients, mostly with inter- mittent or intermediate variants, the metabolic defect can be cor- rected by thiamine (‘thiamine-responsive’ variant). Effective doses vary from 10 mg up to 300 mg per day. Diagnosis Maple syrup urine disease is strongly suggested when an odour of maple syrup is present (most noticeably in the ear wax). Immediate confirmation by positive 2,4-dinitrophenylhydrazine testing is suf- ficient justification to initiate treatment in families at high risk. Diagnosis is confirmed by detection of increased plasma con- centrations of leucine, isoleucine, and valine and/or by increased urinary excretion of α-keto and hydroxy acids. The detection of l-alloisoleucine is diagnostic. Reduced enzyme activity of the branched-chain α-keto acid dehydrogenase complex in leuco- cytes, lymphoblasts, cultured fibroblasts, or amniocytes confirms the diagnosis. Except for the common Mennonite mutation, the 2-Oxoisocaproate 2-OH-isocaproate 3-OH-isovalerate 3-Methylcrotonyl- glycine 3-OH-isovalerate Isovalerylglycine Isoleucine 2-Oxo- 3-methylvalerate 2-Methylbutyryl-CoA Tiglyl-CoA Aminotransferase BCKDH MBD Hydratase Valine 2-Oxo- isovalerate Isobutyryl-CoA Methylacrylyl-CoA Aminotransferase BCKDH Hydratase Leucine 2-Oxo- isocaproate Isovaleryl-CoA 3-Methyl- crotonyl-CoA Aminotransferase BCKDH IVD MCC MHBD Deacylase Hydratase 3-Oxothiolase DH HMG-CoA lyase 2-Methyl- 3-OH-butyryl-CoA 3-OH-isobutyryl-CoA 3-Methyl- glutaconyl-CoA 2-Methyl- acetoacetyl-CoA 3-OH-isobutyrate 3-OH-3-methyl- glutaryl-CoA Methylmalonate semialdehyde IBD Propionyl-CoA Acetyl-CoA Acetoacetate Tiglyl- glycine 3-OH-propionate Methylcitrate Methylmalonyl-CoA Succinyl-CoA Krebs cycle Mutase 2-OH- isovalerate 3-Methylglutarate Carboxylase Alloisoleucine DH Fig. 12.2.2 Metabolism of branched-chain amino acids. BCKDH, branched chain α-keto acid dehydrogenase (deficient in MSUD); DH, dehydrogenase; hydratase, 3-methylglutaconyl-CoA hydratase (deficient in 3-methylglutaconic aciduria type I); IVD, isovaleryl-CoA dehydrogenase (deficient in isovaleric academia); MCC, 3-methylcrotonyl-CoA carboxylase (deficient in methylcrotonylglycinuria); MCM, methylmalonyl CoA mutase (deficient in methylmalonic aciduria); MHBD, 2-methyl-3-hydroxybutyryl-CoA dehydrogenase (deficient in 2-methyl-3-hydroxybutyryl-CoA dehydrogenase

deficiency); PCC, propionyl- CoA carboxylase (deficient in propionic aciduria). Accumulating pathologic metabolites are shown in italics. Source data from Zschocke J, Hoffmann GF (2011). Vademecum metabolicum. Manual of metabolic paediatrics, 3rd edition. Schattauer, Stuttgart.
Fig. 12.2.3 Opisthotonic hypertonic comatose infant with maple syrup urine disease.

SECTION 12 Metabolic disorders 1956 molecular genetics of maple syrup urine disease are too complex for diagnostic use. Prenatal testing is available by enzymatic ana- lysis of amniotic cells.
Treatment and outcome Emergency treatment aims to reduce branched-chain amino acids, particularly leucine. To induce anabolism, high calorie intake is re- quired. Most importantly, glucose stimulates endogenous insulin secretion activating protein synthesis. If required, insulin should be started early. In parallel, supplements free of branched-chain amino acids should be administered by nasogastric drip feeding. Since low plasma concentrations of isoleucine and valine limit protein syn- thesis, cautious supplementation to decrease leucine concentrations is mostly required. Extracorporeal detoxification (haemodialysis, haemofiltration) may be required if leucine exceeds 20 mg/dl (1500 µmol/litre). Liver transplantation may be considered a reasonable treatment option for patients with classic maple syrup urine disease. The decision of medical treatment versus transplantation, however, is very complex and must be reached for each patient individually. Long-term treatment of maple syrup urine disease is based on dietary restriction of branched-chain amino acids and supplemen- tation of thiamine, if proven beneficial. Management requires close and lifelong regulation of diet. Children with the classic form of maple syrup urine disease have a satisfactory prognosis only if they are diagnosed and treated before symptom onset; for this reason MS/MS-based newborn screening has been introduced in some countries.
Isovaleric aciduria Aetiology/pathophysiology Isovaleric aciduria was described by Tanaka in 1966. It is caused by deficiency of isovaleryl-CoA dehydrogenase, an enzyme located proximally in the catabolic pathway of the essential branched-chain amino acid leucine (Fig. 12.2.2). The encoding IVD gene is local- ized on 15q14–q15. Due to the metabolic block, isovaleryl-CoA accumulates, and the pathognomonic metabolite isovalerylglycine is formed by conjugation of isovaleryl-CoA to the amino group of glycine through the activity of the mitochondrial enzyme glycine- N-acylase. It is suggested that accumulating acyl-CoA esters sequester CoA, thereby disturbing energy metabolism. Specifically, isovaleryl- CoA inhibits pyruvate dehydrogenase and N-acetylglutamate syn- thase causing lactic acidosis and hyperammonaemia. Furthermore, isovaleric acid inhibits granulopoiesis and occurs during metabolic decompensations.
Clinical presentation Half of the patients with isovaleric aciduria present in the neonatal period with severe metabolic crises that may lead to coma and death, whereas the remainder experience chronic intermittent disease with episodes of metabolic acidosis and psychomotor retardation. Both phenotypes can occur within the same family suggesting a modi- fying role of environmental and epigenetic factors. A mild, poten- tially asymptomatic phenotype exists due to a common mutation (c.932C>T; p.A282V). This mutation was detected in one-half of mutant alleles in patients identified by newborn screening and also in older, healthy siblings. During metabolic crises, patients present with the typical features of classic organic acid disorders, that is, acidosis, ketosis, vomiting, progressive alteration of consciousness, and, finally, overwhelming illness, deep coma, and death if not given appropriate therapy. Clinical abnormalities often develop within the first days of life. A pathognomonic foul odour reminiscent of sweaty feet, caused by isovaleric acid, occurs. Abnormalities of the haematopoietic system such as thrombocytopenia, neutropenia, or pancytopenia develop; hyperammonaemia is usually mild. In the chronic intermittent form, children slide into recur- rent metabolic crises because of a high intake of protein or minor infections inducing a catabolic state.

Cytopenias develop as described earlier, and hyperglycaemia may develop, most likely due to stress-induced counter-regulatory hormonal ef- fects. Pancreatitis may be a complication of isovaleric aciduria. Older patients may have normal psychomotor development or mild to severe learning difficulties, depending on the frequency of decompensation and the age of diagnosis and institution of treatment. Diagnosis The clinical symptoms of isovaleric aciduria resemble other or- ganic acidaemias; even the suggestive odour may be shared by similar disorders (Boxes 12.2.1 and 12.2.2). The combination of ketoacidosis, dehydration, and hyperglycaemia has led to erro- neous diagnosis of diabetic ketoacidosis, and persistent vomiting in infancy to the wrong suggestion of hypertrophic pyloric sten- osis and unnecessary surgery. A reliable way to accomplish the diagnosis is quantitative analysis of urinary organic acids and acylglycines by GC/MS or the analysis of acylcarnitine profiles by MS/MS. During metabolic decompensation, the urinary organic acid profile reveals high excretion of isovalerylglycine which remains elevated. 3-Hydroxyisovaleric acid only increases during metabolic decom- pensation. Isovalerylcarnitine is the characteristic acylcarnitine of this disease and its urinary excretion increases following supple- mentation with l-carnitine. The diagnosis of isovaleric aciduria can be confirmed by enzyme analysis in fibroblasts or mutation analysis in specialized laboratories. Several methods have been successfully used for prenatal diagnosis including stable isotope dilution ana- lysis of isovalerylglycine, MS/MS detection of isovalerylcarnitine in amniotic fluid, or macromolecular labelling from (1-14C)-isovaleric acid in cultured amniocytes. Molecular diagnosis is only available in a research setting. Treatment and outcome Total natural protein intake is restricted according to the patient’s leucine tolerance and is adjusted to age-specific requirements. To provide a complementary source of the other amino acids, a leucine- free formula is available. Beyond childhood, a protein-restricted diet allowing a moderate restriction of leucine intake is usually suf- ficient. In addition, urinary excretion of isovaleryl-CoA as nontoxic carnitine conjugates is activated by supplementation with carnitine (50–100 mg/kg per day). During acute decompensation, isovaleric aciduria is treated fol- lowing the general principles for other organic acid disorders (see ‘Emergency treatment’).

 Aspirin is contraindicated in patients with isovaleric aciduria be- cause salicylic acid is a competing substrate for glycine-N-acylase, interfering with isovalerylglycine synthesis. Most children will survive the first life-threatening episode if correct treatment is set in place early. If effective treatment can be installed before any severe metabolic decompensation, it will sig- nificantly improve outcome. Therefore, in some countries isovaleric aciduria is screened for in newborns using MS/MS. 3-Methylcrotonylglycinuria 3-Methylcrotonylglycinuria is an inborn error of leucine catab- olism due to deficiency of 3-α-methylcrotonyl-CoA carboxylase (Fig. 12.2.2). It appears to be the most frequent inborn organic acid disorder, with a frequency of 1 in 50 000 newborns. The 3-methylcrotonylglycinuria enzyme requires biotin as a cofactor, and the isolated enzymatic defect must be differentiated from pri- mary deficiencies in the biotin pathway (see ‘Biotinidase deficiency’ and ‘Holocarboxylase synthetase deficiency’). As a consequence of 3-methylcrotonylglycinuria deficiency, 3-hydroxyisovaleric acid, 3-hydroxyisovalerylcarnitine, 3-methylcrotonylcarnitine, and 3-methylcrotonylglycine accumulate. Clinical presentation From the follow-up of individuals identified by newborn screening it has become evident that deficiency of 3-methylcrotonylglycinuria is a genetic condition with low clinical expressivity and penetrance, representing largely (c.90%) a nondisease. Less than 10% of affected individuals may develop mostly mild neurological symptoms which are often not clearly attributed to 3-methylcrotonylglycinuria de- ficiency. However, a few patients may develop acute metabolic de- compensation (ketoacidosis, hypoglycaemia,

hyperammonaemia, Reye-like syndrome) precipitated by febrile illness during infancy; this may be fatal if untreated. Diagnosis The diagnosis is confirmed biochemically by identification of 3-hydroxyisovaleric acid and 3-methylcrotonylglycine in urine (GC/MS) or 3-hydroxyisovalerylcarnitine in dried blood spots or plasma (MS/MS whereas in patients with additionally increased 3-hydroxypropionic, methylcitric, or lactic acids multiple carb- oxylase deficiency or biotinidase deficiency should be considered. In particular, 3-hydroxyisovalerylcarnitine concentrations which spontaneously decrease to normal values in follow-up inves- tigations of any neonate should prompt the investigation of 3-methylcrotonylglycinuria deficiency in the mother. Significantly reduced enzyme activity in fibroblasts or leucocytes or mutation analysis confirms the diagnosis. It is important to ex- clude multiple carboxylase deficiency by demonstrating normal en- zyme activities of propionyl-CoA carboxylase, pyruvate carboxylase, as well as biotinidase. Prenatal diagnosis is possible by stable isotope dilution analysis of amniotic fluid or by enzymatic and molecular analyses in cultivated amniocytes or chorionic villi. Treatment and outcome Most affected individuals do not require specific treatment, with the exception of carnitine supplementation if secondary carnitine depletion is found. However, moderate protein restriction and ad- ministration of leucine-free amino acid supplements has been tried. 3-Methylcrotonylglycinuria is usually unresponsive to biotin whereas, in those with the p.R385S mutation, biotin responsiveness has been reported. If acute metabolic decompensation occurs, af- fected patients are treated as with other organic acid disorders (see ‘Emergency treatment’). Most affected individuals remain asymp- tomatic without specific treatment and thus the benefit of newborn screening remains to be elucidated. 3-Methylglutaconic acidurias Increased urinary excretion of 3-methylglutaconic acid is the biochemical hallmark of a heterogeneous group of inborn errors termed 3-methylglutaconic acidurias types including a primary defect in leucine catabolism, primary mitochondrial disorders, for example, Pearson’s syndrome and ATP synthase deficiency, and patients with Smith–Lemli–Opitz syndrome, a cholesterol biosyn- thesis disorder. Whereas in 3-methylglutaconyl-CoA hydratase de- ficiency elevated 3-methylglutaconic is caused by a primary defect in leucine degradation, in all other diseases with 3-methylglutaconic aciduria the increase of this metabolite is thought to be secondary to mitochondrial membrane biosynthesis, maintenance, and phospholipid remodelling or disturbed cholesterol biosynthesis. Interestingly, leucine degradation is linked to cholesterol biosyn- thesis via the Popjak shunt and the 3-hydroxy-3-methylglutaryl- CoA salvage pathway. With recognition of an increasing number of underlying defects in recent years, the initial nomenclature of 3- methylglutaconic acidurias has been revised. In the following, both old (type I–V) and new nomenclature (specifying the syndrome and affected gene) are given. Primary 3-methylglutaconic aciduria 3-Methylglutaconic aciduria type I Aetiology/pathophysiology 3-Methylglutaconic aciduria type I is caused by deficiency of 3-methylglutaconyl-CoA hydratase (Fig. 12.2.2) required for the conversion of 3-methylglutaconyl- CoA to 3-hydroxy-3-methylglutaryl-CoA in leucine catabolism. The hydratase is identical to an RNA-binding protein (designated AUH) possessing enoyl-CoA hydratase activity. The defect leads to an accumulation of 3-methylglutaconic, 3-methylglutaric, and 3-hydroxyisovaleric acids. Clinical presentation The clinical phenotype of affected individ- uals is variable and also includes an asymptomatic disease course. Patients present with neurological symptoms including delayed speech and motor development. Metabolic decompensation with hypoglycaemia and metabolic acidosis is rare but can occur fol- lowing a catabolic state. The recent discovery of the disorder in adult-onset patients with slowly progressive ataxia, dementia, and leukoencephalopathy may point to the long-term nature and mani- festations of this disease. Diagnosis Urinary excretion of large amounts of 3-

methylglutaconic, 3-methylglutaric, and 3-hydroxyisovaleric acids but normal excretion of 3-hydroxy-3-methylglutaric acid points to hydratase deficiency. Increased 3-hydroxyisovalerylcarnitine is a hint for either type of 3-methylglutaconic aciduria. The definitive diagnosis is made by en- zyme analysis in fibroblasts or by mutation analysis. Treatment and outcome The need for treatment has not been es- tablished, especially for dietary treatment. The outcome appears

 favourable as a significant number of untreated patients have never developed symptoms. Secondary 3-methylglutaconic acidurias TAZ defect or Barth's syndrome (formerly,
3-methylglutaconic aciduria type II) Aetiology/pathophysiology The molecular basis of Barth's syndrome is deficiency of tafazzin which is localized in the inner mitochondrial membrane affecting phospholipid metabolism, in particular cardiolipin. The origin of elevated levels of 3-methylglutaconic and 3-methylglutaric acids in Barth's syndrome is unknown. The identification of the causative gene allowed the retrospective classification of different families labelled in the past as X-linked endocardial fibrosis, severe X-linked cardiomyopathy, or Barth's syndrome. All these entities have been shown to share the same mo- lecular pathology. Clinical presentation In 1983, Barth and colleagues described an X-linked neuromuscular disease characterized by dilated cardiomyopathy, skeletal myopathy, retarded growth, and neutropenia. Patients may present at birth or during the first weeks of life, usually with congestive cardiac failure. With long-standing cardiac disease, endocardial fibroelastosis may develop. Delayed gross motor mile- stones, myopathic facies, a waddling gait, and a positive Gower's sign are common. Occasionally patients may show moderate lactic acidosis. Postnatal growth retardation may be severe, and beyond 2 years of age patients are usually very stunted but with normal head circumferences. Diagnosis Barth's syndrome should be considered in any male presenting with dilated cardiomyopathy. If neutropenia, idiopathic myopathy, and growth retardation are also present, the diagnosis of Barth's syndrome is almost certain. Biochemically, increased 3- methylglutaconic acid is usually found in urine but is not a constant feature. 2-Ethylhydracrylic acid may be also elevated. Muscle dis- ease and lactic acidaemia may initiate a work-up for mitochondrial disorders. Muscle biopsy may reveal involvement of deficient re- spiratory chain complexes I and IV. The diagnosis is confirmed by cardiolipin analysis in thrombocytes or mutation analysis. Mutation analysis makes prenatal diagnosis now available. Treatment and outcome Children affected by Barth's syndrome need to be carefully managed mainly by expert cardiologists; im- munologists and neurologists should also be involved. Cardiac ar- rhythmias carry a poor prognosis and may require implantation of an internal cardiac defibrillator. Successful heart transplantation has been carried out. Due to increased susceptibility to severe bacterial infections, infectious diseases need to be treated promptly and ag- gressively. Protein restriction and carnitine supplementation has been employed with unclear benefit. About 25% of patients with Barth's syndrome succumb during infancy and early childhood due to cardiac complications or overwhelming bacterial infections. OPA3 defect or Costeff's syndrome (formerly,
3-methylglutaconic aciduria type III) Aetiology/pathophysiology Costeff's syndrome is caused by mu- tations in the OPA3 gene resulting in a defect of a putative mito- chondrial protein with yet unknown function. The origin of elevated levels of 3-methylglutaconic and 3-methylglutaric acids is also un- known. So far the disorder has only been reported in Iraqi Jews. Clinical presentation The determining clinical presentation is early-onset optic atrophy, which may be accompanied by nystagmus. In later childhood or adolescence, patients may develop extrapyr- amidal signs and

