Branched-chain Organic Acidurias/Acidaemias

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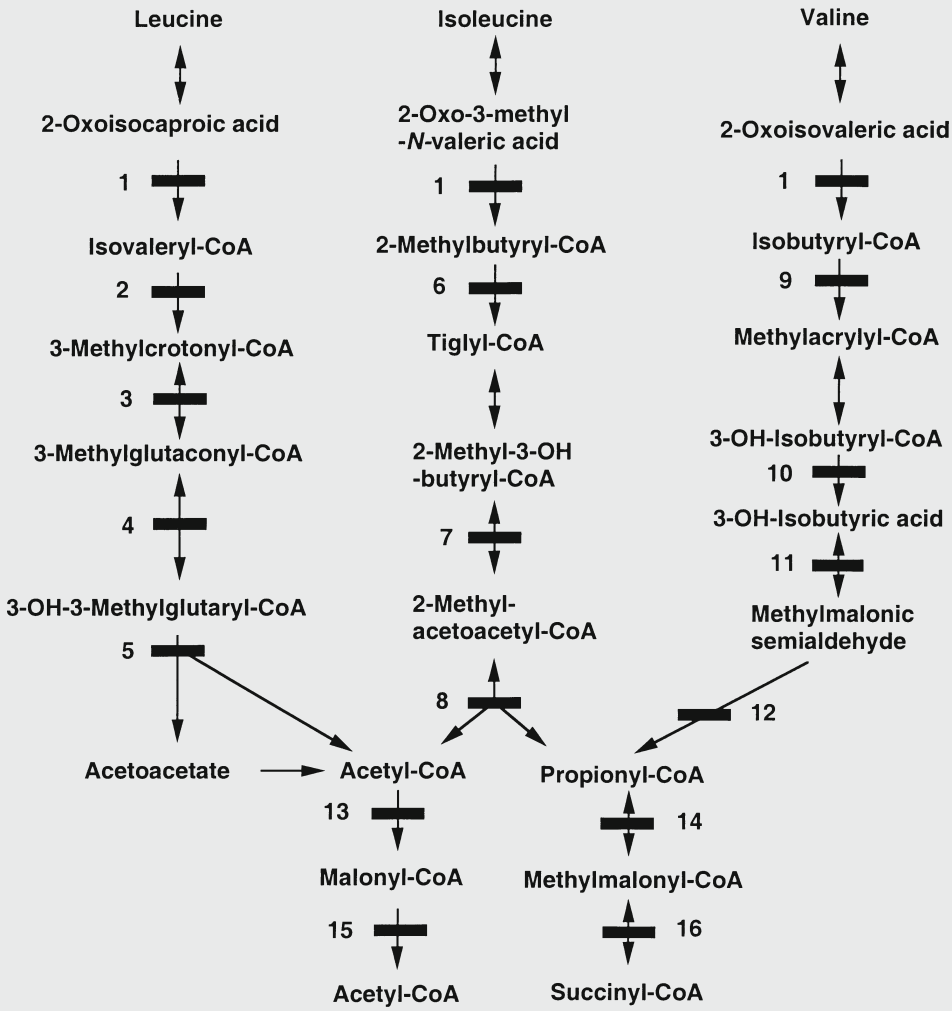
Catabolism of Branched-chain Amino Acids

The three essential branched-chain amino acids (BCAAs), leucine, isoleucine and valine, are initially catabolised by a common pathway (◘ Fig. 19.1). The first reaction, which occurs primarily in muscle, involves reversible transamination to 2-oxo- (or keto)acids and is followed by oxidative decarboxylation to coenzyme A (CoA) derivatives by branched-chain oxo- (or keto)acid dehydrogenase (BCKD). The latter enzyme is similar in structure to pyruvate dehydrogenase (◘ Fig. 12.2). Subsequently, the degradative pathways of BCAA diverge. Leucine is catabolised to acetoacetate and acetyl-CoA, which enters the Krebs cycle. The final step in the catabolism of isoleucine involves cleavage into acetyl-CoA and propionyl-CoA, which also enters the Krebs cycle via conversion into succinyl-CoA. Valine is also ultimately metabolised to propionyl-CoA. Methionine, threonine, fatty acids with an odd number of carbons, the side chain of cholesterol, and bacterial gut activity also contribute to the formation of propionyl-CoA.



◘ **Fig. 19.1.** Pathways of branched-chain amino acid catabolism. **1**, Branched-chain 2-ketoacid dehydrogenase complex; **2**, isovaleryl-coenzyme A (CoA) dehydrogenase; **3**, 3-methylcrotonyl-CoA carboxylase; **4**, 3-methylglutaconyl-CoA hydratase; **5**, 3-hydroxy-3-methylglutaryl-CoA lyase; **6**, short-/branched-chain acyl-CoA dehydrogenase; **7**, 2-methyl-3-hydroxybutyryl-CoA dehydrogenase; **8**, 2-methylacetoacetyl-CoA thiolase; **9**, isobutyryl-CoA dehydrogenase; **10**, 3-hydroxyisobutyryl-CoA deacylase; **11**, 3-hydroxyisobutyric acid dehydrogenase; **12**, methylmalonic semialdehyde dehydrogenase; **13**, acetyl-CoA carboxylase (cytosolic); **14**, propionyl-CoA carboxylase; **15**, malonyl-CoA decarboxylase; **16**, methylmalonyl-CoA mutase. Enzyme defects are indicated by *solid bars*

Branched-chain organic acidurias or organic acidaemias are a group of disorders that result from an abnormality of specific enzymes involving the catabolism of branched-chain amino acids (BCAAs). Collectively, the most commonly encountered are maple syrup urine disease (MSUD), isovaleric aciduria (IVA), propionic aciduria (PA) and methyl malonic aciduria (MMA). They can present clinically as a severe neonatal-onset form of metabolic distress, an acute and intermittent late-onset form, or a chronic progressive form presenting as hypotonia, failure to thrive, and developmental delay. Other rare disorders involving leucine, isoleucine, and valine catabolism are 3-methylcrotonyl glycinuria, 3-methylglutaconic (3-MGC) aciduria, short-/branched-chain acyl-CoA dehydrogenase deficiency, 2-methyl-3-hydroxybutyryl-CoA dehydrogenase deficiency, isobutyryl-CoA dehydrogenase deficiency, 3-hydroxyisobutyric aciduria, and malonic aciduria. All these disorders can be diagnosed by identifying acylcarnitines and other organic acid compounds in plasma and urine by gas chromatography-mass spectrometry (GC-MS) or tandem MS and all can be detected by newborn screening using tandem MS.

19.1 Maple Syrup Urine Disease, Isovaleric Aciduria, Propionic Aciduria, Methylmalonic Aciduria

19.1.1 Clinical Presentation

Children with maple syrup urine disease (MSUD), isovaleric aciduria (IVA), propionic aciduria (PA), or methylmalonic aciduria (MMA) have many clinical and biochemical symptoms in common. There are three main clinical presentations:

1. A severe neonatal-onset form with acute metabolic decompensation and neurological distress.
2. An acute, intermittent, late-onset form also with recurrent episodes of metabolic decompensation.
3. A chronic, progressive form presenting as hypotonia, failure to thrive, and developmental delay.

In addition, prospective data gathered by newborn screening programmes, mainly using tandem MS and the systematic screening of siblings of subjects with an abnormal newborn screening result, have demonstrated the relative frequency of asymptomatic forms.

■ Severe Neonatal-onset Form

■■ General Presentation

The general presentation of this form is that of a toxic encephalopathy with either ketosis or ketoacidosis (type I or II in the classification of neonatal inborn errors of metabolism in ► Chapter 1). An extremely evocative clinical setting is that of a full-term baby born after a normal pregnancy and delivery who, after an initial symptom-free period, undergoes relentless deterioration with no apparent cause and is unresponsive to symptomatic therapy. The interval between birth and clinical symptoms may range from hours to weeks, depending on the nature of the defect, and may be related to the timing of the sequential catabolism of carbohydrates, proteins, and fats. Typically, the first signs are poor feeding and drowsiness, followed by unexplained progressive coma. There may be cerebral oedema with a bulging fontanelle, arousing suspicion of a central nervous system (CNS) infection. At a more advanced stage, neurovegetative dysregulation with respiratory distress, hiccups, apnoeas, bradycardia, and hypothermia may appear. In the comatose state, most patients have characteristic changes in muscle tone and exhibit involuntary movements. Generalised hypertonic episodes with opisthotonus, boxing or pedalling movements, and slow limb elevations, spontaneously or upon stimulation, are frequently observed. Another pattern is that of axial hypotonia and limb hypertonia with large-amplitude tremors and myoclonic jerks, which are often mistaken for convulsions. In contrast, true convulsions occur late and inconsistently. The electroencephalogram may show a burst-suppression pattern. In addition to neurological signs, patients may present with dehydration and mild hepatomegaly.

■■ Specific Signs

Maple Syrup Urine Disease. Concomitantly with the onset of the symptoms, the patient emits an intense (sweet, malty, caramel-like) maple-syrup-like odour. In general, neonatal (classic) MSUD does not lead to pronounced abnormalities seen on routine laboratory tests. Patients are not severely dehydrated, have no metabolic acidosis, no hyperammonaemia or only a slight elevation (<130 μmol/l), and no blood lactate accumulation, and the blood cell count is normal. The main laboratory abnormalities are greatly increased branched-chain amino acids (BCAAs) in plasma and the presence of 2-ketoacids rapidly detectable in urine with organic acid analysis or with the 2,4-dinitrophenylhydrazine (DNPH) test.

Isovaleric Aciduria, Propionic Aciduria and Methylmalonic Aciduria. In contrast to MSUD, dehydration is a frequent finding in patients with IVA, PA, or MMA, and moderate hepatomegaly may be observed. They have metabolic acidosis (pH <7.30) with increased anion gap and ketonuria (Acetest 2-3 positive). However, ketoacidosis can be moderate and is often responsive to symptomatic therapy. Hyperammonaemia is a constant finding. When the ammonia level is very high (>500 μmol/l) it can induce respiratory alkalosis and lead to the erroneous diagnosis of a urea-

cycle disorder. Moderate hypocalcaemia (<1.7 mmol/l) and hyperlactataemia (3-6 mmol/l) are frequent findings. The physician should be wary of attributing marked neurological dysfunction merely to these. Blood glucose can be normal, reduced, or elevated. When blood glucose level is very high (20 mmol/l) and is associated with glucosuria, ketoacidosis, and dehydration it may mimic neonatal diabetes. Neutropenia, thrombocytopenia, nonregenerative anaemia, and pancytopenia can occur and are frequently confused with sepsis. Among these disorders, IVA is easily recognized by its unpleasant sweaty feet odour.

In some cases, the combination of vomiting, abdominal distension, and constipation may suggest gastrointestinal obstruction. Cerebral haemorrhages have been described in a few neonates, a complication that may be linked to inappropriate correction of acidosis and may explain some poor neurological outcomes.

■ Acute Intermittent Late-onset Form

In approximately one fourth of the patients the disease presents after a symptom-free period, which is commonly longer than 1 year and sometimes even lasts until adolescence or adulthood. Recurrent attacks may be frequent and, between them, the child may seem entirely normal. Onset of an acute attack may arise during catabolic stress such as can occur with infections or following increased intake of protein-rich foods, but sometimes there may be no overt cause.

