35 Disorders of Purine and Pyrimidine Metabolism

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Purine Metabolism

Purine nucleotides are essential cellular constituents which intervene in energy transfer, metabolic regulation, and synthesis of DNA and RNA. Purine metabolism can be divided into three pathways:

- The biosynthetic pathway, often termed *de novo*, starts with the formation of phosphoribosyl pyrophosphate (PRPP) and leads to the synthesis of inosine monophosphate (IMP). From IMP, adenosine monophosphate (AMP) and guanosine monophosphate (GMP) are formed. Further metabolism (not illustrated) leads to their di- and triphosphates, to their corresponding deoxyribonucleotides, and to RNA and DNA.
- The catabolic pathway starts from GMP, IMP and AMP, and produces uric acid, a poorly soluble compound, which tends to crystallize once its plasma concentration surpasses 6.5–7 mg/dl (0.38–0.47 mmol/l).
- The salvage pathway utilizes the purine bases, guanine, hypoxanthine and adenine, which are provided by food intake or the catabolic pathway, and reconverts them into, respectively, GMP, IMP and AMP. Salvage of the purine nucleosides, adenosine and guanosine, and their deoxy counterparts, catalyzed by kinases, also occurs.

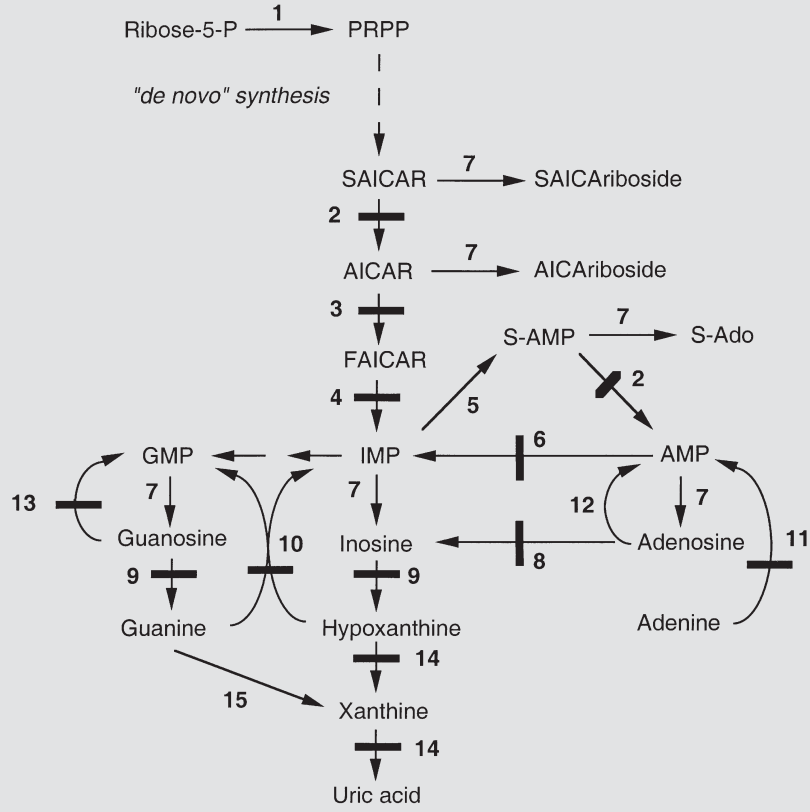


Fig. 35.1. Pathways of purine metabolism. *AICAR*, aminoimidazolecarboxamide ribotide; *AMP*, adenosine monophosphate; *FAICAR*, formylaminoimidazolecarboxamide ribotide; *GMP*, guanosine monophosphate; *IMP*, inosine monophosphate; *P*, phosphate; *PRPP*, phosphoribosyl pyrophosphate, *S-Ado,* succinyladenosine; *SAICAR*, succinylaminoimidazolecarboxamide ribotide; *S-AMP*, adenylosuccinate, *XMP*, xanthosine monophosphate.
1, PRPP synthetase; **2**, adenylosuccinase (adenylosuccinate lyase); **3**, AICAR transformylase; **4,** IMP cyclohydrolase (3 and 4 form ATIC); **5**, adenylosuccinate synthetase; **6**, AMP deaminase; **7**, 5′-nucleotidase(s), **8**, adenosine deaminase; **9**, purine nucleoside phosphorylase; **10**, hypoxanthine-guanine phosphoribosyltransferase; **11**, adenine phosphoribosyltransferase; **12**, adenosine kinase; **13**, guanosine kinase; **14**, xanthine oxidase (dehydrogenase). Enzyme defects are indicated by *solid bars* across the arrows

Inborn errors exist of the biosynthetic, catabolic, and salvage pathways of purine and pyrimidine metabolism, which are depicted in ◘ Fig. 35.1 and 35.3, respectively. The major presenting signs and laboratory findings in these inborn errors are listed in ◘ Table 35.1.

35.1 Inborn Errors of Purine Metabolism

Inborn errors of purine metabolism comprise errors of:

- *purine nucleotide synthesis*: phosphoribosylpyrophosphate (PRPP) synthetase superactivity, adenylosuccinase (ADSL) deficiency, AICA-ribosiduria caused by ATIC deficiency;
- *purine catabolism*: the deficiencies of muscle AMP deaminase (AMP-DA, also termed myoadenylate deaminase), adenosine deaminase (ADA), purine nucleoside phosphorylase (PNP) and xanthine oxidase;
- *purine salvage*: the deficiencies of hypoxanthine-guanine phosphoribosyltransferase (HGPRT) and adenine phosphoribosyltransferase (APRT). The deficiency of deoxyguanosine kinase causes mitochondrial DNA depletion (► also Chap. 15).

With the exception of muscle AMP-DA deficiency, all these enzyme defects are very rare.

35.1.1 Phosphoribosyl Pyrophosphate Synthetase Superactivity

Clinical Presentation

The disorder is mostly manifested by the appearance, in young adult males, of gouty arthritis and/or uric acid lithiasis, potentially leading to renal insufficiency [1, 2]. Uricemia can be very high, reaching 10–15 mg/dl (0.60–0.90 mmol/l) [normal adult values: 2.9–5.5 mg/dl (0.17–0.32 mmol/l)]. The urinary excretion of uric acid is also increased, reaching up to 2400 mg (14 mmol)/24 h, or 2.5 mmol/mmol creatinine [normal adult values: 500–800 mg (3-4.7 mmol)/24 h, or 02–0.3 mmol/mmol creatinine].