moderate cognitive impairment. In about one-half of the patients, spasticity develops and progresses over years. Diagnosis Costeff's syndrome should be suspected in patients pre- senting with early-onset optic atrophy if additional neurological symptoms develop. 3-Methylglutaconic aciduria is a biochemical indicator of Costeff's syndrome, which may now be proven by mo- lecular analysis. Treatment and outcome Effective treatment has not been reported. Treatment is symptomatic and focuses on the prevention of disabil- ities due to progressive spasticity. The disease appears stationary but the long-term outcome is unknown. Specified and not otherwise specified 3-methylglutaconic aciduria type IV Aetiology/pathophysiology 3-Methylglutaconic aciduria type IV is undoubtedly the most heterogeneous and increasing group of 3-methylglutaconic acidurias. As unexplained 3-methylglutaconic aciduria (i.e. type IV) was also found incidentally in asymptomatic adults, it appears doubtful that this biochemical feature by itself is of pathophysiological relevance. Diagnosis Patients are identified by elevated urinary con- centrations of 3-methylglutaconic and 3-methylglutaric acids. Classification of type IV methylglutaconic aciduria is made by ex- clusion of known causes of 3-methylglutaconic aciduria (see other subsections), primary mitochondrial disorders (e.g. Pearson's syn- drome), and Smith–Lemli–Opitz syndrome. Four clinical pheno- typic groups have been delineated including patients with an encephalopathic, hepatocerebral, cardiomyopathic, and myopathic disease form. Genetic testing in these patients has elucidated a high number of disease-causing mutations in the POLG1, SUCLA2, TMEM70, and RYR1 genes which have been associated with known diseases. Furthermore, MEGDEL syndrome due to mutations in the SERAC1 gene was recently described. Based on this, the mo- lecular cause of 3-methylglutaconic aciduria type IV can be iden- tified in many patients. In patients with a known molecular basis of 3-methylglutaconic aciduria, 3-methylglutaconic aciduria type IV shall be replaced by a more specific terminology, for example, SERAC1 defect or MEGDEL syndrome, or TMEM70 defect. Treatment and outcome No effective treatment has been re- ported. Treatment is symptomatic and focuses on the prevention of neurological deterioration. The identification of type IV 3- methylglutaconic aciduria is not yet of prognostic relevance. DNAJC19 defect or dilated cardiomyopathy with ataxia (DCMA) syndrome (formerly, 3-methylglutaconic aciduria type V) Aetiology/pathophysiology Another type of 3-methylglutaconic aciduria has recently been elucidated in 18 patients of the Canadian Dariusleut Hutterite population. It is an autosomal recessive con- dition caused by a mutated DNAJC19 gene. Proteins of the DNAJ

 domain are involved in molecular chaperone systems, DNAJ19 having been localized to the inner mitochondrial membrane. Clinical presentation Inherited DNAJ19 deficiency leads to clin- ical presentation which initially resembles Barth's syndrome (3- methylglutaconic aciduria type II) with early-onset severe dilated (or noncompaction) cardiomyopathy with conduction defects. However, it also leads to with nonprogressive cerebellar ataxia, tes- ticular dysgenesis, and growth failure. Diagnosis The diagnosis can be made by genetic testing in patients with a suggestive clinical presentation and 3-methylglutaonic aciduria. Therapy and outcome No effective treatment has been reported. Treatment is symptomatic and focuses on the prevention of cardiac deterioration. 2-Methyl-3-hydroxybutyryl-CoA dehydrogenase deficiency Aetiology/pathophysiology 2-Methyl-3-hydroxybutyryl-CoA dehydrogenase deficiency is a rare cerebral organic acid disorder. This mitochondrial enzyme is involved in the catabolism of isoleucine and branched-chain fatty acids (Fig. 12.2.2). Retrospectively, patients were misdiagnosed as having 3-oxothiolase deficiency until Zschocke and colleagues (2000) recognized the separate distinct clinical and biochemical

presentation. Inheritance is X-chromosomal semidominant (fe- males may be symptomatic). Disease-causing mutations were iden- tified in the HSD17B10 gene. The pathophysiology of this disease is unknown. The enzyme is identical to an amyloid β-peptide-binding protein which is implicated in Alzheimer's disease. Clinical presentation 2-Methyl-3-hydroxybutyryl-CoA dehydrogenase deficiency mostly results in a progressive neurodegenerative disease. Regression usu- ally becomes obvious in late infancy or early childhood but is vari- able. Affected boys usually develop truncal hypotonia with spasticity of the limbs, dyskinesia and athetosis, a horizontal nystagmus, and retinal blindness. Motor and mental skills are completely lost, as are sensory modalities. Epilepsy is frequently found and is usually dif- ficult to treat. When hypertrophic cardiomyopathy was diagnosed, deterioration was rapid with death due to progressive heart failure. Neuroimaging documents progressive generalized atrophy, basal ganglia injury, periventricular white matter abnormalities, and oc- cipital infarctions in individual cases. Heterozygous female patients may be asymptomatic or may have variable stationary psychomotor retardation with impaired hearing. Diagnosis The disease should be considered in children presenting with early-onset progressive encephalopathy, especially if X-linked in- heritance is suggested. The biochemical hallmark of this disease is increased urinary excretion of 2-methyl-3-hydroxybutyric acid and tiglylglycine. Elevations of 2-ethylhydracrylic acid and 3- hydroxyisobutyric acid in urine may also be found. These abnor- malities may be subtle. Treatment and outcome No effective rational treatment is known. Care of patients with this disease should repeatedly entail (1) assessment of muscle and cardiac function, (2) neurological examination including elec- troencephalography and MRI, and (3) assessment of visual and hearing system. The prognosis is mostly poor, with death in early childhood. Propionic aciduria Aetiology/pathophysiology In 1961, Childs and coworkers described the index patient with pro- pionic aciduria. Since ketosis and hyperglycinaemia were the bio- chemical hallmarks recognized, the disorder was lumped together with methylmalonic acidurias as 'ketotic hyperglycinaemia' to dis- tinguish it from nonketotic hyperglycinaemia. Implementation of GC/MS analysis to metabolic diagnostic work-up allowed the differentiation of these disorders in the 1970s. Propionic aciduria is caused by an autosomal recessive inherited deficiency of biotin- dependent duodecameric propionyl-CoA carboxylase, the first step in propionate metabolism, in which propionyl-CoA is converted to methylmalonyl-CoA (Fig. 12.2.2). Over 100 disease-causing muta- tions have been identified in the PCCA gene (13q32) and the PCCB gene (3q21–22). Propionyl-CoA is formed from the catabolism of isoleucine, threonine, methionine, valine, odd-numbered fatty acids, and the side chain of cholesterol, and from gut bacteria. Deficiency of propionyl-CoA carboxylase gives rise to accumulation of propionyl-CoA and metabolites of alternative propionate oxidation such as 2-methylcitric acid, 3-hydroxypropionic acid, tiglic acid, propionylcarnitine, and propionylglycine. All of these can be de- tected and quantified by GC/MS (urine, plasma) or MS/MS (dried blood spots, plasma). Elevated propionyl-CoA and its pathological derivatives inter- fere with a variety of metabolic pathways including inhibition of (1) the glycine cleavage enzyme resulting in hyperglycinaemia, (2) N-acetylglutamate synthase resulting in hyperammonaemia, and (3) pyruvate dehydrogenase complex as well as several enzymes of the tricarboxylic acid cycle resulting in lactic acidaemia and hyperketosis, and severe impairment of energy metabolism. Clinical presentation Propionic aciduria usually presents with severe neonatal meta- bolic decompensation characterized clinically by multiorgan failure and biochemically by hyperammonaemia, metabolic acidosis, hyperketosis, lactic acidaemia, hyperglycinaemia, and hyperalaninaemia. Propionic aciduria may be misinterpreted as sepsis or ventricular haemorrhage. Acute metabolic decompen- sation and long-term complications usually involve organs with a high energy demand, including the brain, heart

and skeletal muscle, liver, and bone marrow. Frequent signs and symptoms are failure to thrive, microcephaly, mild to severe motor disabilities and learning difficulties, truncal hypotonia, extrapyramidal symptoms (dystonia, chorea), seizures, cardiomyopathy, myopathy, hepatomegaly, acute or chronic pancreatitis, leucopenia, thrombocytopenia, anaemia, or pancytopenia, whereas renal complications are uncommon. Metabolic decompensations in infancy or childhood are similar to those in the neonatal period. The first symptom is often vomiting; this has led to erroneous diagnosis of pyloric stenosis or duodenal obstruction, resulting in a number of pyloromyotomies or other explorations. Basal ganglia injury, mostly affecting the putamen,

 occurs (Fig. 12.2.4); generalized cerebral atrophy and white matter disease is common. A small subgroup of patients exhibit almost exclusively encephal- opathy and progressive neurological disease, resembling a lysosomal storage disorder. A milder form of propionic aciduria reported in Japan manifests from childhood with mild learning difficulties or extrapyramidal symptoms, and only occasionally with metabolic acidosis. Finally, some individuals remain asymptomatic until teenage years and are identified during family studies. Diagnosis The method of diagnosis is GC/MS analysis of organic acids (urine) or MS/MS analysis of acylcarnitines (dried blood spots, plasma, urine). Characteristic metabolites are 2-methylcitric acid, 3-hydroxypropionic acid, tiglic acid, propionylglycine, and propionylcarnitine. The absence of methylmalonic acid excludes methylmalonic acidurias, and the absence of β-hydroxyisovaleric acid and β-methylcrotonylglycine rules out multiple carboxylase de- ficiency. In plasma and urine, increased concentrations of glycine and ketone bodies may be present. Confirmation of diagnosis is made by enzyme analysis in leucocytes or fibroblasts, or by mutation analysis. Prenatal diagnosis can be made by mutation analysis, enzyme ana- lysis, or quantitative GC/MS analysis of 2-methylcitric acid. Treatment and outcome Prevention of metabolic decompensation is the most important de- terminant of outcome. During acute decompensation, propionic aciduria is treated like other organic acid disorders (see ‘Emergency treatment’). Long-term treatment is based on lifelong dietary restric- tion of the precursors isoleucine, valine, methionine, and threonine, as well as by supplementation with l-carnitine. As significant propi- onate production occurs in the gut, intermittent decontamination (10–14 days/month) with metronidazole or colistin as well as meas- ures preventing constipation are often used. Some patients exhibit recurrent or almost chronic hyperammonaemia, especially during infancy. This may necessitate additional supplementation with ar- ginine or citrulline and/or administration of sodium benzoate or phenylbutyrate. However, benzoate treatment may aggravate the depletion of free carnitine and CoA. Biotin responsiveness in propi- onic aciduria is very rare, if present at all. More than 20 children with propionic aciduria have undergone orthotopic liver transplantation, but the outcome is mixed. Auxiliary as well as living-related liver transplantations have been successfully performed, but liver trans- plantation in propionic aciduria seems to be more complicated than in patients with urea cycle defects. Patients with neonatal onset of symptoms still have a poor out- come. Patients with late onset of symptoms reach adulthood but often have physical and mental disabilities; nonetheless, some patients can survive to adulthood with normal intellects. The phenotypic vari- ation of patients with propionic aciduria as well as evidence-based recommendations for diagnosis, treatment, and follow-up have re- cently been reported by an international consortium of experts. Methylmalonic aciduria Aetiology/pathophysiology Methylmalonic aciduria is the biochemical hallmark of a heteroge- neous group of inborn metabolic errors with a cumulative prevalence of at least 1 in 100 000 newborns in Europe. Index patients were first described in 1967 by Oberholzer and Stokke. This section focuses on isolated methylmalonic aciduria caused by mutations in the MUT gene localized on 6p21

encoding the apoenzyme methylmalonyl- CoA mutase. Methylmalonyl-CoA mutase can alternatively be im- paired by defects in the biosynthesis of 5′-deoxyadenosylcobalamin, deficient cobalamin transport, or by acquired cobalamin deficiency as in pernicious anaemia. In infancy, severe progressive disease may develop in breastfed infants of mothers who have (undiagnosed) pernicious anaemia or adhere to a strict vegan diet. Methylmalonic acid is a more reliable index of body stores of cobalamin than cobalamin levels in blood. d-Methylmalonyl-CoA is formed in propionate metabolism by carboxylation of propionyl-CoA. l-Methylmalonyl-CoA is formed from d-methylmalonyl-CoA by d-methylmalonyl-CoA racemase and, subsequently, is converted to succinyl-CoA by the dimeric 5′-deoxyadenosylcobalamin-dependent mitochondrial enzyme methylmalonyl-CoA mutase (Fig. 12.2.2). As with propionic aciduria (see ‘Propionic aciduria’), impairment of energy metabolism by propionyl-CoA and 2-methylcitric acid plays a key role in the pathophysiology of methylmalonic acidurias, resulting in multiorgan failure. In addition, methylmalonic acid may exert additional toxic effects. Clinical presentation Patients with severe methylmalonyl-CoA mutase deficiency (mut0) usually present with neonatal metabolic crises which are clinically Fig. 12.2.4 Transverse MRI image of a 7-year-old girl, who had been diagnosed with propionic aciduria in infancy and had been successfully treated since then. While in good metabolic control, she suddenly became comatose. Massive infarction of the basal ganglia had occurred, and the child died a few days later. Spin echo technique. Courtesy of Drs R. Haas and W.L. Nyhan, Department of Pediatrics, University of California, San Diego, USA.

 1961 and biochemically (except for methylmalonic acid) indistinguish- able from those of patients with propionic aciduria. In patients with residual methylmalonyl-CoA mutase activity (mut−), the onset of symptoms is more variable. Neonatal onset of symptoms is found as is a chronic intermittent form, that is, precipitation of recurrent metabolic crises in infancy and children following a high intake of protein or a catabolic state. Long-term complications are frequent, in particular in mut0 patients. These include failure to thrive, chronic neurological symptoms such as extrapyramidal movement disorder, motor disabilities, learning difficulties, and epilepsy, cardiomyop- athy, myopathy, and pancreatitis. Neuroradiological studies demon- strate lesions of globus pallidus, generalized cerebral atrophy, and white matter disease. The development of chronic renal failure in a large proportion of patients appears inevitable. Diagnosis A reliable way to make the diagnosis is GC/MS analysis of urinary organic acids or MS/MS analysis of acylcarnitines showing ele- vated concentrations of methylmalonic acid as well as of metab- olites of alternative propionate oxidation (e.g. propionylglycine, 3-hydroxypropionic acid, 2-methylcitric acid, propionylglycine, and propionylcarnitine; as in propionic aciduria). These biochemical abnormalities have a considerable interday and intraday variation and are influenced by responsiveness to cobalamin and metabolic state. Differential diagnosis of methylmalonic aciduria is acquired cobalamin depletion or inherited cobalamin deficiencies, transient mild methylmalonic acidurias of unknown origin in infants, and methylmalonic encephalopathy due to deficiency of succinyl-CoA synthase. Concomitant megaloblastic anaemia and an increase of plasma homocysteine indicates disturbed cobalamin metabolism as the cause of methylmalonic aciduria. Standardized criteria to define responsiveness to hydroxocobalamin are not established. The determination of methylmalonyl-CoA mutase activity in fibroblast extracts, mutation analysis or the in- vestigation of labelled propionate incorporation following transfec- tion by a vector containing cloned mutase cDNA in intact patients’ fibroblasts may be required to differentiate primary defects of methylmalonyl-CoA mutase (mut0, mut−) from primary defects of 5′- deoxyadenosylcobalamin (cblA and cblB defects). Prenatal diagnosis is

available by enzyme or mutation analyses as well as by quantitative stable isotope dilution assay of 2-methylcitric acid. Treatment and outcome Metabolic maintenance and emergency treatment follows the treat- ment principles for organic acid disorders in general and propionic aciduria in particular (see ‘Propionic aciduria’). In addition, substi- tution with cobalamin may be beneficial, since partial or complete response to cobalamin has been demonstrated (except for mut0 pa- tients). In neonates and infants, intramuscular hydroxocobalamin is required; children and adults may be treated with oral cyano- cobalamin. Chronic renal failure may progress, necessitating haemodialysis or peritoneal dialysis. Kidney transplantation has been performed in these patients. Liver transplantation can provide enzyme activity to ameliorate the metabolic defect and the idea of combined liver–kidney or isolated liver transplantation has emerged. The benefit remains doubtful, however, as mortality is significant; in addition, liver transplantation does not reliably protect against se- vere neurological and renal complications. The phenotypic variation of patients with methylmalonic aciduria as well as evidence-based recommendations for diagnosis, treatment, and follow-up have re- cently been reported by an international consortium of experts. 3-Hydroxyisobutyryl-CoA hydrolase deficiency Aetiology/pathophysiology 3-Hydroxyisobutyryl-CoA hydrolase (HIBCH) catalyses the fifth step of valine catabolism converting 3-hydroxyisobutyryl- CoA to 3-hydroxyisobutyrate and is due to biallelic mutations of the HIBCH gene which is located on 2q32.2. HIBCH de- ficiency is biochemically characterized by accumulation of 3-hydroxyisobutyrylcarnitine deriving from 3-hydroxyisobutyryl- CoA and S-2-carboxypropyl-L-cysteine and -cysteamine deriving from methyacrylyl-CoA. Whereas 3-hydroxyisobutyrylcarnitine can be eliminated via urinary excretion, methylacrylyl-CoA is a highly reactive compound which readily undergoes addition reac- tion with sulphhydryl groups. Inactivation of sulhydryl-containing enzymes such as respiratory chain complexes and cofactors is con- sidered as the major pathomechanism. Clinical presentation So far this disease has rarely been described. Patients presented with a (Leigh-like) mitochondrial encephalopathy starting in infancy, delayed global development, muscular hypotonia, poor feeding, and multiple malformations (dysmorphic facial features, vertebral anomalies, tetralogy of Fallot, agenesis of cingulate gyrus and corpus callosum) in one patient. Neurological symptoms are progressive. Diagnosis The diagnosis is based on metabolic tests demonstrating elevated 3-hydroxyisobutyrylcarnitine by tandem mass spectrometry and 2-methyl-2,3-dihydroxybutyric acid and 2-hydroxyisovaleric acid by organic acid analysis. S-2-carboxypropyl-L-cysteine and - cysteamine can be determined by specific HPLC analysis. Enzymatic testing of the deficient enzyme in fibroblast and molecular genetic testing confirms the diagnosis. Analysis of respiratory chain en- zymes in muscle biopsy often show decreased activity of pyruvate dehydrogenase complex and/or multiple deficiencies of respiratory chain complexes. Treatment and outcome Since this disease is thought to be caused by accumulation of toxic metabolites of the valine catabolic pathway, a low-valine diet should be considered as a treatment option. Carnitine supplementation prevents secondary carnitine depletion. However, the efficacy of this therapeutic approach has not yet been systematically studied. Short-chain enoyl-CoA hydratase deficiency Aetiology/pathophysiology Mitochondrial short-chain enoyl-CoA hydratase (ECHS1) is a multispecific enzyme that catalyses the hydration of chain-shortened α,β-unsaturated enoyl-CoA thioesters in the β-oxidation spiral of fatty acids as well as in the catabolic pathways of valine, isoleucine, tryptophan, and lysine. Deficiency of ECHS1, which is coded by the ECHS1 gene located on 10q26.3, induces a very similar biochem- ical phenotype as in HIBCH deficiency. In contrast to HIBCH defi- ciency, however, tiglylglycine but not 3-hydroxyisobutyrylcarnitine