■■ Neurological Presentation

Recurrent attacks of either coma or lethargy with ataxia are the main presentations of these acute late-onset forms. The most frequent variety of coma is that presenting with ketoacidosis, but in exceptional cases this may be absent.

Hypoglycaemia may occur in patients with MSUD, while in the other disorders blood glucose levels are low, normal, or high. Mild hyperammonaemia can be present in IVA, PA, and MMA patients. Although most recurrent comas are not accompanied by focal neurological signs, some patients may present with acute hemiplegia, hemianopsia, or symptoms and signs of cerebral oedema mimicking encephalitis, a cerebrovascular accident, or a cerebral tumour. These acute neurological manifestations have frequently been preceded by other premonitory symptoms that had been missed or misdiagnosed. They include acute ataxia, unexplained episodes of dehydration, persistent and selective anorexia, chronic vomiting with failure to thrive, hypotonia, progressive developmental delay and abnormal behaviour.

■■ Hepatic Forms

Some patients may present with a Reye syndrome-like illness characterised by onset of coma, cerebral oedema, hepatomegaly, liver dysfunction, hyperammonaemia and even macro- or microvesicular fatty infiltration of the liver. These observations emphasise the importance of complete metabolic investigations in such situations.

■■ Haematological and Immunological Forms

Severe haematological manifestations are frequent, mostly concomitant with ketoacidosis and coma, and are sometimes the presenting problem. Neutropenia is regularly observed in both neonatal and late-onset forms of IVA, PA and MMA. Thrombocytopenia occurs mostly in infancy, and anaemia occurs only in the neonatal period. Various cellular and humoural immunological abnormalities have been described in patients presenting with recurrent infections, leading to erroneous diagnosis and management.

■ Chronic, Progressive Forms

■■ Gastrointestinal Presentation

Persistent anorexia, chronic vomiting, aversion to protein-rich food, failure to thrive and osteoporosis (evidence of a long-standing GI disturbance) are frequent manifestations. In infants, this presentation is easily misdiagnosed as gastro-oesophageal reflux, cow's milk protein intolerance, coeliac disease, late-onset chronic pyloric stenosis or hereditary fructose intolerance, particularly if these symptoms start after weaning and diversification of food intake. Later in life, recurrent vomiting with ketosis may occur. These patients may remain undetected until an acute neurological crisis with coma leads to the diagnosis.

■■ Chronic Neurological Presentation

Some patients present with severe hypotonia, muscular weakness and poor muscle mass that can simulate congenital neurological disorders or myopathies. Nonspecific developmental delay, progressive psychomotor retardation, dementia, seizures and movement disorders may also be observed during the course of the disease. However, these rather nonspecific findings are rarely the only presenting symptoms [1].

■ Complications

■■ Neurological Complications

Maple Syrup Urine Disease. Acute cerebral oedema is a well-recognised complication in newborn infants with MSUD and encephalopathy. Brain ultrasonography displays a characteristic pattern that may be of help in the diagnosis [2]. In older patients with metabolic decompensation it may cause brain stem compression and unexpected death, particularly following intensive rehydration [3]; it may also develop slowly due to long-standing elevations of BCAAs. Additionally, demyelination can

occur over time in those patients with poor biochemical control and persistently raised BCAAs. The areas most commonly affected are the periventricular white matter of the cerebral hemispheres, the deep cerebellar white matter, the dorsal part of the brain stem, the cerebral peduncles, the dorsal limb of the internal capsule and the basal ganglia. The severity of dysmyelination does not correlate with signs of acute neurotoxicity, and the changes are reversible with appropriate treatment [4, 5]. Acute axonal neuropathy may complicate late-onset decompensation [6].

Propionic Aciduria and Methylmalonic Aciduria. An increasing number of patients with PA and MMA have presented with an acute or progressive extrapyramidal syndrome associated with increased signal within the basal ganglia (mostly the globus pallidus in MMA). The basal ganglia involvement may be due to oedema that evolves to necrosis. In addition, magnetic resonance imaging (MRI) studies indicate cerebral atrophy and delayed myelination [7, 8]. These dramatic complications are arguments for adequate life-long dietary control even if the patient is free of symptoms. Even in well-treated patients with PA who are clinically and metabolically stable, brain lactate is elevated; this might indicate that aerobic oxidation is persistently impaired from elevated intracellular propionic metabolites [8]. Late-onset optic neuropathy with visual dysfunction is another insidious complication [9].

▪▪ Renal Complications

Renal tubular acidosis associated with hyperuricaemia may be an early and presenting sign in some late-onset patients with MMA. This condition partially improves with metabolic control. Chronic renal failure is increasingly recognised in patients older than 10 years [10]. The renal lesion is a tubulo-interstitial nephritis with type-4 tubular acidosis and adaptative changes secondary to the reduced glomerular filtration rate [11]. The course of the disease is usually indolent, but end-stage renal failure may develop, and dialysis and renal transplantation are likely to be necessary by the end of the 2nd decade of life in many patients [12]. If the nephropathy is the complication of a chronic glomerular hyperfiltration secondary to excessive MMA excretion, minimising and deceleration of renal injury may require strict metabolic control.

▪▪ Skin Disorders

Large, superficial desquamation, alopecia, and corneal ulceration may develop in the course of late and severe decompensations in MSUD, PA or MMA. These skin lesions have been described as a staphylococcal scalded-skin syndrome with epidermolysis or as acrodermatitis enteropathica-like syndrome [13]. In many cases, these complications occur together with diarrhoea and can be ascribed to acute protein malnutrition, especially to isoleucine deficiency.

▪▪ Pancreatitis

Acute, chronic or recurrent pancreatitis may complicate this group of organic acidaemias. It has been the presenting illness in patients with late-onset forms of IVA. The pathophysiological mechanism is unknown. However, ketoacidosis is assumed to play a role, as pancreatitis also complicates diabetic ketoacidosis. The condition may be difficult to diagnose and must be considered in the assessment of patients with acute deterioration. However, elevation of serum lipase and amylase alone does not confirm the diagnosis. Pancreatitis, being defined by inflammation on pancreas imaging, implies specific dietary therapy with a low-fat diet. In contrast, isolated hyperamylasaemia and hyperlipasaemia would normalise with the correction of the metabolic status [14].

▪▪ Cardiomyopathy

Cardiomyopathy is one of the major complications in PA and may be responsible for rapid deterioration or death. It may develop as part of an acute decompensation or as a chronic deterioration even in patients who are metabolically stable. Both dilated and hypertrophic types have been reported, with an estimated prevalence of 23% in one cohort [15]. In another cohort, 70% of patients beyond infancy were found to have developed disturbance in cardiac electrophysiology that could contribute to cardiac complications [16]. The mechanism is uncertain but may result from energy deprivation or toxic accumulation. Investigation and follow-up may be useful to prevent irreversible damage and to help in decisions on therapeutic measures, as recovery with renal replacement therapies or with orthotopic liver transplantation has been described in rare cases [15, 17].

19.1.2 Metabolic Derangement

▪ Maple Syrup Urine Disease

MSUD is caused by a deficiency of the branched-chain 2-ketoacid dehydrogenase (BCKD) complex, the second common step in the catabolism of the three BCAAs (◘ Fig. 19.1, enzyme 1). Like the other 2-ketoacid dehydrogenases, BCKD is composed of three catalytic components (◘ Fig. 12.2): a decarboxylase (E1), composed of E1α- and E1β-subunits and requiring thiamine pyrophos-

phate as a coenzyme, a dihydrolipoyl acyltransferase (E2) and a dihydrolipoamide dehydrogenase (E3). A deficiency of the E1 or E2 component can cause MSUD, whereas a deficiency of the E3 component produces a specific syndrome (dihydrolipoamide dehydrogenase [E3] deficiency) with congenital lactic acidosis, branched-chain 2-ketoaciduria and 2-ketoglutaric aciduria (► Chapter 12). However, E3 deficiency, particularly the neonatal-onset forms, may present with lactic acidaemia alone, with elevation of branched-chain amino acids only becoming apparent weeks or months later.

The enzyme defect results in marked increases in the branched-chain 2-ketoacids in plasma, urine and cerebrospinal fluid (CSF). Owing to the reversibility of the initial transamination step, the BCAAs also accumulate. Smaller amounts of the respective 2-hydroxy acids are formed. Alloisoleucine is invariably found in the blood of all classic MSUD patients and in those with variant forms, at least in those still without dietary treatment. This compound is endogenously formed and is a diastereomer of isoleucine.

Among the BCAA metabolites, leucine and 2-ketoisocaproic acid appear to be the most neurotoxic. In MSUD, they are always present in approximately equimolar concentrations in plasma, and may cause acute brain dysfunction when their plasma concentrations rise above 1 mmol/l. Isoleucine and valine are of lesser clinical significance. Their 2-ketoacid-to-amino acid ratios favour the less toxic amino acids, and cerebral symptoms do not occur even when the blood levels of both amino acids are extremely high.

▪ Isovaleric Aciduria

IVA is caused by a deficiency of isovaleryl-CoA dehydrogenase (IVD; ◘ Fig. 19.1, enzyme 2), an intramitochondrial flavoenzyme which, in a similar way to the acyl-CoA dehydrogenases (◘ Fig. 13.1), transfers electrons to the respiratory chain via the electron transfer flavoprotein (ETF)/ETF-ubiquinone oxidoreductase (ETF-QO) system. Deficiencies of the ETF/ETFQO system result in multiple acyl-CoA-dehydrogenase deficiency (MADD; synonym: glutaric aciduria type II) (► Chapter 13).

The enzyme defect results in the accumulation of derivatives of isovaleryl-CoA, including free isovaleric acid, which is usually increased in both plasma and urine (although normal levels have been reported), 3-hydroxyisovaleric acid (3-HIVA) and *N*-isovalerylglycine. This glycine conjugate is the major derivative of isovaleryl-CoA, owing to the high affinity of the latter for glycine *N*-acylase. Conjugation with carnitine (catalysed by carnitine *N*-acylase) results in the formation of isovalerylcarnitine.

▪ Propionic Aciduria

PA is caused by a deficiency of the mitochondrial enzyme propionyl-CoA carboxylase (PCC; ◘ Fig. 19.1, enzyme 14), one of the four biotin-dependent enzymes. PCC is a multimeric protein composed of two different sorts of PCC subunits, α- (which bind biotin) and β-PCC subunits. So far, all patients with isolated PA have been biotin resistant.

PA is characterised by greatly increased concentrations of free propionic acid in blood and urine and the presence of multiple organic acid by-products, among which propionylcarnitine, 3-hydroxypropionate and methylcitrate are the major diagnostic metabolites. The first is formed by acylation to carnitine. The second is formed by either β- or ω-oxidation of propionyl-CoA. Methylcitrate arises by condensation of propionyl-CoA with oxaloacetate, which is catalysed by citrate synthase. During ketotic episodes, 3-HIVA is formed by condensation of propionyl-CoA with acetyl-CoA, followed by chemical reduction. Low concentrations of organic acids derived from a variety of intermediates of the isoleucine catabolic pathway, such as tiglic acid, tiglylglycine, 2-methyl-3-hydroxybutyrate, 3-hydroxybutyrate and propionylglycine, can also be found. Owing to an abnormal biotin metabolism, propionyl-CoA accumulation also occurs in multiple carboxylase deficiency (biotinidase deficiency, holocarboxylase synthetase (HCS) deficiency), resulting in defective activity of all four biotin-dependent carboxylases (► Chapter 27).