A few patients have been reported in which clinical signs of uric acid overproduction already appeared in infancy and were accompanied by neurologic abnormalities, mainly sensorineural deafness, particularly for high tones, but also hypotonia, locomotor delay, ataxia and autistic features [2].

Metabolic Derangement

The enzyme forms phosphoribosyl pyrophosphate (PRPP) from ribose-5-phosphate and ATP (◘ Fig. 35.1). PRPP is the first intermediate of the *de novo* synthesis of purine nucleotides (not shown in full detail in ◘ Fig. 35.1), which leads to the formation of inosine monosphosphate (IMP), from which the other purine compounds are derived. PRPP synthetase is highly regulated. Various genetic regulatory and catalytic defects [1, 2] lead to superactivity, resulting in increased generation of PRPP. Because PRPP amidotransferase, the rate-limiting enzyme of the *de novo* pathway, is physiologically not saturated by PRPP, the synthesis of purine nucleotides increases, and hence the production of uric acid. PRPP synthetase superactivity is one of the few known examples of an hereditary anomaly of an enzyme which enhances its activity. The mechanism of the neurological symptoms is unresolved.

Genetics

The various forms of PRPP synthetase superactivity are inherited as X-linked traits. In the families in which the anomaly is associated with sensorineural deafness, heterozygous females have also been found with gout and/or hearing impairment [2]. Studies of the gene in six families revealed a different single base change in each of them [3].

Diagnostic Tests

Diagnosis requires extensive kinetic studies of the enzyme, which are performed on erythrocytes and cultured fibroblasts in a few laboratories in the world. The disorder should be differentiated from partial HGPRT deficiency, which gives similar clinical signs.

Treatment and Prognosis

Patients should be treated with allopurinol, which inhibits xanthine oxidase, the last enzyme of purine catabolism (◘ Fig. 35.1). This results in a decrease of the production of uric acid and in its replacement by hypoxanthine, which is about 10-fold more soluble, and xanthine, which is slightly more soluble than uric acid. Initial dosage of allopurinol is 10–20 mg/kg per day in children and 2–10 mg/kg per day in adults. It should be adjusted to the minimum required to maintain normal uric acid levels in plasma, and reduced in subjects with renal insufficiency. In rare patients with a considerable increase in *de novo* synthesis, xanthine calculi can be formed during allopurinol therapy [4]. Consequently, additional measures to prevent cristallization are recommended. These include a low purine diet (free of organ meats, fishes such as anchovy, herring, mackerel, salmon, sardines and tuna, dried beans and peas), high fluid intake and, since uric acid and xanthine are more soluble at alkaline than at acid pH, administration of sodium bicarbonate, potassium citrate or citrate mixtures to bring urinary pH to 6.0-6.5. Adequate control of the uricemia prevents gouty arthritis and urate nephropathy, but does not correct the neurological symptoms.

Table 35.1. Main presenting clinical signs and laboratory data in inborn errors of purine and pyrimidine metabolism

Clinical signs	Diagnostic possibilities
Arthritis	PRPP synthetase superactivity
	HGPRT deficiency (partial)
Ataxia	PNP deficiency
	HGPRT deficiency (complete)
	Cytosolic 5′-nucleotidase superactivity
Autistic features	PRPP synthetase superactivity
	Adenylosuccinase deficiency
	Dihydropyrimidine dehydrogenase deficiency
	Cytosolic 5′-nucleotidase superactivity
Congenital blindness	AICA-ribosiduria (ATIC deficiency)
Convulsions	Adenylosuccinase deficiency
	Combined xanthine and sulfite oxidase deficiency
	Dihydropyrimidine dehydrogenase deficiency
	Dihydropyrimidinase deficiency
	Cytosolic 5′-nucleotidase superactivity
Deafness	PRPP synthetase superactivity
Dysmorphic features	AICA-ribosiduria (ATIC deficiency)
Growth retardation	Adenylosuccinase deficiency
	ADA deficiency
	UMP synthase deficiency
	Dihydropyrimidine dehydrogenase deficiency
	Cytosolic 5′-nucleotidase superactivity
Hypotonia	Adenylosuccinase deficiency
	Muscle AMP deaminase deficiency
	Ureidopropionase deficiency
Kidney stones:	
Uric acid	PRPP synthetase superactivity
	HGPRT deficiency (complete or partial)
Xanthine	Xanthine oxidase deficiency (isolated or combined with sulfite oxidase deficiency)
2,8-Dihydroxyadenine	APRT deficiency
Orotic acid	UMP synthase deficiency
Muscle cramps	Muscle AMP deaminase deficiency
Muscle wasting	Adenylosuccinase deficiency
Psychomotor delay	PRPP synthetase superactivity
	Adenylosuccinase deficiency
	AICA-ribosiduria (ATIC deficiency)
	Combined xanthine and sulfite oxidase deficiency
	HGPRT deficiency (complete)
	UMP synthase deficiency
	Dihydropyrimidine dehydrogenase deficiency
	Dihydropyrimidinase deficiency
	Ureidopropionase deficiency
	Cytosolic 5′-nucleotidase superactivity
Recurrent infections	ADA deficiency
	PNP deficiency
	Cytosolic 5′-nucleotidase superactivity
Renal insufficiency	PRPP synthetase superactivity
	HGPRT deficiency (complete or partial)
	APRT deficiency
Self-mutilation	HGPRT deficiency (complete)
Laboratory data	**Diagnostic possibilities**
Anemia	
Megaloblastic	UMP synthase deficiency
Hemolytic	ADA superactivity
	Pyrimidine 5′-nucleotidase deficiency
Hyperuricemia	PRPP synthetase superactivity
	HGPRT deficiency (complete or partial)
Hypouricemia	PNP deficiency
	Xanthine oxidase deficiency (isolated or combined with sulfite oxidase deficiency)
Lymphopenia	
B and T-cells	ADA deficiency
T-cells	PNP deficiency
Orotic aciduria	UMP synthase deficiency

ADA, adenosine deaminase; *APRT*, adenine phosphoriboysltransferase; *ATIC*, AICAR transformylase/IMP cyclohydrolase; *HGPRT*, hypoxanthine-guanine phosphoribosyltransferase; *PNP*, purine nucleoside phosphorylase; *PRPP*, phosphoribosyl pyrophosphate; *UMP*, uridine monophosphate.