SECTION 12 Metabolic disorders 1962 is elevated. Inconsistently, there is evidence of mildly impaired mitochondrial oxidation of short-chain fatty acids. The biochemical derangement highlights that in analogy to HIBCH deficiency, im- pairment of valine catabolism and thus accumulation of the toxic metabolite methacrylyl-CoA is most important. Clinical presentation Patients present with (Leigh-like) mitochondrial encephalopathy, dystonia, epilepsy, optic nerve atrophy and cardiomyopathy. Onset of symptoms is usually found in the newborn period or during in- fancy. The disease course is progressive and in its severest form it might be fatal in infancy or childhood. Diagnosis Diagnosis of this disease should be considered in patients with a combination of mitochondrial encephalopathy and cardiomyop- athy. Urinary excretion of 2-methyl-2,3-dihydroxybutyric acid is determined by organic acid analysis, S-2-carboxypropyl-L-cysteine and -cysteamine can be analysed using specific HPLC methods. Enzymatic testing of the deficient enzyme in fibroblast and mo- lecular genetic testing confirm the diagnosis. Analysis of respira- tory chain enzymes in muscle biopsy often show decreased activity of pyruvate dehydrogenase complex and/or multiple deficiencies of respiratory chain complexes. Treatment and outcome In analogy to HIBCH deficiency, a low-valine diet should be con- sidered a treatment option. It remains to be elucidated whether this therapeutic intervention is able to improve the disease course. Malonic aciduria Aetiology/pathophysiology First described in 1984, very few patients with malonic aciduria have been delineated until now. Malonic aciduria is caused by malonyl- CoA decarboxylase deficiency leading to a disturbed fatty acid me- tabolism. Malonyl-CoA is the first committed intermediate of fatty acid synthesis. In addition, it regulates carnitine acyltransferases among other enzymes steering fatty acid metabolism. The cytocolic enzyme is found most often in the liver, brain, heart, and skeletal muscle. Clinical presentation The clinical presentation is variable but mostly involves acute meta- bolic episodes with progressive lethargy, hypotonia, and hepato- megaly associated with metabolic acidosis. Hypoglycaemia, lactic acidosis, and/ or mild hyperammonaemia can also develop. Cardiac involvement is present in about 40% of patients with cardiomy- opathy which can progress to cardiac failure. Other patients were identified with less specific symptoms such as developmental delay, hypotonia, seizures, and short stature. Diagnosis Urinary organic acids identify increased malonic acid, sometimes in combination with fumaric acid, malic acid, and aethylmalonic acid. During metabolic decompensations ketosis develops with elevated dicarboxylic acids. Total and free carnitine levels are reduced due to the formation of malonylcarnitine, which may allow population newborn screening. Treatment and outcome A clearly effective therapeutic regimen has not been established. Carnitine supplementation as well as a diet high in carbohydrates and low in long-chain triglycerides improved the clinical symp- toms as well as metabolic disturbances in patients. In some patients, medium-chain triglycerides supplements appeared helpful. Little is known about the long-term prospects of this disorder. Patients were stable on the different treatment options at least until adolescence. Defects of lysine, hydroxylysine, and tryptophan metabolism The common catabolic pathway of lysine, hydroxylysine, and trypto- phan is summarized in Fig. 12.2.5. Hyperlysinaemia I/hyperlysinaemia II or saccharopinuria Hyperlysinaemia/saccharopinuria is caused by a recessive deficiency of the bifunctional protein 2-aminoadipic semialdehyde synthase. As hyperlysinaemia/saccharopinuria is considered a nondisease, af- fected individuals do not require specific treatment. 2-Amino-/2-oxoadipic aciduria Disease-causing mutations in the DHTKD1 gene cause autosomal recessive 2-amino-/2-oxoadipic aciduria. This gene encodes for the E1 subunit of a 2-oxoglutarate dehydrogenase complex-like pro- tein in the lysine degradative pathway. Most affected individuals re- main asymptomatic, whereas others may present with variable mild neurological symptoms. Glutaric aciduria type I Aetiology/pathophysiology Glutaric aciduria type I was described in 1975. It

occurs with an estimated frequency of 1 in 100 000 newborns, but which is Acetyl-CoA Kynurenine 2-Aminoadipic semialdehyde 2-Aminoadipic acid 2-Oxoadipic acid Glutaryl-CoA Lysine Tryptophan Saccharopine Phospho- hydroxylysine 3-OH-kynurenine 2-Oxoadipic acid 2-Amino- adipic acid Reductase Dehydrogenase Aminotransferase Dehydrogenase GCDH Crotonyl-CoA SCHAD Hydroxylysine Kinase Dioxygenase Kynureninase Cytosol Mito- chondrion Transport in/out mitochondria Fig. 12.2.5 Catabolic pathway of lysine, tryptophan, and hydroxylysine. 2-Aminoadipic semialdehyde synthase (deficient in hyperlysinaemia/ saccharopinuria); 2-aminoadipate aminotransferase (deficiency has not yet been reported), 2-oxoglutarate dehydrogenase-like complex (deficient in in 2-amino-/2-oxoadipic aciduria); glutaryl-CoA dehydrogenase (GCDH; deficient in glutaric aciduria type I). Source data from Zschocke J, Hoffmann GF (2011). Vademecum metabolicum. Manual of metabolic paediatrics, 3rd edition. Schattauer, Stuttgart.

12.2 Protein-dependent inborn errors of metabolism 1963 considerably higher (up to 1 in 300) in some communities (e.g. the Amish in Pennsylvania, United States of America, and the Oji-Cree First Nations in Canada). Glutaric aciduria type I is caused by de- ficiency of flavin adenine dinucleotide-dependent glutaryl-CoA dehydrogenase, a mitochondrial enzyme in the catabolic pathway common to tryptophan, lysine, and hydroxylysine (Fig. 12.2.5). Glutaryl-CoA dehydrogenase is encoded by the GCDH gene lo- calized on 19p13.2. More than 200 disease-causing mutations have been described. There is no genotype–phenotype correlation. As a consequence of glutaryl-CoA dehydrogenase deficiency, glutaric, 3-hydroxyglutaric, and (inconsistently) glutaconic acids as well as glutarylcarnitine accumulate. The limited permeability of the blood–brain barrier to dicarboxylic acids (such as glutaric acid) leads to their accumulation in the brain (trapping hypothesis). Some of these metabolites are neurotoxins. Candidate mechanisms are stimulation of excitotoxic cell damage via activation of N-methyl-d- aspartate receptors, and inhibition of 2-oxoglutarate dehydrogenase and the dicarboxylate shuttle between astrocytes and neurons.
Clinical presentation Newborns are often asymptomatic but may present with transient and subtle neurological symptoms such as truncal hypotonia or asymmetric posturing. (Progressive) macrocephaly occurs in 75% of patients. Neuroimaging in infancy often reveals hypoplasia of the temporal pole, subependymal pseudocysts, and delayed myelin- ation; subdural fluid collections may be found which may be mis- taken as nonaccidental trauma. The prognostically relevant event of glutaric aciduria type I is the onset of an acute encephalopathic crisis which is usually precipi- tated by a catabolic state (e.g. febrile illness) during infancy and early childhood. Encephalopathic crises characteristically result in acute striatal injury and, subsequently, dystonia. Approximately 15% of patients with glutaric aciduria type I follow a chronic disease course and develop the same neurological symptoms as the acutely injured children over the first 2 years of life without overt crisis (insidious- onset variant) or during adolescence/adulthood presenting with leukoencephalopathy (late-onset variant). Asymptomatic indi- viduals occur occasionally. Neuroradiological abnormalities are frequently found, including widening of the sylvian fissure due to re- duced opercularization (Fig. 12.2.6a), ventriculomegaly and striatal lesions which develop after the encephalopathic crisis (Fig. 12.2.6b), and leukoencephalopathy which is mostly periventricular but may also affect subcortical U fibres (Fig. 12.2.6c). Diagnosis Glutaric aciduria type I should be suspected in patients with macro- cephaly and an extrapyramidal movement disorder starting in in- fancy or childhood. The diagnostic process can be guided by further clinical features. Diagnosis is ascertained by GC/MS detection of glutaric and 3-hydroxyglutaric acids in organic acid analysis (urine, plasma, or cerebrospinal fluid) or by MS/MS detection of elevated glutarylcarnitine (dried blood spots, plasma, urine). Confirmation by enzymatic analysis in

leucocytes or fibroblasts or demonstration of two pathogenic mutations is advisable. A subgroup of patients presents with a mild biochemical phenotype (low excretors) and thus may be missed if diagnostic work-up does not include quan- titative methods (e.g. stable isotope dilution assay). Examination of the carnitine status usually reveals low total and free carnitine. Prenatal diagnosis is possible by determining glutaric acid with stable isotope dilution techniques and by enzymatic and/or mo- lecular testing. Treatment and outcome The principal aim of treatment is the prevention of encephalopathic crises and neurological deterioration. Strict adherence to the emer- gency protocol is especially important (see ‘Emergency treatment’). During the vulnerable period (i.e. until age 6 years), lysine-restricted dietary treatment (including lysine-free amino acid supplements) and carnitine supplementation is recommended. Riboflavin is widely used but is of doubtful benefit. Treatment efficacy of movement dis- orders is still poor. Baclofen, benzodiazepines, and trihexyphenidyl are widely used to treat dystonia. Botulinum toxin and intrathecal baclofen are valid additions. If patients are diagnosed while they are asymptomatic, treatment prevents brain degeneration in the majority of patients. Notably, best outcome results (≥90% remain healthy) were achieved for patients following international guide- line recommendations including a low-lysine diet and carnitine supplementation for maintenance treatment and immediate emer- gency treatment during any putatively threatening episode such as intercurrent infectious diseases. Deviation from this combined metabolic treatment increases the risk of motor disability such as in untreated patients. More than 90% of untreated patients are thought to develop neurological disabilities. Life expectancy is markedly re- duced following the manifestation of dystonia. Hyperornithinaemia (ornithine-5-aminotransferase): gyrate atrophy Autosomal recessive hyperornithinaemia associated with gyrate atrophy of the choroid and retina is caused by deficiency of ornithine-5-aminotransferase. Clinical presentation Progressive myopia is the first clinical symptom, followed by pro- gressive chorioretinal degeneration with night blindness starting late in the first decade. Loss of peripheral vision proceeds to tunnel vision and eventually blindness by the third or fourth decade. The principal abnormality is an atrophy of choroid and retina. Cataracts also develop but optic discs, cornea, and iris remain normal. A few patients develop mild proximal muscle weakness. Diagnosis Severe isolated hyperornithinaemia is usually discovered by amino acid analysis with plasma ornithine concentrations ranging from 400 to 1400 µmol/litre (normal <200 µmol/litre). The disease can be confirmed enzymatically by decreased activity of ornithine- 5-aminotransferase in fibroblasts as well as by identification of disease-causing mutations in the OAT gene, but the diagnosis is usu- ally evident. Treatment and prognosis Permanent reduction of plasma ornithine into the normal range (<200 µmol/litre) is required to stop or at least slow chorioretinal degeneration. Only a small proportion of patients respond to pharmacological doses of the ornithine-5-aminotransferase co- factor pyridoxine. Additional therapeutic approaches to reduce or- nithine are the augmentation of renal losses by administration of

 pharmacological doses of l-lysine or α-aminoisobutyric acid (which is not metabolized), or substrate deprivation by dietary arginine re- striction. Combined treatment appears to be necessary since no single therapy is unequivocally effective. Multiple carboxylase deficiency The water-soluble vitamin biotin is a cofactor of four important carboxylases that take part in gluconeogenesis, fatty acid synthesis, and the catabolism of several amino acids and odd-chain fatty acids (Fig. 12.2.7). The covalent binding of biotin with apocarboxylases forming the active holocarboxylases is catalysed by biotin holocarboxylase synthetase. In the biotin cycle, biotin is recycled after proteolytic degradation of holocarboxylases (Fig. 12.2.8). Biotin in small

amounts is widely present in natural foods. Within the body, biotin bound to holocarboxylases represents the major source. In dietary and in endogenous sources, biotin is protein-bound as (a) (b) (c) Fig. 12.2.6 (a) Axial T2-weighted MRI spin echo image of a 2½-year-old boy with glutaryl-CoA dehydrogenase deficiency. He was diagnosed neonatally, never suffered an encephalopathic crisis, and developed no major neurological deficit. Extension of sylvian fissures which was mild during early infancy had slowly regressed. He did not develop characteristic frontotemporal atrophy and showed a normal myelination. (b) Axial T2-weighted spin echo image of a 15-month-old boy with glutaryl-CoA dehydrogenase deficiency 2 weeks after acute encephalopathic crisis. In addition to extension of sylvian fissures, hyperintensity of putamen, caudate, and pallidum are obvious. (c) T2-weighted axial and coronal MRIs of a 66-year-old man with glutaryl-CoA dehydrogenase deficiency demonstrating confluent white matter changes, wide temporopolar and insular cerebrospinal fluid spaces, and cortical atrophy, but normal signal of basal ganglia. The previously healthy man presented from the age of 50 with slowly progressive neurological disease, including seizures, dementia, and speech problems. Aggressive behaviour as well as acoustic and visual hallucinations led to the suggestion of psychiatric disease. (c) Reproduced with permission from Külkens et al. 2005.

12.2 Protein-dependent inborn errors of metabolism 1965 biocytin or short biotinyl peptides. Liberation of biotin from its pro- tein conjugates is catalysed by biotinidase. Biotinidase deficiency Aetiology/pathophysiology Biotinidase regenerates biotin from endogenous sources and liber- ates protein-bound biotin, which derives from natural foodstuffs and the holocarboxylases. Free biotin is recycled and used for the reformation of holocarboxylases by the action of holocarboxylase synthetase through the biotin cycle (Fig. 12.2.8). The primary bio- chemical defect in most patients with late-onset multiple carb- oxylase deficiency was shown in 1983 to be a profound deficiency of serum biotinidase encoded by the BTD gene (3p25). The metabolic abnormalities caused by deficiency of the respective biotin-dependent carboxylases are as follows: lactic acidosis due to pyruvate carb- oxylase deficiency; hyperammonaemia and accumulation of me- tabolites of alternative propionate metabolism (see also ‘Propionic aciduria’) due to propionyl-CoA carboxylase deficiency; and eleva- tion of 3-hydroxyisovaleric acid, 3-methylcrotonylglycine, and 3-hydroxyisovalerylcarnitine (see also ‘3-Methylcrotonylglycinuria’) due to methylcrotonyl-CoA carboxylase deficiency. Clinical presentation Onset of first symptoms is variable, ranging from 1 week to 10 years of age. The mean age of presentation is between 3 and 6 months. Provision of biotin by the mother in utero delays symptoms and bio- chemical abnormalities in newborns with biotinidase deficiency. The most frequent symptoms are lethargy, hypotonia, seizures, and ataxia often in combination with stridor, episodes of hyperventi- lation, and apnoea. If undiagnosed and untreated, progression of the disease can be potentially fatal (Fig. 12.2.9). In older children, progressive neurological disease is often the leading presentation, including ataxia, (myoclonic) epileptic encephalopathy, and devel- opmental delay. Neurosensory hearing loss and ophthalmic dis- orders, such as optic atrophy, develop in most untreated patients. Skin rash and/or alopecia are hallmarks of the disease. Diagnosis Urinary organic acid analysis is useful for differentiating isolated carboxylase deficiencies from the multiple carboxylase deficien- cies that occur in biotinidase deficiency and holocarboxylase syn- thase deficiency. However, metabolic abnormalities are highly variable and are absent at birth when the patient is not biotin de- pleted. Whereas accumulation of abnormal organic acid metabol- ites may show characteristic metabolites of propionic aciduria (see also ‘Propionic aciduria’), pyruvate carboxylase deficiency, and 3-methylcrotonylglycinuria (see also ‘3-Methylcrotonylglycinuria’) (Fig. 12.2.2), only 3-

