Cerebral Organic Acid Disorders and Other Disorders of Lysine Catabolism

Stefan Kölker and Georg F. Hoffmann

Contents

J.-M. Saudubray et al. (eds.), *Inborn Metabolic Diseases*, https://doi.org/10.1007/978-3-662-63123-2_22

Catabolism of Lysine, Hydroxylysine, and Tryptophan

Species-, organ- and organelle-specific differences in the enzymes involved in the catabolism of lysine, hydroxylysine and tryptophan are not yet completely unravelled, and this synopsis is therefore partially hypothetical as far as human metabolism is concerned. Lysine, hydroxylysine, and tryptophan are thought to be degraded within the mitochondrium, initially via separate pathways, which converge into a common pathway at the point of 2-aminoadipic-6-semialdehyde (hydroxylysine catabolism and pipecolic acid pathway of lysine catabolism) and at the point of 2-oxoadipic acid (tryptophan catabolism; ◘ Fig. 22.1). The major route of lysine catabolism in most tissues is via the saccharopine pathway starting with the bifunctional enzyme 2-aminoadipic-6-semialdehyde synthase (enzyme 1). A small amount of lysine is catabolised via pipecolic acid and the peroxisomal key enzyme pipecolic acid oxidase (enzyme 2); this pipecolic acid pathway, however, might be an important route of lysine catabolism in the brain. Unlike in bacteria, however, the human origin of pipecolic acid is not yet fully understood. An orthograde production of pipecolic acid from lysine but also retrograde production from 2-aminoadipic-6-semialdehyde was shown. In addition, the microbiome should be considered as an alternative source. Hydroxylysine enters the pathway after phosphorylation by hydroxylysine kinase (enzyme 3. 2-Aminoadipic-6-semialdehyde is converted into 2-aminoadipic acid by 2-aminoadipic-6-semialdehyde dehydrogenase (antiquitin, enzyme 4, which is then converted to 2-oxoadipic acid by 2-aminoadipate aminotransferase (enzyme 5. 2-Oxoadipic acid is primarily converted to glutaryl-CoA by the dehydrogenase E1 and transketolase domains-containing protein 1 (DHTKD1), an enzyme with 2-oxoadipic dehydrogenase activity, forming the 2-oxoadipate dehydrogenase complex (OADHc, enzyme 6a) together with dihydrolipoamide S-succinyltransferase (DLST) and dihydrolipoamide dehydrogenase (DLD) similar to the 2-oxoglutarate dehydrogenase complex of the Krebs cycle. The E1 subunit of the 2-oxoglutarate dehydrogenase complex, 2-oxoglutarate dehydrogenase (OGDH), has a higher affinity for 2-oxoglutarate, but can alternatively accept 2-oxoadipic acid as a substrate (enzyme 6b). DHTKD1 and OGDH, which display substrate overlap, form a mitochondrial megacomplex with DLST and DLD. Glutaryl-CoA is dehydrogenated and decarboxylated to crotonyl-CoA by glutaryl-CoA dehydrogenase (enzyme 7). This enzyme transfers electrons to flavin adenine dinucleotide (FAD) and hence to the respiratory chain via electron transfer protein (ETF)/ETF-dehydrogenase (ETF-DH). Crotonyl-CoA is subsequently converted to 3-hydroxybutyryl-CoA by short-chain enoyl-CoA hydratase 1 (ECHS1, enzyme 8, ► Chap. 18). This enzyme is multispecific and also acts as a crotonase in the degradative pathways of valine, isoleucine, and short-chain fatty acids. 3-Hydroxybutyryl-CoA is converted to acetoacetyl-CoA by 3-hydroxyacyl-CoA dehydrogenase (enzyme 9, ► Chap. 13). Glutaric acid, which may derive from the intestinal microbiome, spontaneous disintegration of glutaryl-CoA or other sources, is reactivated by succinyl-CoA-dependent conversion of succinate-hydroxymethylglutarate CoA transferase to glutaryl-CoA (enzyme 10). From the six distinct enzyme deficiencies identified in the degradation of lysine, only enzymes 4, 6b, 7, and 8 have clinically proven relevance as metabolic disorders. Glutaric aciduria type I is caused by deficient glutaryl-CoA dehydrogenase (enzyme 7). Glutaric aciduria type II, caused by ETF/ETF-DH deficiencies, is discussed in ► Chap. 12. Pipecolic acid oxidase (enzyme 2) is discussed in the context of peroxisomal disorders in ► Chap. 42, 2-aminoadipic-6-semialdehyde dehydrogenase (antiquitin, enzyme 4) deficiency in ► Chap. 29, 2-oxoglutarate dehydrogenase deficiency (enzyme 6b) in ► Chap. 11, and ECHS1 deficiency (enzyme 8) in ► Chap. 18, since its major pathogenic effect is located in the valine catabolic pathway. Finally, several recent findings point to new functions for different short-chain lysine acylations of mitochondrial proteins (non-enzymatic acylations) and histones (enzyme-mediated) as important posttranslational modifications that regulate various cellular processes. Human inborn errors of these processes are yet to be discovered.

L-2- and D-2-Hydroxyglutaric aciduria type I are caused by deficiencies of specific FAD-dependent dehydrogenases, whereas D-2-hydroxyglutaric aciduria type II is caused by gain-of-function mutations of mitochondrial isocitrate dehydrogenase 2 and D-2-/L-2-hydroxyglutaric aciduria by inherited deficiency of the mitochondrial citrate carrier which mediates transport of dicarboxylic metabolites between the mitochondrion and the cytosol (◘ Fig. 22.2). Aspartoacylase (aminoacylase 2) irreversibly splits *N*-acetylaspartic acid (NAA), a brain-specific compound where its concentration reaches approximately 20 mM, into acetate and aspartate in oligodendrocytes (not illustrated). Deficiency of this enzyme causes *N*-acetylaspartic aciduria (Canavan disease). Deficiency in *N*-acetyltransferase (NAT), which catalyses NAA synthesis, causes hypoacetylaspartia.

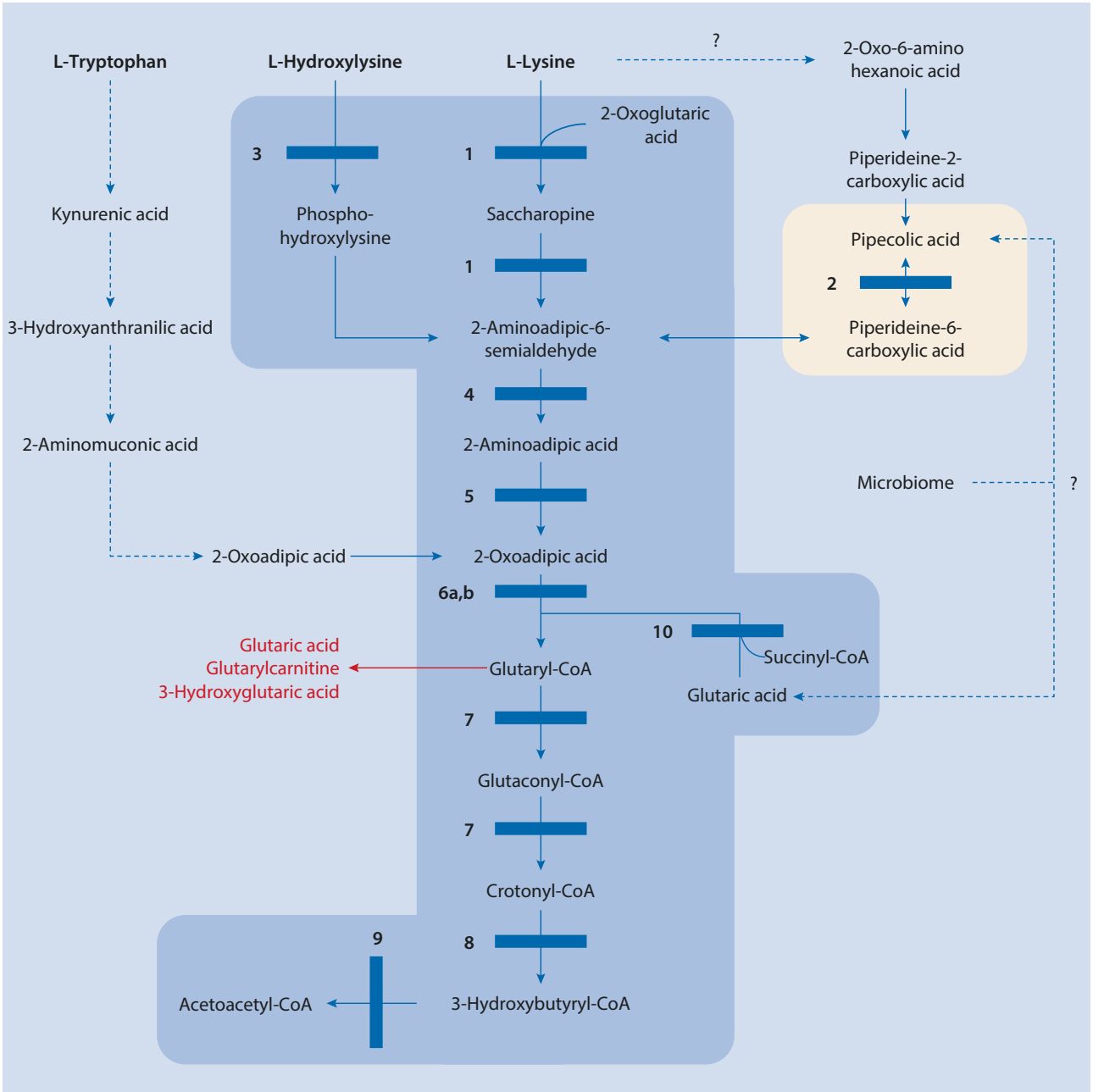

Fig. 22.1 Tryptophan, hydroxylysine and lysine catabolic pathways. *1*, 2-aminoadipic-6-semialdehyde synthase; *2*, pipecolic acid oxidase; *3*, hydroxylysine kinase; *4*, 2-aminoadipic-6-semialdehyde dehydrogenase (antiquitin); *5*, 2-aminoadipate aminotransferase); *6a*, 2-oxoadipate dehydrogenase complex with DHTKD1 as E1 subunit; *6b*, 2-oxoglutarate dehydrogenase complex, an enzymatic complex of the Krebs cycle, accepts 2-oxoadipic acid as an alternative substrate to 2-oxoglutarate; *7*, glutaryl-CoA dehydrogenase; *8*, short-chain enoyl-CoA hydratase 1 (crotonase); *9*, 3-hydroxyacyl-CoA dehydrogenase; *10*, succinate-hydroxymethylglutarate-CoA transferase. Enzyme deficiencies are indicated by solid bars across the arrows. Question marks indicate current uncertainties in the human lysine catabolic pathway. Note that the pathways are compartmentalized. The blue shaded area depicts the mitochondrial part, while the yellow box contains the peroxisomal part and the colourless area the cytosolic part of the pathways (with minor simplifications). Red coloured metabolites are elevated in glutaric aciduria type I