35.1.2 Adenylosuccinase Deficiency

Clinical Picture

In the first reported presentation, often referred to as type I, patients display moderate to severe psychomotor retardation, frequently accompanied by epilepsy after the first years, and by autistic features (failure to make eye-to-eye contact, repetitive behavior, temper tantrums), seldom by severe growth retardation associated with muscular wasting [5, 6]. Rare patients, referred to as type II, are only mildly retarded [6], or display profound muscle hypotonia accompanied by slightly delayed motor development [7]. Other patients have been reported with convulsions starting within the first days to weeks of life [8, 9]. The marked clinical heterogeneity justifies systematic screening for the deficiency in unexplained, profound as well as mild psychomotor retardation, and in neurological disease with convulsions and/or hypotonia.

Metabolic Derangement

Adenylosuccinase (ADSL, also named adenylosuccinate lyase), catalyzes two steps in purine synthesis (Fig. 35.1): the conversion of succinylamino-imidazole carboxamide ribotide (SAICAR) into AICAR, along the *de novo* pathway, and that of adenylosuccinate (S-AMP) into AMP. Its deficiency results in accumulation in cerebrospinal fluid and urine of the succinylpurines, SAICA riboside and succinyladenosine (S-Ado), the products of the dephosphorylation, by 5′-nucleotidase(s), of the two substrates of the enzyme. Present evidence indicates that the more severe presenta-

tions of ADSL deficiency tend to be associated with S-Ado/SAICA riboside ratios around 1, whereas in milder clinical pictures these ratios are comprised between 2 and 4. This suggests that SAICA riboside is the offending compound, and that S-Ado could protect against its toxic effects. The ADSL defect is marked in liver and kidney, and variably expressed in erythrocytes, muscle, and fibroblasts [5, 6, 9]. The higher S-Ado/SAICA riboside ratios might be explained by a more profound loss of activity of the enzyme toward S-AMP than toward SAICAR, as compared with a parallel deficiency in severely affected patients [9]. The symptoms of the deficiency remain unexplained, but positron emission tomography reveals a marked decrease of the uptake of fluorodeoxyglucose in the cortical brain areas [10].

Genetics

The deficiency is transmitted as an autosomal recessive trait [5, 6]. Studies of the ADSL gene, localized on chromosome 22, have led to the identification of about 40 mutations [11-13] (ADSL mutations database home page, http://www.icp.ucl.ac.be/adsldb/). Most are missense mutations but a splicing error [12] and a mutation in the 5′UTR [14] have also been identified. Most frequently encountered, particularly in The Netherlands, and accounting for about one-third of the alleles investigated, is a R462H mutation. Most other mutations are found in single families, in which most patients are compound heterozygotes.

Diagnostic Tests

Diagnosis is based on the presence in cerebrospinal fluid and urine of SAICA riboside and S-Ado, which are normally undetectable. These can be recognized by various techniques. For systematic screening, a modified Bratton-Marshall test [15], performed on urine, appears most practical. False positive results are, however, recorded in patients who receive sulphonamides, for the measurement of which the test was initially devised. Several thin-layer chromatographic methods are also available [16]. Final diagnosis requires HPLC with UV detection [5]. Prenatal diagnosis of ADSL deficiency can be performed by mutation analysis on chorion villi [17].

Treatment and Prognosis

With the aim to replenish hypothetically decreased concentrations of adenine nucleotides in ADSL-deficient tissues, some patients have been treated for several months with oral supplements of adenine (10 mg/kg per day) and allopurinol (5-10 mg/kg per day). Adenine can be incorporated into the adenine nucleotides via adenine phosphoribosyltransferase (APRT, ◘ Fig. 35.1). Allopurinol is required to avoid conversion of adenine by xanthine oxidase, into minimally soluble 2,8-dihydroxyadenine, which forms kidney stones. No clinical or biochemical improvement was recorded, with the exception of weight gain and some acceleration of growth [6]. Oral administration of ribose (10 mmol/kg per day) has been reported to reduce seizure frequency in an ADSL-deficient girl [18]. Uridine (2 mmol/kg per day) also had a slight beneficial effect [19].

The prognosis for survival of ADSL-deficient patients is very variable. Mildly retarded patients have reached adult age, whereas several of those presenting with early epilepsy have died within the first months of life.

35.1.3 AICA-Ribosiduria

In a female infant [20] with profound mental retardation, marked dysmorphic features (prominent forehead and metopic suture, brachycephaly, wide mouth with thin upper lip, low-set ears, and prominent clitoris due to fused labia majora), and congenital blindness, a positive urinary Bratton-Marshall test led to the identification of a massive excretion of 5-amino-4-imidazolecarboxamide (AICA)-riboside, the dephosphorylated counterpart of AICAR (◘ Fig. 35.1). Assay of ATIC, the bifunctional enzyme catalyzing the two last steps of *de novo* purine biosynthesis, revealed a profound deficiency of AICAR transformylase, and a partial deficiency of IMP cyclohydrolase. Sequencing of the ATIC gene showed a K426R change in the transformylase region in one allele, and a frameshift in the other. The discovery of this novel inborn error of purine synthesis reinforces the necessity to perform a Bratton-Marshall test [15] in all cases of unexplained mental retardation and/or neurological symptoms.