hydroxyisovaleric acid may be found ele- vated, especially in the early stages of the disease. Notably, 3-hydroxyisovaleric acid is also the most commonly elevated urinary metabolite in holocarboxylase synthetase deficiency, 3-methylcrotonyl-CoA carboxylase deficiency, and acquired biotin deficiency. Biotin is decreased in plasma and urine and biocytin is increased in urine. Diagnosis is made by analysis of serum biotinidase activity. Enzymatic activity less than 10% is classified as profound biotinidase deficiency and activity between 10 and 30% as partial biotinidase deficiency. Furthermore, few patients with decreased affinity of biotinidase for biocytin (Km variants) exist. They may show erro- neously high residual activity on in vitro testing. Prenatal diagnosis is feasible by measurement of biotinidase activity but may not be necessary because of effective treatment and favourable clinical out- come. Newborn screening for biotinidase deficiency is now estab- lished in many countries. Treatment and outcome Biotinidase deficiency is effectively treated by daily oral adminis- tration of pharmacological doses of biotin. Restriction of protein is not necessary. Administration of 5 to 10 mg of oral biotin per day promptly reverses or prevents all clinical and biochemical abnor- malities. Biotin treatment has to be maintained lifelong and has no Succinate Oxaloacetate 3* 4* 2* 1* Citrate Acetyl-CoA Pyruvate Methylmalonyl-CoA Propionyl-CoA Malonyl-CoA Fatty acids 3-Methylglutaconyl-CoA 3-Methylcrotonyl-CoA Leucine Glucose Valine Isoleucine Threonine Odd-chain fatty acids Fig. 12.2.7 Important carboxylases in amino acid metabolism. Asterisked enzymes are 1, 3-methylcrotonyl coenzyme A carboxylase; 2, propionyl coenzyme A carboxylase; 3, pyruvate carboxylase; and 4, acetyl coenzyme A carboxylase. Biotin Apocarboxylases (PCC, MCC, PC, ACC) Holocarboxylases Biocytin, short biotinylpeptides Holocarboxylase synthetase Proteolysis Biotinidase Protein-bound biotin (diet) Lysine Lysylpeptides Biotinidase Fig. 12.2.8 The biotin cycle. Biotin is cleaved from biocytin (biotinyl- lysine) or small peptides by biotinidase. Activation of the apoenzymes resulting in functioning carboxylases (3-methylcrotonyl-CoA, propionyl-CoA, acetyl-CoA, and pyruvate carboxylases) is carried out by holocarboxylase synthetase. ACC, acetyl-CoA carboxylase; MCC, 3-methylcrotonyl-CoA carboxylase; PC, pyruvate carboxylase; PCC, propionyl-CoA carboxylase. Source data from Zschocke J, Hoffmann GF (2011). Vademecum metabolicum. Manual of metabolic paediatrics, 3rd edition. Schattauer, Stuttgart.

 side effects. Most patients with biotinidase deficiency known today were detected by newborn screening. Patients with Km variants have an increased risk of becoming biotin deficient and thus must also be treated with biotin. After early detection and consequent treatment, the outcome of biotinidase deficiency is excellent. Holocarboxylase synthetase deficiency Aetiology/pathophysiology Holocarboxylase synthetase deficiency is a rare, autosomal reces- sive disease. Several disease-causing mutations have been identified in the HLCS gene (21q22.1). Only about 40 patients have been re- ported. Residual activity has been observed in all affected individuals suggesting that complete enzyme deficiency may be lethal in utero. The coenzyme biotin is attached to the various apocarboxylases by the enzyme holocarboxylase synthetase. The carboxyl group of biotin is linked by an amide bond to an ε-amino group of a specific lysine residue of the apoenzymes. Deficiency of holocarboxylase synthetase leads to failure of synthesis of all carboxylases, causing biochemical and clinical abnormalities attributable to the dysfunc- tion of each respective carboxylase. Clinical presentation Although holocarboxylase synthetase deficiency was initially termed early-onset multiple carboxylase deficiency, the age of onset of symptoms varies widely, from a few hours after birth to 6 years of age. Nevertheless, about one-half of patients present acutely in the first days of life with severe metabolic decompensation, leth- argy, hypotonia, vomiting, seizures, and hypothermia. Patients with early-

onset presentation exhibit severe metabolic acidosis with lactic acidaemia, ketosis, and hyperammonaemia in analogy to biotinidase deficiency (see also ‘Biotinidase deficiency’). The meta- bolic derangement may quickly progress from lethargy to coma and early death. Skin rashes, feeding difficulties, vomiting, muscular hypotonia and hypertonia, seizures, and the odour of male cat urine are other symptoms. Ataxia, tremor, hyporeflexia, or hyperreflexia are neurological manifestations of the disease. Diagnosis Biochemical abnormalities of holocarboxylase synthetase deficiency are analogous to those described for patients with biotinidase de- ficiency (see also ‘Biotinidase deficiency’). Importantly, plasma biotin is normal in holocarboxylase synthetase deficiency as is serum biotinidase activity. Holocarboxylase synthetase is charac- terized by deficient activities of carboxylases in peripheral blood leucocytes prior to biotin administration; the activities of these enzymes increase to near-normal or normal values after biotin treatment. Indirect confirmation of holocarboxylase synthetase de- ficiency and differentiation from biotinidase deficiency is feasible by measurement of activities of the mitochondrial carboxylases in skin fibroblasts showing residual activity of 0 to 30% when incu- bated in low-biotin (10−10 mol/litre) medium and an increase, some- times to normal values in biotin-supplemented medium (10−6–10−5 mol/litre). In biotinidase deficiency, the activity of mitochondrial carboxylases in fibroblasts is normalized even under low-biotin conditions. Definite diagnosis of holocarboxylase synthetase de- ficiency is not routinely available. Prenatal diagnosis is feasible ei- ther by demonstrating decreased carboxylase activities in cultured amniocytes or by demonstration of elevated 3-hydroxyisovaleric acid and/or methylcitrate in amniotic fluid. Prenatal molecular diagnosis can be offered in families with previously known disease- causing mutations in the HLCS gene. Treatment and outcome Holocarboxylase synthetase deficiency can be treated effectively with pharmacological doses of biotin. The required dose of biotin is dependent on the severity of the enzyme defect and has to be (a) (b) Fig. 12.2.9 Two T2-weighted images of a 7-month-old boy with biotinidase deficiency. (a) The image displays absence of normal myelin signal in the cerebellum as well as hyperintense signal in both pyramidal tracts. (b) The image shows absence of normal myelin signal, cerebral atrophy, and symmetrical hyperintense lesions of both thalami. Courtesy of Dr. T. Bast, Department of Pediatric Neurology, University of Heidelberg, Heidelberg, Germany.

 assessed individually. In most patients, 10 to 20 mg of biotin per day is sufficient, but some need higher doses, that is, 40 to 100 mg/ day. In spite of apparently complete recovery, biochemical and clin- ical abnormalities persist in some patients owing to the high Km for biotin in the defective holocarboxylase synthetase. In case of acute decompensation, treatment according to the emergency protocol in organic acidurias (see ‘Emergency treatment’) has to start without delay. It is unclear whether prenatal treatment with biotin is bene- ficial. The prognosis is good if treatment is initiated immediately, except for affected individuals with Km variants. Other organic acidurias d-2-Hydroxyglutaric aciduria type I and II Aetiology/pathophysiology d-2-Hydroxyglutaric aciduria is an aetiologically heterogeneous cerebral organic acid disorder first described by Chalmers and col- leagues in 1980. d-2-Hydroxyglutaric aciduria type I is caused by deficiency of d-2-hydroxyglutarate dehydrogenase, a mitochon- drial enzyme converting d-2-hydroxyglutarate to 2-oxoglutarate. Pathogenic mutations have been identified in the D2HGDH gene on 2p25.3. Recently, autosomal dominant germline mutations of the IDH2 gene located on 15q26.1 causing increased conversion of 2-oxoglutaric acid to d-2-HG using NADPH by isocitrate dehydro- genase 2 were identified as molecular cause for d-2-hydroxyglutaric aciduria type II. Neurodegeneration in d-2-hydroxyglutaric aciduria is explained by

activation of N-methyl-d-aspartate recep- tors and inhibition of respiratory chain complexes (cytochrome c oxidase, ATP synthase) by d-2-hydroxyglutaric acid. Clinical presentation Patients with d-2-hydroxyglutaric aciduria exhibit variable pheno- types. They have been divided into two subgroups based on clin- ical, neuroradiological, and molecular findings. Patients with d-2-hydroxyglutaric aciduria type I are moderately affected and usu- ally follow a mild clinical course with variable symptoms including learning difficulties, muscular hypotonia, and macrocephaly. Rarely individuals remain almost asymptomatic, that is, presenting only with well-treatable oligoepilepsy or even with no neurological symp- toms. The clinical presentation of patients with d-2-hydroxyglutaric aciduria type II is usually more severe than in patients with type I. Patients present with encephalopathy of early infantile onset, demonstrating a combination of catastrophic epilepsy, muscular hypotonia, cerebral visual failure, and severe psychomotor retard- ation. Facial dysmorphism, macrocephaly, and cardiomyopathy may also be present. Neuroimaging findings in these patients show ventriculomegaly, enlarged subarachnoid spaces, subdural effusions, subependymal cysts, and delayed cerebral maturation (Fig. 12.2.10). Recently, agenesis of the corpus callosum, bilateral involvement of the striatum, and cerebral artery infarctions were added to the spectrum. Diagnosis The biochemical hallmark of this disease is the accumulation of d-2-hydroxyglutaric acid in all body fluids. Type I patients excrete lower concentrations of d-2-hydroxyglutarate than type II patients. Demonstration of elevated levels of 2-hydroxyglutaric acid must be followed up by differential quantitation of the two isomers l- and d- 2-hydroxyglutaric acid. 2-Oxoglutaric acid and other tricarboxylic acid cycle intermediates are usually also elevated in urine. γ- Aminobutyric acid (GABA) and total protein concentrations may be elevated in cerebrospinal fluid. d-2-Hydroxyglutaric acid can also be elevated in multiple acyl-CoA dehydrogenase deficiency, succinic semialdehyde dehydrogenase deficiency, and following bacterial overgrowth of the urine specimen. However, due to characteristic additional parameters these differential diagnoses are usually easy to exclude. Prenatal diagnosis can be performed either through genetic testing or by metabolite determination in amniotic fluid by stable isotope dilution GC/MS assay. Treatment and outcome No specific therapy exists to date. Long-term care of patients should entail regular evaluation of cardiomyopathy and the progression of neurological disease. The prognosis of d-2-hydroxyglutaric aciduria is extremely variable. Severely affected children may die in infancy, while moderately affected patients have a better prognosis up to an unimpaired life. l-2-Hydroxyglutaric aciduria Aetiology/pathophysiology l-2-Hydroxyglutaric aciduria is a rare, autosomal recessively in- herited cerebral disorder. The disease is caused by deficiency of the flavin adenine dinucleotide-dependent mitochondrial enzyme l-2- hydroxyglutarate dehydrogenase converting l-2-hydroxyglutarate to 2-oxoglutarate. This enzyme is encoded by the L2HGDH gene on 14q22.1. The pathophysiology of this disease is unknown. Clinical presentation l-2-Hydroxyglutaric aciduria was first described by Duran and coworkers in 1980. It is characterized by progressive loss of Fig. 12.2.10 Axial T1-weighted spin echo image of a 2-month-old girl with d-2-hydroxyglutaric aciduria type II. The lateral ventricles are highly dilated, occipital more than frontal, the cerebral maturation is delayed. Reproduced with permission from Kölker et al. 2002.

 myelinated arcuate fibres and a spongiform encephalopathy. In the first 2 years of life, mental and psychomotor development may be normal or slightly delayed. Febrile seizures, nonspecific develop- mental delay, and muscular hypotonia are the presenting symptoms. Progressive ataxia, variable extrapyramidal and pyramidal signs, epilepsy, and progressive learning difficulties eventually develop. By adolescence, patients are usually bedridden and severely mentally disabled (IQ 40–50). Two patients have developed cerebral

tumours. Two patients presented at birth with depressed vital signs, severe epileptic encephalopathy, and an abnormal CT scan showing cere- bellar involvement; however, the disease course is usually slowly progressive without metabolic decompensation. The neuroimaging findings in l-2-hydroxyglutaric aciduria are unique and mostly uniform comprising a progressive loss of arcuate fibres combined with progressive cerebellar atrophy and signal changes in globus pallidus and the dentate nuclei (Fig. 12.2.11). Diagnosis l-2-hydroxyglutaric aciduria results in a rather homogeneous clin- ical picture and characteristic abnormalities on neuroimaging. Clinical or neuroradiological suspicion should prompt GC/MS analysis of urinary organic acids followed by differentiation of l- 2- and d-2-stereoisomers. Lysine is often increased both in plasma and cerebrospinal fluid. Prenatal diagnosis is based on the analysis of l-2-hydroxyglutaric acid in amniotic fluid samples or molecular analysis. Treatment and outcome No specific therapy exists to date. Epilepsy can generally be controlled by antiepileptic medications. Patients with l-2-hydroxyglutaric aciduria can be expected to reach adult life. The oldest known pa- tients are close to 40 years of age, bedridden, and severely disabled. Combined d-2- and l-2-hydroxyglutaric aciduria Aetiology/pathophysiology The molecular basis of combined d-2- and l-2-hydroxyglutaric aciduria has recently been unravelled. The disease is caused by homozygous or compound heterozygous mutations in the SLC25A1 gene (gene locus 22q11.21) resulting in a dysfunction of the mito- chondrial citrate carrier and thus in impaired mitochondrial citrate efflux. Clinical presentation In a similar manner to patients with d-2-hydroxyglutaric aciduria type II, patients with the combined d-2- and l-2-hydroxyglutaric aciduria usually present with a severe clinical manifestation in the newborn period. This includes epileptic encephalopathy, mus- cular hypotonia, respiratory insufficiency, extrapyramidal move- ment disorders, cortical visual failure, microcephaly, and severe developmental delay. Agenesis of corpus callosum and optic nerve hypoplasia may be present. Otherwise, brain MRI may be similar to patients with d-2-hydroxyglutaric aciduria type II. Diagnosis Clinical and neuroradiological suspicion should prompt GC/MS analysis of urinary organic acids and differentiation of l-2- and d-2- stereoisomers. The diagnosis can be confirmed by molecular genetic analysis. Treatment and outcome Treatment is symptomatic. Patients with a severe onset and intract- able epileptic seizures have a poor prognosis: eight of twelve recently reported cases died between 1 month and 5 years of age. N-Acetylaspartic aciduria (Canavan’s disease) Aetiology/pathophysiology N-Acetylaspartic aciduria is a devastating infantile neuro degenerative disorder. In 1931, a child with spongy matter (a) (b) Fig. 12.2.11 (a) Axial T2-weighted spin echo image of an 8½-year- old boy with l-2-hydroxyglutaric aciduria. Subcortical white matter is severely deficient with much less involvement of the internal capsule and the periventricular white matter. Please note signal changes in the putamen. (b) Axial T2-weighted spin echo image of an 8½-year-old boy with l-2-hydroxyglutaric aciduria. Please note hyperintense lesions in both dentate nuclei. (a) Reproduced with permission from Kölker et al. 2002.

 degeneration was described by Canavan. In 1986, it was rec- ognized that N-acetylaspartic aciduria was caused by deficient aspartoacylase in a child with a similar clinical presentation. In 1988, aspartoacylase deficiency was definitely linked to Canavan’s disease. Canavan’s disease is found in all ethnic populations but re- veals a much higher frequency in Ashkenazi Jews (1 in 5000 to 1 in 14 000 newborns). The frequent missense mutation p.E285A in the aspartoacylase gene, localized on 17p13-pter, accounts for more than 80% of alleles in Ashkenazi Jews and for 60% of alleles in pa- tients of non-Jewish origin. In healthy individuals, high concen- trations of N-acetylaspartic acid (8 mmol/g tissue) are exclusively

found in brain tissue. Aspartoacylase is localized in oligodendrocytes catalysing the deacetylation of N-acetylaspartic acid to produce acetate, a substrate for the synthesis of myelin lipids including cholesterol. It has been proposed that N-acetyl-l-aspartate may function as a molecular water pump in myelinated neurons, transporting water against its gradient from neurons to oligodendrocytes. Thus aspartoacylase de- ficiency may cause both accumulation of metabolic water causing spongiform white matter changes, and deficiency of acetyl groups needed for cholesterol biosynthesis, causing demyelination; both are characteristic of Canavan's disease.
Clinical presentation Canavan's disease mostly manifests at age 2 to 4 months with delayed development. Hypotonia with prominent head lag, epilepsy, loss of previously acquired skills, as well as progressive megalencephaly are regularly found. Seizures and optic nerve atrophy develop during the second year of life. As the disease progresses, affected children develop pyramidal signs, and finally decerebration. Neuroimaging reveals characteristic symmetrical leukodystrophic changes with loss of arcuate fibres; histology demonstrates spongi- form degeneration, in particular of the cortex and subcortical white matter (Fig. 12.2.12) with less involvement in the cerebellum and brainstem. In infancy, changes may be subtle and misinterpreted as delayed myelination or periventricular leukomalacia. Variant Canavan's disease has been described and partially been proven to be caused by the same metabolic defect.
Diagnosis Muscular hypotonia, head lag, and progressive megalencephaly in infancy are the classic clinical triad of Canavan's disease. The identification of the accumulating N-acetylaspartic acid by GC/MS analysis and confirmation of the suspected diagnosis by en- zyme analysis (skin fibroblasts) or mutation analysis has obviated the need for brain biopsy for the diagnosis of Canavan's disease. Prenatal diagnosis is possible by quantitative GC/MS analysis of N-acetylaspartic acid in amniotic fluid or by mutation analysis. In contrast, enzyme activity is unsuitable for reliable prenatal diagnosis.
Treatment and outcome Management is symptomatic (antiepileptics) and palliative. Special care is needed to prevent recurrent aspirations. Many patients need tube or gastrostomy feeding. Dietary therapies have not been shown to be beneficial and are potentially harmful. A promising protocol for gene therapy was published in 2002 involving the transfer of human aspartoacylase cDNA intraventricularly; however, the clinical changes were not pronounced and were relatively tran- sient. The prognosis of infantile Canavan's disease is rapidly fatal, whereas milder disease has been described with survival beyond the teenage years.
Ethylmalonic encephalopathy
Aetiology/pathophysiology Ethylmalonic encephalopathy is a devastating, infantile, autosomal recessive neurometabolic disorder affecting the brain, gastrointes- tinal tract, and peripheral veins. The underlying metabolic defect was identified in a β-lactamase-like, iron-coordinating metalloprotein of the mitochondrial matrix encoded by the ETHE1 gene. Only re- cently, it was elucidated using Ethe1-deficient mice that the deficient protein is a mitochondrial sulphur dioxygenase which is involved in the catabolism of sulphide in ethylmalonic encephalopathy. As a consequence, toxic levels of sulphide and thiosulphide are found causing powerful inhibition of cytochrome c oxidase, short-chain fatty acid oxidation, and exerting vasoactive and vasotoxic effects. This explains deficient mitochondrial energy metabolism, the ab- normal accumulation short-chain organic acids, acylglycines and acylcarnitines, as well as microangiopathy.
Clinical presentation Ethylmalonic encephalopathy is characterized biochemically by ethylmalonic aciduria and methylsuccinic aciduria, lactic acidaemia, and clinically by severe psychomotor retardation, acrocyanosis, pe- techiae, and chronic diarrhoea. Newborns present with muscular hypotonia followed by pro- gressive neurological deterioration, especially pyramidal dys- function, learning difficulties, orthostatic acrocyanosis with distal Fig. 12.2.12 Axial fast spin echo image of a 6½-year-old girl with aspartoacylase deficiency. Note the marked discrepancy between the

severely affected subcortical white matter and the relatively spared central white matter, at least frontally. Reproduced with permission from Kölker et al. 2002.