▪ Methylmalonic Aciduria

MMA is caused by a deficiency of methylmalonyl-CoA mutase (MCM; ◘ Fig. 19.1, enzyme 16), a vitamin B_{12}-dependent enzyme. Deficient activity of the MCM-apoenzyme leads to MMA: Because the apomutase requires adenosylcobalamin (AdoCbl), disorders that affect AdoCbl formation cause variant forms of MMA (► Chapter 28).

The deficiency of MCM leads to the accumulation of methylmalonyl-CoA, resulting in greatly increased amounts of methylmalonic acid in plasma and urine. Owing to secondary inhibition of PCC, propionic acid also accumulates, and other propionyl-CoA metabolites, such as propionylcarnitine, 3-hydroxypropionic acid, methylcitrate and 3-HIVA, are usually also found in urine. However, some mildly affected or asymptomatic patients, identified through urine organic acids screening in neonates but showing only slightly increased methylmalonic acid in blood and urine, have not shown constant excretion of metabolites derived from propionyl-CoA.

Recently, novel variants of MMA, also characterised by mild MMA, have been identified (◘ below).

Vitamin-B_{12} deficiency must be excluded when excessive urinary methylmalonic acid is found, particularly in

a breast-fed infant whose mother either is a strict vegetarian or suffers from subclinical pernicious anaemia.

- **Secondary Metabolic Disturbances Common to PA and MMA**

The accumulation of propionyl-CoA results in inhibitory effects on various pathways of intermediary metabolism, in increased levels of acylcarnitines (particularly propionyl carnitine) in blood and urine leading to a relative carnitine deficiency and in enhanced synthesis of odd-numbered long-chain fatty acids. Inhibition of various enzymes may explain some features such as hypoglycaemia, hyperlactataemia, hyperammonaemia and hyperglycinaemia. The abnormal ketogenesis that is a major cause of morbidity is not fully understood. Several pathomechanisms (e.g. accumulation of putatively toxic organic acids, inhibition of mitochondrial energy metabolism) have been evoked to explain acute and long-term organ damage [18].

Propionate, essentially in the form of propionyl-CoA, is produced in the body from three main sources: (1) catabolism of the amino acids isoleucine, valine, methionine and threonine, (2) anaerobic fermentation in the gut and (3) mobilisation and oxidation of odd-chain fatty acids during prolonged fasting states. It has been estimated that catabolism of amino acids contributes approximately 50% to the total propionate production, anaerobic gut bacteria, 20% and odd-chain fatty acids, 30% [19]. These data, which are largely from stable isotope turnover studies, are based on a number of unproven assumptions and have not been reproduced in a more systematic manner. They are therefore questionable (for critical reviews, see [12, 20]).

19.1.3 Genetics

- **Maple Syrup Urine Disease**

MSUD is an autosomal-recessive disorder, with an incidence of 1 in 120,000 to 1 in 500,000. It is highly prevalent in the inbred Mennonite population in Pennsylvania, occurring in approximately 1 in 176 newborns. In countries where consanguineous marriages are common the frequency is also higher (about 1 in 50,000 in Turkey). About 75% of those affected suffer from the severe classic form, and the remainder suffer from the milder intermediate or intermittent variants. Over 150 different causal mutations scattered among the three *E1α, E1β* and *E2* genes give rise to either classic or intermediate clinical phenotypes [21].

- **Isovaleric Aciduria**

IVA is an autosomal recessive disorder, with extreme clinical variability for reasons that are unknown. Reported mutations in the *IVD* gene are highly heterogeneous, and generally no phenotype/genotype correlation has been established. However, children with IVA diagnosed by newborn screening and carrying a 932C>T mutant allele can exhibit a milder, potentially asymptomatic phenotype [22].

- **Propionic Aciduria**

PA is an autosomal recessive disorder with an incidence of less than 1 in 100,000. PA can result from mutations in the *PCCA* or *PCCB* genes encoding the α- and β-subunits, respectively, of propionyl-CoA carboxylase.

To date, more than 50 different allelic variations in the *PCCB* gene and more than 30 in the *PCCA* gene have been identified in different populations [23, 24]. Following the introduction of the newborn screening programme in Japan a number of infants with an apparently mild phenotype and the Y435C mutation in the *PCCB* gene have been reported. The natural history of this phenotype is not yet clarified [25]. Particularly in PA, knowledge of the phenotype-genotype correlations may provide important information for the prediction of the metabolic outcome and for the implementation of treatments tailored to individual patients.

- **Methylmalonic Aciduria**

Isolated MMA can be caused by mutations in the *MUT* locus encoding the methylmalonyl CoA mutase (MCM) apoenzyme, or by those in genes required for provision of its cofactor, 5'-deoxyadenosylcobalamin (AdoCbl). Isolated MMA is classified into several genotypic classes and complementation groups. These are designated either mut^- or mut^0 (together termed mut), according to whether there is minimal or no apoenzyme activity, respectively, or cobalamin A or B (Cbl A/B) for cofactor defects. To date more than 50 disease-causing mutations in patients with $mut^{0/-}$ MMA have been identified at the *MUT* locus [26]. MMA is an autosomal recessive disorder. The incidence of both benign and severe forms is about 1 in 50,000. Approximately one half to two thirds of patients have a mutase apoenzyme defect; the remaining patients have cobalamin variants. Genes *MMAA* and *MMAB* for the Cbl A and Cbl B complementation groups have been cloned and deleterious mutations in CblA and CblB patient cell lines, identified [27-29]. It is speculated that the *MMAA* gene product is a component of a transporter or an accessory protein that is involved in the translocation of vitamin B_{12} into mitochondria. The gene product of the *MMAB* gene is a cob(I)alamin adenosyltransferase (see ► Chapter 28 for further details).

Newly described MMA variants include defects in succinyl-CoA synthase and methylmalonyl-CoA epim-

erase. Succinyl-CoA synthase catalyses the conversion of succinyl-CoA to succinate in the Krebs cycle. Its deficiency causes mild MMA, variable lactic acidosis, accumulation of succinyl-carnitine and mitochondrial DNA depletion (► Chapter 15). Succinyl-CoA synthase is composed of an α-subunit (encoded by *SUCLG1*) and two β-subunits (encoded by *SUCLA2* and *SUCLG2*). Several patients with different genetic backgrounds have been found to have mutations in *SUCLA2*, but to date only two families have been reported with mutations in *SUCLG1* [30-34]. The clinical picture in *SUCLA2* patients is highly homogeneous and comprises early-onset encephalomyopathy, dystonia, deafness and Leigh-like MRI abnormalities. Patients with *SUCLG1* mutations are clinically heterogeneous, showing either a severe form with neonatal multiorgan failure and early death or a phenotype similar to that of *SUCLA2* mutation.

A deficiency in methylmalonyl-CoA epimerase has been reported in a subject with mild MMA. This defect has a questionable clinical impact [35, 36].

19.1.4 Diagnostic Tests

Only MSUD can be diagnosed by using plasma amino acid chromatography alone. IVA, PA and MMA are diagnosed by their specific urinary organic acid profiles using GC-MS or abnormal acylcarnitines on tandem MS, while amino acid chromatography displays nonspecific abnormalities, such as hyperglycinaemia and hyperalaninaemia. Owing to acidosis and its impact on glutamine metabolism, hyperammonaemia associated with organic acidurias leads to normal or even low plasma glutamine levels [37, 38]. Whatever the clinical presentation, the diagnosis can be made by sending filter-paper blood specimens, fresh or frozen urine samples or 1- to 2-ml samples of fresh or frozen plasma to an experienced laboratory for analysis. Specific loading tests are not necessary. Newborn screening for this group of organic acidurias can be performed by tandem MS [39-41]. An increased leucine/isoleucine peak in blood spots taken at 24 or 36 h of age requires immediate notification to the sender. The abnormal acylcarnitine found in PA and MMA is propionylcarnitine (C3-carnitine) and that in IVA is isovalerylcarnitine (C5-carnitine) [40].

Enzymatic studies are useful for diagnostic confirmation. Around the 14th week of gestation (2nd trimester), reliable and rapid prenatal diagnosis of IVA, PA, and MMA can be performed by the direct measurement of metabolites in amniotic fluid using GC-MS, stable-isotope dilution techniques, or tandem MS. First-trimester diagnosis using direct enzyme assay or assays of the DNA in families in which the mutations are known can be performed in fresh or cultured chorionic villi. This can also be done in cultured amniotic cells taken in the 2nd trimester. Prenatal diagnosis of MSUD relies exclusively on enzyme assays in chorionic villi or in cultured amniocytes and on mutational analysis.

19.1.5 Treatment and Prognosis

CNS dysfunction can be prevented or at least minimised by early diagnosis and emergency treatment. Neonatal-onset forms frequently require early toxin removal (► Chapter 4). Thereafter dietary restriction, which is necessary to limit the production of organic acids and their metabolites and other specific treatments, is required both for survivors of the early-onset forms and for those with late-onset disease. For both groups it is essential that episodes of metabolic decompensation are recognised and treated sufficiently early; parents must be taught to recognise early warning signs and manage their child appropriately.

▪ Principles of Long-term Dietary Treatment

Long-term dietary treatment is aimed at reducing the accumulation of toxic metabolites while, at the same time, maintaining normal physical development and nutritional status and preventing catabolism. Some patients tolerate normal foods; others need only minimal restriction or can even regulate the diet themselves. However, many need very specific food allowances, implying stringent dietary restrictions that will be necessary for life.

The cornerstone of treatment is the limitation of one or more essential amino acids which, if present in excess, are either toxic or precursors of organic acids. Precise prescriptions are established for the daily intake of amino acids, protein and energy. The diet must provide the recommended daily allowance (RDA) and the estimated safe and adequate daily dietary intakes of minerals and vitamins and follow the principles of paediatric dietetics [42].

▪ Protein/Amino Acid Prescriptions

Requirements for BCAAs and protein vary widely from patient to patient and in the same patient, depending on the nature and severity of the disorder, other therapies prescribed (stimulation of an alternate pathway), growth rate, state of health and feeding difficulties. Individual requirements must be estimated for each child by frequent monitoring of clinical and metabolic status. The balance between protein malnutrition and metabolic disequilibrium can be difficult to maintain in severe PA and MMA

and needs to be kept under regular review, especially after an acute metabolic decompensation or after a change in the diet.

Within this group of organic acidurias, only in MSUD is the diet directly related to the intake of an amino acid, which is leucine in milligram amounts. Natural protein, which contains leucine, must be severely restricted in an age-dependent manner to only one tenth to a half of the normal recommended daily requirement. Consequently, in order to meet the protein RDA for the patient's age, a large supplement of BCAA-free amino acid mixture as a protein substitute is necessary. In IVA it is sufficient to restrict natural protein to the recommended minimum daily requirements or just somewhat more; a special amino acid mixture free of leucine is rarely needed. In neonatal PA and MMA dietary protein is generally restricted to the adequate age-related safe levels. Restriction of specific amino acids has not been proved useful. Although controversial, a limited, relatively small, amount of an amino acid mixture free of valine, isoleucine, methionine and threonine can be added to the diet to supply additional nitrogen and other essential and nonessential amino acids in order to promote a protein-sparing anabolic effect [43].