35.1.4 Muscle AMP Deaminase Deficiency

Clinical Picture

The deficiency of muscle AMP deaminase (AMP-DA, frequently referred to as *myoadenylate deaminase* in the clinical literature) is present in 1-2% of the Caucasian population. Most deficient individuals are asymptomatic. Nevertheless, some subjects, in whom the AMP-DA defect is termed primary, present with isolated muscular weakness, fatigue, cramps or myalgias following moderate to vigorous exercise, sometimes accompanied by an increase in serum creatine kinase and minor electromyographic abnormalities [21]. Muscular wasting or histological abnormalities are absent. Primary AMP-DA deficiency was initially detected in young adults, but later on wide variability was observed with respect to the age (1.5-70 years) of onset of the symptoms [22, 23]. Moreover, the enzyme defect has been detected in patients with hypotonia and/or cardiomyopathy, and in asymptomatic family members of subjects with the disorder. Secondary AMP-DA deficiency is found in association with several neuromuscular disorders amongst which amyotrophic lateral sclerosis, fascioscapulohumeral myopathy, Kugelberg-Welander syndrome, polyneuropathies, and Werdnig-Hoffmann disease [22, 23].

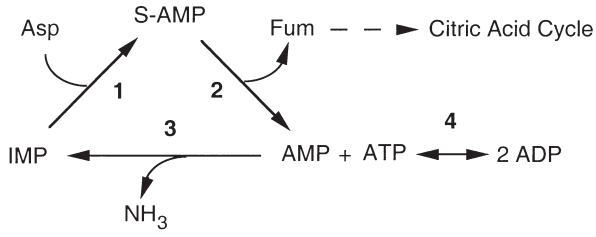
Metabolic Derangement

AMP-DA, adenylosuccinate synthetase and adenylosuccinase form the purine nucleotide cycle (◘ Fig. 35.2). Numerous functions have been proposed for this cycle in muscle (reviewed in [24]): (a) removal of AMP formed during exercise, in order to favor the formation of ATP from ADP by myokinase (adenylate kinase); (b) release of NH_3 and IMP, both stimulators of glycolysis and hence of energy production; (c) production of fumarate, an intermediate of the citric acid cycle, which also yields energy. It has therefore been proposed that the muscle dysfunction observed in primary AMP-DA deficiency is caused by impairment of energy production for muscle contraction. However, this does not tally with the vast number of asymptomatic AMP-DA-deficient individuals, and suggests that the deficiency might have a synergistic effect in association with other hitherto unidentified disorder(s).

It should be noted that muscle, liver and erythrocytes contain different isoforms of AMP-DA. A regulatory mutation of liver AMP-DA has been proposed as a cause of primary gout with overproduction of uric acid [25]. Individuals with a complete, although totally asymptomatic deficiency of erythrocyte AMP-DA have been detected in Japan, Korea and Taiwan [26].

Genetics

Primary AMP-DA deficiency is apparently transmitted as an autosomal recessive trait. *AMPD1*, the gene encoding muscle AMP-DA, is located on chromosome 1. In most individuals with the primary deficiency the defect is caused by a nonsense c.34C→T mutation resulting in a stop codon [27]. Population studies show that this mutant allele is found with a high frequency in Caucasians. This accords with the finding that about 2% of diagnostic muscle biopsies are AMP-DA deficient, and suggests that the mutation arose in a remote Western European ancestor. More recently, other more rare mutations of the *AMPD1* gene have been identified in AMP-DA deficient individuals. Interestingly, mutations of the *AMPD1* gene seem associated with improved outcome in heart diseases [28].



◘ **Fig. 35.2.** The purine nucleotide cycle. *IMP*, inosine monophosphate; *S-AMP*, adenylosuccinate; *AMP*, adenosine monophosphate; *ADP*, adenosine diphosphate; *ATP*, adenosine triphosphate; *Asp*, aspartate; *Fum*, fumarate. **1**, Adenylosuccinate synthetase; **2**, adenylosuccinase; **3**, AMP deaminase; **4**, also shown is myokinase (adenylate kinase)

Diagnostic Tests

Screening for the defect can be performed by an exercise test (► Chap. 3). A several-fold elevation of venous plasma ammonia, seen in normal subjects, is absent in AMP-DA deficiency. Final diagnosis is established by histochemical or biochemical assay in a muscle biopsy. In the primary defect, the activity of AMP-DA is below 2% of normal, and little or no immunoprecipitable enzyme is found. In the secondary defect, the activity is 2–15% of normal, and usually appreciable immunoreactivity is present [29]. In several large series of muscle biopsies for diagnostic purposes, low enzyme activities were found in about 2% of all specimens [22, 23].

Treatment and Prognosis

Patients may display a gradual progression of their symptoms, which may lead to the point that even dressing and walking a few steps lead to fatigue and myalgias. They should be advised to exercise with caution to prevent rhabdomyolysis and myoglobinuria. Administration of ribose (2–60 g per day orally in divided doses) has been reported to improve muscular strength and endurance [30].

35.1.5 Adenosine Deaminase Deficiency

Clinical Picture

The majority of patients display, within the first weeks or months after birth, a profound impairment of both humoral and cellular immunity, known as *severe combined immunodeficiency disease* (SCID). Multiple, recurrent infections rapidly become life-threatening [31, 32]. Cases with delayed infantile onset, later childhood onset, and even adult onset have, nevertheless, been reported. Caused by a broad variety of organisms, infections are mainly localized in the skin, the respiratory and the gastrointestinal tract. In the latter they often lead to intractable diarrhea, malnutrition and growth retardation. In affected children over 6 months of age, hypoplasia or apparent absence of lymphoid tissue is a suggestive sign. Bone abnormalities, clinically evident as prominence of the costochondral rib junctions, and radiologically as cupping and flaring thereof, are found in about half of the patients. In a few affected children neurological abnormalities are found, including spasticity, head lag, movement disorders, nystagmus and inability to focus. Hepatic dysfunction has also been reported [32, 33].

SCID can be confirmed by relatively simple laboratory tests: lymphopenia (usually less than 500 total lymphocytes per mm^3) involving both B and T cells, as well as hypogammaglobulinemia are almost invariably present. Whereas the IgM deficiency may be detected early, the IgG deficiency becomes manifest only after the age of 3 months, when the maternal supply has been exhausted. More elaborate tests show a deficiency of antibody formation following specific immunization and an absence or severe diminution of the

lymphocyte proliferation induced by mitogens. The disease is progressive, since residual B- and T-cell function which may be found at birth, disappears later on.