SECTION 12 Metabolic disorders 1970 swelling, chronic diarrhoea, and recurrent petechiae (Fig. 12.2.13). Haematuria is often present. MRI scans show signal changes in cere- bellar white matter and lesions in the basal ganglia, the latter ap- pearing suddenly. Diagnosis The biochemical hallmark is increased urinary excretion of ethylmalonic and methylsuccinic acids associated with abnormal excretion of C4- and C5- (n-butyryl-, isobutyryl-, isovaleryl-, and 2- methylbutyryl-) acylglycines and acylcarnitines as well as intermit- tent lactic acidosis. Since primary mitochondrial disorders are an important differential diagnosis, enzymatic analyses of respiratory chain enzymes in muscle biopsy specimen have been performed in some patients revealing secondary cytochrome c oxidase deficiency. Mutation analysis of the ETHE1 gene provides the definitive diag- nosis including prenatal diagnosis. Increased ethylmalonate in urine is also found in multiple- and short-chain acyl-CoA dehydrogenase deficiencies, primary respiratory chain deficiencies, and Jamaican vomiting sickness. Treatment and outcome No effective treatment is known. The prognosis is poor and ethylmalonic encephalopathy is usually lethal in early childhood. Defects of phenylalanine and tyrosine metabolism Phenylketonuria The hyperphenylalaninaemias are a group of disorders characterized by defective hydroxylation of phenylalanine to tyrosine resulting in plasma phenylalanine values above the normal fasting range of 40 to 80 μmol/litre. PKU was first identified by the Norwegian Asbjørn Følling in 1934 in several severely disabled individuals. Følling de- termined the urinary excretion of phenylpyruvic acid which led to the previously used term ‘phenylpyruvic oligophrenia’. In 1947, Jervis localized the metabolic error as an inability to oxidize phenyl- alanine to tyrosine. In 1953, Bickel and colleagues demonstrated that a phenylalanine-restricted diet was beneficial, and was thus the first successful treatment of an inborn error of metabolism and one which led the way to early diagnosis by newborn screening and treat- ment. The worldwide overall incidence of PKU is approximately 1 in 10 000, with a large national and ethnic variability. Aetiology/pathophysiology PKU is an autosomal recessive disorder caused by a severe defect of phenylalanine hydroxylase which converts phenylalanine into tyrosine (Fig. 12.2.14). Tetrahydrobiopterin is required as a cofactor and thus hyperphenylalaninaemia may also be caused by inappro- priate generation of tetrahydrobiopterin. Through mechanisms still not completely understood, the excess phenylalanine is toxic to the central nervous system. Phenylalanine competes with the transport of large neutral amino acids through the blood–brain barrier using the sodium-independent system L and induces cerebral depletion of these amino acids and, subsequently, reduced synthesis of pro- teins and neurotransmitters (large neutral amino acid hypothesis of PKU). In addition, phenylalanine competes with glycine and glu- tamate at their binding sites in N-methyl-d-aspartate and α-amino- 3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors, thus impairing glutamate signalling and, subsequently, synapse forma- tion and cognitive function. Furthermore, phenylalanine inhibits the rate-limiting enzyme of cholesterol biosynthesis, 3-hydroxy-3- methylglutaryl-CoA reductase, and switches forebrain oligodendro-cytes to a nonmyelinating state. Clinical presentation Untreated, PKU almost invariably causes severe learning difficulties. Newborns with PKU are asymptomatic since fetal phenylalanine is metabolized by the mother’s liver. On regular intake of natural pro- tein, phenylalanine levels quickly rise. Constitutional abnormal- ities (80–100% of patients) such as hypopigmentation of the skin and hair (fair) and iris (blue) develop rapidly because synthesis of Fig. 12.2.13 Patient with ethylmalonic encephalopathy. Phenylalanine Phenyl pyruvate BH4 BH2 4* 3* BH4 BH2 3* 5* 6* BH4 BH2 3* 5OH Tryptophan 5-Hydroxytryptamine 7* Tryptophan 1* 2* Tyrosine

Dihydroxyphenylalanine Dopamine Noradrenaline Adrenaline Phenylacetate Phenylacetylglutamine p-Hydroxyphenyl pyruvate Homogentisate Maleylacetoacetate Fumarylacetoacetate Fumarate + acetoacetate Fig. 12.2.14 The metabolism of phenylalanine and tyrosine and the role of tetrahydrobiopterin. The asterisked enzymes are 1, phenylalanine hydroxylase; 2, tyrosine hydroxylase; 3, dihydrobiopterin reductase; 4, tyrosine aminotransferase; 5, homogentisic acid oxidase; 6, fumaryl acetoacetate hydrolyase; and 7, tryptophan hydroxylase.

12.2 Protein-dependent inborn errors of metabolism 1971 melanin from tyrosine is impaired. Elevated phenylacetate excretion gives the urine an odour reminiscent of mice and can cause an ec- zematous skin eruption. Delayed psychomotor development may become evident from the third month of life. It has been estimated that one IQ point is lost for each week of delay in diagnosis and treatment. Cognitive function is severely compromised in untreated children (IQ <40). Microcephaly and movement disorders are frequent, as are hyperexcitability as well as hypoexcitability and seizures; some pa- tients develop autistic behaviour or aggressiveness. Most patients with untreated PKU cannot be managed by their families and re- quire institutional care. Diagnosis In many countries, newborns are screened for increased phenyl- alanine levels in dried blood spots during the first days of life (new- born screening). Originally, newborn screening of phenylalanine was performed by a bacterial inhibition assay (Guthrie test). The implementation of MS/MS techniques has, however, significantly improved the early identification of affected individuals by new- born screening. Confirmation of a positive screening result is per- formed by quantitative amino acid analysis and mutation analysis. Liver biopsy and subsequent determination of the hepatic activity of phenylalanine hydroxylase is not indicated. Defects in the metabolism of tetrahydrobiopterin (BH4), the cofactor of phenylalanine hydroxylase, have to be differentiated from classic PKU by urinary pterin analysis and enzyme ana- lysis of dihydropteridine reductase in dried blood spots. In many centres, an oral dose of 20 mg/kg BH4 is administered. To per- form this test accurately, the initial plasma phenylalanine con- centration should be greater than 400 µmol/litre (6.7 mg/dl). Following BH4 administration, plasma samples are collected for phenylalanine and tyrosine analysis at defined time points as well as urine samples for pterin analysis. Notably, BH4 normalizes phenylalanine concentrations in patients with a primary disorder of BH4 (see ‘Defects of biopterin metabolism’). This test has the advantage that it may also identify BH4-responsive individuals with PKU. Treatment and outcome The most important therapeutic intervention in PKU is phenylalanine-restricted dietary treatment. Regular phenylalanine determinations are used for monitoring. Unfortunately, recom- mendations for PKU treatment differ considerably with regard to cut-off levels to begin dietary treatment, age-dependent recom- mendations for phenylalanine concentrations, frequency of clinical examinations, and phenylalanine monitoring (Table 12.2.4). There is no rational explanation for this. The concept of dietary treatment has four components: (1) com- plete avoidance of food containing abundant phenylalanine (e.g. meat, fish, milk, etc.); (2) calculated intake of natural food with a low phenylalanine/protein ratio (e.g. vegetables and fruit) and low- protein products; (3) adequate intake of energy substrates; and (4) calculated intake of phenylalanine-free amino acid supplements, vitamins, minerals, and trace elements. During catabolic states phenylalanine concentrations may increase, which is counteracted by dietary reduction of phenylalanine intake. In contrast, during growth spurts in childhood and adolescence the requirement for phenylalanine may transiently increase. When a very strict diet is begun early and is well maintained, affected children can expect normal development. Regression of IQ and development of neurological symptoms when diets were stopped in later childhood have led to continuation of dietary treat-

ment into the teenage years and adulthood. Patients generally have not suffered when the diet was stopped at or after 15 or 16 years of age. However, there is no follow-up with respect to IQ change of a substantial number who have been off diet for 20 years or more. Most recommendations and centres have adopted a philosophy of ‘diet for life’. However, the urgent need for more detailed informa- tion remains. Maternal PKU In 1980, Lenke and Levy reported the severe effects of maternal hyperphenylalaninaemia in the fetus (Table 12.2.5). The clinical features are similar to the fetal alcohol syndrome, and the severity of manifestations depends on the maternal phenylalanine level. In addition to learning difficulties and behavioural disorders, the ad- verse effects include malformations such as cardiac defects (usu- ally conotruncal), microcephaly, dysmorphic features, intrauterine growth retardation, neuronal migration disorders, and agenesis of the corpus callosum. Treatment and outcome Because of active placental transport, the ratio of fetal to maternal phenylalanine plasma levels is 1.5 to 1.7. Maternal phenylalanine values should be between 120 and 360 µmol/litre, which requires a strict diet and very careful monitoring twice weekly. Microcephaly and congenital heart disease in the offspring of mothers returning to diet at the seventh or eighth week emphasizes the need for pre- conception diet and training. Lowering maternal plasma phenyl- alanine concentrations during pregnancy to a level between 120 and 360 µmol/litre results in a favourable outcome in virtually all cases. Defects of biopterin metabolism In the hydroxylation of phenylalanine, the cofactor BH4 is con- sumed and must be regenerated. BH4 is formed in a three-step pathway from guanosine triphosphate. The first and rate-limiting reaction is catalysed by guanosine triphosphate cyclohydrolase and leads to the production of dihydroneopterin triphosphate. A defi- ciency of BH4 does not only impair phenylalanine hydroxylase in the liver, resulting in hyperphenylalaninaemia, but also tyrosine hydroxylase, tryptophan hydroxylase, as well as nitric oxide syn- thases (Fig. 12.2.15). Tyrosine hydroxylation is needed for the synthesis of noradrenaline and dopamine, and tryptophan hydrox- ylation for the production of serotonin. BH4 is therefore crucial to the production of neurotransmitters. The supply of this coenzyme is impaired in five recessively inherited enzyme defects. Most produce hyperphenylalaninaemia, which may not be marked. All but pterin- 4α-carbinolamine dehydratase deficiency cause progressive neuro- logical disease. In less than 1% of newborns a raised phenylalanine value detected by newborn screening is due to a defect of biopterin metabolism. The enzyme defects lead to reduced levels of BH4 within the cen- tral nervous system without significantly affecting phenylalanine metabolism in the liver (normal plasma phenylalanine). However,

 Table 12.2.4 Guidelines for treatment and monitoring of PKU: international comparison Germany 1999 UK 1993 USA 2014 Indication for dietary treatment

> “ 600 µmol/litre 400 µmol/litre 360 µmol/litre Start of dietary treatment As soon as possible ≤ day 20 of life ≤ day 7 of life Recommendations for phenylalanine levels and frequency of phenylalanine monitoring Germany 1999 UK 1993 USA 2014 Age Germany 1999 UK 1993 USA 2014 40–240 µmol/litre (0.7–4 mg/dl) 120–360 µmol/litre (2–6 mg/dl) 120–360 µmol/litre (2–6 mg/dl) 0 2–4×/month 4×/month 4×/month 1 1–2×/month 2×/month 2 3 4 5 2×/month School age: 6 120–480 µmol/litre (2–8 mg/dl) 7 8 9 40–900 µmol/litre (0.7–15 mg/dl) 10 1×/month 1×/month 11 Adolescence and adulthood Adolescence and adulthood

> 12 120–700 µmol/litre (2–11.7 mg/dl) 120–360 µmol/litre (2–6 mg/dl) 13 1×/month 14 15 40–1200 µmol/litre (0.7–20 mg/dl) 16 4–6 ×/year 17 120–360 µmol/litre (2–6 mg/dl) 18+ Recommendations for clinical monitoring Germany 1999 Germany 2004 UK 1993 USA 2000 Dietary training Amino acid profile Nutrition No details Anthropometric data Blood count Growth Health status Minerals, trace elements General health status Neurological status Calcium and phosphorus metabolism Psychological development Enzymes: AP, GOT, GPT Vitamins and serum lipid status

12.2 Protein-dependent inborn errors of metabolism 1973 turnover of serotonin and the catecholamines in the brain can still become severely compromised. Fasting plasma phenylalanine levels are always normal in the dominantly inherited guanosine triphos- phate cyclohydrolase deficiency (Segawa's disease) and the auto- somal recessive sepiapterin reductase deficiency. Clinical presentation Except for pterin-4α-carbinolamine dehydratase (PCBD1) defi- ciency, autosomal recessive defects of biopterin metabolism re- sult in severe encephalopathies. Common but variable symptoms are progressive learning difficulties, dystonia, chorea, oculogyric crises, convulsions, tremor, spasticity, microcephaly, growth re- tardation, swallowing difficulties, and depressive and aggressive be- haviour. Diurnal variation is often present. Onset of symptoms is in the first months of life with hypotonia; sometimes affected new- borns have difficulties in postnatal adaptation. Signs of autonomic dysfunction include hypersalivation, temperature instability, leth- argy, hypersomnolence, and episodes of sweating and pallor. Less frequently reported are 'bulbar' signs (drooling, dysarthria, ab- normal tongue movements), 'ataxia', probably not cerebellar ataxia or sensory ataxia but dystonic gait, and Gower's sign. PCBD1 is a bifunctional protein that acts as an enzyme in the regeneration of BH4 and as a dimerization cofactor of the transcription factors HNF1A and HNF1B, which are important in liver, pancreas, and kidney development and function. Mutations in PCBD1 have re- cently been reported to cause early-onset nonautoimmune diabetes mellitus highlighting that PCBD1 activity is required for early pan- creatic development. In addition, adult patients with PCBD1 defi- ciency and hypomagnesaemia due to renal magnesium wasting have been identified demonstrating that PCBD1 also plays an important role in the kidney, in particular in the distal convoluted tubule. In later infancy and childhood, defects in the metabolism of the biogenic monoamines may be suspected in patients with (fluc- tuating) extrapyramidal disorders, in particular parkinsonism dystonia or more general 'athetoid cerebral palsy', and vegetative disturbances. A severe epileptic encephalopathy and progressive learning difficulties may be present. Diagnosis Every infant with hyperphenylalaninaemia detected in a population newborn screening programme or in the course of other diagnos- tics later in life must be carefully investigated for possible defects of biopterin metabolism (see also 'Phenylketonuria'). Differential diag- nosis requires the analysis of pterins in urine or from Guthrie cards as well as the determination of enzyme activity of dihydropteridine reductase in dried blood spots. If the initial plasma phenylalanine concentration is above 400 µmol/litre (6.7 mg/dl), oral loading with BH4 (20 mg/kg) will result in normalization of phenylalanine values within 4 to 8 h. Urinary biopterin and neopterin values are low in the guanosine triphosphate cyclohydrolase deficiency, whereas 6-pyruvoyltetrahydrobiopterin synthase deficiency has high neopterin values and low biopterin values. In patients with dihydropteridine reductase deficiency, neopterin is normal or slightly elevated and biopterin very high. After the biochemical diagnosis, all defects should be ascertained

enzymatically and, if available, by mutation analysis. Following a diagnosis of a defect of biopterin metabolism, a lumbar puncture becomes necessary for analysis of the neurotrans- mitter metabolites 5-hydroxyindoleacetic acid and homovanillic acid as well as neopterin, biopterin, and 5-methyltetrahydrofolic acid. This allows differentiation between severe and mild forms of BH4 deficiencies and sets the indication for treatment with the neurotransmitter precursors l-dopa and 5-hydroxytryptophan. In patients with suggestive encephalopathies and normal phenyl- alanine values, analysis of neurotransmitters in cerebrospinal fluid is the only way of diagnosis. Treatment and outcome Blood phenylalanine concentrations should be more rigidly con- trolled than in classic PKU patients. In patients with guanosine triphos- phate cyclohydrolase deficiency and 6-pyruvoyl-tetrahydrobiopterin deficiency, administration of BH4 appears to be the most efficient therapy in controlling blood phenylalanine levels. Patients with dihydropteridine reductase deficiency need a low-phenylalanine diet as in PKU. Deficiency of neurotransmitters requires treatment with the neurotransmitter precursors l-dopa (3–15 mg/kg per day) and 5-hydroxytryptophan (2–9 mg/kg per day) in combination with carbidopa (10 or 25% of l-dopa). Lumbar punctures must be repeated regularly to adjust doses. In patients revealing l-dopa-induced peak- dose dyskinesia slow-release forms of drugs can be used, and reaching the upper therapeutic limits of l-dopa may be an indication for the use of monoamine oxidase and/or catechol-O-methyltransferase Table 12.2.5 Incidences (%) of abnormalities in the offspring of mothers affected with classical PKU Congenital abnormalities Maternal PKU Unaffected mothers Mental disability 92 5 Microcephaly 73 4.8 Intrauterine retardation 40 9.6 Congenital heart defects 12 0.8 Source data from Lenke R, Levy HL (1980). Maternal PKU and hyperphenylalaninemia: an international study of treated and untreated pregnancies. N Engl J Med, 303, 1202–8. GTP BH4 PTPS Neopterin SR BH2 Biopterin PAH, TYH, TPH, NOS PCD DHPR GTPCH Fig. 12.2.15 Biopterin metabolism. BH4 is synthesized and regenerated by five enzymes. BH4 is consumed as a cofactor in the hydroxylation of tyrosine and tryptophan as well as phenylalanine (see also PKU) and nitric oxide synthase (NOS). BH2, dihydrobiopterin. Relevant enzyme defects: DHPR, dihydropteridine reductase; GTPCH, GTP cyclohydrolase; PCD, pterin carbinolamine dehydratase; PTPS, 6-pyruvoyl- tetrahydropterin synthase; SR, sepiapterin reductase. Source data from Zschocke J, Hoffmann GF (2011). Vademecum metabolicum. Manual of metabolic paediatrics, 3rd edition. Schattauer, Stuttgart.