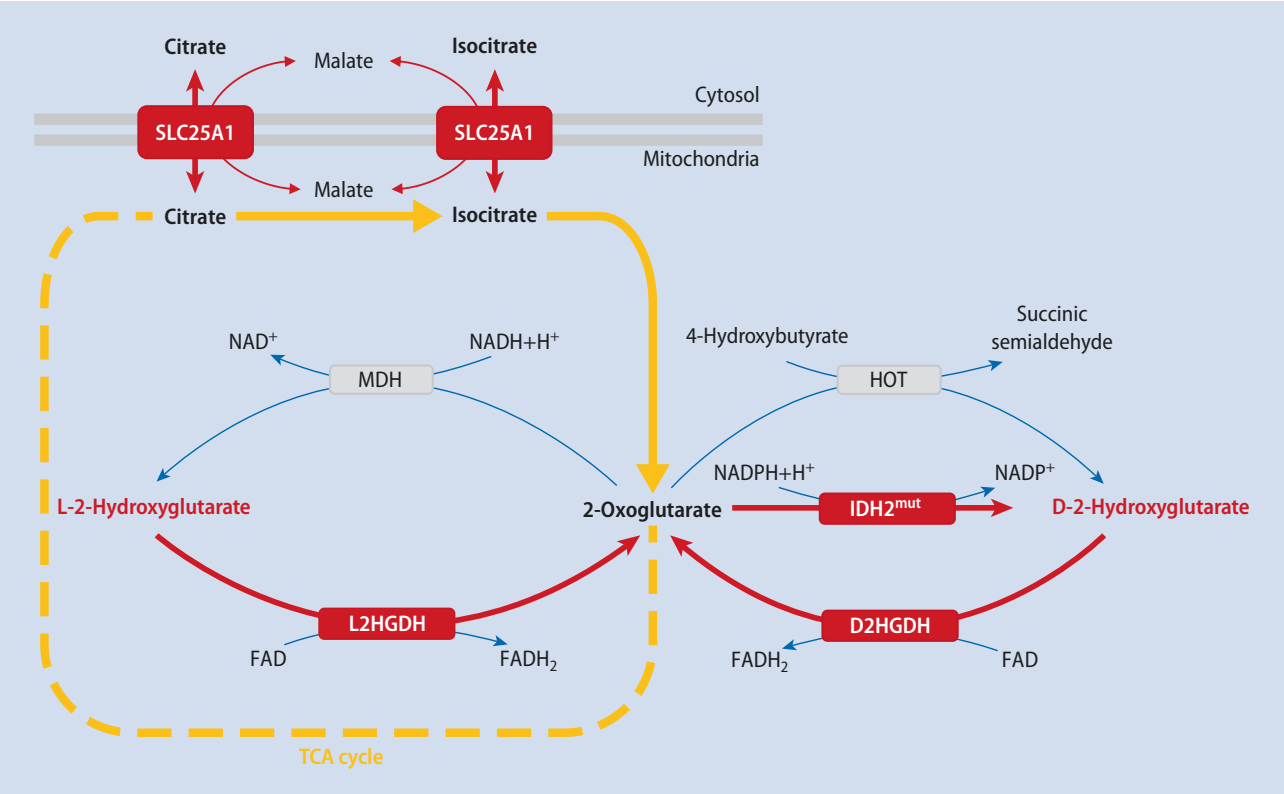

Fig. 22.2 Molecular origin of 2-hydroxyglutaric acidurias. The tricarboxylic acid (TCA) cycle intermediate 2-oxoglutarate is key to the understanding of 2-hydroxyglutaric acidurias. L-2-hydroxyglutarate is formed from 2-oxoglutarate by a side reaction of mitochondrial L-malate dehydrogenase (MDH) and, subsequently, this "faulty" metabolite is reconverted by L-2-hydroxyglutarate dehydrogenase (L2HGDH), a proof-reading enzyme, deficient in L-2-hydroxyglutaric aciduria. 2-Oxoglutarate is also used as a substrate by hydroxyacid-oxoacid transhydrogenase (HOT) for the conversion of 4-hydroxybutyrate to succinic semialdehyde, forming D-2-hydroxybutyrate and hence coupling TCA cycle and GABA metabolism. D-2-hydroxyglutarate is reconverted to 2-oxoglutarate by D-2-hydroxyglutarate dehydrogenase (D2HGDH), which is deficient in D-2-hydroxyglutaric aciduria type I. Although this enzyme is functional in D-2-hydroxyglutaric aciduria type II its capacity is exceeded by the conversion of 2-oxoglutarate to D-2-hydroxyglutarate by the neomorphic isocitrate dehydrogenase 2 due to a gain-of-function mutation. The above discussed enzymes are all functional in combined L-2- and D-2-hydroxyglutaric aciduria, which is caused by inherited deficiency of the mitochondrial tricarboxylate transporter, also called mitochondrial citrate carrier (SLC25A1), mediating the exchange transport of malate against citrate and isocitrate between mitochondria and cytosol. Deficiency of this transporter increasingly shunts mitochondrial citrate and isocitrate towards 2-oxoglutarate, the substrate for both L-2- and D-2-hydroxyglutarate synthesis by MDH and HOT, respectively

Introduction

Twelve inborn errors of metabolism are described in this chapter. Glutaric aciduria type I, L-2-hydroxyglutaric aciduria, D-2-hydroxyglutaric aciduria (type I and II), D-2-/L-2-hydroxyglutaric aciduria, N-acetylaspartic aciduria, and hypoacetylaspartia are all associated with neurological disease of varying severity whereas hyperlysinaemia/saccharopinuria, hydroxylysinuria, 2-aminoadipic and 2-oxoadipic aciduria, aminoacylase 1 deficiency and glutaric aciduria type III are likely non diseases or have an unclear clinical significance.

A group of organic acid disorders predominantly presents with (progressive) neurological symptoms of ataxia, epilepsy, myoclonus, pyramidal symptoms reflecting white matter disease, extrapyramidal symptoms due to basal ganglia lesions, and macrocephaly [1]. The core cerebral organic acid disorders are glutaric aciduria type I, D-2-hydroxyglutaric aciduria (types I and II), L-2-hydroxyglutaric aciduria, D-/L-2-hydroxyglutaric aciduria, succinic semialdehyde dehydrogenase deficiency (▶ Chap. 30), and *N*-acetylaspartic aciduria. Strikingly, in all these disorders the pathological compounds that accumulate either are odd-chain dicarboxylic acids (D-2-, L-2-, 3-hydroxyglutarate, glutarate) sharing the same carbon backbone with the excitatory amino acid glutamate (2-amino-glutarate), or have been suggested to be neurotransmitters/–modulators (γ-hydroxybutyrate, *N*-acetylaspartylglutamate). Evidence is accumulating from in vitro and in vivo studies showing that these acyl-CoA esters and accompanying carbonic acids indeed interfere with important pathways of cerebral metabolism, including glutamatergic

or gamma amino butyric acid (GABA)-ergic neurotransmission, cerebral energy metabolism, and myelin metabolism. Delayed myelination or progressive white matter disease, basal ganglia injury and cerebellum pathology, the main pathologies in cerebral organic acid disorders, are also characteristic of mitochondrial disorders, suggesting at least partial common pathological mechanisms. In L-2-hydroxyglutaric aciduria, the risk of developing cerebral neoplasms is increased.

Among this disease group, only glutaric aciduria type I forms characteristic acylcarnitines (i.e. glutarylcarnitine), which can be used for mass screening of newborns by tandem mass spectrometry. Metabolic hallmarks such as hypoglycaemia, metabolic acidosis, lactic acidaemia, or hyperammonaemia, the usual concomitants of branched-chain, 'classic' organic acid disorders (▶ Chap. 18), are generally absent, and hence the correct diagnosis requires an increased awareness of referring physicians and biochemists. Diagnostic clues can be derived from neuroimaging findings (◘ Figs. 22.3, and 22.4). Progressive disturbances of myelination, cerebellar atrophy, cortical atrophy, signal changes and/or atrophy of the basal ganglia and any symmetrical (fluctuating) pathology apparently independent of defined regions of vascular supply are suggestive.

In contrast to the cerebral organic acid disorders and pyridoxine-dependent epilepsy due to 2-aminoadipic-6-semialdehyde dehydrogenase deficiency (▶ Chap. 29), the other known defects of lysine and hydroxylysine degradation all appear to be rare biochemical variants of human metabolism with low clinical significance.

Increasing evidence points to a close link between metabolism and cell signalling via short-chain lysine acylations of metabolic proteins and histones such as acetylation, succinylation, malonylation, and glutarylation. Lysine acylation modifies mitochondrial function, enzyme activity, and enables concerted adaptation to environmental changes and hence is considered an important posttranslational modification.

22.1 Hyperlysinaemia (2-Aminoadipic Semialdehyde Synthase Deficiency)/Saccharopinuria

22.1.1 Clinical Presentation

About half of the identified individuals were detected incidentally and were healthy [2]. Symptoms include developmental delay, epilepsy, spasticity, ataxia, short stature, joint laxity, and spherophakia, respectively. Overall, the associated phenotypes appeared to be random rather than causally linked.

22.1.2 Metabolic Derangement

Hyperlysinaemia/saccharopinuria is caused by deficiency of the bifunctional protein 2-aminoadipic semialdehyde synthase (enzyme 1 in ◘ Fig. 22.1). This is the first enzyme of the mitochondrial saccharopine pathway, which is the main route of lysine degradation in most tissues [3]. The two functions of this enzyme, lysine:2-oxoglutarate reductase and saccharopine dehydrogenase, may be affected differently by gene variations. Most often, both activities are decreased, resulting in predominant hyperlysinaemia and hyperlysinuria with relatively mild saccharopinuria (hyperlysinaemia I). In hyperlysinaemia II/saccharopinuria, saccharopine dehydrogenase activity is more decreased than lysine:2-oxoglutarate reductase activity, resulting in a predominant excretion of saccharopine.

Failure to remove the ε-amino group is thought to result in an overflow of the minor lysine degradation pathway, with removal of the α-amino group by oxidative deamination. The oxoacid cyclises and is reduced to pipecolic acid. As a consequence, hyperpipecolataemia is regularly observed in hyperlysinaemia. Unlike in bacteria, however, this orthograde production of pipecolic acid from lysine is not generally accepted, since the enzyme initiating this pathway has not yet been identified in man.

Hyperlysinuria can also result from impaired renal tubular transport, often as part of a genetic transport defect of dibasic amino acids (▶ Chap. 25). In this situation it occurs without hyperlysinaemia.

22.1.3 Genetics

Hyperlysinaemia/saccharopinuria is caused by bi-allelic variations in *AASS* [3].