Metabolic Derangement

The deficiency results in the accumulation in body fluids of adenosine, normally nearly undetectable (◘ Fig. 35.1), and deoxyadenosine (not shown in ◘ Fig. 35.1), another substrate of adenosine deaminase (ADA), derived from the catabolism of DNA. Inside lymphocytes, deoxyadenosine excess leads to accumulation of dATP which inhibits ribonucleotide reductase, an essential enzyme for the synthesis of DNA which has to proceed at a high rate during lymphocyte development and differentiation. More recently, dATP has also been reported to provoke thymic T-cell apoptosis [34]. Deoxyadenosine has moreover been shown to inactivate S-adenosylhomocysteine hydrolase [32], an enzyme which intervenes in methyl transfer, but how this affects lymphocyte function remains elusive.

Genetics

Approximately 1/3 of the cases of inherited SCID are X-linked, whereas 2/3 are autosomal recessive. ADA deficiency is found only in the latter group, where it accounts for about 50% of the patients. The frequency of the deficiency is estimated at 1 per 100,000-500,000 births. Studies of the ADA gene, located on chromosome 20, have hitherto revealed over 70 mutations, the majority of which are single nucleotide changes, resulting in an either inactive or unstable enzyme [32]. Most patients carry two different mutations on each chromosome 20, but others, mainly from inbred communities, are homozygous for the mutation. Spontaneous in vivo reversion to normal of a mutation on one allele, as observed in tyrosinemia type I (► Chap. 18), has been reported [35].

Diagnostic Tests

The diagnosis is mostly performed on red blood cells. In general, severity of disease correlates with the loss of ADA activity: children with neonatal onset of SCID display 0–1% residual activity; in individuals with later onset, 1–5% of normal ADA activity are found [32]. It should be noted that only about 15% of the patients with the clinical and hematologic picture of inherited SCID are ADA-deficient. In the remaining patients, SCID is caused by other mechanisms. A few subjects have been described with ADA deficiency in red blood cells, but normal immunocompetence [32]. This is explained by the presence of residual ADA activity in their lymphocytes.

Treatment and Prognosis

Untreated, ADA deficiency as a rule invariably led to death, usually within the first year of life, unless drastic steps were taken, such as rearing in strictly sterile conditions from birth on. Treatment became possible with the advent of bone marrow transplantation. This remains the first choice provided an histocompatible donor is available, and gives a good chance for complete cure, both clinically and immunologically [36]. The graft provides stem cells, and hence T and B cells, which have sufficient ADA activity to prevent accumulation of adenosine and deoxyadenosine. Survival is, however, much lower with HLA-mismatched transplants.

If no histocompatible bone marrow donor is found, enzyme replacement therapy can be given. Repeated partial exchange transfusions with normal erythrocytes, irradiated before use to prevent graft-versus-host disease, result in marked clinical and immunological improvement in some patients, but in most response is poor or not sustained [36]. A much more effective enzyme replacement therapy is achieved with polyethylene glycol-modified ADA (PEG-ADA). Covalent attachment of PEG to bovine ADA results in marked extension of its half-life, and reduction of immunogenicity. Weekly to bi-weekly intramuscular injections of 15–30 units of PEG-ADA per kg result in mostly marked clinical improvement. In vitro immune function also significantly improves [37].

The first approved clinical trial of gene therapy was performed in 1990 in two girls with ADA deficiency [38]. Their peripheral blood T cells were collected, cultured with interleukin-2, corrected by insertion of the ADA gene by means of a retroviral vector, and reinfused. Because lymphocytes live only a few months, 11 or 12 infusions were given over two years to each patient. The number of T cells normalized, as did many cellular and humoral immune responses, no adverse events were observed and, remarkably, 10 years after the last cell infusion expression of the retroviral gene was still present [39]. Since as a precaution, patients continued to receive PEG-ADA although at reduced doses, benefits cannot be attributed unequivocally to gene therapy.

More recently, successful correction of ADA deficiency has been accomplished by gene therapy into hematopoietic stem cells which in theory have an unlimited life span, without concomitant PEG-ADA treatment, and with addition of a low-intensity, nonmyeloablative conditioning regimen [40]. It should be mentioned that gene therapy in X-linked, not ADA deficient SCID, although highly effective, as been placed on hold due to the development of leukemia in some patients [41].

35.1.6 Adenosine Deaminase Superactivity

A hereditary, approx. 50-fold elevation of red cell ADA, has been shown to cause non-spherocytic hemolytic anaemia [42]. The latter can be explained by an enhanced catabolism of the adenine nucleotides, including ATP, owing to the increased activity of ADA.

36.1.7 Purine Nucleoside Phosphorylase Deficiency

Clinical Picture

Recurrent infections are usually of later onset, starting from the end of the first year to up to 5-6 years of age, and are initially less severe than in ADA deficiency [43, 44]. A strikingly enhanced susceptibility to viral diseases, such as varicella, measles, cytomegalovirus and vaccinia has been reported, but severe candida and pyogenic infections also occur. One third of the patients have anemia, and two thirds display neurologic symptoms, including spastic tetra- or diplegia, ataxia and tremor. Immunological studies reveal an increasing deficiency of cellular immunity, reflected by a marked reduction in the number of T-cells. B-lymphocyte function is deficient in about one third of the patients.

Metabolic Derangement

The deficiency provokes an accumulation in body fluids of the 4 substrates of the enzyme which are normally nearly undetectable, namely guanosine, inosine (◘ Fig. 35.1), and their deoxycounterparts (not shown in ◘ Fig. 35.1), the latter derived from DNA breakdown. Formation of uric acid is thus severely hampered. The profound impairment of cellular immunity, characterizing the disorder, has been explained by an accumulation, particularly in T-cells, of excess dGTP. It is formed from deoxyguanosine, inhibits ribonucleotide reductase, and hence cell division.

Genetics

The deficiency is inherited in an autosomal recessive fashion. Studies of the PNP gene, located on chromosome 14, have revealed a number of molecular defects, among which a R234P mutation was most common [45].

Diagnostic Tests

Patients often display a striking decrease of the production of uric acid: plasma uric acid is usually below 1 mg/dl and may even be undetectable. However, in patients with residual PNP activity, uricemia may be at the borderline of normal. The urinary excretion of uric acid is usually also markedly diminished. Other causes of hypouricemia such as xanthine oxidase deficiency (► below), and drug administration (acetylsalicylic acid, thiazide diuretics), should be ruled out. Enzymatic diagnosis of PNP deficiency is usually performed on red blood cells.