 inhibitors. Patients with dihydropteridine reductase deficiency, in addition, need administration of folinic acid to restore normal cere- brospinal fluid folate concentrations. Normal long-term psychomotor development can be achieved but outcome strongly depends on the age when the diagnosis is made and how rigidly therapy is followed, especially in early life. Dominantly inherited guanosine triphosphate cyclohydrolase deficiency Clinical presentation Dominantly inherited guanosine triphosphate cyclohydrolase deficiency, often called Segawa’s disease, is an eminently treat- able condition. Early recognition is therefore of crucial import- ance. Presentation in children usually occurs within the first decade of life with a mean age of onset of symptoms being about 7 years (range 16 months to 13 years). The first symptom is usu- ally postural dystonia of one leg with progression to all limbs followed by action dystonia and hand tremor within the next 10 to 15 years, during which time cognition remains intact. Occasionally, in older children, the first signs may start in the arms with torticollis or writer’s cramp (focal dystonia). The dys- tonia is frequently asymmetrical and accompanied by reduced facial expression or slowing of fine finger movements. Diurnal fluctuation is often present, with symptoms improving after night- time sleep or bed rest. The variation in presenting symptoms is large. Penetrance is reduced and many carriers of a mutant gene are asymptomatic. Diagnosis In

classic cases with prominent dystonia of the lower limbs, marked diurnal variation, as well as worsening of the symptoms after ex- ercise, the clinical diagnosis of the deficiency is easily made, in particular in the presence of dramatic and sustained response to l-dopa. However, the diagnosis can be a real challenge in atypical cases, in which it can be ascertained by determining BH4, and de- creased levels of neopterin and homovanillic acid in cerebrospinal fluid. Confirmation of the diagnosis can be achieved by enzyme ana- lysis in cultured skin fibroblasts or by mutation analysis. Treatment and outcome Treatment relies on l-dopa in combination with 10 to 25% carbidopa. Amounts administered have varied between 3 and 10 mg/kg per day divided into one to four doses with the effectiveness of treatment being monitored by the clinical outcome. The long-term prognosis is usually excellent. Tyrosinaemias The steps in tyrosine metabolism starting with the rate-limiting step—the conversion to p-hydroxyphenylpyruvic acid by tyrosine aminotransferase—are outlined in Fig. 12.2.14. Intermediates of this tyrosine metabolism are used for production of catecholamines, dopamine, and the principal pigment of hair and skin, melanin. Tyrosinaemia type I (fumarylacetoacetase deficiency) Clinical presentation Tyrosinaemia type I is also known as hepatorenal tyrosinosis. About one-third of patients present acutely in the early weeks of life with failure to thrive, vomiting, hepatomegaly, fever, oedema, and epistaxis; by the end of the first year of life 90% have developed symptoms. The disease can progress rapidly and death from hepatic failure often occurs in infancy. A milder more chronic presentation is compatible with survival for several years with chronic liver disease, a renal tubular Fanconi’s syndrome with hypophosphataemic rickets, and episodic abdom- inal pain and neuropathy suggestive of acute porphyria. The most serious complication is hepatocellular carcinoma which develops in early childhood in one-third of untreated patients. Diagnosis Raised plasma tyrosine (often together with methio- nine), succinylacetone, and 5-aminolaevulinic acid excretion as well as renal Fanconi’s syndrome are the biochemical markers of tyrosinaemia type I caused by a deficiency of fumarylacetoacetate hydrolyase, the last enzyme in the pathway of tyrosine degradation (Fig. 12.2.14). Serum α-fetoprotein is usually strikingly elevated. Succinylacetone, formed from fumarylacetoacetate, is the most spe- cific diagnostic metabolite. Plasma tyrosine values may be normal, resulting in insufficient specificity of this parameter for newborn screening. Fumarylacetoacetate hydrolyase can be assayed in lymphocytes or fibroblasts. It is nonspecifically depressed in the liver in a var- iety of liver diseases. The measurement of succinylacetone in amni- otic fluid and activity of fumarylacetoacetate hydrolyase in cultured amniocytes or chorionic villus samples forms the basis of prenatal diagnosis, if informative mutations are not available. Treatment and outcome Restricted intake of tyrosine and phenyl- alanine may reduce the excretion of succinylacetone and produce regression of the Fanconi tubular defects, but does not cure the liver disease. The risk of hepatocellular carcinoma remains and early liver transplantation was the treatment of choice until nitisinone (2-(2-nitro-4-trifluoromethylbenzoyl)1–3-cyclohexanedione) was introduced by Lindstedt and colleagues in 1991. Nitisinone almost completely blocks 4-hydroxyphenylpyruvate dioxygenase thus turning tyrosinaemia type I into tyrosinaemia type III and redu- cing the production of toxic metabolites. Treatment with nitisinone should start as soon as the diagnosis is made with a dose of 1 mg/ kg per day. In most patients there is a rapid improvement in liver and renal function; succinylacetone should disappear from the urine within 1 week of treatment. Patients need to be treated with a diet low in phenylalanine and tyrosine at the same time as introducing nitisinone. Plasma levels of tyrosine should be kept between 250 and 500 μmol/litre. The long-term results of nitisinone treatment are encouraging with greatly reduced incidence of liver damage and hepatic car- cinoma. Liver transplantation remains the treatment of choice for a few patients who do not respond to nitisinone and if there is any suggestion of

malignant change. Tyrosinaemia type II (tyrosine aminotransferase deficiency) Clinical presentation Corneal erosions and dendritic ulcers may form within a few months of birth with later scarring, nystagmus, and glaucoma. Skin lesions may begin after the eye lesions with blis- tering, painful palms and soles, and hyperkeratosis. Tongue changes have been described. Learning difficulties are an inconstant feature in about 50% of patients, but language defects may be more common with possible impaired coordination and self-mutilation.

12.2 Protein-dependent inborn errors of metabolism 1975 Diagnosis Tyrosine aminotransferase, which is deficient, cata- lyses the formation of p-hydroxyphenylpyruvic acid (Fig. 12.2.14). Plasma tyrosine values reach 20 times normal (normal 40–100 µmol/ litre) in younger patients and 10 times normal in others. There is increased excretion of tyrosine, N-acetyltyrosine, tyramine, and of phenolic acids; there is no Fanconi's syndrome and no increase in succinylacetone. The clinical features and amino acid analyses are usually sufficient for diagnosis, which may be confirmed either by measuring the en- zyme activity in liver or by molecular genetic studies. Treatment and outcome A low-tyrosine, low-phenylalanine diet has been used to produce rapid improvement of skin and eye mani- festations. Corneal transplants can be valuable. The neurological symptoms appear to improve less. The degree of dietary control needed to sustain clinical improvement is uncertain. Plasma tyro- sine concentrations less than 500 µmol/litre are considered desirable. Tyrosinaemia type III (4-hydroxyphenylpyruvate
dioxygenase deficiency) 4-Hydroxyphenylpyruvate dioxygenase deficiency (Fig. 12.2.14) ap- pears to be very rare and possibly without clinical pathology, that is, a nondisease. It may be associated with learning difficulties and pos- sibly other neurological complications. The biochemical findings are similar to those in tyrosinaemia type II, but the plasma values of tyrosine are usually less than 1200 µmol/litre. Enzyme and mo- lecular genetic studies can prove the diagnosis. Most patients are treated with a low-tyrosine, low-phenylalanine diet. Alkaptonuria Clinical presentation In 1902, alkaptonuria was the first disorder to be recognized as an inborn error of metabolism by Garrod. It is caused by a deficiency of homogentisate dioxygenase resulting in the accumulation of homogentisic acid and its oxidized derivative benzoquinone acetic acid. The latter can then be polymerized to form a dark pigment which is deposited in connective tissue. The disorder is extremely rare in most populations but occurs with greatly increased fre- quency in the Dominican Republic and in Slovakia. Presentation in infancy occurs only if discoloration of the urine is noticed. It is usu- ally normal when passed but darkens on standing (more rapidly at alkaline pH) to deep brown or almost black. Back pain begins in the second and third decade with increasing stiffness due to interverte- bral disc degeneration. Involvement of the hips, knees, and shoul- ders follows. Greyish discoloration of cartilage is seen in the pinna, and pigment is deposited in the sclera. Abnormal pigmentation is seen in the heart valves and joint cartilages, and pigmented stones are common in the prostate. Valvular calcification is prominent, es- pecially in the coronary arteries. Recent studies of the natural course of alkaptonuria indicate that it is associated with premature heart disease and premature death with long-standing impairment of quality of life. Pigment deposition with involvement of the fibrolipid components of atherosclerotic plaques cause calcific stenosis of the aortic valve. In 58 patients studied by Phornphutkul and colleagues (2002), life-table analysis showed that joint replacement occurred at a mean age of 55 years, renal stones at 64 years, and cardiac valve involvement at 54 years; coronary calcification occurred at a mean age of 59 years. Diagnosis Homogentisic acid can be demonstrated by urinary organic acid analysis. Enzymatic as well as molecular confirmation is possible. Plasma tyrosine concentrations are normal. Treatment and outcome So far no treatment has been shown to prevent the long-term

com- plications. The prognosis for the joints is poor. By the fifth decade, the lumbar spine is likely to be rigid and other joints will be ser- iously affected. Patients often require large amounts of analgesic and risk the complications of long-term consumption of nonsteroidal anti-inflammatory agents, which may exacerbate incipient coronary heart disease. Homogentisic acid can be decreased by a low-protein diet. It is very probable that specifically designed low-phenylalanine and low- tyrosine diets would lower the production still further. Nitisinone, the triketone inhibitor of 4-hydroxyphenylpyruvate dioxygenase introduced by Lindstedt in 1991, greatly reduces overproduction of homogentisic acid in alkaptonuria. Early studies from Gahl's group at the National Institutes of Health, United States of America, showed that in adults of both sexes with alkaptonuria, an oral dose of 1.05 mg twice daily reduced urinary homogentisic acid excretion from a mean of 4 g to 0.2 g per day. More than 220 patients with her- editary tyrosinaemia type I have received the drug at daily doses of 0.5 to 2.0 mg/kg body weight and even at these doses it is generally well tolerated, apart from mild blood cytopenias. In alkaptonuria nitisinone, as predicted, may elevate the plasma tyrosine concentra- tions (in the early trial from c.70 to 760 µmol/litre) and there is thus a theoretical risk of lens opacities, which can be avoided by careful slit-lamp monitoring, plasma amino acid measurement, and dietary adjustment. In alkaptonuria, the outcome of nitisinone treatment will take many years to evaluate fully, but comprehensive therapeutic study is justified by the clear relationship between overproduction of a single metabolite and life-shortening tissue manifestations with disabling joint disease. Neurotransmitter diseases and related disorders Monogenic defects of neurotransmission have become recog- nized as a cause of early-onset, severe, progressive, and often treat- able encephalopathies. The diagnosis is based on the quantitative determination of the neurotransmitters or their metabolites in cerebrospinal fluid, that is, glycine, serine, and GABA, the acidic me- tabolites of the biogenic monoamines, and individual pterin species (Box 12.2.6). Determinations of metabolites in blood or urine are neither sensitive nor specific. In contrast to inborn errors in cata- bolic pathways, neurotransmitter defects are determined by the Box 12.2.6 Cerebrospinal fluid: investigation for neurotransmitter disorders • Cells, protein, immunoglobulin classes, and glucose (plus plasma glu- cose and evaluation of blood–brain barrier) • Lactate and pyruvate • Amino acids (plus plasma obtained simultaneously) • Biogenic monoamine metabolites • Individual pterin species • 5-Methyltetrahydrofolate

 interplay of biosynthesis, degradation, and receptor status. Even borderline abnormalities can be diagnostic and their recognition requires a strictly standardized sampling protocol and adequate age- related reference values. Disorders of monoamine metabolism Defects in the metabolism of the biogenic monoamines affect sero- tonin and/or catecholamine (dopamine and noradrenaline) metab- olism (Fig. 12.2.16). They present from infancy or childhood with (fluctuating) extrapyramidal disorders, in particular parkinsonian dystonia or more general 'athetoid cerebral palsy', and vegetative disturbances, most noticeably hypoglycaemia. A severe epileptic en- cephalopathy and progressive learning difficulties may be present. Tyrosine hydroxylase deficiency Tyrosine hydroxylase catalyses the hydroxylation of l-tyrosine to l-dopa, the rate-limiting step in the biosynthesis of the catechol- amines dopamine, noradrenaline, and adrenaline (Fig. 12.2.16). The iron-containing mixed function oxidase requires molecular oxygen and the cofactor BH4. Tyrosine hydroxylase is expressed only in catecholaminergic neurons and the adrenal medulla. Tyrosine hydroxylase deficiency has become incorporated into concepts and classifications of dystonias as the cause of reces- sive l-dopa-responsive dystonia, but can also present as l-dopa- nonresponsive dystonia or progressive early-

onset encephalopathy. Clinical presentation Clinical symptoms often develop between 3 and 7 months of age. Most patients show a substantial clin- ical improvement already on low doses of l-dopa together with the decarboxylase inhibitor carbidopa, although in contrast to l-dopa-responsive dystonia due to haploinsufficiency of guanosine triphosphate cyclohydrolase I, often neither the neurological status nor the catecholamine levels in cerebrospinal fluid can be com- pletely normalized in most patients. At the severe end of the spectrum, virtually no movements are observed, not even dystonic movements. Some patients are more severely affected and present with a progressive neurometabolic disorder from early infancy with a progressive infantile enceph- alopathy characterized by abnormal extrapyramidal movements and affecting several cerebral and possibly cerebellar systems. It is important to stress that such patients also show symptoms of sig- nificant catecholamine deficiency, such as hypoglycaemia and in- adequate stress responses. There is an obvious tendency to preterm birth with troublesome cardiorespiratory perinatal adaptation. Most infants with tyrosine hydroxylase deficiency develop sur- prisingly normally until an arrest of motor development with a characteristic combination of neurological symptoms later in infancy. Hypokinesia, marked truncal hypotonia, a mask face, oculogyric crises, myoclonic jerks, and an extrapyramidal tremor can progressively develop. The last three symptoms can be mis- taken as epileptic phenomena. Oculogyric crises are present but, as with the miosis, may go undiagnosed because of prominent ptosis. Contractures, failure to thrive, and immobilization may develop. It appears likely that life expectancy is significantly reduced; (dys- tonic) cerebral palsy is a likely descriptive (mis-)diagnosis. Some patients did not develop extrapyramidal symptoms in the first year of life, were able to walk independently, and followed a clin- ical course best summarized as spastic paraplegia. Their symptoms fully resolved following l-dopa supplementation, and they are now healthy adults living independently. 5-HIAA noradrenaline DβH adrenaline PNM 3-O-methyldopa vanillyllactic acid COMT DOPAC MAO COMT 3MT COMT COMT MAO HVA NM M VMA MHP G ALD MAO + aldd. MAO + alcd. COMT COMT COMT MAO tryptophan tyrosine 5-OH-tryptophan serotonin dopamine L -DOPA AADC + B6 TR + BH4 TH + BH4 AADC + B6 Fig. 12.2.16 Metabolism of biogenic monoamines. 5- HIAA, 5-hydroxyindolacetic acid; AADC, aromatic l-aminoacid decarboxylase; alcd., alcohol dehydrogenase; ALD, intermediate aldehyde (3-methoxy-4-hydroxyphenyl- hydroxyacetaldehyde); aldd., aldehyde dehydrogenase; BH4, tetrahydrobiopterin; COMT, catechol-ortho-methyltransferase; DbH, dopamine-b-hydroxylase; DOPAC, 3,4-dihydroxyphenylacetic acid; HVA, homovanillic acid; M, metanephrine; MAO, monoaminooxidase; MHPG, 3-methoxy-4-hydroxy-phenylglycol; MT, 3-methoxytyramine; NM, normetanephrine; PNM, phenylethanolamine-N-methyltransferase; TH, tyrosin hydroxylase; TR, tryptophan hydroxylase; VMA, vanillylmandelic acid; . . ., several steps involved.