22.1.4 Diagnostic Tests

The initial observation in individuals with hyperlysinaemia/saccharopinuria is an impressive lysinuria with up to 15,000 mmol/mol creatinine (controls <70). Detailed amino acid analysis reveals additional accumulation of saccharopine, homoarginine, 2-amino adipic acid, and pipecolic acid. Elevations of the same metabolites can be documented in other body fluids, such as plasma and cerebrospinal fluid (CSF), with high lysine as the predominant abnormality (up to 1700 μmol/l in plasma, controls <200, and up to 270 μmol/l in CSF, controls <28). Secondary hyperlysinaemias due to mitochondrial shortage of 2-oxoglutarate are also observed in urea cycle disorders, pyruvate carboxylase deficiency,

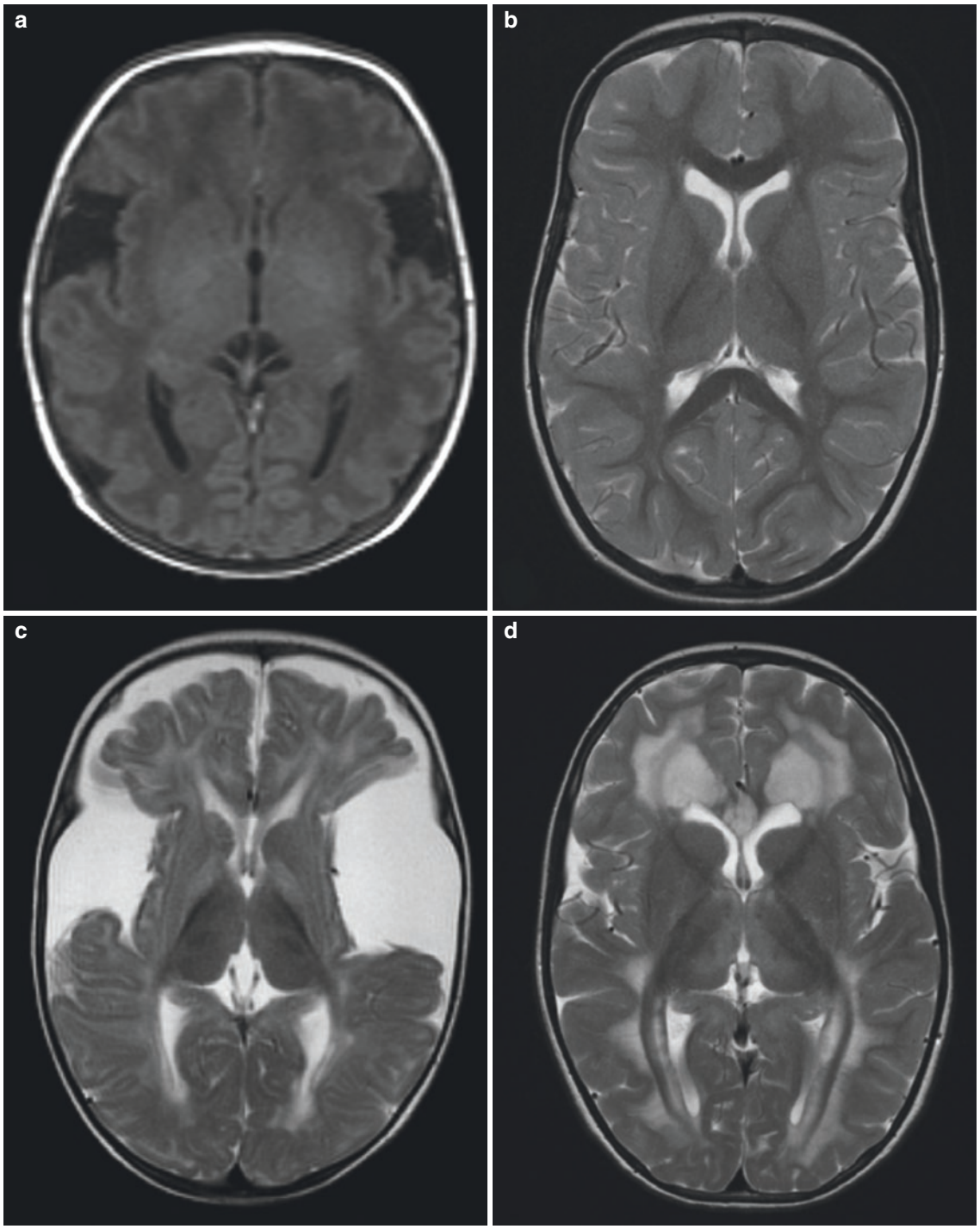

Fig. 22.3 MRI findings in patients with glutaric aciduria type I. **a,** T1-weighted axial MRI of an asymptomatic male newborn with glutaric aciduria type I, showing enlargement of temporopolar and frontopolar CSF spaces and an immature gyration pattern. **b,** T2-weighted axial MRI of an asymptomatic 2-year-old girl identified by newborn screening. Previously dilated external CSF spaces and temporal hypoplasia have normalised. There is no pathology of the basal ganglia or anywhere else. **c,** T2-weighted axial MRI at age 7.5 months showing striatal atrophy and markedly dilated temporopolar and frontopolar CSF spaces. Signal abnormalities of globus pallidus, thalamus, and supratentorial white matter are also found. This child presented with moderate axial hypotonia, which progressed after a delay in the start of emergency treatment during an infectious disease. After a further 4 weeks, the child developed dystonia of all extremities. **d,** T2-weighted axial MRI of a girl at age 11 years with suspected late-onset disease variant showing marked hyperintensity of the supratentorial white matter sparing the U fibres and mild to moderate signal changes of the caudate, thalamus, and dentate nuclei (not shown). The girl presented with nausea and vertigo at 10 years of age, which has improved following the start of carnitine supplementation and a protein-controlled diet. Motor and cognitive function is normal. (By courtesy of Dr. Inga Harting and Dr. Angelika Seitz)

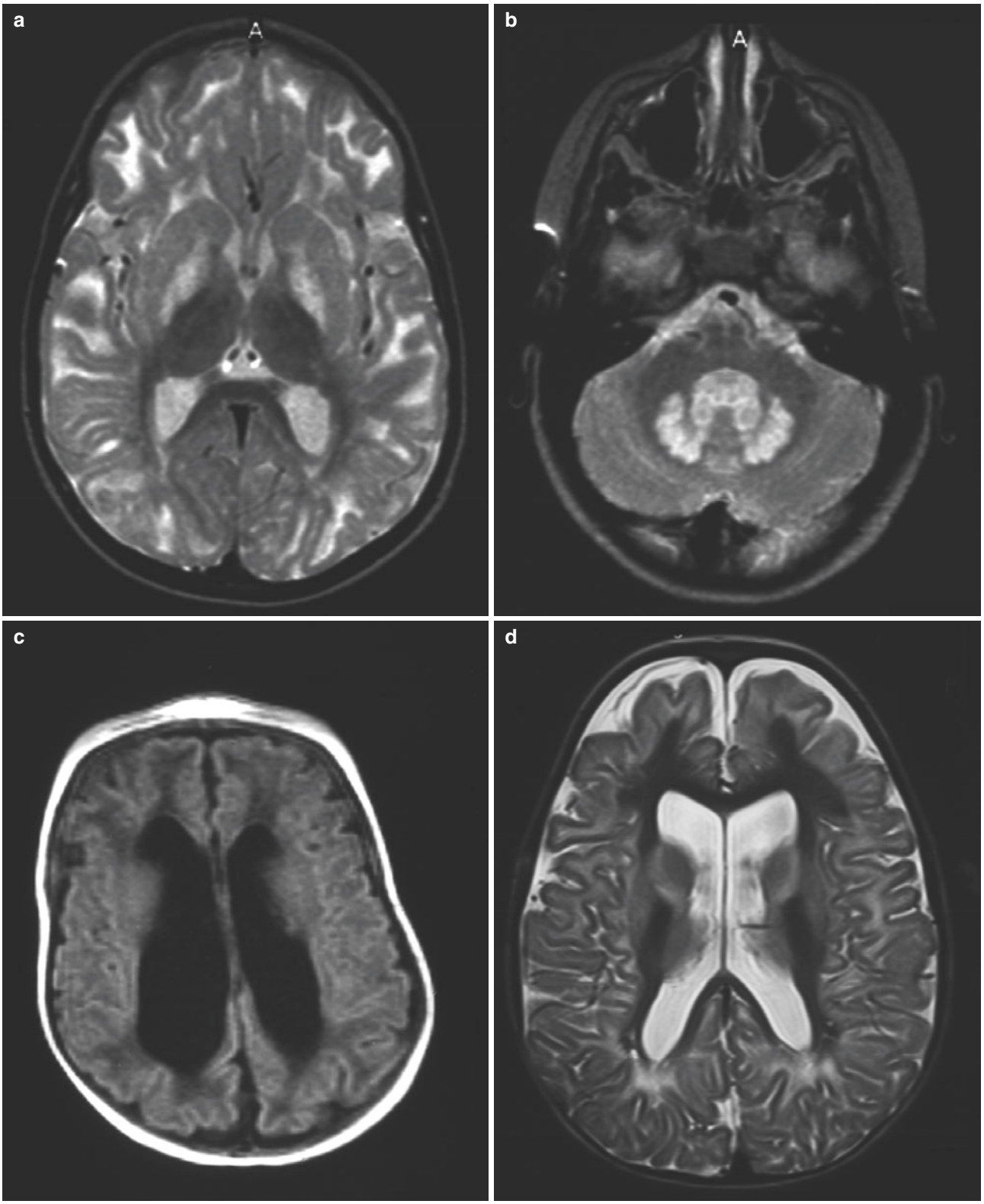

Fig. 22.4 MRI findings in patients with other cerebral organic acidurias. **a & b,** Axial T2-weighted MRI of a 8.5-year-old boy with L-2-hydroxyglutaric aciduria, illustrating characteristic involvement of subcortical white matter (also affecting the U fibres) and globus pallidus (**a**), and symmetrical involvement of the dentate nuclei (**b**). **c,** Axial MRI of a 2-month-old girl with D-2-hydroxyglutaric aciduria type I. Note the delayed myelination and occipitally pronounced enlargement of lateral ventricles. **d,** Axial fast spin echo image of a 6.5-year-old girl suffering from *N*-acetylaspartic aciduria. Note the marked discrepancy between the severely affected subcortical white matter and the relatively spared central white matter, at least frontally

methylmalonic and propionic acidurias [4], and L-2-hydroxyglutaric aciduria.

The deficiency of 2-aminoadipic semialdehyde synthase can be confirmed by molecular genetic studies and by determining the overall degradation of [1-^{14}C] lysine to $^{14}CO_2$ or the specific activity of lysine:2-oxoglutarate reductase and saccharopine dehydrogenase, respectively, in fibroblasts [3].