The prescribed amounts of leucine or natural protein are provided by natural foods. Breast milk or standard infant formula is used in young infants. For toddlers and children solids are introduced, using serving lists and lists of amino acid content in foods. In all protein-restricted diets, high-protein foods (eggs, meat, dairy products), apart from milk, are generally avoided, since the lower percentage of amino acids in vegetable protein (compared with that in animal protein) makes it easier to satisfy the appetite of children.

■ Energy and Micronutrient Prescriptions

Energy requirements vary widely and may be greater than normal to ensure that essential amino acids are not degraded to provide energy or nitrogen for the biosynthesis of nitrogenous metabolites. Reduction of energy intake below the individual's requirements results in a decreased growth rate and a metabolic imbalance. The energy requirement is met through natural foods, special amino acid formulas and additional fat and carbohydrates from other sources, including protein-free modular feeds. Distribution of energy intake from protein, carbohydrates and lipids should approach the recommended percentages. The diet must be assessed for minerals, vitamins and trace elements and, if incomplete, supplemented with an appropriate commercial preparation. Hyperosmolarity of formulas should be avoided by offering sufficient fluids.

■ Evaluation of Clinical and Nutritional Status

This comprises regular evaluation of weight, length and head circumference, which should all follow growth percentiles appropriate for the patient's age. Nutritional status is also judged by blood cell count, haemoglobin and haematocrit, plasma protein and albumin, iron and ferritin, evaluation of calcium/phosphate metabolism and plasma amino acid profile. The metabolic and nutritional statuses are both evaluated weekly during the 1st month of therapy, once a month during the 1st year, and later every 3-6 months. In patients treated with a low-protein diet without an added amino acid mixture, measurement of urea excretion is an easy means to evaluate anabolism [43]. Regular assessment of developmental progress provides the opportunity for psychological support, as social and emotional needs are major elements of the overall therapy of the affected child and of the family's wellbeing.

■ Specific Adjustments

■■ Maple Syrup Urine Disease

Acute Phase Management in the Newborn. Exogenous toxin removal procedures such as haemodialysis and haemofiltration together with high-energy dietary treatment are usually advised for the reversal of acute metabolic decompensation in symptomatic newborns with the classic form of MSUD [44]. With these measures the plasma leucine level is reduced to 1 mmol/l or less within hours. During the recovery interval, oral intake of BCAA-free formula (tube feeding) should be started early and BCAA intake adjusted according to the plasma levels, which are monitored daily until the optimal equilibrium is attained. During this stage, plasma concentrations of valine and isoleucine may fall below normal and become rate limiting for protein synthesis, a situation which requires generous valine and isoleucine supplements in doses of 300-400 mg/day. Newborn screening for MSUD by tandem MS allows for early diagnosis and intervention and in some cases obviates the need for extracorporeal detoxification. In affected newborns found positive on screening the oral intake of BCAA-free formula (tube feeding) with adequate calorie supply (glucose polymer) and supplementation with isoleucine and valine (300-400 mg/day) can be sufficient to stimulate protein synthesis and to normalise plasma leucine levels within 2-3 days [3, 45].

Long-term Management. Management of MSUD comprises a life-long strict and carefully adjusted semisynthetic diet, as well as acute-phase treatment during episodes of catabolic stress. The dietary treatment of MSUD differs from that of other organic acidurias, since the condition results in elevated plasma BCAA levels. In that

respect MSUD can be regarded as an aminoacidopathy, and the principles of dietary treatment are essentially those that apply to phenylketonuria. The diet consists of measured proportions of BCAA-containing foods (as natural protein) and a synthetic BCAA-free amino acid supplement, which in most preparations also contains the recommended requirements for minerals and vitamins and other essential nutrients. Additional fat and carbohydrate are provided by protein-free products and additional supplements. The aim of such treatments is to maintain the 2-3 h postprandial plasma BCAAs at near-normal concentrations (leucine: 80-200 μmol/l; isoleucine: 40-90 μmol/l; valine: 200-425 μmol/l). Since leucine is the most toxic precursor, the diet can be based on the leucine requirement, with frequent adjustment according to plasma leucine levels.

In newborns with the classic severe form of MSUD, the leucine requirement is 300-400 mg/day (80-110 mg/kg/day), which is approximately 50-60% of the leucine intake in healthy newborns. Minimum valine and isoleucine requirements are 200-250 mg/day. Apart from considerable interindividual variation, children, adolescents and adults with the classic form of MSUD tolerate about 500-700 mg of leucine per day. Individuals with variant forms tolerate greater amounts, and some do well on a low-protein diet.

Serial monitoring of blood BCAA levels is essential in the treatment of MSUD, and intakes of BCAAs must frequently be titrated against plasma concentrations. Occasionally, small amounts of free valine and isoleucine must be added to those provided by natural protein, because the tolerance for leucine is lower than that for the other two. When the plasma leucine levels are high and those of valine and isoleucine low, a rapid fall of leucine can only be achieved by combining a reduced leucine intake with a temporary supplement of valine and isoleucine.

In MSUD, unlike other organic acidurias, no abnormal acylcarnitines are formed and there is no increased carnitine loss; consequently no carnitine supplement is required. Although treatment with thiamine has often been advocated, its efficacy has not been confirmed in any form of MSUD.

Emergency Regimen. During maintenance treatment minor illnesses such as fever, vomiting, or diarrhoea result in an increase in catabolism and amino acid release from muscle protein. Neurotoxic levels of BCAAs and BCKAs are reached within hours, and patients may present with apathy, ataxia, hallucinations and, eventually, with fasting hypoglycaemia and convulsions. High energy intake and temporary removal of natural protein from the diet, and continuing supplements of BCAA-free amino acids (with the early addition of valine and isoleucine supplements) help to limit accumulation of the branched-chain compounds. Owing to its anabolic effect, intravenous insulin (0.15-0.20 IU/kg body weight/h), combined with large amounts of glucose and with continued enteral BCAA-free amino acids, can be successfully used to treat severe catabolic episodes. Such therapy may prevent metabolic decompensation following major surgery and trauma and can obviate the necessity for extracorporeal toxin removal in critically ill children.

Maternal MSUD. In a woman with MSUD, maintaining the plasma leucine level between 100 and 300 μmol/l and plasma valine and isoleucine in the upper normal ranges resulted in the delivery of a healthy infant. Leucine tolerance increased progressively from the 22nd week of gestation from 350 to 2100 mg/day. The risk of metabolic decompensation in the mother during the catabolic postpartum period can be minimised by careful monitoring after delivery in a metabolic referral centre [46].

Liver Transplantation. Liver replacement results in a clear increase in whole-body BCKD activity to at least the level seen in the very mild MSUD variant; following transplant patients no longer require protein-restricted diets and the risk of metabolic decompensation during catabolic events is apparently abolished [47, 48].

Prognosis. Patients with MSUD are now expected to survive; they are generally healthy between episodes of metabolic imbalance, and some attend regular schools and have normal IQ scores. However, the average intellectual performance is clearly below that of normal subjects [45, 49]. The intellectual outcome is inversely related to how long after birth plasma leucine levels remain above 1 mmol/l and is dependent on the quality of long-term metabolic control [50]. This suggests that inclusion of MSUD in neonatal screening programmes by tandem-mass spectrometry may improve the prognosis in this disease. Normal development and normal intellectual outcome and performance can be achieved at least in prospectively treated patients [3] and if average long-term plasma leucine levels are not more than 1.5-2 times normal [50]. However, some patients may present mental health problems despite good metabolic control. Children may have inattention and hyperactivity, and older patients may show generalised anxiety, panic or depression, resulting in poor educational and social achievement. Both types of disorders may require specific treatment [45]. In addition, timely evaluation and intensive treatment of minor illnesses at any age is essential, as late death attributed to recurrence of metabolic

crises with infections has occurred [3]. Assiduous care is also indicated for patients with variant forms, in order to prevent further ketoacidotic crises after they have been diagnosed and to retain the relatively good prognosis.

■■ Isovaleric Aciduria

Acute Phase Management in the Newborn. Intensive treatment with nonspecific measures (glucose infusion to provide calories and reduce endogenous protein catabolism, possibly bicarbonate infusion to control the acidosis) including exogenous toxin (and ammonia) removal may be needed in newborns, who are often in a poor clinical condition precluding the effective use of alternate pathways to enhance the removal of isovaleryl-CoA. In these circumstances, the administration of intravenous L-carnitine (100-400 mg/kg/day) and oral L-glycine (250-600 mg/kg/day) are effective means of treatment. Glycine can be provided as a 100-mg/ml water solution delivered in four to eight separate doses.

Dietary Therapy. The aim of treatment is to reduce the isovaleric acid burden to a minimum and to keep the urine free of IVA and 3-hydroxy-IVA. Such a therapy consists of a low-protein diet with supplemental glycine and carnitine and should be started as soon as possible after birth. In most patients the amount of protein tolerated meets the official protein requirements, and a special amino acid mixture free of leucine is rarely needed. Excessive protein intake should be avoided.

Carnitine and Glycine Therapy. For supplemental therapy either oral L-carnitine (50-100 mg/kg/day) or oral L-glycine (150-300 mg/kg/day) can be used. Under stable conditions the need for both supplementations is still controversial, but it can be useful during metabolic stress when toxic isovaleryl-CoA accumulation increases the need for detoxifying agents [51]. Supplementation with large doses of carnitine gives rise to an unpleasant odour in many IVA patients.

Prognosis. Prognosis is better than for the other organic acidurias. Even when a patient is compliant with treatment, metabolic crises can occur during catabolic stress, making a short hospitalisation for intravenous fluid (glucose/electrolytes/buffer) necessary. With puberty metabolic crises no longer occur. Growth is normal; intellectual prognosis depends on early diagnosis and treatment and, subsequently, on long-term compliance [52]. According to this, inclusion of IVA into neonatal screening programmes by tandem MS should improve the prognosis. So far (only one pregnancy published) there is no evidence that uncomplicated maternal IVA has any adverse effect on the unborn child [53].

In asymptomatic individuals identified by newborn screening and showing a mild biochemical phenotype it is crucial to follow the course of the inherited metabolic disturbance prospectively, as far as possible without any therapeutic regimen in order to find out the natural history.

■■ Propionic Aciduria and Methylmalonic Aciduria

Acute Phase Management in the Newborn. The urinary excretion of propionic acid is negligible, and no alternate urinary pathway is sufficient to effectively detoxify newborns with PA. However, this does not mean that exogenous toxin removal procedures are inevitably required. Extracorporal detoxification such as haemo(dia) filtration and haemodialysis (peritoneal dialysis is far less efficient), together with measures to promote anabolism, should be considered when neonatal illness is accompanied by severe hyperammonaemia (>400 μmol/l). In contrast to PA, the efficient removal of toxin in MMA takes place via urinary excretion, because of the high renal clearance of methylmalonic acid (22±9 ml/min per 1.73 m^2), which allows excretion of as much as 4-6 mmol/day. Thus, emergency treatment of the newborn with MMA, if not complicated by very high ammonia levels, mainly comprises rehydration and promotion of anabolism [54].