Treatment and Prognosis

Until recently, most patients have died from overwhelming viral or bacterial infections, although at a later age than untreated ADA-deficient children. Treatments consisted of bone marrow transplantation and repeated transfusions of normal, irradiated erythrocytes [36, 44]. More recently, successful matched bone marrow transplantation has been reported [46]. Enzyme and gene therapy might become available in the near future.

35.1.8 Xanthine Oxidase Deficiency

Clinical Picture

Two deficiencies of xanthine oxidase (or dehydrogenase) are known: an isolated form [47],also termed hereditary *xanthinuria*, and a combined xanthine oxidase and *sulfite oxidase* deficiency [48]. Isolated xanthine oxidase deficiency can be completely asymptomatic, although in about one third of the cases kidney stones are formed. Most often not visible on X-ray, they may appear at any age. Myopathy may be present, associated with crystalline xanthine deposits. In the combined deficiency, the clinical picture of sulfite oxidase deficiency (which is also found as an isolated defect [49], ► Chap. 21) dominates that of the xanthine oxidase deficiency. The symptoms include neonatal feeding difficulties and intractable seizures, myoclonus, increased or decreased muscle tone, eye lens dislocation and severe mental retardation.

Metabolic Derangement

The deficiency results in the near total replacement of uric acid by hypoxanthine and xanthine as the end products of purine catabolism (◘ Fig. 35.1). Hereditary xanthinuria can result from a deficiency of xanthine oxidase (type I) or of both xanthine oxidase and aldehyde oxidase (type II). The latter is a closely related enzyme that metabolizes synthetic purine analogues such as allopurinol. In combined xanthine oxidase and sulfite oxidase deficiency there is in addition an accumulation of sulfite and of sulfur-containing metabolites, and a diminution of the production of inorganic sulfate. The combined defect is caused by the deficiency of a *molybdenum cofactor*, which is required for the activity of both xanthine oxidase and sulfite oxidase.

Genetics

The inheritance of both isolated xanthine oxidase deficiency and combined xanthine oxidase and sulfite oxidase deficiency is autosomal recessive. Studies of the xanthine oxidase gene, localized on chromosome 2, have led to the identification in hereditary xanthinuria type I of two mutations, resulting in a nonsense substitution and a termination codon, respectively [50]. Xanthinuria type II might be caused by mutation of a molybdenum cofactor sulferase gene [51]. More than 30 different mutations in three molybdenum cofactor biosynthetic genes have been identified in combined xanthine oxidase and sulfite oxidase deficiency [52].

Diagnostic Tests

Both in isolated and combined xanthine oxidase deficiency, plasma concentrations of uric acid below 1 mg/dl (0.06

mmol/L) are measured; they may decrease to virtually undetectable values on a low-purine diet. Urinary uric acid is reduced to a few percent of normal and replaced by hypoxanthine and xanthine. In the combined defect, these urinary changes are accompanied by an excessive excretion of sulfite and other sulfur-containing metabolites, such as S-sulfocysteine, thiosulfate and taurine. The enzymatic diagnosis requires liver or intestinal mucosa, the only human tissues which normally contain appreciable amounts of xanthine oxidase. Sulfite oxidase and the molybdenum cofactor can be assayed in liver and fibroblasts.

Treatment and Prognosis

Isolated xanthine oxidase deficiency is mostly benign but in order to prevent renal stones a low purine diet should be prescribed and fluid intake increased. The prognosis of combined xanthine oxidase and sulfite oxidase deficiency is very poor. So far, all therapeutic attempts, including low-sulfur diets, the administration of sulfate and molybdenum [48], and trials to bind sulfite with thiol-containing drugs, have been unsuccessful.

35.1.9 Hypoxanthine-Guanine Phosphoribosyltransferase Deficiency

Clinical Picture

The disorder can present under two forms. Patients with complete or near-complete deficiency of hypoxanthine-guanine phosphoribosyltransferase (HGPRT) display the Lesch-Nyhan syndrome [53]. Affected children generally appear normal during the first months of life. At 3 to 4 months of age, a neurological syndrome evolves, which includes delayed motor development, choreo-athetoid movements, and spasticity with hyperreflexia and scissoring. Over the years, the patients develop a striking, compulsive self-destructive behavior, involving biting of their fingers and lips, which leads to mutilating loss of tissue. Speech is hampered by athetoid dysarthria. Whereas most patients have IQ's around 50, some display normal intelligence. Approximately 50% of the patients have seizures. Soon or later they form uric acid stones. Mothers of Lesch-Nyhan patients have reported the finding of orange crystals on their affected son's diapers during the first few weeks after birth. Untreated, the uric acid nephrolithiasis progresses to obstructive uropathy and renal failure during the first decade of life. The latter clinical picture may, exceptionally, also be observed in early infancy.

Partial HGPRT deficiency is found in rare patients with gout. Most of them are normal on neurological examination, but occasionally spasticity, dysarthria and a spinocerebellar syndrome are found [54]. Whereas most patients with the Lesch-Nyhan syndrome do not develop gouty arthritis, this finding is common in partial HGPRT deficiency.

Metabolic Derangement

The considerable increase of the production of uric acid is explained as follows: PRPP, which is not utilized at the level of HGPRT (◘ Fig. 35.1), is available in increased quantities for the rate limiting, first enzyme of the *de novo* synthesis, PRPP amidotransferase (not shown in ◘ Fig. 35.1). Since the latter is normally not saturated with PRPP, its activity increases and the ensuing acceleration of the *de novo* synthesis results in the overproduction of uric acid.

The pathogenesis of the neurological symptoms is still not satisfactorily explained. A number of studies point to dopaminergic dysfunction, involving decreases of the concentration of dopamine and of the activity of the enzymes required for its synthesis, although dopaminergic drugs are not useful. Positron emission tomography of the brain with F-18 fluorodopa, an analogue of the dopamine precursor levodopa, has revealed a generalized decrease of the activity of dopa decarboxylase [55]. How the HGPRT defect leads to the deficit of the dopaminergic system, and how the latter results in the characteristic neuropsychiatric manifestations of the Lesch-Nyhan syndrome, remains to be clarified.