22.1.5 Treatment and Prognosis

Long-term dietary restriction of lysine has no proven benefit. As affected individuals do not suffer from metabolic decompensations, specific interventions during intercurrent illnesses are not recommended. Hyperlysinaemia/saccharopinuria has not been associated with an increased risk of mortality.

22.2 Hydroxylysinuria (Hydroxylysine Kinase Deficiency)

Hydroxylysinuria and concomitant hydroxylysinaemia has been identified in a few patients, all of whom showed some degree of cognitive disability [5]. No further clinical and/or biochemical studies were reported. The abnormality can be assumed to be caused by a defect of hydroxylysine kinase (enzyme 3 in ◘ Fig. 22.1).

22.3 2-Aminoadipic and 2-Oxoadipic Aciduria (DHTKD1 Deficiency)

22.3.1 Clinical Presentation

2-Aminoadipic and 2-oxoadipic aciduria is thought to be of low or even no clinical significance. Over 20 individuals are known, more than half of whom are asymptomatic [6]. Symptoms include psychomotor retardation, muscular hypotonia, epilepsy, ataxia, and failure to thrive, but it is likely that these are coincidental findings. However, a heterozygous nonsense mutation in *DHTKD1* (p.Tyr485Xaa) has been associated with Charcot-Marie-Tooth disease type 2Q in a large Chinese pedigree [7].

22.3.2 Metabolic Derangement

The metabolic profile is heterogeneous, with most patients showing elevations of 2-aminoadipic, 2-oxoadipic and 2-hydroxyadipic acid, whereas some excrete only 2-aminoadipic acid. Isolated excretion of 2-aminoadipic acid may be caused by antiepileptic therapy with vigabatrin, which inhibits 2-aminoadipate aminotransferase. 2-Aminoadipic acid is deaminated to 2-oxoadipic acid by a mitochondrial 2-aminoadipate aminotransferase. 2-Oxoadipic acid is also formed from the degradation of tryptophan, but this is not yet fully understood in humans. 2-Oxoadipic acid is further metabolised to glutaryl-CoA via two distinct enzyme complexes: The major pathway involves the 2-oxoadipate dehydrogenase complex (OADHc) which contains DHTKD1 as E1 subunit, a close homolog to 2-oxoglutarate dehydrogenase (OGDH). DHTKD1 has a high affinity for 2-oxoadipic acid. Alternatively, 2-oxoadipic acid can be handled by the oxoglutarate dehydrogenase complex (OGDHc), containing OGDH as E1 subunit which prefers 2-oxoglutaric acid as a substrate but can alternatively handle 2-oxoadipic acid if DHTKD1 is blocked or deficient. Evidence is increasing that OGDH, DHTKD1, DLST, and DLD can form a hybrid 2-oxo acid dehydrogenase complex [8].

22.3.3 Genetics

Autosomal recessive inheritance is implied by the pedigrees and by the finding that parents cannot be biochemically differentiated from controls. In 2012, pathogenic mutations in *DHTKD1* localized on 10p14 were identified as molecular cause of 2-aminoadipic and 2-oxoadipic aciduria [9].

22.3.4 Diagnostic Tests

Affected individuals are diagnosed by demonstrating variable elevations of 2-aminoadipic acid on amino acid chromatography and/or of 2-oxoadipic and 2-hydroxyadipic acids on urinary organic acid analysis. Plasma lysine may be twofold elevated and urinary glutaric acid up to 50 mmol/mol of creatinine (controls <9). The suspected diagnosis is confirmed by identification of two disease-causing *DHTKD1* variants.

22.3.5 Treatment and Prognosis

Autosomal recessive 2-aminoadipic and 2-oxoadipic aciduria is likely to be a non-disease, while individuals with a specific heterozygous nonsense variant may develop Charcot Marie Tooth disease type 2Q. Individuals with 2-aminoadipic and 2-oxoadipic aciduria as well as with Charcot Marie Tooth disease type 2Q do not suffer from metabolic decompensations,

and specific interventions during intercurrent illnesses do not appear necessary. Administration of pharmacological doses of vitamins B_1 (thiamine) and B_6 (pyridoxine) as well as low lysine diet had no effect on the levels of pathological metabolites and no proven clinical benefit.

22.4 Glutaric Aciduria Type I (Glutaryl-CoA Dehydrogenase Deficiency)

22.4.1 Clinical Presentation

Glutaric aciduria type I should be seriously considered in the differential diagnosis of any infant who has macrocephaly combined with hypoplasia of the temporal cortex and concomitantly enlarged Sylvian fissures, striatal lesions (putamen > caudate > > globus pallidus), and variable signal changes of periventricular white matter on magnetic resonance imaging (MRI) (◘ Fig. 22.3) and/or a complex movement disorder with predominant dystonia, orofacial dyskinesia and dysarthria superimposed on axial hypotonia. Chorea may also be observed [10, 11]. In 75% of patients, macrocephaly is present at or shortly after birth, peaking at the age of 3–6 months, preceding the severe neurological disease and possibly a clue to diagnosis in countries without extended newborn screening programs. Furthermore, affected babies often present with potentially reversible muscular hypotonia which may slow motor development in the first year of life. Neuroimaging studies have been performed in a number of asymptomatic newborns and infants, revealing the characteristic findings of temporal hypoplasia (95% of all patients; (◘ Fig. 22.3), wide anterior temporal and sylvian CSF spaces, an immature gyration pattern, delayed myelination, and isolated T_2 hyperintensity in the globus pallidus [12]. These non-striatal MRI abnormalities may completely resolve if treatment is started in the newborn period (◘ Fig. 22.3). The clinical significance of enlarged subdural fluid spaces in infants with glutaric aciduria type I is the unprotected crossing of these spaces by bridging veins. Such infants are prone to suffer acute subdural haemorrhages, which may be accompanied by retinal haemorrhages, after only minor head trauma, particularly around the first birthday when starting to walk. Parents of children with glutaric aciduria type I have been wrongly accused for abusive head trauma [13]. Alternatively, vascular abnormalities have been explained by altered haemodynamics and endothelial dysfunction [14].

At a median age of 9–10 months, the majority of untreated patients suffer an acute brain injury, usually associated with febrile infectious disease, but this acute encephalopathic crisis may also be precipitated by any other episode that induces catabolism, including undesirable reactions following routine immunisations [11]. MRI reveals striatal injury spreading in a dorsoventral direction (◘ Fig. 22.3), starting at the dorsolateral aspects of the putamen. Almost all reported encephalopathic crises have occurred before 36 months of age. They have not yet been described at school age, during adolescence, or in adulthood. Acquired motor skills are often acutely lost, including the ability to sit, pull up to standing, to suck and swallow, and head control. The infants appear alert with profound muscular hypotonia of the trunk and a mobile dystonia of the extremities which develops over days to months following striatal injury. Usually there are no metabolic derangements as in "classic" organic acidurias. If the underlying metabolic disorder remains undiagnosed, additional cerebral systems are slowly but progressively affected. Impaired chewing and swallowing, vomiting and aspiration, plus increased energy demand due to increased muscle tone frequently results in failure to thrive and malnutrition. Kyphoscoliosis and chest wall dystonia can cause restrictive lung disease. Early death (40–50% of symptomatic patients by the age of 20 years) may occur in the course of pneumonia and respiratory failure, during hyperpyrexic crises, or suddenly without apparent cause [11].

Although the majority of patients present with characteristic symptoms and disease course, the natural history of glutaric aciduria type I can be variable even within families. With the implementation of extended newborn screening programs a growing number of patients with a so-called insidious onset disease variant have been identified. These patients develop dystonia over weeks and months without a preceding episode that is known to precipitate striatal injury; however, more subtle symptoms like hand tremor and mild oral dyskinesia could also be the first manifesting signs. Striatal injury and dystonia tend to be less severe than in individuals with acute encephalopathic crises. In neonatally screened individuals, the insidious-onset variant is most frequently found in individuals not receiving or adhering to the recommended low lysine diet [15, 16]. A few individuals, mainly diagnosed in adolescence or adulthood during family studies, or previously undiagnosed women with glutaric aciduria type I, identified through false positive newborn screening results of their non-affected children, have not developed striatal injury despite never having been treated. Finally, previously unaffected adolescent and adult patients can present with progressive signal changes in the white matter but unaffected basal ganglia [12]. Although this was initially suggested to represent a late-onset disease variant, recent studies have not supported the notion of a distinct disease course (◘ Fig. 22.3). Recent MRI studies

demonstrate that white matter changes are a common finding in glutaric aciduria type I. They progress with age and are commonly found in high excretor patients [16, 17], highlighting the risk of long-term neurotoxicity due to cerebral accumulation of glutarate and 3-hydroxyglutarate and concomitantly progressive neuroaxonal compromise.

Chronic kidney disease has recently been detected as the first non-neurologic presentation in a growing number of patients, even in those identified by newborn screening, and does not seem to be impacted by recommended therapy [16].

Finally, three patients with glutaric aciduria type I with poor adherence or late start of recommended therapy and malignant brain tumors have been reported [18]. More information is required and careful clinical and neuroradiological follow-up is required to understand whether patients with glutaric aciduria type I bear an increased risk of developing brain neoplasms similar as in L-2-hydroxyglutaric aciduria.

22.4.2 Metabolic Derangement

Glutaric aciduria type I is caused by a deficiency of glutaryl-CoA dehydrogenase, a mitochondrial flavin adenine dinucleotide (FAD)-requiring enzyme, which catalyses the dehydrogenation of glutaryl-CoA as well as the subsequent decarboxylation of glutaconyl-CoA to crotonyl-CoA (enzyme 7 in Fig. 22.1). In glutaric aciduria type I, part of the accumulating glutaryl-CoA in mitochondria is esterified with carnitine to glutarylcarnitine by carnitine acyltransferase, leading to an increased ratio of acylcarnitines to free carnitine in plasma and urine. Glutarylcarnitine is excreted, contributing to secondary carnitine deficiency.

The mechanisms of age-specific destruction of specific cerebral structures in glutaric aciduria type I is complex. Evidence points to impaired brain energy metabolism and production of reactive oxygen species induced by accumulating glutaric acid, 3-hydroxyglutaric acid, and glutaryl-CoA: glutaryl-CoA inhibits the 2-oxoglutarate dehydrogenase complex, glutaric acid impairs the dicarboxylic acid shuttle and hence the metabolic coupling between astrocytes and neurons, and 3-hydroxyglutaric acid promotes excitotoxic mechanisms [19]. Accumulation of these putatively dicarboxylic neurotoxins in the brain is facilitated by the low permeability of the blood-brain barrier for dicarboxylic acids, causing ‘entrapment’ of these metabolites in the brain compartment of patients [20]. It has been suggested that disturbed cerebral haemodynamics, such as disturbed autoregulation and regional perfusion pressure gradients, adds to the metabolic toxicity of this disease [14]. Recently, enhanced glutarylation of lysine residues and concomitantly impaired function of mitochondrial proteins, particularly of glutamate dehydrogenase and carbonic anhydrase 5b, was demonstrated in glial cells [21]. The functional consequence of this finding needs to be further elucidated.