 Diagnosis The diagnosis of tyrosine hydroxylase deficiency can only be made via cerebrospinal fluid analysis following a stand- ardized lumbar puncture protocol. A characteristic metabolite constellation is found: low concentrations of metabolites of dopa- minergic neurotransmission homovanillic acid and 3-methoxy- 4-hydroxyphenylethyleneglycol in the presence of normal concentrations of metabolites belonging to the serotonin neurotrans- mission system such as 5-hydroxyindoleacetic acid (Fig. 12.2.16). Urinary determinations of catecholamines and homovanillic acid turned out to be inconclusive in several affected individuals. Enzyme analysis is not possible in tyrosine hydroxylase deficiency because tissues expressing enzyme activity—brain and adrenal medulla—are difficult to obtain. Thus mutation analysis is the only way to confirm the diagnosis. Treatment and outcome Therapeutic interventions with l-dopa together with the decarboxylase inhibitor carbidopa and selegiline were

able to improve and/or even normalize the clinical picture in most patients but not all. Despite all therapeutic interventions, the disease course can be lethal. Treatment with l-dopa has to be started slowly and carefully, with doses as low as 0.5 mg/kg per day in two to six divided doses to avoid dyskinesias due to hypersensitivity and up-regulation of dopamine receptors in dopamine-deficient patients. In such patients, l-dopa can only be increased very slowly, sometimes over several years. Slow-release preparations may be useful to ensure constant l-dopa levels. In general, incremental steps of l-dopa/carbidopa should not be more than 1 mg/kg per day. Aromatic l-amino acid decarboxylase deficiency Aromatic l-amino acid decarboxylase deficiency is caused by autosomal recessively inherited mutations in the DDC gene. The enzyme is required for the synthesis of both serotonin and the catecholamines. Clinical presentation Clinical symptoms are indistinguishable from those of patients with tyrosine hydroxylase deficiency. The se- verity seems to fall into two groups. About half of the patients pre- sent with feeding difficulties, autonomic dysfunction, and hypotonia in the neonatal period. In the first few months of life dystonia or intermittent limb spasticity, axial and truncal hypotonia, extreme irritability, oculogyric crises, and psychomotor retardation be- come obvious. More mildly affected patients may initially develop unremarkably or only slightly delayed and present with motor re- tardation, hypokinesia, rigidity, and truncal hypotonia from early childhood. Diagnosis The enzyme deficiency leads to accumulation of 3- O-methyldopa, 5-hydroxytryptophan, and l-dopa together with low concentrations of the end products homovanillic acid and 5- hydroxyindoleacetic acid (Fig. 12.2.16). 3-O-Methyldopa is formed by methylation of l-dopa. Confirmation of the diagnosis is by en- zyme assay in plasma and finally by mutation analysis. Treatment and outcome Different approaches using dopamine agonists (pergolide, pramipexole, bromocriptine, and ropinirole) and/or nonselective monoamine oxidase inhibitors (tranyl cypromine, phenelzine) have been attempted. Response to treat- ment is variable but outcome appears to be better in more mildly affected and later-presenting patients. The overall prognosis is guarded. About half of the patients improve on individual treatment regimens and acquire different degrees of motor and psychosocial skills. Others do not show any improvements. Dopamine β-hydroxylase deficiency Clinical presentation Recessively inherited mutations in the dopa- mine β-hydroxylase gene lead to lowered levels of noradrenaline within central and autonomic noradrenergic neurons (Fig. 12.2.16). The disorder is characterized by sympathetic noradrenergic de- nervation and adrenomedullary failure. The central consequences appear minimal. Syndromes become obvious in adolescence with noradrenergic failure, severe orthostatic hypotension, and ptosis of the eyelids. During childhood fatigue, episodes of fainting, syn- copes, and exercise intolerance are generally present. Physical and cognitive function is normal. In males, autonomic neuropathy leads to retrograde ejaculation. Diagnosis Dopamine β-hydroxylase deficiency is classified as a pri- mary autonomic neuropathy. Conditions that lead to chronic failure of the autonomic nervous system are, therefore, the primary differ- ential diagnosis. Biochemically, dopamine β-hydroxylase deficiency is different from other conditions with orthostatic hypotension or autonomic dysfunction. Failure to produce noradrenaline and the consequent lack of end-product inhibition of tyrosine hydroxylase leads to a noradrenaline/dopamine ratio of less than 0.1, and such a finding is pathognomonic for the disease. An increase in blood pressure and correction of the orthostatic hypotension in response to dihydroxyphenylserine is also diagnostic. Some 3 to 4% of the normal adult population have near zero levels of the enzyme in plasma, therefore plasma enzyme determination alone cannot be used to confirm the diagnosis, it requires mutation analysis. Treatment and outcome Dopamine β-hydroxylase deficiency is treated with dihydroxyphenylserine. This compound is decarb- oxylated by l-amino acid decarboxylase to form noradrenaline. Administration of 250 to 500 mg twice daily

results in an increase in blood pressure and sustained relief of the orthostatic symptoms. Without appropriate treatment postural hypotension can lead to sig- nificant injuries or even death.
Disorders of pyridoxine metabolism In 1954, Hunt and colleagues described a patient with a seizure dis- order that was successfully treated solely by administration of pyri- doxine (vitamin B6) and coined the term ‘pyridoxine dependency’. It became good clinical practice to test for pyridoxine responsive- ness in every child with ‘difficult-to-treat’ seizures starting before 2 years of age. Later, a similar therapeutic response was described in the same clinical constellation for folinic acid. Finally, the enzymatic defect has been pinpointed to the α-aminoadipic semialdehyde de- hydrogenase located in the lysine degradation pathway in the brain, which results in the accumulation of the intermediate piperideine-6- carboxylate scavenging pyridoxal phosphate.
A similar pathogenic mechanism again leading to intractable seizures is responsible for pyridoxal deficiency in hyperprolinaemia type II and during treat- ment with the tuberculostatic drug isoniazid. Another monogenic defect in humans is directly located within the synthesis of pyridoxal 5′-phosphate: pyridox(am)ine 5′-phosphate oxidase deficiency resulting in pyridoxal phosphate-responsive seiz- ures (Fig. 12.2.17).

 Each newborn with severe neonatal/infantile epileptic enceph- alopathy should have a lumbar puncture and then immediately receive consecutive therapeutic trials with vitamin B6, pyridoxal 5′-phosphate, and folinic acid.
Aetiology/pathophysiology Pyridoxine-dependent epilepsy and folinic acid-responsive seiz- ures are treatable causes of neonatal epileptic encephalopathy. The genetic base of both conditions is autosomal recessive inher- itance of pathogenic mutations in the ALDH7A1 (antiquitin) gene causing deficiency of the enzyme α-aminoadipic semialdehyde dehydrogenase located in the pipecolic acid pathway, the major route of cerebral lysine oxidation. As a consequence of accumu- lating α-aminoadipic semialdehyde and the cyclic compound Δ1- piperideine 6-carboxylate, which spontaneously forms an adduct with pyridoxal phosphate via a Knoevenagel reaction, pyridoxal phosphate is inactivated resulting in cerebral depletion of pyridoxal phosphate. Pyridoxal phosphate-dependent enzymes such as glu- tamate dehydrogenase, GABA transaminase and aromatic l-amino acid dehydrogenase are inactivated by pyridoxal phosphate deple- tion causing significant disturbance in the metabolism of the neuro- transmitters dopamine, serotonin, glutamate and GABA and thus a severe epileptic encephalopathy. The conversion of pyridoxine, pyri- doxal, and pyridoxamine to pyridoxal phosphate however remains unaffected.
Clinical presentation Pyridoxine-dependent epilepsy can be heterogeneous in its presen- tation, and sometimes idiopathic epilepsies respond to treatment with high-dose pyridoxine. Classical patients with pyridoxine- dependent epilepsy present with an intractable seizure disorder within the first 2 days of life, and at the latest within 28 days. In some patients intrauterine convulsions are reported. There is no consistent electrographic pattern. Continuous and discontinuous backgrounds, suppression burst-like patterns, and hypsarrhythmia have all been observed. There are additional atypical presentations: (1) late onset, that is, later than 28 days; (2) neonatal onset but with an initial response to conventional anticonvulsant therapy; (3) neonatal onset with initially negative, but a later sustained positive response to pyridoxine. Folinic acid-sensitive seizures have been an enigmatic clinical and biochemical entity until it has been elucidated recently that they are alleic to pyridoxine-dependent epilepsy. Patients present with myo- clonic or clonic seizures, apnoea, and irritability within 5 days after birth. The electroencephalogram shows a discontinuous background pattern with multifocal spikes and sharp waves. Without specific treatment seizures will only be partially controlled. Psychomotor development will become severely impaired. It is

therefore recom- mended that all patients with ‘difficult-to-treat’ seizures starting be- fore 2 years should have a trial of pyridoxine and folinic acid (usually given orally in this circumstance). Diagnosis The diagnosis of pyridoxine-dependent epilepsy and folinic acid- responsive seizures should be suspected clinically in patients with neonatal epileptic encephalopathy or ‘difficult-to-treat’ seizures starting before 2 years of age who respond to pyridoxine and/ or folinic acid. Because it is a treatable condition, a high index of suspicion is warranted. Both pyridoxine and pyridoxal phosphate may cause apnoea and prolonged cerebral depression after the ini- tial dose, and resuscitation equipment and intensive care facilities should be available. The suspected diagnosis can be confirmed by measurement of α- aminoadipic semialdehyde in body fluids. Elevated CSF and plasma pipecolic acid is also used as a biomarker. Furthermore, CSF analysis may reveal a monoamine pattern similar to l-amino acid dehydro- genase deficiency, elevated glutamate, and decreased GABA concen- trations. Enzyme assay and mutation analysis of the ALDH7A1 gene is the most definitive proof of diagnosis. Treatment and outcome Treatment requires 5 to 30 mg/kg body weight per day of pyridoxine in one dose. Successful treatment with folinic acid can be achieved with 3 to 5 mg/kg body weight per day of folinic acid given in three doses. Doses need to be increased and adjusted to body weight during growth. Breakthrough seizures are an obvious criterion for increasing the dose. There is evidence that lower doses of pyridoxine and folinic acid, while controlling seizures, may still not prevent the development of cognitive impairment. High doses of pyridoxine carry the risk of developing skin photosensitivity as well of a per- ipheral sensory neuropathy. Doses up to 1 g/day can be regarded as safe in older children. Serial cognitive assessment is therefore recommended. If there is a positive family history of pyridoxine- dependent seizures, maternal treatment in utero is indicated. Since pyridoxine-dependent epilepsy and folinic acid-sensitive seizures appear to be genetically and biochemically identical, this new understanding requires a re-evaluation of optimal strategies such as the combined use of pyridoxine and folinic acid as well as of a low-lysine diet aiming to reduce the accumulation of α-aminoadipic semialdehyde and Δ1-piperideine 6-carboxylate. Hyperprolinaemia type II: l-Δ1-pyrrolines-5-carboxylate dehydrogenase deficiency Clinical presentation For a long time hyperprolinaemia type I, which has no clinically relevant phenotype, was not separ- ated from hyperprolinaemia type II. Also, as individuals with PK PNPO PNPO PK PK Membrane-associated phosphatases Cellular uptake PK Pyridoxamine Pyridoxine Pyridoxamine-P Pyridoxine-P Pyridoxal-P Pyridoxal Intracellular pyridoxal-phosphate Fig. 12.2.17 Pyridoxine metabolism. Pyridoxal phosphate (PALP; vitamin B6) is cofactor of transamination and decarboxylation reactions in various pathways including serotonin and dopamine biosynthesis. It is synthesized from dietary pyridoxal, pyridoxamine, and pyridoxine; enzymes involved include pyridoxal kinase (PK) and pyridox(am)ine
5-phosphate oxidase (PNPO). Source data from Zschocke J, Hoffmann GF (2011). Vademecum metabolicum. Manual of metabolic paediatrics, 3rd edition. Schattauer, Stuttgart.

12.2 Protein-dependent inborn errors of metabolism 1979 hyperprolinaemia type II often have no clinical manifestations, hyperprolinaemia was considered a nondisease. However, on inves- tigation of larger cohorts of affected individuals it became obvious that hyperprolinaemia type II can lead to epilepsy in more than 50% of patients. The epilepsy usually disappears in adulthood. Diagnosis Plasma concentrations of proline are highly elevated, ex- ceeding 1500 µmol/litre. Whereas proline is the only amino acid ele- vated in plasma and cerebrospinal fluid, glycine and hydroxyproline are also found elevated in urine as these three amino acids share a common renal tubular transport system. Hyperprolinaemia type II must be distinguished from hyperprolinaemia type I by demon-

stration of elevated levels of l-Δ1-pyrroline-5-carboxylate and/or by enzyme assay or molecular analysis. Treatment and outcome Unless a seizure disorder is present, no specific treatment is required. In a child with a seizure disorder, treatment with 5 to 30 mg/kg body weight per day of pyridoxine in one dose should be started. There are usually no adverse sequelae. Pyridoxal phosphate-responsive seizures: pyridox(am)ine
5′-phosphate oxidase deficiency Clinical presentation Pyridoxal phosphate responsive seizures due to pyridox(am)ine 5′-phosphate oxidase deficiency (Fig. 12.2.17) result in a most severe early neonatal encephalopathy with con- vulsions, myoclonus, rotatory eye movements, and sudden clonic contractions. Seizures are resistant to conventional anticonvulsant therapy. Many patients are born prematurely, and fetal distress is common, including ‘signs of asphyxia’ and low Apgar scores. Early (lactic) acidosis and hypoglycaemia may be observed. Thus pyri- doxal phosphate-responsive seizures must enter the differential diagnosis of hypoxic–ischaemic encephalopathy in prematurely born infants. Diagnosis The deficiency of pyridox(am)ine 5′-phosphate oxidase results in combined deficiencies of l-amino acid decarboxylase, threonine dehydratase, ornithine δ-aminotransferase, and the gly- cine cleavage enzyme with the concomitant biochemical findings. In addition, some patients display variable lactic acidaemia as well as a tendency to hypoglycaemia. However, no biochemical abnor- mality is 100% specific or sensitive, and a positive response to the drug remains the most reliable indication of pyridoxal phosphate- responsive seizures. The diagnosis is confirmed by mutation analysis. Treatment and outcome Pyridoxal 5′-phosphate given by nasogas- tric tube is dramatically effective in stopping seizures and improving the appearances of the electroencephalogram. Long-term treat- ment requires 30 to 60 mg/kg body weight per day of pyridoxal 5′- phosphate in four doses. Doses need to be increased and adjusted to body weight during growth. Patients probably require lifelong supplementation. Breakthrough seizures are an obvious criterion for increasing the dose. So far many questions remain open with regards to prognosis. Serial cognitive assessment is recommended. Defects of glycine and serine metabolism Nonketotic hyperglycinaemia Nonketotic hyperglycinaemia is the second most common dis- order of amino acid metabolism, second to PKU, with an overall worldwide frequency estimated at 1 in 60 000 births. It is caused by deficient activity of the glycine cleavage system which represents the main catabolic route of glycine (Fig. 12.2.18) and is present at high levels in liver, brain, and placenta. In brain, it keeps glycine levels very low, resulting in a typically low cerebrospinal fluid to plasma glycine ratio. Glycine is connected to multiple biochemical pathways. Most im- portant is the generation of methylenetetrahydrofolate. The glycine cleavage system is made up of four mitochondrial proteins, P, H, T, and L. The P protein is a decarboxylase requiring pyridoxal phos-phate. The heat-resistant H protein contains lipoic acid and carries the aminomethyl moiety. Both proteins are needed to generate CO2 from the carbon-1 of glycine. The T protein requires tetrahydrofolate and produces methylenetetrahydrofolate from carbon-2 of glycine. The fourth protein (L protein) is needed to transfer hydrogen from the lipoic acid moiety of the H protein to nicotinamide adenine diphosphate. Clinical presentation Symptoms of nonketotic hyperglycinaemia are exclusively neurological. Pregnancy and delivery are generally uneventful. Hiccupping in utero maybe recognized retrospectively. Lethargy, convulsions, anorexia, poor feeding, and vomiting pro- gress to coma and unresponsiveness 24 to 48 h after birth. Patients are severely hypotonic. Seizures with hiccupping and myoclonic spasms are prominent, and there is a burst suppression pattern on electroencephalography. Apnoea worsens during the third day of life, mostly requiring ventilation. The mortality rate at this stage is high, especially, if the children are not ventilated. After 2 to 3 weeks the patients improve slightly and no longer require intensive care. However, intellectual development does not occur in survivors, seiz- ures persist, and tendon reflexes are

increased. Microcephaly, poor head control, profound retardation, and a picture of spastic cerebral palsy develop. Up to 15% of patients with neonatal presentation have a better recovery after the neonatal period. They have a milder seizure dis- order, usually controlled by benzoate therapy or by a single anticon- vulsant. Most of these patients make some developmental progress, but they are still mentally disabled with developmental quotients varying between 10 and 60. Variant forms of nonketotic hyperglycinaemia present in later in- fancy or childhood with severe seizures, spastic paraparesis, clonus, and extensor plantar responses with modestly raised plasma and cerebrospinal fluid glycine values. Optic atrophy with cerebellar signs has also been described. The outcome is similar to that of pa- tients with the severe form of neonatal nonketotic hyperglycinaemia. Glycine Serine 3-Phosphoserine 3-Phosphohydroxypyruvate 3-Phosphoglycerate Methylene tetrahydrofolate Glycine + H2O Tetrahydrofolate CO2 + H2O Fig. 12.2.18 Reversible glycine cleavage to carbon dioxide and water is illustrated together with reversible interconversion of serine and glycine. These reactions also serve to generate one-carbon units. 3-phosphoglycerate (glycolysis) is the ultimate source.