Genetics

Both the Lesch-Nyhan syndrome and the partial deficiencies of HGPRT are transmitted in a X-linked recessive manner. Studies of the HGPRT gene in large groups of unrelated patients have revealed a variety of defects, ranging from point mutations provoking single amino acid substitutions and henceforth enzymes with altered stability and/or kinetic properties, to extensive deletions resulting in suppression of enzyme synthesis [56]. These studies have contributed a great deal to the understanding of the clinical variation observed in human inherited disease, and provided support for the concept that, in X-linked disorders, new mutations constantly appear in the population. Presently, over 250 mutations of the HGPRT gene have been described, and molecular studies have led to precise prenatal diagnosis and efficient carrier testing of at-risk females [57].

Diagnostic Tests

Patients excrete excessive amounts of uric acid, ranging from 25 to 140 mg (0.15 to 0.85 mmol)/kg of body weight per 24 h, as compared to an upper limit of 18 mg (0.1 mmol)/kg per 24 h in normal children. Determination of the ratio of uric acid to creatinine (mg/mg) in morning samples of urine provides a screening test. This ratio is much higher in HGPRT deficiency than the normal upper limits of 2.5, 2.0, 1.0 and 0.6 for infants, 2 years, 10 years and adults, respectively [58]. Increased ratios are also found in other disorders with uric acid overproduction, such as PRPP synthetase superactivity, glycogenosis type I, lymphoproliferative diseases, and after fructose loading. The overproduction of

uric acid is as a rule accompanied by an increase of serum urate, which may reach concentrations as high as 18 mg/dl (1 mmol/L). Occasionally, however, particularly before puberty, uricemia may be in the normal or high normal range.

Patients with the Lesch-Nyhan syndrome display nearly undetectable HGPRT activity in red blood cells [59]. In partial deficiencies, similar low or higher values may be found [60]. Rates of incorporation of hypoxanthine into the adenine nucleotides of intact fibroblasts correlate better with the clinical symptomatology than HGPRT activities in erythrocytes: patients with the complete Lesch-Nyhan syndrome incorporated less than 1.2% of normal, those with gout and neurological symptoms 1.2–10% of normal, and those with isolated gout, 10–55% of normal [60].

Treatment and Prognosis

Allopurinol, as detailed under *PRPP synthetase superactivity*, is indicated to prevent urate nephropathy. Allopurinol, even when given from birth, has, however, no effect on the neurological symptoms, which have sofar been resistant to all therapeutic attempts. Adenine has been administered, together with allopurinol, with the aim to correct a possible depletion of purine nucleotides. However, no or minimal changes in neurological behavior were recorded [61]. Patients should be made more comfortable by appropriate restraints, including elbow splints, lip guards and even tooth extraction, to diminish self-mutilation. Diazepam, haloperidol and barbiturates may sometimes improve choreoathetosis.

In a 22-year-old patient, bone marrow transplantation restored erythrocyte HGPRT activity to normal, but did not change neurological symptoms [62]. Recently, disappearance of self-mutilation was obtained by chronic stimulation of the globus pallidus [63].

35.1.10 Adenine Phosphoribosyltransferase Deficiency

Clinical Picture

The deficiency may become clinically manifest in childhood [64], even from birth [65], but also remain silent for several decades. Symptoms include urinary passage of gravel, small stones and crystals, frequently accompanied by abdominal colic, dysuria, hematuria and urinary tract infection. Some patients may even present with acute anuric renal failure [66]. The urinary precipitates are composed of 2,8-dihydroxyadenine, radiotranslucent, and undistinguishable from uric acid stones by routine chemical testing.

Metabolic Derangement

The deficiency results in suppression of the salvage of adenine (◘ Fig. 35.1), provided by food and by the polyamine pathway (not shown in ◘ Fig. 35.1). Consequently, adenine is oxidized by xanthine oxidase into 2,8-dihydroxyadenine, a very poorly soluble compound (solubility in urine, at pH 5 and 37°C, is about 0.3 mg/dl as compared to 15 mg/dl for uric acid).

The deficiency can be complete or partial. The partial deficiency is only found in the Japanese, among whom it is quite common [67]. Activities range from 10 to 30% of normal at supraphysiological concentrations of PRPP, but a 20- to 30-fold decrease in the affinity for PRPP results in near inactivity under physiological conditions.

Genetics

APRT deficiency is inherited as an autosomal recessive trait. All the type II Japanese patients carry the same c.2069T →C substitution in exon 5, resulting in a M136T change [67]. Approximately 80% are homogenous, with two other mutations accounting for nearly all the other cases. In Caucasians, approximately 30 mutations have been identified, some of which seem more common, also suggesting founder effects [68].

Diagnostic Tests

Identification of 2,8-dihydroxyadenine requires complex analyses, including UV and infrared spectrography, mass spectrometry and X-ray cristallography [64, 65]. It is therefore usually easier to measure APRT activity in red blood cells.

Treatment and Prognosis

In patients with symptoms, allopurinol should be given, as detailed under *PRPP synthetase superactivity*, to inhibit the formation of 2,8-dihydroxyadenine. Both in patients with stones and in those without symptoms, dietary purine restriction and high fluid intake are recommended. Alkalinization of the urine is, however, not advised: unlike that of uric acid, the solubility of 2,8-dihydroxyadenine does not increase up to pH 9 [64].

Ultimate prognosis depends on renal function at the time of diagnosis: late recognition may result in irreversible renal insufficiency requiring chronic dialysis, and early treatment in prevention of stones. Of note is that kidney transplantation has been reported to be followed by recurrence of microcrystalline deposits and subsequent loss of graft function [69].