22.4.3 Genetics

Glutaric aciduria type I is an autosomal recessive disorder caused by pathogenic variants in *GCDH*. Results of newborn screening programs in various regions and cohorts worldwide give an estimated mean frequency of about 1:125,000 [16]. The disease is much more frequent in certain communities, such as the Amish people in Pennsylvania (homozygous for p.Ala421Val, incidence of 1 in 300–400 newborns), the Oji-Cree First Nations in Canada (homozygous for the splice site mutation IVS-1 + 5 g > t, incidence of 1 in 300 newborns), and the Irish travellers (homozygous for p.Glu365Lys).

About 200 different disease-causing mutations in *GCDH* have been identified so far [22]. There is a correlation between genotype and biochemical phenotype in that specific *GCDH* variations with residual enzyme activity of 3–30% are associated with low excretions of metabolites, while loss of enzymatic activity predicts a high excretor phenotype. However, no correlation between genotype and acute or insidious onset of striatal injury until age 36 months has yet been found [11], while white matter changes are much more prevalent in patients with the high excretor phenotype [17]). Single common founder mutations have been identified in high-risk populations (see above), but glutaric aciduria type I is, in general, genetically quite heterogeneous with a high frequency of private mutations: the most frequent mutation in Caucasians, p.Arg402Trp, has been identified in 10–20% of alleles [22].

22.4.4 Diagnostic Tests

Before the introduction of extended newborn screening programs, patients with glutaric aciduria type I have been diagnosed by (quantitative) urinary organic acid analysis [23]. Since urinary concentrations may be (intermittently) normal in low excreting patients, the diagnosis has been challenging or even unsuccessful in some, and successful diagnosis often required repetitive metabolic testing. Additional diagnostic hints are carnitine deficiency with concomitantly increased acylcarnitines due to elevated glutarylcarnitine (C5DC) in plasma and urine. Increased C5DC concentrations can be specifically detected by tandem mass spectrometry

(MS/MS) [24], which has led to the inclusion of glutaric aciduria type I into MS/MS-based newborn screening programs in a growing number of countries. While patients with a high excretor status can be reliably identified by C5DC screening, this test has a lower sensitivity for patients with a low excretor phenotype [16].

Elevated urinary excretion of glutaric acid is found in a number of other disease states, such as glutaric aciduria type II and III, mitochondrial dysfunction, and renal failure. Quantitative analysis of 3-hydroxyglutaric acid in urine has a high sensitivity including patients with the low excretor phenotype and those having secondary carnitine depletion [23]. However, it is known that 3-hydroxyglutaric acid is also elevated in patients with short-chain 3-hydroxyacyl-CoA dehydrogenase deficiency (▶ Chap. 12) and severe ketosis.

Loading tests, e.g. with lysine, or prolonged fasting tests provoking catabolism may be extremely harmful and should be avoided. Demonstration of two known pathogenic *GCDH* variants or significantly decreased glutaryl-CoA dehydrogenase activity in leukocytes (or cultured skin fibroblasts) ultimately confirms glutaric aciduria type I. These confirmatory tests are particularly important in diagnostically problematic cases and are also recommended for individuals with high clinical and/or neuroradiological suspicion but unremarkable metabolic test results. Evidence-based recommendations for the diagnosis of glutaric aciduria type I have been published and revised twice [25, 26].

22.4.5 Treatment and Prognosis

More than 700 patients have been identified worldwide and major progress has been achieved in the prevention of striatal necrosis occurring within the first 36 months of age. A favorable neurologic outcome critically depends on two factors: (1) newborn screening (or any other diagnostic approach that allows the identification of asymptomatic individuals) and (2) adherence to recommended, evidence-based therapy. However, up to one-third of neonatally screened individuals still do not or only partially benefit from early diagnosis and start of therapy [10, 16], due to differences in the therapeutic management. In addition, low-excreting individuals missed by newborn screening are still confronted with high mortality and morbidity, as in the pre-screening era, since post-symptomatic start of metabolic therapy cannot reverse striatal damage.

The following therapeutic measures are recommended and should be introduced and carefully evaluated and adapted by an experienced multi-professional team to minimize the risk of neurological impairment [25, 26].

Emergency Treatment

Emergency treatment during every intercurrent illness must start immediately and before the onset of neurological signs. Gastrointestinal infections are especially dangerous. Treatment should consist of frequent high carbohydrate feeds and increased carnitine supplementation. If feeds are not tolerated high-dose intravenous glucose and carnitine must be given [25, 26]. If lysine-free amino acid supplements are used, these are offered orally, in addition. If the temperature rises above 38.5 °C (101 °F) antipyretics should be administered liberally. All patients should be supplied with an emergency card. Frequent visits and regular information and training of parents may help to prevent lapses or mistakes. This concept should be strictly followed for the first 6 years of life. After this age emergency treatment is individually adjusted. Emergency treatment is thought to be the most effective component of current treatment strategies to prevent acute striatal injury [16].

Oral Supplementations with Carnitine and Riboflavin

Carnitine should be supplemented lifelong to prevent secondary carnitine depletion and to foster the formation of non-toxic C5DC. The lack of carnitine supplementation has been associated with increased mortality [11]. Riboflavin responsiveness and the therapeutic benefit of riboflavin remain unproven. Furthermore, there is no standardised protocol to test for riboflavin responsiveness.

Dietary Treatment

Application of a low lysine diet aims to reduce the quantitatively most relevant precursor amino acid of the putatively neurotoxic glutaric and 3-hydroxyglutaric acids. Dietary treatment involves reduced intake of natural protein, preferably with supplementation of lysine-free, tryptophan-reduced, arginine-fortified amino acid mixtures, at least until age 6 years (◘ Table 22.1), to minimise the risk of malnutrition. To continue low lysine diet is not generally recommended beyond the vulnerable period for striatal injury, i.e. the first 6 years of life [25, 26]. After age 6 years, protein-controlled diet with avoidance of protein excesses is recommended. Special efforts to supply adequate calories are often necessary in patients with dystonia and swallowing difficulties. This may require nasogastric or gastrostomy feeding.

Treatment of the Complex Movement Disorder

The complex movement disorder is difficult to treat, and the efficacy of a drug cannot be predicted precisely for an individual patient [25, 26]. Baclofen (1–2 mg/kg daily) and/or diazepam (0.1–1 mg/kg

Table 22.1 Maintenance therapy in patients with glutaric aciduria type I

		Patient age				
Treatment		0–6 mo	7–12 mo	1–3 y	4–6 y	>6 y
1. Low lysine diet						
Lysine (from natural protein)[1]	mg/kg/d	100	90	80–60	60–50	Controlled protein intake using natural protein with a low lysine content and avoiding lysine-rich food
Amino acid supplements (protein)[2]	g/kg/d	1.3–0.8	1.0–0.8	0.8	0.8	
Energy	kcal/kg/d	100–80	80	94–81	86–63	
2. Micronutrients	%	≥100	≥100	≥100	≥100	>100
3. Carnitine	mg/kg/d	100	100	100	100–50	50–30

After [25, 26]
[1]Using natural protein with a low lysine content. [2]Lysine-free, tryptophan-reduced, arginine-fortified. Consider an individualisation of treatment if normal growth is not achieved

daily) are commonly used to reduce involuntary movements and improve motor function, mostly through muscle relaxation. In some patients their use and dosage are limited by worsening of axial hypotonia and sedative effects. Trihexiphenidyl may improve dystonia, especially in adolescent and adult patients, but it may also be effective in children if the dosage is increased slowly. Botulinum toxin type A may help to prevent hip dislocation and reduce limb dystonia. Antiepileptics such as vigabatrin, carbamazepine, and valproate (which is even contraindicated) as well as L-DOPA, and amantadine are ineffective to control movement disorders in glutaric aciduria type I. The long-term benefits of intrathecal baclofen administration and neurosurgical interventions such as pallidotomy and deep brain stimulation (globus pallidus internus) are uncertain and, since they involve a significant risk of neurological deterioration, these interventions should be decided upon very cautiously and individually.

Although early diagnosis by newborn screening in combination with metabolic treatment has significantly improved the neurologic outcome by decreasing the frequency of striatal injury and untimely death [15, 16], up to one-third of screened patients still develops neurological symptoms of variable degree. Furthermore, longitudinal observational studies of screened individuals unravelled progressive white matter disease with unclear clinical significance, particularly in high excretor patients, the development of chronic kidney disease which does not seem to be impacted by recommended therapy, and the manifestation of malignant brain tumours in a few patients with poor adherence to or late start of therapy. This highlights the need for safe and more effective therapies and careful long-term follow-up.

22.5 Glutaric Aciduria Type II (Multiple Acyl-CoA Dehydrogenase Deficiency)

For historical reasons, multiple acyl-CoA dehydrogenase deficiency, is still termed glutaric aciduria type II; however, the name-giving finding of glutaric aciduria/acidemia is rather a biochemical epiphenomenon than a pathomechanistic explanation of this complex disease involving electron transfer in the mitochondrial respiratory chain. A detailed description of the disease is found in ▶ Chap. 12.

22.6 Glutaric Aciduria Type III (Succinate Hydroxymethylglutarate CoA-Transferase Deficiency)

22.6.1 Clinical Presentation

Glutaric aciduria type III is an autosomal recessive metabolic abnormality with unknown incidence. It is likely a clinically benign condition [27].

22.6.2 Metabolic Derangement

Individuals with glutaric aciduria type III present with isolated glutaric acid accumulation, without the elevated levels of 3-hydroxyglutaric acid and glutarylcarnitine that are found in glutaric aciduria type I. This indicates absence of elevated glutaryl-CoA. Deficiency of succinate hydroxymethylglutarate CoA-transferase causes glutaric aciduria type III (enzyme 10 in ◘ Fig. 22.1). This enzyme converts glutaric acid to glutaryl-CoA using succinyl-CoA as a coenzyme donor [28]. The origin of glutaric acid as substrate for this enzyme remains to be elucidated. Bacterial production in the intestine and spontaneous breakdown of glutaryl-CoA might be considered as a source.

22.6.3 Genetics

Bi-allelic variations in *C7orf10* located on 7p14.1 are causative for glutaric aciduria type III [29].

22.6.4 Diagnostic Tests

Patients with glutaric aciduria type III are diagnosed by urinary organic acid analysis; mutation analysis of *C7orf10* can confirm the diagnosis.

22.6.5 Treatment and Prognosis

Since this is a biochemical abnormality with minor or even no clinical significance, there is no indication for treatment. The prognosis of affected individuals is likely to be favourable.