When conservative measures with high energy supply are sufficient, hyperammonaemia (especially in PA) may be controlled by use of sodium benzoate and/or carbamoylglutamate [55]. Metabolic decompensation in PA may be complicated by severe lactic acidosis due to thiamine deficiency, requiring vitamin supplementation [56].

Long-term Management. The goal of treatment is to reduce the production of methylmalonic or propionic acid by means of

- Natural protein restriction
- Maintaining an optimal calorie intake
- Carnitine supplementation (100 mg/kg/day)
- Reduction of intestinal production of propionate by metronidazole

Dietary Management. The aim of dietary treatment is to reduce the production of propionate by both the restriction of precursor amino acids using a low-protein diet and avoidance of prolonged fasting to limit oxidation of odd-chain fatty acids, which are liberated from triglyceride stores during lipolysis. The low-protein diet must provide at least the minimum amount of protein, nitrogen and essential amino acids to meet requirements for normal growth. Figures for estimates of safe levels of protein intake for infants, children and adolescents are available [42], which can be used as a guide for low-protein diets.

In early childhood this is often 1-1.5 g/kg/day. To improve the quality of this diet it may be supplemented with a relatively small amount of synthetic amino acids free from the precursor amino acids. However, the long-term value of these supplements remains uncertain, and metabolic balance can be achieved without them [12, 42, 43]. Some studies have shown that the addition of a special amino acid mixture to a severely restricted diet has no effect on growth or metabolic status and that these amino acids are mostly broken down and excreted as urea [43].

The diet must be nutritionally complete, with adequate energy intake and sufficient vitamins and minerals, in order to save the patient from serious complications associated with poor nutrition. Long fasts should be avoided. In order to prevent fasting at night nocturnal tube feeding may be used in the early years of management.

In children with severe forms of PA and MMA, anorexia and feeding problems are almost invariably present, and in order to maintain a good nutritional state feeds have to be given via nasogastric tube or gastrostomy at some stage. This is essential to provide adequate dietary intake, to prevent metabolic decompensation and to help the parents to cope with a child who is difficult to feed [12, 42, 43].

Most patients with a late-onset form are easier to manage. Individual protein tolerance can be quite high. Even though their individual tolerance allows a less rigid protein restriction and leads to a lower risk of malnutrition, these patients must be taught to reduce their protein intake immediately during intercurrent illness to prevent metabolic imbalance.

Vitamin Therapy. Every patient with MMA should be tested for responsiveness to vitamin B_{12}. Some late-onset forms (and, more rarely, neonatal-onset forms) of MMA are responsive to vitamin B_{12}; thus, parenteral vitamin therapy, starting with hydroxycobalamin 1000-2000 μg/day for about 10 days, must be carefully tried during a stable metabolic condition. During this period 24-h urine samples are collected for an organic acid analysis. Vitamin-B_{12} responsiveness leads to a prompt and sustained decrease of propionyl-CoA byproducts. However, as biochemical results may be difficult to assess, they must later be confirmed by in vitro studies. Most B_{12}-responsive patients need only mild protein restriction or none at all. Vitamin B_{12} is either given orally once per day or administered once a week (1000-2000 μg i.m.). In some cases, i.m. hydroxycobalamin therapy can be kept in reserve for intercurrent infections.

Carnitine Therapy. Chronic oral administration of L-carnitine (100 mg/kg/day) appears to be effective not only in preventing carnitine depletion but also in allowing urinary propionylcarnitine excretion and in this way reducing propionate toxicity [12].

Metronidazole Therapy. Microbial propionate production can be suppressed by antibiotics. Metronidazole, an antibiotic that inhibits anaerobic colonic flora, has been found to be specifically effective in reducing urinary excretion of propionate metabolites by 40% in MMA and PA patients. Long-term metronidazole therapy (at a dose of 10-20 mg/kg once daily for 10 consecutive days each month) may be of significant clinical benefit [12]. This intermittent administration may prevent the known side effects of the drug, such as leukopenia, peripheral neuropathy and pseudomembranous colitis.

Growth Hormone. Growth hormone (GH) induces protein anabolism. It is contraindicated in the acutely ill patient but potentially useful in the long term for those in whom growth is poor [12]. There is a place for recombinant human GH treatment as an adjuvant therapy in some patients with MMA and PA, mainly in those with reduced linear growth, but controlled long-term studies are needed [57].

Biochemical Monitoring. During the course of decompensation, plasma ammonia, blood gases, electrolytes, calcium, phosphate, lactate, glucose, uric acid, amylase, lipase and ketones in urine should be monitored. Some groups prefer also to measure urea in urine [43]. Regular amino acid analysis (all essential amino acids, and in particular isoleucine) is important. Furthermore, methylmalonic acid in plasma or urine should be controlled in order to define the lowest possible level in each individual patient on treatment. There may be little practical use for the measurement of acylcarnitines and of odd-chain fatty acids in terms of directing clinical management.

Prognosis. Around 15% of patients with MMA are vitamin B_{12} responsive and have mild disease and a good long-term outcome [10, 58, 59]. Conversely, both vitamin B_{12}-unresponsive patients with MMA and those with PA have severe disease and many encephalopathic episodes, mainly due to intercurrent infections [60]. Among all patients with all forms of MMA, mut^0 patients have the poorest prognosis, and vitamin B_{12}-responsive CblA and mut^- patients, the best [10, 58]. Owing to earlier diagnosis and better treatment, outcomes for PA and MMA patients have improved in the last decade [43, 59, 60]. Survival rates into early and mid-childhood can now exceed 70%. However, morbidity, in terms of cognitive development, remains high, with a majority of pa-

tients having DQ/IQ in the mildly to moderately retarded range. With the improved management the frequency of growth retardation has decreased, and now most patients with PA and MMA have growth curves within the normal range [43]. Abnormal neurological signs (mainly movement disorders, chorea, dystonia) continue to increase with age [10, 58-60]. Chronic progressive impairment of renal function is a frequent and serious complication that manifests in older patients with high methylmalonic acid excretion [10, 58]. Renal transplantation is likely to be necessary for many patients with MMA who survive into adolescence [61]. Including PA and MMA into newborn screening programmes by tandem MS may make it possible to identify the late-onset forms of the diseases in the newborn period and contribute to a further improvement in the outcome in this group. Decreased early mortality, less severe symptoms at diagnosis and more favourable short-term neurodevelopmental outcomes were recorded in patients identified through expanded newborn screening. However, the short duration of follow-up so far does not allow us to draw final conclusions about the effects of newborn screening on long-term outcome [60].

There are only a few reports of female patients with MMA who have carried a pregnancy to term. The outcome was favourable despite high levels of methylmalonic acid in blood and urine [62, 63].

Liver/KidneyTransplantation. In view of the poor long-term prognosis associated with a high risk of complications, liver or combined liver-kidney transplantations have been performed in a growing group of patients of different ages (from early infancy to adulthood) [64, 65]. After successful transplantation most patients have returned to a liberalised diet. A few patients with PA had remission of cardiomyopathy following liver transplantation [15, 17]. Despite sometimes only slight improvement in the levels of circulating propionyl-CoA metabolites, life-threatening episodes of ketoacidosis disappeared or were reduced to some degree. However, some patients have developed acute decompensation and basal ganglia necrosis years after liver transplantation and while on a normal diet. Today, it is recommended that such patients be maintained on a relaxed diet and with continued carnitine supplementation. There is ample experience that progressive renal failure and neurological dysfunction, including metabolic stroke, are not always prevented. Successful isolated kidney transplantation has been performed in some MMA patients in end-stage renal failure, with a very significant improvement in their metabolic control [66].

Management of Intercurrent Decompensations. Acute intercurrent episodes are prevented or minimised by awareness of the situations that may induce protein catabolism. These include intercurrent infections, immunisation, trauma, anaesthesia and surgery and dietary indiscretion. In all cases, the main response comprises a reduction in protein intake. All patients should have detailed instructions, including information on a semi-emergency diet, in which natural protein intakes are reduced by half, and an emergency diet, in which it is stopped. In both, energy supply is augmented using carbohydrates and lipids, such as solutions based on protein-free formula base powder or a mixture of glucose polymer and lipids diluted in an oral rehydration solution. For children treated with specific amino acid mixtures the usual supplements can be added, though one should be aware that they increase osmolarity and that their taste renders nasogastric tube feeding unavoidable. Their use is contraindicated in MMA and PA in cases of severe hyperammonaemia. At home, the solution is given in small, frequent drinks during day and night or by nasogastric tube [42]. After 24-48 h, if the child is doing well the usual diet is resumed within 2 or 3 days.

In cases of clinical deterioration with anorexia and/or gastric intolerance or if the child is obviously unwell, the patient must be hospitalised to evaluate the clinical status, to search for and treat intercurrent disease and to halt protein catabolism. Emergency therapy depends on the presence of dehydration, acidosis, ketosis and hyperammonaemia. Most often, intravenous rehydration for 12-24 h results in sufficient clinical improvement to allow for progressive renutrition with continuous enteral feeding. During this step enough natural protein to at least cover the minimal dietary requirements should be introduced into the feeds. The energy intakes are supplied with carbohydrates and lipids, applying the same rules as for the treatment of late-onset forms. During this stage of management close metabolic evaluation is advised, as the condition is labile and may deteriorate, requiring adjustment of the therapy. Conversely, if the patient's condition improves quickly normal feeding should be restored without delay.

During periods when enteral feeding is contraindicated or poorly tolerated, as can occur with severe or prolonged decompensation, the use of total parenteral nutrition may be an effective means of improving metabolic control and preventing further deterioration.

19.2 3-Methylcrotonyl Glycinuria

19.2.1 Clinical Presentation

The clinical phenotype ranges from neonatal onset with severe neurological involvement and even death to complete lack of any symptoms in adults. On the whole, symp-

tomatic patients present either in the neonatal period or later in childhood. Some infants present with intractable seizures from the 1st days of life, others with feeding difficulties, failure to thrive, and hypotonia within the 1st weeks after weaning, and some have recurrent seizures resulting in microcephaly and developmental delay. Most patients, however, present with a Reye-like syndrome following intercurrent illness or a protein-enriched diet within the first 2 years of life, developing neurological manifestations along with hypoglycaemia, ketoacidosis, hyperammonaemia and very low plasma carnitine. Additional manifestations in late-onset patients include muscular hypotonia, seizures, psychomotor retardation, hemiparesis ('metabolic stroke'), signs of 'metabolic leukodystrophy' and dilated cardiomyopathy. A few adult women diagnosed following newborn screening of their infants have complained of muscle weakness.

19.2.2 Metabolic Derangement

In 3-methylcrotonyl glycinuria, leucine catabolism is blocked by deficiency of 3-methyl crotonyl CoA carboxylase (3-MCC) (◘ Fig. 19.1, enzyme 3). This enzyme is one of the four biotin-containing carboxylases known in humans. Accumulation of 3-methylcrotonylglycine also occurs in multiple carboxylase deficiency, but in contrast to 3-MCC is found together with lactic acid and derivatives of propionyl-CoA (► Chapter 27).