 Diagnosis Confirmation of diagnosis by enzyme assay and/or mo- lecular analysis is highly advisable and should be pursued to facili- tate future prenatal diagnosis. Biochemically, nonketotic hyperglycinaemia is characterized by elevated glycine in plasma and in cerebrospinal fluid, with glycine being more elevated in cerebrospinal fluid than in plasma. Plasma glycine is elevated to values of 600 to 1200 µmol/litre but may vary throughout the day, and can be normal at times. Normal values for cerebrospinal fluid levels of glycine are around 4 to 5 µmol/litre, the normal cerebrospinal fluid to plasma ratio being less than 0.04. In nonketotic hyperglycinaemia patients, the cerebrospinal fluid to plasma glycine ratio is between 0.07 and 0.30. Great care must be taken to obtain simultaneous plasma and cere- brospinal fluid samples. Diagnostic pitfalls can arise due to postpran- dial blood sampling, blood contamination of the cerebrospinal fluid, profound liver dysfunction, and treatment with valproate. Urine or- ganic acids must be determined to exclude propionic aciduria and methylmalonic aciduria, as well as glyceric aciduria. Activity of the glycine cleavage system can only be reliably measured on liver biop- sies and in direct uncultured chorionic villi for prenatal diagnosis. So far the molecular structures of the P protein, the T protein, and the H protein have been elucidated, allowing molecular diagnosis of defects of these three proteins. Molecular studies have demonstrated a defect of the P protein in about 50 to 60% of patients and in the T protein in about 30% of patients; a few patients were found to have mutations in the GLDC gene leaving approximately 15% of patients with no mutations found after all three genes had been analysed. Treatment and outcome Therapeutic interventions are unsatisfac- tory. Some damage to the central nervous system may be prenatal. Withdrawal of artificial ventilation and intensive care support should be discussed with the parents of neonates in the apnoeic phase. Once breathing resumes, most patients survive for many years. Plasma glycine can be lowered by exchange transfusion or peri- toneal dialysis but without clinical improvement. Low-protein diets have only a limited effect on decreasing plasma glycine concentra- tions. Supplying one-carbon units in the form of methionine or N-formyltetrahydrofolate has not helped. The combination of so- dium benzoate to increase glycine excretion and diazepines, which compete for inhibitory glycine receptors in the central nervous system, has lowered plasma and cerebrospinal fluid levels of glycine and reduced seizures. Doses up to 600 to 750 mg/kg per day may be required to lower glycine sufficiently to values between 120 and 280 µmol/litre. At such high, potentially toxic doses monitoring of benzoate levels is advised, nevertheless gastric irritation is very fre- quent and gastric protection with H2-

antihistamine or proton pump inhibitors is preventively recommended. Most patients need gastric tube feeding or gastrostomy. Gastro- oesophageal reflux develops frequently, and many patients benefit from a Nissen fundoplication. Recurrent bronchitis is a major problem and bronchopneumonia is frequently the cause of death. For patients with mild nonketotic hyperglycinaemia, management of the hyperactivity can be a major challenge. 3-Phosphoglycerate dehydrogenase deficiency Serine is synthesized from the glycolytic intermediate 3-phosphoglycerate by 3-phosphoglycerate dehydrogenase yielding 3-phosphohydroxypyruvate (Fig. 12.2.18). Deficiency of this en- zyme leads to serine deficiency. Clinical presentation Patients with serine deficiency due to 3- phosphoglycerate dehydrogenase deficiency have congenital micro-cephaly. They develop severe psychomotor retardation with spastic tetraparesis and severe microcephaly. Seizures usually start in in- fancy as West's syndrome with hypsarrhythmia. The MRI scan is characterized by striking delayed or absent myelination, with subse- quent cortical and subcortical atrophy. Variable symptoms include cataract, hypogonadism, megaloblastic anaemia, and nystagmus. Diagnosis Serine deficiency in 3-phosphoglycerate dehydrogenase deficiency is most reliably diagnosed in cerebrospinal fluid with values less than 14 µmol/litre (normal cerebrospinal fluid serine 42–86 µmol/litre in infancy). Serine values in fasting plasma are also reduced (28–64 µmol/litre, controls 70–187 µmol/litre). However, nonfasting plasma levels can be normal. Treatment and outcome l-Serine should be administered orally until normalized (300–500 mg/kg per day). If seizures persist, gly- cine should be added up to 300 mg/kg per day. A very satisfactory outcome was achieved by antenatal treatment in one patient. Defects of γ-aminobutyric acid metabolism GABA is formed from glutamate in the brain by the cytosolic en-zyme glutamate decarboxylase, which requires pyridoxal phos- phate (Fig. 12.2.19). Glutamate can be regenerated from GABA by transamination with ketoglutarate (GABA transaminase), which is also pyridoxal phosphate dependent. The other product is succinic semialdehyde, which is dehydrogenated to succinate and enters the citric acid cycle. Deficiency of succinic semialdehyde dehydrogenase leads to formation and excretion of 4-hydroxybutyric acid. GABA transaminase deficiency Some patients with GABA transaminase deficiency presented with a fatal neonatal encephalopathy, characterized by seizures, hypotonia, hyperreflexia, a high-pitched cry (cat cry), and accelerated growth. The diagnosis can be suspected from significantly elevated levels of GABA (both free and total), as well as β-alanine and homocarnosine Fig. 12.2.19 Synthesis and catabolism of 4-aminobutyric acid (GABA). The enzymes recognized for known monogenic disorders in humans are shown in boxes: GAD, glutamic acid decarboxylase deficiency, GT, GABA transaminase deficiency, SSADH, succinic semialdehyde dehydrogenase deficiency. The cofactor vit. B6 (vitamin B6) is underlined. Source data from Zschocke J, Hoffmann GF (2011). Vademecum metabolicum. Manual of metabolic paediatrics, 3rd edition. Schattauer, Stuttgart.

 in cerebrospinal fluid. Plasma levels of these amino acids are also increased, but not as significantly. The diagnosis must be confirmed by enzyme assay and possibly mutation analysis, both of which can also be used for prenatal diagnosis. Unfortunately, there is no rational treatment available. Recently, in a family suffering from encephalomyopathic mitochondrial DNA depletion syndrome, the underlying molecular defect was detected in GABA transaminase encoded by 4-aminobutyrate aminotransferase (ABAT). Apparently, GABA transaminase connects GABA and nucleoside metabolism resulting in a neurometabolic disorder including mitochondrial DNA depletion. Succinic semialdehyde dehydrogenase deficiency
(4-hydroxybutyric aciduria) Clinical presentation The clinical presentation of succinic semialdehyde

dehydrogenase deficiency is highly heterogeneous, even within sibships. The cardinal manifestations are complex and rather nonspecific: hypotonia and delay of motor, mental, and fine motor skills and language. Ataxia and/or seizures occur in about half of the patients. Hyperkinesis and aggressive and autistic behaviour are additional features. MRI studies show bilateral globus pallidus abnormalities but again not constantly. Diagnosis Diagnosis is usually suspected by demonstrating in- creased levels of γ-hydroxybutyrate by organic acid analysis. It is confirmed by enzyme assay and preferentially additional mutation analysis. Treatment and outcome A common treatment for succinic semialdehyde dehydrogenase deficiency is the antiepileptic drug vigabatrin. The results have been encouraging in some patients, but of little value or even detrimental in others. Seizures respond to con- ventional anticonvulsants. A ketogenic diet shows promise. Succinic semialdehyde dehydrogenase deficiency is a slowly progressive en- cephalopathy in childhood; it eventually stabilizes in most patients. Defects of trans-sulphuration and remethylation The trans-sulphuration pathway transfers the sulphur of methio- nine to serine, thus generating cysteine (Fig. 12.2.20). Methionine adenosyltransferase, with widely distributed isoenzyme forms, produces S-adenosylmethionine, the donor in a variety of methy- lation reactions. S-Adenosylhomocysteine is cleaved to homocyst- eine, the sulphydryl compound that exists in reversible equilibrium with its disulphide homocystine. Half of the homocysteine formed goes through the trans-sulphuration pathway and the other half takes a methyl group from betaine (betaine methyltransferase) or 5-methyltetrahydrofolic acid (methionine synthase). The latter is a cobalamin-dependent enzyme which is functionally impaired in de- fects of vitamin B12 metabolism. In addition, methionine synthase reductase is necessary to keep the methionine synthase-bound co- balamin in a functional state. The remethylation of homocysteine is also impaired if the activity of the reductase that generates 5- methyltetrahydrofolate is inadequate. When accumulation of homo- cysteine and its products homocystine and the also formed mixed disulphide results from defects of homocysteine remethylation, plasma methionine concentrations are low. They are high when homocystine accumulates from impaired activity of cystathionine β-synthase. Classic homocystinuria: cystathionine β-synthase deficiency Clinical presentation Untreated classic homocystinuria is a slowly progressive, devastating multiorgan disorder. First symptoms in childhood are a rapidly pro- gressive myopia and lens dislocation. Lens dislocation usually oc- curs in preschool years, but later dislocation is well recognized in pyridoxine-responsive patients, and a few have not developed it even in adult life. Monocular and binocular blindness has been rela- tively frequent due to secondary glaucoma, staphyloma formation, buphthalmos, and retinal detachment. In the older child, skeletal abnormalities and learning difficul- ties become obvious. Genu valgum and pes cavus are usually the first signs of skeletal changes, which include osteoporosis and spon- taneous crush vertebral fractures. The common abnormalities seen in Marfan’s syndrome—high-arched palate, pectus excavatum or carinatum, genu valgum, pes cavus or planus, scoliosis—are all well recognized in homocystinuria. Arachnodactyly is less common and the fingers not infrequently (and elbows occasionally) show mild flexion contractures. Skeletal disproportion with a crown– pubis length less than the pubis–heel length is usual (Fig. 12.2.21). Learning difficulties affect two-thirds of patients. Patients responsive to pyridoxine (vitamin B6) (see following ‘Treatment and outcome’ subsection) have generally higher IQ values than nonresponsive patients. Seizures affect about one-fifth of patients and a few show extrapyramidal features, sometimes with severe involuntary move- ments. Psychiatric disturbances have also been described. Thromboembolism is a major cause of morbidity and the main cause of high premature mortality. Thromboses have been described in a wide variety of arteries and veins: cerebral, coronary, mesen- teric, renal, and peripheral. –CH3 –CH3 3* Tetrahydrofolate Glycine SO4 Cysteine

Cystathionine Serine Homocysteine *1 S-adenosyl homocysteine S-adenosyl methionine Methionine 4 Dimethyl glycine Methyltetrahydrofolate Methylene tetrahydrofolate Betaine –CH3 CH2 2*
Fig. 12.2.20 The trans-sulphuration pathway from methionine to cysteine is shown on the right and the remethylation of homocysteine on the left. Asterisked enzymes are: 1, cystathionine β-synthase; 2, methylene tetrahydrofolate reductase, 3, methionine synthase; and 4, betaine methyltransferase.

SECTION 12 Metabolic disorders 1982 Diagnosis Elevated plasma methionine values between 100 and 500 µmol/ litre (sometimes higher) are seen with plasma total homocysteine values of 50 to 200 µmol/litre. A mixed disulphide (half homocyst- eine, half cysteine) is always present at concentrations somewhat below those of homocystine. Diagnosis requires the determination of fasting quantitative plasma amino acids, as well as plasma total homocysteine. Total homocysteine measured by HPLC includes both homocysteine moieties of homocystine, the homocysteine moiety of the mixed disulphide, and the homocysteine bound to plasma proteins. The urine gives a positive nitroprusside test (it is also positive in cystinuria). However, this test can be falsely negative. Unfortunately, methionine elevation is unreliable in the early days of life, hampering the possibility of newborn screening. This can be reliably performed by screening for homocystinuria but still exclu- sively detects the more severely pyridoxine nonresponsive patients. Confirmation of the diagnosis can be performed by enzyme assay using cultured skin fibroblasts and/or mutation analysis, which al- lows prenatal diagnosis. Treatment and outcome Optimal outcome of treatment depends on its earliest possible intro- duction. Treatment is focused on correcting homocysteine levels; lifelong monitoring is essential. In about one-half of the patients, oral pyridoxine rapidly reduces methionine and homocysteine to near-normal values. The first treatment should be to try using doses from as low as 50 mg in infants to 1000 mg/day in older children or adults and reducing the dose if a response is achieved; 5 to 10 mg/ day of folic acid should also be given. Very large sustained doses (1000 mg/day or more) in adults can cause peripheral neuropathy. Those responding only partially or not at all to pyridoxine require a very low-protein diet supplemented with a methionine-free amino acid supplement, minerals, and vitamins. Biochemical control may only be achieved in older children and adults on natural protein in- takes of 5 to 10 g/day. Plasma cystine should be maintained in the normal range and supplementation should be considered. Both folic acid (5–10 mg/day) and betaine (up to 12 g/day) can further re- duce plasma homocysteine levels but may produce large elevations of plasma methionine. Low red-cell folate values occur and even megaloblastic anaemia. Low serum vitamin B12 values also occur and should be corrected. Treatment started early can prevent or re- duce the clinical sequels and lower the incidence of vascular events throughout life; many patients have a normal life expectancy. Methylene tetrahydrofolate reductase deficiency Clinical presentation Neurological features predominate with psychomotor retardation, seizures, abnormalities of gait, and psychiatric disturbance. The age of symptom development varies widely from infancy with a pro- gressive encephalopathy with apnoea, seizures, and microcephaly to adulthood with ataxia, motor abnormalities, psychiatric symptoms, subacute degeneration of spinal cord, and cerebrovascular events. Demyelination occurs and the changes may resemble the classic findings of subacute combined degeneration seen in vitamin B12 de- ficiency. The risk of vascular disease is high. Diagnosis Plasma methionine concentrations are below normal and plasma homocysteine concentrations are in the range 20 to 200 µmol/litre with an elevated excretion of 15 to 600 µmol/day. As homocysteine is easily missed on amino acid analysis, quantitative determination of total homocysteine by HPLC is the most important clue to diag- nosis. There is no megaloblastic anaemia. The enzyme can be as- sayed in

liver, leucocytes, lymphocytes, or fibroblasts also allowing prenatal diagnosis. Treatment and outcome Betaine in large doses (20–150 mg/kg per day) effectively lowers plasma homocysteine and raises plasma methionine. Other treat- ments tried alone or in combination include folinic acid, vitamin B12, pyridoxine, and methionine. Some have suggested a cocktail of all these treatments. It is difficult to be sure of clinical success. Deficiencies of methionine synthase reductase (cobalamin E defect) and methionine synthase (cobalamin G defect) Clinical and biochemical findings of methionine synthase reduc- tase (cobalamin E defect) and methionine synthase (cobalamin G defect) deficiencies are virtually identical. Characteristic find- ings are developmental delay and megaloblastic anaemia, but the onset may be in later in childhood with dementia and spasticity. Retinal degeneration, cardiac defects, and haemolysis have been described. Megaloblastic anaemia occurs in almost all patients. Biochemical findings include low plasma methionine and raised homocysteine as well as homocystine in plasma and urine. Methylmalonic acid should be measured in urine to exclude other cobalamin defects (see ‘Methylmalonic aciduria’). Methionine synthase can be as- sayed in liver or fibroblasts and antenatal diagnosis has been carried out on cultured amniocytes. Cells with the cobalamin E defect re- quire specific reducing conditions to demonstrate the deficient en- zyme activity. Molecular diagnosis is possible for both conditions. Treatment involves large doses of hydroxocobalamin with betaine Fig. 12.2.21 A patient with cystathionine synthase deficiency. Note the kyphosis and disproportionate short trunk.

 1983 and possibly folinic acid. Success of therapy and outcome is variable and often unfavourable. Other defects of sulphur amino acid metabolism Among several additional defects known, cystathioninuria due to cystathionase deficiency is probably clinically harmless. Cystathionine in excess of 1 g/day may be excreted at clearance values close to the glomerular filtration rate. Methionine adenosyltransferase deficiency causes raised plasma methionine levels (up to 1200 µmol/litre; normal 15–30 µmol/litre) and appears to be harmless in most patients. The enzyme defect is partial. Severe deficiency of methionine adenosyltransferase I/III may be associated with demyelination and neurological features. In such patients, treatment with S-adenosylmethionine (400 mg of the toluene sulphonate, twice daily) is an option. Glycine N-methyltransferase deficiency is very rare and was dem- onstrated in children with mild liver disease. Biochemical findings included elevated plasma methionine and S-adenosylmethionine levels. Similarly rare appear to be patients affected with S-adenosylhomocysteine hydrolase. Pathology and clinical findings are significant in liver, muscle, and the nervous system. Biochemical findings are complex, with elevated plasma methionine, S-adenosylhomocysteine, and S-adenosylmethionine levels. Total homocysteine and cystathionine may also be slightly elevated. FURTHER READING Ando T, et al. (1971). Propionic acidemia in patients with ketotic hyperglycinemia. J Pediatr, 78, 827–32. Besse A, et al. (2015) The GABA transaminase, ABAT, is essential for mitochondrial nucleoside metabolism. Cell Metab, 21, 417–27. Bickel H, Gerrard J, Hickmans E (1953). Influence of phenylalanine on PKU. Lancet, 2, 812–13. Baumgartner MR, et al. (2014). Proposed guidelines for the diag- nosis and management of methylmalonic and propionic acidemia. Orphanet J Rare Dis, 9, 130. Blau N, et al. (eds) (2014). Physician’s guide to the diagnosis, treatment, and follow-up of inherited metabolic disease, 2nd edition. Springer, Heidelberg. Brown GK, et al. (1984). Malonyl coenzyme A decarboxylase defi- ciency. J Inherit Metab Dis, 7, 21–6. Bursell MK, et al. (2011). Adenosine kinase deficiency disrupts the methionine cycle and causes hypermethioninemia, encephalopathy, and abnormal liver function. Am J Hum Genet, 78, 507–15. Canavan MM (1931). Schilder’s encephalitis perioxalis

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