22.7 L-2-Hydroxyglutaric Aciduria (L-2-Hydroxyglutaric Dehydrogenase Deficiency)

22.7.1 Clinical Presentation

Most patients with L-2-hydroxyglutaric aciduria follow a characteristic disease course [30]. In infancy and early childhood mental and psychomotor development appears normal or only slightly retarded. Thereafter seizures, progressive ataxia, pyramidal tract signs, slight extrapyramidal signs, and progressive mental retardation become the most obvious clinical findings. Progressive macrocephaly is present in about half of the patients. Cognitive disability is frequent and is often significant, with an IQ of about 40–50 in adolescents and adults. Sometimes mental deterioration is rapidly progressive, and a fatal neonatal outcome has been rarely described.

In L-2-hydroxyglutaric aciduria the pattern of neuroradiologic abnormalities is pathognomonic [30]. The subcortical white matter appears mildly swollen with some effacement of gyri. The progressive loss of arcuate fibres is combined with severe cerebellar atrophy and increased signal densities of dentate nuclei and globi pallidi (◘ Fig. 22.3) on T_2-weighted images. Patients with this disease bear an increased risk for developing malignant brain tumours, such as medulloblastoma, glioblastoma multiforme, astrocytoma, and primitive neuroectodermal tumour [31].

22.7.2 Metabolic Derangement

The disorder is caused by an inherited deficiency of FAD-linked 2-hydroxyglutarate dehydrogenase, a mitochondrial enzyme converting L-2-hydroxyglutarate to 2-oxoglutarate [32] (◘ Fig. 22.2). L-2-hydroxyglutarate is increased in CSF, plasma, and urine [33]. In addition, a number of hydroxydicarboxylic acids (glycolate, glycerate, 2,4-dihydroxybutyrate, citrate, and isocitrate) are elevated in CSF. Another consistent biochemical finding is an increase of lysine in blood and CSF.

L-2-Hydroxyglutarate has no known functions, but its formation results from a side reaction of L-malate dehydrogenase on 2-oxoglutarate, the structural homologue of oxaloacetate, to L-2-hydroxyglutarate, which is converted back to 2-oxoglutarate by L-2-hydroxyglutarate dehydrogenase [34]. 2-Oxoglutarate is an important Krebs cycle intermediate but has many other functions. It is also required for the first step of mitochondrial lysine oxidation, i.e. the formation of saccharopine, which explains elevated lysine concentrations. L-2-Hydroxyglutaric aciduria is considered a disease of the growing group of disorders of metabolite proofreading.

22.7.3 Genetics

L-2-Hydroxyglutaric aciduria is caused by bi-allelic variants in *L2HGDH* [35].

22.7.4 Diagnostic Tests

L-2-Hydroxyglutarate is found elevated in all body fluids [30]. In addition, lysine is slightly increased in CSF, as is protein, the latter occurring in the absence of pleocytosis. Confirmation of the suspected diagnosis is usually done genetically.

22.7.5 Treatment and Prognosis

Riboflavin has led to a partial improvement of neurological symptoms in a few patients and reduced urinary excretion of L-2-hydroxyglutarate in some [36] but not in others (G. F. Hoffmann, personal observation). Epilepsy can generally be controlled by standard medications. No causal therapy is currently known which could stop or prevent the progression of neurological symptoms and tumorigenesis, and thus the prognosis is usually poor.

22.8 D-2-Hydroxyglutaric Aciduria Type I (D-2-Hydroxyglutarate Dehydrogenase Deficiency) and Type II (Isocitrate Dehydrogenase 2 Deficiency)

22.8.1 Clinical Presentation

Two genetic causes of D-2-hydroxyglutaric aciduria, type I and II, have been delineated [37, 38]. Although both types share some clinical overlap, patients with type I are usually less severely affected and develop a more variable clinical phenotype than those with type II. Frequent clinical findings are developmental delay, muscular, hypotonia, and epilepsy. Some individuals with type I develop an attenuated phenotype with mild developmental delay or even remain asymptomatic. In contrast, type II patients more often present with a neonatal onset with epileptic encephalopathy, severe muscular hypotonia, lack of psychomotor development, cortical blindness, (dilated) cardiomyopathy), and early death.

In the severely affected individuals, neuroimaging uniformly reveals disturbed and delayed gyration, myelination and opercularisation, ventriculomegaly, more pronounced of the occipital horns, and cysts over the head of the caudate nucleus (◘ Fig. 22.4).

22.8.2 Metabolic Derangement

Patients show moderately (type I) to highly (type II) elevated levels of D-2-hydroxyglutarate in all body fluids. In addition, Krebs cycle intermediates are found to be elevated in the urine of some patients, as well as GABA in CSF. Type I is caused by deficient D-2-hydroxyglutarate dehydrogenase, an enzyme that converts D-2-hydroxyglutarate to 2-oxoglutarate [37], while type II originates from mutated mitochondrial isocitrate dehydrogenase 2 [38] (◘ Fig. 22.2). The neomorph enzyme gains the ability to convert 2-oxoglutarate into D-2-hydroxyglutarate, which is in contrast to its normal function, the NADPH-producing oxidative decarboxylation of D-isocitrate to 2-oxoglutarate and hence the control of mitochondrial redox balance and mitigation of cellular oxidative defense.

A similar mechanism like in D-2-hydroxyglutaric aciduria type II explains D-2-hydroxyglutaric aciduria observed in patients with malignant gliomas and acute myeloid leukaemia due to somatic mutations in isocitrate dehydrogenase 1 (cytosolic) or 2 (mitochondrial).

22.8.3 Genetics

Bi-allelic mutations in *D2HGDH* (2q37.3) are the molecular cause of D-2-hydroxyglutaric aciduria type I [37], while autosomal dominant variations of *IDH2* located on 15q26.1 [38] cause D-2-hydroxyglutaric aciduria type II, with a high frequency of de novo variations.

22.8.4 Diagnostic Tests

Elevation of 2-hydroxyglutaric acid can be identified by conventional gas chromatography/mass spectrometry analysis, but cannot be differentiated in the L-2- and D-2-isomers, which requires chromatographic separation using derivatisation with a chiral reagent or a chiral stationary phase. D-2-hydroxyglutaric acid is found elevated in urine, plasma, and CSF. In addition, GABA is often elevated in CSF, and intermediates of energy metabolism are elevated in urine (lactic, succinic, malic, and 2-oxoglutaric acids). The suspected diagnosis is usually confirmed genetically.

D-2-Hydroxyglutaric acid can also be elevated in multiple acyl-CoA dehydrogenase deficiency (glutaric aciduria type II), and – rarely – in patients with glutaric aciduria type I and succinic semialdehyde dehydrogenase deficiency, but these can be readily distinguished by the urine organic acid profile (► Chaps. 12 and 30).

22.8.5 Treatment and Prognosis

To date there is no rational therapy for D-2-hydroxyglutaric aciduria type I and II; riboflavin and L-carnitine supplementation has not been of benefit. Seizures can be very difficult to control, and patients have died early with profound developmental delay. The clinical phenotype does not appear to progress rapidly in type I disease, if affected children do not develop an early onset epileptic encephalopathy. The course of type II disease is usually progressive with early death in

childhood in about 50%. Specific inhibition of the neomorphic isocitrate dehydrogenase 2 by a small molecule rescued cardiomyopathy and improved survival in a mouse model for this disease. However, it remains to be elucidated whether this strategy is safe and effective in affected individuals [39].

22.9 D-2- and L-2-Hydroxyglutaric Aciduria (Mitochondrial Citrate Carrier or SLC25A1 Deficiency)

22.9.1 Clinical Presentation

D-2- and L-2-Hydroxyglutaric aciduria has been described as a devastating neurometabolic disorder in more than 50 patients. Affected individuals display a severe clinical phenotype with neonatal onset metabolic encephalopathy, infantile epilepsy refractory to antiepileptic drug therapy, severe global developmental retardation, muscular hypotonia, cortical blindness, and early death [40]. Recently, a milder disease variant presenting primarily with a neuromuscular junction defect causing congenital myasthenic syndrome type 23 has been reported, extending the clinical phenotype.

22.9.2 Metabolic Derangement

The major biochemical finding is accumulation of D-2- and L-2-hydroxyglutaric acids in body fluids with a predominance of D-2-hydroxyglutaric acid. Krebs cycle intermediates including 2-oxoglutarate, malate, fumarate, and succinate are also elevated accompanied by decreased concentrations of citrate and isocitrate [41]. SLC25A1, the mitochondrial citrate carrier, exchanges cytosolic malate for mitochondrial citrate and isocitrate (Fig. 22.2). If this exchange transport is disrupted this has negative consequence for mitochondrial and cytosolic metabolism: (1) In mitochondria, concentrations of Krebs cycle intermediates downstream of isocitrate, such as 2-oxoglutarate, are permanently increased, resulting in increased formation of both D-2- and L-2-hydroxyglutarate, whose synthesis requires 2-oxoglutarate. (2) In the cytosol, concomitant citrate depletion leads to decreased formation of NADPH+H and fatty acid and sterol synthesis.

22.9.3 Genetics

Bi-allelic variations in *SLC25A1* (located on 22q11.21) encoding the mitochondrial citrate carrier, cause D-2- and L-2-hydroxyglutaric aciduria [42] and congenital myasthenic syndrome 23 [41].

22.9.4 Diagnostic Tests

The metabolic work-up is performed in analogy to D-2- and L-2-hydroxyglutaric acidurias (see above).

22.9.5 Treatment and Prognosis

The prognosis in the hitherto described patients was poor. Treatment with citrate may result in biochemical and clinical improvement to some extent (Fig. 22.5) [43].

22.10 N-Acetylaspartic Aciduria (Aspartoacylase or Aminoacylase 2 Deficiency) (Canavan Disease)

22.10.1 Clinical Presentation

N-Acetylaspartic (NAA) aciduria mostly manifests at 2–4 months of age with head lag, muscular hypotonia and macrocephaly, progressing to marked developmental delay, seizures, optic nerve atrophy, progressive spasticity, and opisthotonic posturing [44]. At birth the head circumference may not be remarkably increased; however, in the majority of cases it increases pathologically after 6 months of age, crossing the percentiles with obvious macrocephaly by 1 year. In the second year of life seizures often develop, together with irritability and sleep disturbance. Muscular hypotonia gives way to spasticity reminiscent of cerebral palsy. Impaired chewing and swallowing, problems with gastro-oesophageal reflux, vomiting and aspiration can result in recurrent infections and failure to thrive.

The most consistent findings on MRI studies are diffuse abnormalities of white matter [45]. Although not always present and not uniform, MRI usually shows symmetric diffuse low signal intensity on T_1-weighted images and high signal intensity on T_2-weighted images (Fig. 22.4).