Owing to the enzyme block, 3-methylcrotonyl-CoA and 3-methylcrotonic acid accumulate. Most of the accumulated acyl-CoA is conjugated with glycine to form 3-methylcrotonylglycine (MCG). In contrast, acylation of 3-methylcrotonyl-CoA with carnitine appears to be only a minor pathway. 3-Hydroxyisovalerate (3-HIVA), another major metabolite, is derived through the action of a crotonase on 3-methylcrotonyl-CoA and the subsequent hydrolysis of the CoA-ester. 3-Hydroxyisovalerylglycine has not been found in this condition. However, acylation with carnitine leads to the formation 3-hydroxyisovaleryl carnitine, which is the major abnormal acylcarnitine found in plasma and dried blood by tandem MS techniques.

19.2.3 Genetics

3-MCC is a heteromeric enzyme consisting of α- (biotin-containing) and β-subunits. 3-MCC deficiency results from loss of function mutations in the *MCCA* and *MCCB* genes encoding these subunits. The mutations can be classified into two groups, denoted CGA and CGB. More than 50 mutations have been identified in both genes [67]. They are associated with an almost total lack of enzyme activity in fibroblasts. The apparent biochemical severity of all the MCC mutations contrasts with the variety of the clinical phenotypes, suggesting that there are other unknown cellular and metabolic factors that affect the resulting phenotypes. Although most patients are compound heterozygotes or homozygotes, some are heterozygotes with a dominant negative allele [68]. The introduction of tandem MS into newborn screening has revealed an unexpectedly high incidence of this disorder, which in certain areas appears to be the most frequent organic aciduria, found in 1:40,000 newborns in Germany and Australia [39, 40].

19.2.4 Diagnostic Tests

The diagnosis relies on a characteristic urinary profile of organic acids, with huge excretion of 3-HIVA and 3-methycrotonylglycine and without the lactate, methylcitrate, and tiglylglycine found in multiple carboxlase deficiency (MCD). Supplementation with pharmacological doses of biotin does not alter this pattern. Total and free carnitine concentrations in plasma are extremely low. The presence of 3-hydroxyisovaleryl carnitine (C5OH) in plasma and in dried blood spots is diagnostic for 3-MCC deficiency, since it is not found in IVA. In other disorders, such as MCD, propionylcarnitine (3C) is also seen, and in 3-hydroxy-3-methylglutaryl CoA lyase deficiency glutarylcarnitine is the major finding (► Chapter 3).

Since family studies and newborn screening have identified a number of totally asymptomatic siblings and mothers with MCC deficiency, it is advisable to search in any affected family for other MCC-deficient subjects by analysis of the acylcarnitine profile in blood and organic acids in urine.

19.2.5 Treatment and Prognosis

Long-term treatment of symptomatic infants based on a mildly protein-restricted diet (meeting the recommended requirements) results in a general improvement and a reduction in the number of exacerbations. It is effective in lowering the abnormal excretion of organic acids which, however, never disappears.

Glycine and carnitine therapies directed at increasing the excretion of glycine and carnitine conjugates are complementary rather than competitive means of detoxification. Glycine supplementation (175 mg/kg/day) increases the excretion of 3-MCG. Carnitine supplementation (100 mg/kg/day) corrects the very low plasma carnitine levels

and increases the excretion of 3-HIVA. Family studies and newborn screening have identified a number of totally asymptomatic siblings and mothers with 3-MCC deficiency who have very low carnitine concentrations in blood and have never had any treatment, so that the need for treatment must be doubted. The poor prognosis described in early-onset forms presenting as neonatal seizures could be due to late diagnosis and treatment. In acute late-onset forms presenting as Reye-like syndrome, all but one patient fully recovered. A consensus protocol to assist clinicians in the diagnosis and management of screen-positive newborns for 3-MCC deficiency has been proposed [69](► Chapter 3).

19.3 3-Methylglutaconic Aciduria

3-Methylglutaconic aciduria is found in a number of inborn errors of metabolism, only one of which is associated with a defect in leucine metabolism (for review see [70]). However, for completeness the other disorders are also considered here.

3-Methylglutaconic Aciduria Type I. 3-Methylglutaconic aciduria type I (3-MGA type I) has only been identified in very few individuals, who presented with a wide spectrum of clinical signs of a neurometabolic disease ranging from no symptoms (at 2 years of age) to mild neurological impairment, severe encephalopathy with basal-ganglia involvement, quadriplegia, athetoid movement disorder, severe psychomotor retardation and leukoencephalopathy in a 61-year-old woman.

3-Methylglutaconyl (MGC)-CoA is metabolised to 3-hydroxy-3-methylglutaryl-CoA by 3-MGC-CoA hydratase (◘ Fig. 19.1, enzyme 4). Defective activity leads to 3-MGC aciduria type I, which is characterised by urinary excretion of 3-MGC and 3-methylglutaric acids. Both metabolites derive from accumulated 3-methylglutaconyl-CoA, through hydrolysis and dehydrogenation, respectively. The combined urinary excretion of 3-MGC and 3-methylglutaric acids range from 500 to 1000 mmol/mol creatinine, of which 3-methylglutaric acid represents about 1%. The metabolic pattern also includes 3-HIVA, which differentiates type I from the other secondary types. 3-MGC-CoA Hydratase activity can be measured in fibroblasts. The role of the human 3-MGC-CoA hydratase in leucine metabolism has been elucidated, and different mutations in the *AUH* gene have been identified [71, 72]. No clear therapeutic regimen has been described. Carnitine supplementation may have beneficial effects.

3-Methylglutaconic aciduria type I must be distinguished from many other conditions associated with 3-MGC aciduria, which include Barth syndrome (3-MGA type II), Costeff optic atrophy syndrome (3-MGA type III) and disorders of unknown origin, summarised as 3-MGA type IV.

3-Methylglutaconic Aciduria Type II (Barth Syndrome). This X-linked disorder, characterised by dilated cardiomyopathy, skeletal myopathy, neutropenia and mitochondrial respiratory chain dysfunction, is considered in ► Chapter 35.

3-Methylglutaconic Aciduria Type III. 3-MGA type III (Costeff optic atrophy syndrome) has mostly, but not exclusively, been reported in Iraqi Jewish individuals. It is a neuro-ophthalmological syndrome that consists of early-onset bilateral optic atrophy and later-onset spasticity, extrapyramidal dysfunction and cognitive deficits. 3-Methylglutaconic and 3-methylglutaric acid excretion are increased. The disease is caused by mutations in the *OPA3* gene. The high prevalence of this allele in the Iraqi Jewish population (allele frequency of 1 in 10) suggests a founder effect. [73]. Although the majority of *OPA3* mutations are associated with recessive disease, autosomal dominant inheritance has also been reported.

3-Methylglutaconic Aciduria Type IV. There is a relatively large and heterogeneous group of patients with 3-MGC aciduria, who suffer from variable, multisystem diseases and cannot be classified as having type MCG aciduria types I, II, III or V (3-MGC aciduria type IV, unspecified diseases). Some have been described with respiratory chain disorders [74]. A group of these patients may have quite a well-defined and similar clinical presentation without any clear enzymatic defect [75]. Conversely, mutations in the *TMEM70* gene have been observed in patients with 3-MDC aciduria, hypertrophic cardiomyopathy and mitochondrial ATP synthase deficiency [76].

3-Methylglutaconic Aciduria Type V. 3-MGA aciduria type V, or dilated cardiomyopathy with ataxia (DCMA) syndrome, presents with an early-onset dilated cardiomyopathy with conduction defects and nonprogressive cerebellar ataxia. The disorder, which has been described in 18 patients belonging to the Canadian Dariusleut Hutterite population, is also associated with testicular dysgenesis and growth failure [77]. There are 5- to 10-fold increases in both plasma and urine 3-MGC and 3-MG acid. A mutation in the *DNAJC19* gene, which codes for a protein believed to act as molecular chaperone in the inner mitochondrial membrane, was identified by homozygosity mapping.

19.4 Short-/Branched-chain Acyl-CoA Dehydrogenase Deficiency

Isolated 2-methylbutyrylglycinuria, caused by 2-methylbutyryl-CoA dehydrogenase deficiency (MBD) and encoded by the *ACDSB* gene (◘ Fig. 19.1, enzyme 6), is an autosomal recessive disorder of isoleucine metabolism [78]. A few patients have been diagnosed following various clinical symptoms, and a set of asymptomatic subjects of Hmong descent were identified through newborn screening with elevated C5-acylcarnitine concentrations in blood spots. Detection of MBD deficiency in newborn screening is not limited to this population, and an increasing number of asymptomatic patients have been extensively investigated. Clinical relevance of this disorder remains in doubt and requires careful long-term follow-up of affected individuals. Theoretically, valproic acid should be avoided, as valproyl-CoA could be a substrate of MBD (► Chapter 3).

19.5 2-Methyl-3-Hydroxybutyryl-CoA Dehydrogenase Deficiency

Only a few patients with 2-methyl-3-hydroxybutyryl-CoA dehydrogenase (MHBD) deficiency have been described. All male patients had an unusual neurodegenerative and progressive disease, and some affected females had psychomotor retardation and speech delay. Related women (mothers and grandmothers of patients) have shown mild to moderate developmental delay. In early childhood the severe neurodegenerative symptoms included rigidity, dystonic posturing, spastic diplegia, dysarthria, choreoathetoid movements, restlessness, cortical blindness, myoclonic seizures, brain atrophy, periventricular white matter and basal ganglia abnormalities. All patients identified so far have had a severe progressive neurological phenotype rather than ketoacidotic attacks, in contrast to patients with a defect in the next step of isoleucine degradation attributable to 2-methylacetoacetyl-CoA thiolase deficiency.

MHBD deficiency (◘ Fig. 19.1, enzyme 7) is a defect in the degradation of isoleucine and branched-chain fatty acids. Laboratory findings include marked elevations of urinary 2-methyl-3-hydroxybutyrate and tiglylglycine without elevation of 2-methylacetoacetate. The organic acid excretion is more pronounced after a 100-mg/kg oral isoleucine challenge. Enzyme studies have shown markedly decreased activity of MHBD in fibroblasts and lymphocytes. MHBD deficiency is caused by mutations in the X-chromosomal *HADH2* gene. A short-term stabilisation of neurological symptoms and a biochemical response to an isoleucine-restricted diet have been observed in some patients [79, 80].

The deficiency of 2-methyl-acetoacetyl-CoA thiolase (◘ Fig. 19.1, enzyme 8), also known as 3-ketothiolase or T2, is discussed in ► Chapter 14.

19.6 Isobutyryl-CoA Dehydrogenase Deficiency

The mitochondrial enzyme isobutyryl-CoA dehydrogenase (IBD) catalyses the third step in the degradation of valine (◘ Fig. 19.1, enzyme 9). It is encoded by the *ACAD8* gene [81]. Fewer than 20 patients with IBD deficiency have been described. Only the first patient, a 2-year-old, was diagnosed following the investigation of anaemia and dilated cardiomyopthy. Other patients have been identified following the expansion of newborn screening [41, 81, 82]. This disorder can be detected on the basis of elevated butyrylcarnitine/isobutyrylcarnitine (C4-carnitine) concentrations in newborns' blood spots analysed by tandem MS. The presence of this metabolite, which is also present in short-chain acyl-CoA dehydrogenase deficiency, requires further investigation for precise diagnosis [82]. The possible clinical implication of this enzyme defect is not known, and to date most of the identified patients have remained asymptomatic. However, a few patients have moderate speech delay and careful follow-up is necessary.