The neuropathology of Canavan disease is characterised by a progressive loss of myelinated arcuate fibres [45]. Detailed histopathological descriptions at autopsy have elucidated that white matter is characteristically soft and gelatinous. The spongy or vacuolisation changes are clearly seen in the lower layers of the grey matter and in the subcortical white matter, with the more central white matter relatively spared.

Most patients follow the disease course described above, which is also termed the infantile form. Rare clinical

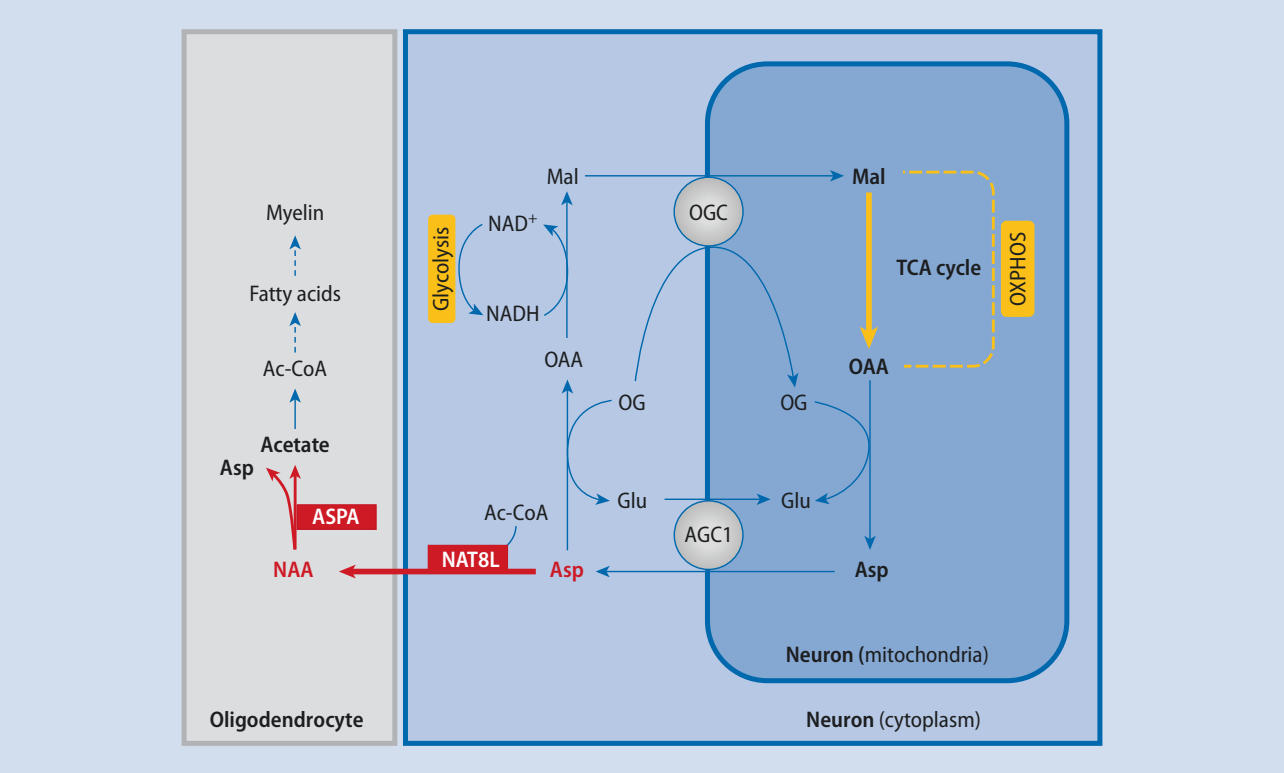

Fig. 22.5 Cerebral *N*-acetylaspartate (NAA) metabolism. The discrepant compartmentation of NAA synthesis by L-aspartate *N*-acetyltransferase (NAT8L) in neurons, deficient in individuals with hypoacetylaspartia, and its subsequent hydrolysis by aspartoacylase (ASPA) in oligodendrocytes, deficient in individuals with Canavan disease, is a mechanism for channeling NAA-associated acetate from neurons to oligodendrocytes, providing a major substrate for myelin synthesis. NAA metabolism is coupled to the cerebral malate (Mal)-aspartate (Asp) shuttle, which affects the redox status of cytoplasm and mitochondria and thus regulates energy production in these compartments. Maintaining a low ratio of NADH to NAD^+ in the cytoplasm, and a high ratio in the mitochondria, provides a driving force for the respiratory chain (OXPHOS) in mitochondria, whereas it favors glycolysis in the cytoplasm. Ac-CoA acetyl-CoA, AGC1 aspartate–glutamate carrier 1, Glu glutamate, OAA oxaloacetate, OG 2-oxoglutarate, OGC oxoglutarate carrier, TCA tricarboxylic acid

variants with different disease courses have been described as congenital, i.e. presenting at or shortly after birth, or as juvenile forms, i.e. presenting after 5 years of age.

22.10.2 Metabolic Derangement

The disease is caused by aspartoacylase (aminoacylase 2) deficiency leading to the accumulation of NAA in brain, CSF, plasma, and urine. In the brain, aspartoacylase is exclusively located in oligodendrocytes hydrolysing its natural substrate NAA, which is formed in neurons from L-aspartate and L-acetate (Fig. 22.5). Defective NAA catabolism is thought to result in reduced brain acetate levels and myelin lipid synthesis. This has been demonstrated in aspartoacylase-deficient mice showing a 30% decrease in total myelin lipids at the time of peak postnatal myelination in the brain [46]. Besides acetate depletion, NAA may also act as an efflux molecular water pump between neurons and oligodendrocytes enabling the removal of neuronal metabolic water produced by glucose oxidation. Decreased NAA catabolism might result in osmotic dysregulation of the brain and, subsequently, spongiform leukodystrophy [47].

22.10.3 Genetics

N-Acetylaspartic aciduria is an autosomal recessive disease caused by bi-allelic variations in *ASPA*. It is a pan-ethnic disease with a much higher frequency among Ashkenazi Jews, most of whom carry two specific mutations, a missense mutation, p.Glu285Ala, accounting for 84% of mutant alleles, and a nonsense mutation, p.Tyr231X, accounting for 13% [48]; the frequency of these two mutations makes carrier screening possible. In non-Jewish patients the mutations are diverse and mostly private.

22.10.4 Diagnostic Tests

The diagnosis is established by determining NAA in the urine by organic acid analysis. Hundredfold elevations are pathognomonic but the disorder should be confirmed by molecular tests and/or enzyme analysis.

22.10.5 Treatment and Prognosis

No effective treatment exists for *N*-acetylaspartic aciduria. Lithium citrate, which induces a mild decrease in brain NAA levels of affected children, is safe but not clinically effective [49]. Because acetate in the form of acetyl-CoA is a building block for lipids, it has been proposed that dietary acetate supplementation with glyceryl triacetate might be a therapeutic option. Although the results of a low-dose safety study were published, the therapeutic efficacy of glyceryl triacetate remains unproven [50]. Recent AAV-ASPA gene therapy studies in aspartoacylase-deficient mice aim to target astro- or oligodendroglia, redirecting NAA metabolism in the brain and normalizing myelination [51]. It remains to be elucidated whether this innovative strategy can be translated to a disease-changing therapy for affected individuals with Canavan disease.

The prognosis for most affected individuals remains very poor, with death usually occurring in the first decade of life although there may be survival into the second decade in a (near) vegetative state.

22.11 Aminoacylase 1 Deficiency

Aminoacylase 1 deficiency is a rare disease with less than 20 patients reported. The clinical relevance of this disorder has not yet been fully elucidated. Although initially considered a non-disease, some patients present with a heterogeneous clinical spectrum including intellectual and motor disability, delayed speech development, muscular hypotonia, and autistic features. However, since detection of aminoacylase 1 deficiency was part of selective screening in symptomatic patients with suspected metabolic disease, a strong selection bias of this cohort is likely.

N-Acetylation of a protein extends its half-life; 50–80% of proteins show formylated or acetylated *N*-termini. Free amino acids can be recycled after protein breakdown by hydrolysis of N-acetylated amino acids using aminoacylases. Aminoacylase 1 catalyzes the release of free amino acids from a variety of *N*-acetylated precursors – except for *N*-acetylaspartate (▶ Sect. 22.10). It has a high tissue-specific activity in kidney and brain. Enzyme deficiency results in increased formation and urinary excretion of acetylated amino acids [52].

Aminoacylase 1 deficiency is an autosomal recessive disorder caused by homozygous or compound heterozygous mutations in *ACY1* [52].

22.11.1 Diagnostic Tests

Elevated urinary exretion of *N*-acetylated amino acids including derivatives of methionine, glutamine, alanine, leucine, glycine, valine, and isoleucine can be detected by gas chromatography/mass spectrometry or NMR spectroscopy [52]. Decreased aminoacylase 1 activity in lymphoblasts or bi-allelic variations of *ACY1* confirms the diagnosis.

22.11.2 Treatment and Prognosis

Since the clinical significance of aminoacylase 1 deficiency remains unclear, there is no clear-cut indication for treatment. Studies systemically evaluating the effect of metabolic treatment are pending. The prognosis of affected individuals is likely to be favourable.

22.12 Hypoacetylaspartia (L-Aspartate N-Acetyltransferase Deficiency)

A single patient with hypoacetylaspartia has been described with marked developmental delay and secondary microcephaly with truncal ataxia, seizures, and behavioural abnormalities on follow-up, in whom ^{1}H-MRS had revealed the absence of NAA signal [53]. A defect of L-aspartate N-acetyltransferase, the enzyme that is required for the synthesis of NAA, was suspected, and a neuron-specific protein, NAT8L, encoded by *NAT8L*, was found to be responsible for NAA synthesis, and was mutated in the patient [54] (◘ Fig. 22.5).

22.13 Malate-Aspartate Shuttle Defects

Recently, defects in 5 of the 6 components of the malate-aspartate shuttle were described. They are presented in ▶ Chap. 11.

References

1. Hoffmann GF, Gibson KM, Trefz FK et al (1994) Neurological manifestations of organic acid disorders. Eur J Pediatr 153:S94–S100
2. Dancis J, Hutzler J, Ampola MG et al (1983) The prognosis of hyperlysinemia: an interim report. Am J Hum Genet 35:438–442
3. Sacksteder KA, Biery BJ, Morell JC et al (2000) Identification of the α-aminoadipic semialdehyde synthase gene, which is defective in familial hyperlysinemia. Am J Hum Genet 66:1736–1743
4. Saudubray JM, Rabier D (2007) Biomarkers identified in inborn errors of lysine, arginine and ornithine. J Nutr 137:1669S–1672S
5. Goodman SI, Browder JA, Hiles RA, Miles BS (1972) Hydroxylysinemia. A disorder due to a defect in the metabolism of free hydroxylysin. Biochem Med 6:344–354
6. Przyrembel H, Bachmann D, Lombeck I et al (1975) Alpha-ketoadipic aciduria, a new inborn error of lysine metabolism; biochemical studies. Clin Chim Acta 58:257–269
7. Xu WY, Gu MM, Sun LH et al (2012) A nonsense mutation in DHTKD1 causes Charcot-Marie-tooth disease type 2 in a large Chinese pedigree. Am J Hum Genet 91:1088–1094
8. Leandro J, Dodatko T, Aten J et al (2020) DHTKD1 and OGDH display substrate overlap in cultured cells and form a hybrid 2-oxo acid dehydrogenase complex in vivo. Hum Mol Genet:ddaa037. https://doi.org/10.1093/hmg/ddaa037. [Epub ahead of print]
9. Danhauser K, Sauer SW, Haack TB et al (2012) DHTKD1 mutations cause 2-aminoadipic and 2-oxoadipic aciduria. Am J hum genet 91:1082–1087; Strauss KA, Williams KB, Carson VJ, et al. (2020) Glutaric acidemia type 1: treatment and outcome of 168 patients over three decades. Mol Genet Metab S1096-7192(20)30198-0. https://doi.org/10.1016/j.ymgme.2020.09.007. Online ahead of print
10. Strauss KA, Williams KB, Carson VJ, Poskitt L, Bowser LE, Young M, Robinson DL, Hendrickson C, Beiler K, Taylor CM, Haas-Givler B, Hailey J, Chopko S, Puffenberger EG, Brigatti KW, Miller F, Morton DH (2020) Glutaric acidemia type 1: Treatment and outcome of 168 patients over three decades. Mol Genet Metab 131(3):325–340. https://doi.org/10.1016/j.ymgme.2020.09.007. Epub 2020 Oct 4. PMID: 33069577
11. Kölker S, Garbade SF, Greenberg CR et al (2006) Natural history, outcome and treatment efficacy in children and adults with glutaryl-CoA dehydrogenase deficiency. Pediatr Res 59:840–847
12. Harting I, Neumaier-Probst E, Seitz A et al (2009) Dynamic changes of striatal and extrastriatal abnormalities in glutaric aciduria type I. Brain 132:1764–1782
13. Morris AAM, Hoffmann GF, Naughten ER et al (1999) Glutaric aciduria and suspected child abuse. Arch Dis Childh 80:404–405
14. Strauss KA, Donelly P, Wintermark M (2010) Cerebral haemodynamics in patients with glutaryl-coenzmye a dehydrogenase deficiency. Brain 133:76–92
15. Heringer J, Boy SPN, Ensenauer R et al (2010) Use of guidelines improves the outcome in glutaric aciduria type I. Ann Neurol 68:743–752
16. Boy N, Mengler K, Thimm et al (2018) Newborn screening: a disease-changing intervention for glutaric aciduria type 1. Ann Neurol 83:970–979
17. Harting I, Boy N, Heringer J et al (2015) (1)H-MRS in glutaric aciduria type 1: impact of biochemical phenotype and age on the cerebral accumulation of neurotoxic metabolites. J Inherit Metab Dis 38:829–838
18. Serrano Russi A, Donoghue S, Boneh A, Manara R, Burlina AB, Burlina AP (2018) Malignant brain tumors in patients with glutaric aciduria type I. J Inherit Metab Dis 125:276–280
19. Sauer SW, Okun JG, Schwab MA et al (2005) Bioenergetics in glutaryl-coenzyme a dehydrogenase deficiency: a role for glutaryl-coenzyme A. J Biol Chem 280:2180–21836
20. Sauer SW, Okun JG, Fricker G et al (2006) Intracerebral accumulation of glutaric and 3-hydroxyglutaric acids secondary to limited flux across the blood-brain barrier constitutes a biochemical risk factor for neurodegeneration in glutaryl-CoA dehydrogenase deficiency. J Neurochem 97:899–910
21. Schmiesing J, Storch S, Dörfler AC et al (2018) Disease-linked glutarylation impairs function and interactions of mitochondrial proteins and contributions to mitochondrial heterogeneity. Cell Rep 24:2946–2956
22. Goodman SI, Stein DE, Schlesinger S et al (1998) Glutaryl-CoA dehydrogenase mutations in glutaric acidemia (type I): review and report of thirty novel mutations. Hum Mutat 12:141–144
23. Baric I, Wagner L, Feyh P et al (1999) Sensitivity and specificity of free and total glutaric and 3-hydroxyglutaric acids measurements by stable isotope dilution assays for the diagnosis of glutaric aciduria type I. J Inherit Metab Dis 22:867–882
24. Lindner M, Kölker S, Schulze A et al (2004) Neonatal screening for glutaryl-CoA dehydrogenase deficiency. J Inherit Metab Dis 27:851–859
25. Kölker S, Christensen E, Leonard JV et al (2011) Diagnosis and management of glutaric aciduria type I--revised recommendations. J Inherit Metab Dis 34:677–694
26. Boy N, Mühlhausen C, Maier EM et al (2017) Proposed recommendations for diagnosing and managing individuals with glutaric aciduria type I: second revision. J Inherit Metab Dis 40:75–101
27. Knerr I, Zschocke J, Trautmann U et al (2002) Glutaric aciduria type III: a distinctive non-disease. J Inherit Metab Dis 25:483–490
28. Mailaire S, van Schaftingen E, Veiga-da-Cunha M (2014) C7orf10 encodes succinate-hydroxymethylglutarate CoA-transferase, the enzyme that converts glutarate to glutaryl-CoA. J Inherit Metab Dis 37:13–19
29. Sherman EA, Strauss KA, Tortorelli S et al (2008) Genetic mapping of glutaric aciduria, type 3, to chromosome 7 and identification of mutations in C7orf10. Am J Hum Genet 83:604–609
30. Barth PG, Hoffmann GF, Jaeken J et al (1993) L-2-Hydroxyglutaric acidemia: clinical and biochemical findings in 12 patients and preliminary report on L-2-hydroxyacid dehydrogenase. J Inherit Metab Dis 16:753–761
31. Moroni I, Bugiani L, D'Incerti C et al (2004) L-2-Hydroxyglutaric aciduria and brain malignant tumors: a predisposing condition? Neurology 62:1882–1884
32. Rzem R, Veiga-da-Cunha M, Noel G et al (2004) A gene encoding a putative FAD-dependent L-2-hydroxyglutarate dehydrogenase is mutated in L-2-hydroxyglutaric aciduria. Proc Natl Acad Sci U S A 101:16849–16854
33. Hoffmann GF, Jakobs C, Holmes B et al (1995) Organic acids in cerebrospinal fluid and plasma of patients with L-2-hydroxyglutaric aciduria. J Inherit Metab Dis 18:189–193
34. Rzem R, Vincent MF, Schaftingen VE et al (2007) L-2-Hydroxyglutaric aciduria, a defect of metabolite repair. J Inherit Metab Dis 30:681–689

35. Topcu M, Jobard F, Halliez S et al (2004) L-2-Hydroxyglutaric aciduria: identification of a mutant gene C14orf160, localised on chromosome 14q22.1. Hum Mol Genet 13:2803–2811
36. Samuraki M, Komai M, Hasegawa Y et al (2008) A successfully treated adult patient with L-2-hydroxyglutaric aciduria. Neurology 70:1051–1052
37. Struys EA, Salomons GS, Achouri Y et al (2005) Mutations in the D-2-hydroxyglutarate dehydrogenase gene cause D-2-hydroxyglutaric aciduria. Am J Hum Genet 76:358–360
38. Kranendijk M, Struys EA, Schaftingen VE et al (2010) IDH2 mutations in patients with D-2-hydroxyglutaric aciduria. Science 330:336
39. Wang F, Travins J, Lin Z et al (2016) A small molecule inhibitor of mutant IDH2 rescues cardiomyopathy in a D-2-hydroxyglutaric aciduria type II mouse model. J Inherit Metab Dis 39:807–820
40. Muntau AC, Röschinger W, Merkenschlager A et al (2000) Combined D-2 and L-2-hydroxyglutaric aciduria with neonatal onset encephalopathy: a third biochemical variant of 2-hydroxyglutaric aciduria? Neuropediatrics 31:137–140
41. Chaouch A, Porcelli V, Cox D et al (2014) Mutations in the mitochondrial citrate carrier SLC25A1 are associated with impaired neuromuscular transmission. J Neuromuscul Dis 1:75–90
42. Nota B, Struys EA, Pop A et al (2013) Deficiency in SLC25A1, encoding the mitochondrial citrate carrier, causes combined D-2- and L-2-hydroxyglutaric aciduria. Am J Human Genet 92:627–631
43. Mühlhausen C, Salomons GS, Lukacs Z et al (2014) Combined D2−/L2-hydroxyglutaric aciduria (SLC25A1 deficiency): clinical course and effects of citrate treatment. J Inherit Metab Dis 37:775–781
44. Matalon R, Michals K, Kaul R (1995) Canavan disease: from spongy degeneration to molecular analysis. J Pediatr 127:511–517
45. Brismar J, Brismar G, Gascon G, Ozand P (1990) Canavan disease: CT and MR imaging of the brain. Am J Neuroradiol 11:805–810
46. Madhavarao CN, Arun P, Moffett JR et al (2005) Defective N-acetylaspartate catabolism reduces brain acetate levels and myelin lipid synthesis in Canavan's disease. PNAS 102:5221–5226
47. Baslow MH (2002) Evidence supporting a role for N-acetyl-l-aspartate as a molecular water pump in myelinated neurons in the central nervous system. An analytical review Neurochem Int 40:295–300
48. Matalon R, Michals-Matalon K (1998) Molecular basis of Canavan disease. Eur J Paediatr Neurol 2:69–76
49. Assadi M, Janson C, Wan DJ et al (2010) Lithium citrate reduces excessive intracerebral N-acetylaspartate in Canavan disease. Eur J Paediatr Neurol 14:354–359
50. Madhavarao CN, Arun P, Anikster Y et al (2009) Glyceryl triacetate for Canavan disease: a low-dose trial in infants and evaluation of a higher dose for toxicity in the tremor rat model. J Inherit Metab Dis 32:640–650
51. Gessler DJ, Li D, Xu H et al (2017) Redirecting N-acetylaspartate metabolism in the central nervous system normalizes myelination and rescues Canavan disease. JCI Insight 2:e90807. https://doi.org/10.1172/jci.insight.90807
52. Sass JO, Mohr V, Olbrich H et al (2006) Mutations of ACY1, the gene encoding aminoacylase 1, cause a novel inborn error of metabolism. Am J Hum Genet 78:401–409
53. Bolthauser E, Schmitt B, Wevers RA et al (2004) Follow-up of a child with hypoacetylaspartia. Neuropediatrics 35: 255–258
54. Wiame E, Tyteca D, Pierrot N et al (2010) Molecular identification of aspartate N-acetyltransferase and its mutation in hypoacetylaspartia. Biochem J 425:127–136