19.7 3-Hydroxyisobutyric Aciduria

A few patients with increased excretion of 3-hydroxyisobutyric acid (3-HIBA), an intermediate of the catabolic pathways of valine and thymidine, have been identified. This condition may be linked to various enzymatic defects. Unfortunately, in most cases described, the enzymatic diagnosis has been speculative.

Clinical presentation is heterogeneous. Some patients present in infancy, with acute metabolic episodes with ketoacidosis, hypoglycaemia or hyperlactataemia. Muscle involvement and hypertrophic cardiomyopathy have been reported. CNS involvement is highly variable, ranging from normal development to brain dysgenesis observed in neonates.

Several enzyme defects may underlie 3-hydroxyisobutyric aciduria. However, only combined deficiency of malonic, methylmalonic and ethylmalonic semialdehyde dehydrogenase (MMSDH, *(MMSDH)* gene) (◘ Fig. 19.1, enzyme 12) [83] and 3-hydroxyisobutyryl-

CoA deacylase deficiency (◘ Fig. 19.1, enzyme 10) have been identified [84].

19.8 Malonic Aciduria

MA is a rare condition, with fewer than 30 cases reported. Deficient malonyl-CoA decarboxylase (MLYCD) is usually expressed in fibroblasts or leukocytes, and various mutations have been reported in the *MLYCD* gene [85].

19.8.1 Clinical Presentation

A neonatal form has been described in some patients, who displayed progressive lethargy, hypotonia, hepatomegaly associated with metabolic acidosis, and mild hyperammonaemia, variously associated with hypoglycaemia and/or hyperlactacidaemia. Cardiac failure due to cardiomyopathy could be present at birth.

In the late-onset forms, most patients present acute metabolic episodes secondary to intercurrent infections. Some of these patients could be previously known to be affected with a mild and nonspecific psychomotor retardation. Other children have been diagnosed following systematic screening for mental retardation and hypotonia. Cardiomyopathy has been present in about 40% of identified patients.

19.8.2 Metabolic Derangement

Malonic aciduria is due to deficiency of MLYCD (◘ Fig. 19.1, enzyme 15). The physiological role of this cytosolic enzyme could be in the regulation of cytoplasmic malonyl-CoA abundance and, thus, of mitochondrial fatty acid uptake and oxidation. Patients with MLYCD deficiency display a number of phenotypes that are reminiscent of mitochondrial fatty acid oxidation disorders [85]. However, in contrast to these, dicarboxylic aciduria together with ketonuria is found during catabolic episodes and the patients exhibit normal ketogenesis on acute fat-loading tests.

19.8.3 Genetics

MLYCD deficiency is an autosomal recessive disorder. More than 20 mutations in the *MLYCD* gene have been reported. No hotspot mutations have been identified. No phenotype-genotype relationship was detected, and siblings may have different presentation/s [85]. Anaother rare disorder presenting with malonic and methylmalonic aciduria has been recently elucidated as secondary to ACSF 3 deficiency, a member of the acyl-CoA synthetase family (86).

19.8.4 Diagnostic Tests

Diagnosis relies on a characteristic profile of urinary organic acids, in which malonic and methylmalonic acids are constant findings. Abnormal succinic aciduria has been found in about half the cases, as have various dicarboxylic and glutaric acidurias.

Total and free carnitine concentrations in plasma are low. Documented accumulation of malonylcarnitine would allow tandem mass spectrometry screening of newborn blood spots. MLYCD has been found to be reduced in cultured fibroblasts and/or leukocytes of most defective cell lines, with residual activity less than 10% of control. Patients with normal enzyme activity in fibroblasts have a similar disorder, with mutations in the *MLYCD* gene [85].

19.8.5 Treatment and Prognosis

No rules for treatment and prognosis have been established. Carnitine supplementation corrects the carnitine deficiency and may improve the cardiomyopathy and muscle weakness. Conversely, some patients have worsened despite carnitine supplementation and have recovered with a long-chain triglyceride-restricted/medium-chain triglyceride-supplemented diet [87]. Long-term prognosis is unknown. Except for the two patients who developed extrapyramidal signs following an acute crisis, most patients have residual mild developmental delay. There are subjects identified by newborn screening who remained asymptomatic at least during preschool age.

References

[1] Gascon GC, Ozand PT, Brismar J (1994) Movement disorders in childhood organic acidurias clinical, neuroimaging, and biochemical correlations. Brain Dev 16:94-103
[2] Fariello G, Dionisi-Vici C, Orazi C et al. (1996) Cranial ultrasonography in maple syrup urine disease. AJNR Am J Neuroradiol 17:311-315
[3] Morton DH, Strauss KA, Robinson DL et al. (2002) Diagnosis and treatment of maple syrup urine disease: a study of 36 patients. Pediatrics 109:999-1008
[4] Treacy E, Clow CL, Reade TR et al. (1992) Maple syrup urine disease: interrelations between branched-chain amino-, oxo- and hydroxyacids; implications for treatment; associations with CNS dysmyelination. J Inherit Metab Dis 15:121-135

[5] Schoenberger S, Schweiger B, Schwahn B et al. (2004) Dysmyelination in the brain of adolescents and young adults with maple syrup urine disease. Mol Genet Metab 82:69-75
[6] Kleopa KA, Raizen DM, Friedrich CA, Brown MJ, Bird SJ (2001) Acute axonal neuropathy in maple syrup urine disease. Muscle Nerve 24:284-287
[7] Brismar J, Ozand PT (1994) CT and MR of the brain in disorders of the propionate and methylmalonate metabolism. AJNR Am J Neuroradiol 15:1459-1473
[8] Chemelli AP, Schocke M, Sperl W et al. (2000) Magnetic resonance spectroscopy (MRS) in five patients with treated propionic acidemia. J Magn Reson Imaging 11:596-600
[9] Williams ZR, Hurley PE, Altiparmark UE et al. (2009) Late onset optic neuropathy in methylmalonic and propionic acidemia. Am J Ophthalmol 147: 929-933
[10] Hörster F, Garbade SF, Zwickler T et al. (2009) Prediction of outcome in isolated methylmalonic acidurias: combined use of clinical and biochemical parameters. J Inherit Metab Dis 32:630-639
[11] Rutledge SL, Geraghty M, Mroczek E et al. (1993) Tubulointerstitial nephritis in methylmalonic acidemia. Pediatr Nephrol 7:81-82
[12] Leonard JV (1995) The management and outcome of propionic and methylmalonic acidaemia. J Inherit Metab Dis 18:430-434
[13] Lane TN, Spraker MK, Parker SS (2007) Propionic acidemia manifesting with low isoleucine generalized exfoliative dermatosis. Pediatr Dermatol 24:508-510
[14] Gilmore A, Bock H-G, Nowicki M (2008) Hyperamylasemia/hyperlipasemia in a child with propionic acidemia. Am J Med Genet A 146A: 3090-3091
[15] Romano S, Valayannopoulos V, Touati G et al. (2010) Cardiomyopathies in propionic aciduria are reversible after liver transplantation. J Pediatr 156:128-134
[16] Baumgartner D, Schöll-Burgi S, Sass JO et al. (2007) Prolonged QTc intervals and decreased left ventricular contractility in patients with propionic acidemia. J Pediatr 150: 192-197
[17] Sato S, Kasahara M, Fukuda A et al. (2009) Liver transplantation in a patient with propionic acidemia requiring extracorporeal membrane oxygenation during severe metabolic decompensation. Pediatr Transplant 13:790-793
[18] Morath MA, Okun JG, Müller IB et al. (2008) Neurodegeneration and chronic renal failure in methylmalonic aciduria a pathophysiological approach. J Inherit Metab Dis 31:35-43
[19] Sbai D, Narcy C, Thompson GN et al. (1994) Contribution of odd-chain fatty acid oxidation to propionate production in disorders of propionate metabolism. Am J Nutr 59:1332-1337
[20] Leonard JV (1996) Stable isotope studies in propionic and methylmalonic acidaemia. Eur J Pediatr 156 [Suppl 1]:S67-S69
[21] Aevarsson A, Chuang JL, Wynn RM et al. (2000) Crystal structure of human branched-chain α-ketoacid dehydrogenase and the molecular basis of multienzyme complex deficiency in maple syrup urine disease. Structure 8:277-291
[22] Ensenauer R, Vockley J, Willard JM et al. (2004) A common mutation is associated with a mild, potentially asymptomatic phenotype in patients with isovaleric acidemia diagnosed by newborn screening. Am J Hum Genet 75:1136-1142
[23] Perez-Cerda C, Clavero S, Perez B et al. (2003) Functional analysis of PCCB mutations causing propionic acidemia based on expression studies in deficient human skin fibroblasts. Biochim Biophys Acta 1638:43-49
[24] Perez B, Desviat LR, Rodriguez-Pombo P et al. (2003) Propionic acidemia: identification of twenty–four novel mutations in Europe and North America. Mol Genet Metab 78:59-67
[25] Yorifuji T, Kawai M, Muroi J et al. (2002) Unexpectedly high prevalance of the mild form of propionic acidemia in Japan: presence of a common mutation and possible clinical implications. Hum Genet 111:161-165
[26] Acquaviva C, Benoist JF, Pereira S et al. (2005) Molecular basis of methylmalonyl-CoA mutase apoenzyme defect in 40 European patients affected by mut (0) and mut (-) forms of methylmalonic acidemia: identification of 29 novel mutations in the MUT gene. Hum Mutat 25:167-176
[27] Lemer-Ellis JP, Dobson CM, Wai T et al. (2004) Mutations in the *MMAA* gene in patients with the cblA disorder of vitamin B12 metabolism. Hum Mutat 24:509-516
[28] Jorge-Finnigan A, Aguado C, Sanchez-Alcudia R et al. (2010) Functional and structural analysis of five mutations identified in methylmalonic acidurie cblB type. Hum Mutat 31:1-10
[29] Martínez MA, Rincón A, Desviat LR, Merinero B, Ugarte M, Pérez B (2005) Genetic analysis of three genes causing isolated methylmalonic acidemia: identification of 21 novel allelic variants. Mol Genet Metab 84:317-325
[30] Elpeleg O, Miller C, Hershkovitz E et al. (2005) Deficiency of the ADP-forming succinyl-CoA synthase activity is associated with encephalomyopathy and mitochondrial DNA depletion. Am J Hum Genet 76:1081-1086
[31] Carrozzo R, Dionisi-Vici C, Steuerwald U et al. (2007) SUCLA2 Mutations are associated with mild methylmalonic aciduria, Leigh-like encephalomyopathy, dystonia and deafness. Brain 130:862-874
[32] Ostergaard E, Hansen FJ, Sorensen N, et al. (2007) Mitochondrial encephalomyopathy with elevated methylmalonic acid is caused by SUCLA2 mutations. Brain 130:853-861
[33] Ostergaard E, Christensen E, Kristensen E et al. (2007) Deficiency of the alpha subunit of succinate-coenzyme A ligase causes fatal infantile lactic acidosis with mitochondrial DNA depletion. Am J Hum Genet 81:383-387
[34] Ostergaard E, Schwartz M, Batbayli M et al. (2010) A novel missense mutation in SUCLG1 associated with mitochondrial DNA depletion, encephalomyopathic form, with methylmalonic aciduria. Eur J Pediatr 169:201-205
[35] Bikker H, Bakker HD, Abeling NG et al. (2006) A homozygous nonsense mutation in the methylmalonyl-CoA epimerase gene (MCEE) results in mild methylmalonic aciduria. Hum Mutat 27: 640-643
[36] Dobson MC, Gradinger A, Longo N et al. (2006) Homozygous nonsense mutation in the MCEE gene and siRNA suppression of methylmalonyl-CoA epimerase expression: a novel cause of mild methylmalonic acidurie. Mol Genet Metab 88: 327-333
[37] Filipowicz HR, Ernst SL, Ashurst CL, Pasquali M, Longo N (2006) Metabolic changes associated with hyperammonemia in patients with propionic acidemia. Mol Genet Metab 88:123-130
[38] Nissim I (1999) New aspects of glutamine/glutamate metabolism: the role of acute pH changes. Am J Physiol 277:F493-497
[39] Wilcken B, Wiley V, Hammond J, Carpenter K (2003) Screening newborns for inborn errors of metabolism by tandem mass spectrometry. N Engl J Med 348:2304-2312
[40] Schulze A, Lindner M, Kohlmüller D et al. (2003) Expanded newborn screening for inborn errors of metabolism by electrospray ionization-tandem mass spectrometry: results, outcome, and implications. Pediatrics 111:1399-1406
[41] Rinaldo P, Tortorelli S, Matern D (2004) Recent developments and new applications of tandem mass spectrometry in newborn screening. Curr Opin Pediatr 16:427-433
[42] MacDonald A, Dixon M, White F (2008) Disorders of amino acid metabolism, organic acidemias and urea cycle defects. In: Shaw

V, Lawson M (eds) Clinical paediatric dietetics, 3rd edn. Blackwell, Oxford, UK, chap 17
[43] Touati G, Valayannopoulos V, Mention K et al. (2006) Methylmalonic and propionic acidurias: management without or with a few supplements of specific amino acid mixtures. J Inherit Metab Dis 29:288-299
[44] Puliyanda DP, Harmon WE, Peterschmitt MJ, Irons M, Somers MJ (2002) Utility of hemodialysis in maple syrup urine disease. Pediatr Nephrol 17:239-242)
[45] Strauss KA, Wardley B, Robinson D et al. (2010) Classical maple syrup urine disease and brain development: principles of management and formula design. Mol Genet Metab 99: 333-345
[46] Grünewald S, Hinrichs F, Wendel U (1998) Pregnancy in a woman with maple syrup urine disease. J Inher Metab Dis 21:89-94
[47] Strauss KA, Mazariegos GV, Sindhi R et al. (2006) Elective liver transplantation for the treatment of classical maple syrup urine disease. Am J Transplant 6: 557-564
[48] Khanna A, Hart M, Nyhan WL et al. (2006) Domino liver transplantation in maple syrup urine disease. Liver Transplant 12: 876-882
[49] Hilliges C, Awiszus D, Wendel U (1993) Intellectual performance of children with maple urine disease. Eur J Pediatr 152:144-147
[50] Hoffmann B, Helbling C, Schadewaldt P, Wendel U (2006) Impact of longitudinal plasma leucine levels in the intellectual outcome in patients with classic MSUD. Pediatr Res 59:17-20
[51] Fries MH, Rinaldo P, Schmidt-Sommerfeld E et al. (1996) Isovaleric acidemia: response to a leucine load after three weeks of supplementation with glycine, l-carnitine, and combined glycine-carnitine therapy. J Pediatr 129:449-452
[52] Vockley J, Ensenauer R (2006) Isovaleric acidemia: new aspects of genetic and phenotypic heterogeneity. Am J Med Genet C Semin Med Genet 142:95-103
[53] Shih VE, Aubry RH, De Grande G et al. (1984) Maternal isovaleric acidemia. J Pediatr 105:77-78
[54] Picca S, Dionisi-Vici C, Abeni D et al. (2001) Extracorporeal dialysis in neonatal hyperammonemia: modalities and prognostic indicators. Pediatr Nephrol 16:862–867
[55] Filippi L, Gozzimi E, Fiorini et al. (2010) *N*-Carbamoylglutamate in emergency management of hyperammonemia in neonatal onset propionic and methylmalonic aciduria. Neonatology 97: 286-290
[56] Matern D, Seydewitz, HH, Lehnert W et al. (1996) Primary treatment of propionic acidemia complicated by acute thiamine deficiency. J Pediatr 129:758-760
[57] Touati G, Ogier de Baulny H, Rabier D et al. (2003) Beneficial effects of growth hormone treatment in children with methylmalonic and propionic acidurias (abstract). J Inherit Metab Dis 26 [Suppl 2]:40
[58] Baumgartner ER, Viardot C (1995) Long-term follow-up of 77 patients with isolated methylmalonic acidaemia. J Inherit Metab Dis 18:138-142
[59] De Baulny HO, Benoist JF, Rigal O et al. (2005) Methylmalonic and propionic acidemias: management and outcome. J Inherit Metab Dis 28:415-423
[60] Dionisi Vici C, Deodato F, Roschinger W et al. (2006) »Classical« organic acidurias, propionic aciduria, methylmalonic aciduria and isovaleric aciduria: long-term outcome and effects of expanded newborn screening using tandem mass spectrometry. J Inherit Metab Dis 29:383-389
[61] Lubrano R, Eli M, Rossi M et al. (2007) Renal transplant in methylmalonic acidemia: could it be the best option? Report on a case at ten years and review of the literature. Pediatr Nephrol 22:1209-1214
[62] Diss E, Iams J, Reed N et al. (1995) Meythylmalonic aciduria in pregnancy: a case report. Am J Obstet Gynecol 172:1057-1059
[63] Deodato F, Rizzo C, Boenzi S et al. (2002) Successful pregnancy in a woman with mut- methylmalonic acidaemia. J Inherit Metab Dis 25:133-134
[64] Kasahara M, Horikawa R, Tagawa M et al. (2006) Current role of liver transplantation for methylmalonic acidemai: a review of literature. Pediatr Transplant 10: 943-947
[65] Barshes NR, Vanatta , Patel AJ et al. (2006) Evaluation and management of patients with propionic acidemia undergoing liver transplantation: a comprehensive review. Pediatr Transplant 10: 773-78
[66] Lubrano R, Elli M, Rossi M et al. (2007) Renal transplant in methylmalonic acidemia: could it be the best option? Report on a case at 10 years and review of the literature. Pediatr Nephrol 22:1209-1214
[67] Stricki M, Suormala T, Fowler B, Valle D, Baumgartner MR (2009) Cryptic exon activation by disruption exon splice enhancer: novel mechanism causing 3-methylcrotonyl-CoA carboxylase deficiency. J Biol Chem 284: 28953-28957
[68] Baumgartner MR, Dantas MF, Suormala T et al. (2004) Isolated 3-methylcrotonyl-CoA carboxylase deficiency: evidence for an allele-specific dominant negative effect and responsiveness to biotin therapy. Am J Hum Genet 75:790-800
[69] Arnold GL, Koeberl DD, Matern D et al. (2008) A Delphi-based consensus clinical practice protocol for the diagnosis and management of 3-methylcrotonyl CoA carboxylase deficiency. Mol Genet Metab 93:363-370
[70] Wortmann SB, Kluijtmans LA, Engelke UF et al. (2010) The 3-methylglutaconic acidurias: what's new ? J Inher Metab Dis Sep 30 (Epub ahead of print)
[71] Ijlst L, Loupatty FJ, Ruiter JP et al. (2002) 3-Methylglutaconic aciduria type I is caused by mutations in AUH. Am J Hum Genet 71:1463-1466
[72] Mack M, Schniegler-Mattox U, Peters V et al. (2006) Biochemical characterization of human 3-methylglutaconyl-CoA hydratase and its role in leucine metabolism. FEBS J 273:2012-2022
[73] Gunay-Aygun M, Gahl WA, Anikster Y (updated 2009) 3-Methylglutaconic aciduria type III. In Pagon RA, Bird TC, Dolan CR, Stephens K (eds) GeneReviews [internet]. University of Washington, Seattle 1993-2006, 28 Jul
[74] Wortmann SB, Rodenburg RJ, Jonckheere A et al. (2009) Biochemical and genetic analysis of 3-methylglutaconic acidurie type IV: a diagnostic strategy. Brain 132:136-146
[75] Di Rosa G, Deodato F, Loupatty FJ et al. (2006) Hypertrophic cardiomyopathy, cataract, developmental delay, lactic acidosis: a novel subtype of 3-methylglutaconic aciduria. J Inherit Metab Dis 29:546-550
[76] Houstek J, Kmoch S, Zeman J (2009) TMEM70-protein – a novel ancillary factor of mammalian ATP synthase. Biochim Biophys Acta 1787:529-532
[77] Davey KM, Parboosingh JS, McLeod DR et al. (2006) Mutation of DNAJC19, a human homologue of yeast inner mitochondrial membrane co-chaperones, causes DCMA syndrome, a novel autosomal recessive Barth syndrome-like condition. J Med Genet 43:385–393
[78] Sass JO, Ensenauer R, Röschinger W et al. (2008) 2-Methylbutyryl-coenzyme A dehydrogenase deficiency: functional and molecular studies on a defect in isoleucine catabolism. Mol Genet Metab 93:30-35
[79] Garcia-Villoria J, Navarro-Sastre A, Fons C et al. (2009) Study of patients and carriers with 2-methyl-3-hydroxybutyryl-CoA dehydrogenase (MHBD) deficiency: difficulties in the diagnosis. Clin Biochem 42:27-33

[80] Yang SY, He XY, Miller D (2007) HSD17B10: a gene involved in cognitive function through metabolism of isoleucine and neuroactive steroids. Mol Genet Metab 92:36–42
[81] Pedersen CB, Bischoff C, Christensen E et al. (2006) Variations in IBD (ACAD8) in children with elevated C4-acylcarnitine detected by tandem mass spectrometry newborn screening. Pediatr Res 60:315-320
[82] Oglesbee D, He M, Majumber N et al. (2007) Development of a newborn screening follow-up algorithm for the diagnosis of isobutyryl-CoA dehydrogenase deficiency. Genet Med 9:108-116
[83] Chambliss KL, Gray RG, Rylance G et al. (2000) Molecular characterization of methylmalonate semialdehyde dehydrogenase deficiency. J Inherit Metab Dis 23 497-504
[84] Brown GK, Huint SM, Scholem R et al. (1982) Hydroxyisobutyryl-coenzyme A deacylase deficiency: a defect in valine metabolism associated with physical malformations. Pediatrics 70:532-538
[85] Salomons GS, Jakobs C, Landegge Pope L et al. (2007) Clinical, enzymatic and molecular characterization of nine new patients with malonyl- coenzyme A decarboxylase deficiency. J Inherit Metab Dis 30: 23-28
[86] Sloan JL, Johnston JJ, Manoli I et al. (2011) Exone sequencing identifies ACSF 3 as a cause of combined malonic and methymlonic aciduria. Nature Genetics 43: 883-886
[87] Footitt EJ, Stafford J, Dixon M et al. (2010) Use of a long-chain triglyceride-restricted/medium-chain triglyceride-supplemented diet in a case of malonyl-CoA decarboxylase deficiency with cardiomyopathy. J Inherit Metab Dis DOI 10.1007/s10545-010-9137-z