35.1.11 Deoxyguanosine Kinase Deficiency

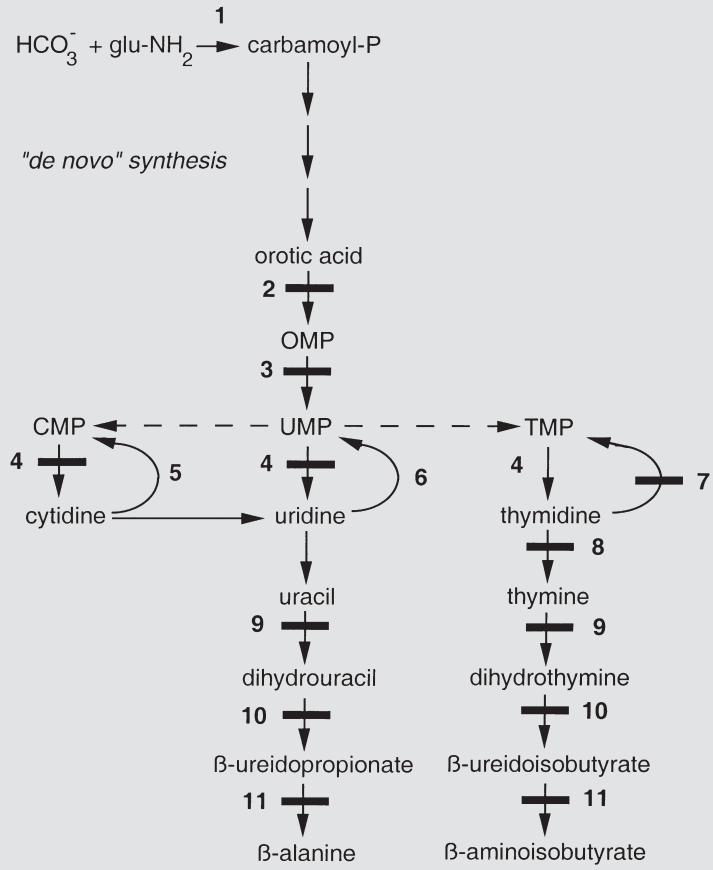
In several patients with the hepatocerebral form of mitochondrial DNA depletion syndrome (► also Chap. 15), characterised by early progressive liver failure, neurological abnormalities, hypoglycemia, and increased lactate, a deficiency of mitochondrial deoxyguanosine kinase

was identified [70]. This enzyme phosphorylates the deoxycounterpart of guanosine (◘ Fig. 35.1) into deoxyGMP, and plays an essential role in the supply of precursors of mitochondrial DNA, particularly in liver and brain that lack a cytosolic form of the enzyme. A single nucleotide deletion in the mitochondrial deoxyguanosine kinase gene segregated with the disease in 19 patients in 3 kindreds [70]. Since then, othere mutations have been identified.

Pyrimidine Metabolism

Similarly to that of the purine nucleotides, the metabolism of the pyrimidine nucleotides can be divided into three pathways:

- The biosynthetic pathway starts with the formation of carbamoylphosphate by cytosolic carbamoylphosphate synthetase (CPS II), which is different from the mitochondrial CPS I which catalyzes the first step of ureogenesis (◘ Fig. 20.1). This is followed by the synthesis of UMP, and hence of CMP and TMP.
- The catabolic pathway starts from CMP, UMP and TMP, and yields β-alanine and β-aminoisobutyrate which are converted into intermediates of the citric acid cycle.
- The salvage pathway, composed of kinases, converts the pyrimidine nucleosides, cytidine, uridine, and thymidine, into the corresponding nucleotides, CMP, UMP, and TMP.



◘ **Fig. 35.3.** Pathways of pyrimidine metabolism. *CMP*, cytidine monophosphate; *glu-NH2*, glutamine; *OMP*, orotidine monophosphate; *PRPP*, phosphoribosylpyrophosphate; *TMP*, thymidine monophosphate; *UMP*, uridine monophosphate. **1**, carbamoylphosphate synthetase; **2**, orotate phosphoribosyltransferase; **3**, orotidine decarboxylase (**2** and **3** form UMP synthase); **4**, pyrimidine (cytosolic) 5′-nucleotidase; **5**, cytidine kinase; **6**, uridine kinase; **7**, thymidine kinase; **8**, thymidine phosphorylase; **9**, dihydropyrimidine dehydrogenase; **10**, dihydropyrimidinase; **11**, ureidopropionase. Enzyme deficiencies are indicated by *solid bars* across the arrows

35.2 Inborn Errors of Pyrimidine Metabolism

Inborn errors of pyrimidine metabolism comprise a defect of the *synthesis* of pyrimidine nucleotides (UMP synthase deficiency), and three inborn errors of pyrimidine *catabolism*: the deficiencies of dihydropyrimidine dehydrogenase (DPD) dihydropyrimidinase (DHP), and pyrimidine 5′-nucleotidase. More recently, superactivity of cytosolic 5′-nucleotidase, a fourth defect of pyrimidine catabolism, ureidopropionase deficiency, and deficiencies of thymidine phosphorylase and thymidine kinase, which cause mitochondrial diseases (► also Chap. 15), have been reported.

35.2.1 UMP Synthase Deficiency (Hereditary Orotic Aciduria)

Clinical Presentation

Megaloblastic anemia, which appears a few weeks or months after birth, is usually the first manifestation [71, 72]. Peripheral blood smears often show anisocytosis, poikilocytosis, and moderate hypochromia. Bone marrow examination reveals erythroid hyperplasia and numerous megaloblastic erythroid precursors. Characteristically, the anemia does not respond to iron, folic acid or vitamin B_{12}. Unrecognized, the disorder leads to failure to thrive and to retardation of growth and psychomotor development.

Metabolic Derangement

Uridine monophosphate (UMP) synthase is a bifunctional enzyme of the *de novo* synthesis of pyrimidines (◘ Fig. 35.3). A first reaction, orotate phosphoribosyltransferase (OPRT), converts orotic acid into OMP, and a second, orotidine decarboxylase (ODC), decarboxylates OMP into UMP. The defect provokes a massive overproduction of orotic acid and a deficiency of pyrimidine nucleotides [72]. The overproduction is attributed to the ensuing decrease of the feedback inhibition exerted by the pyrimidine nucleotides on the first enzyme of their *de novo* synthesis, cytosolic carbamoyl phosphate synthetase II (◘ Fig. 35.3). The deficiency of pyrimidine nucleotides leads to impairment of cell division, which results in megaloblastic anemia and in retardation of growth and development.

Genetics

Hereditary orotic aciduria is inherited as an autosomal recessive trait. The genetic lesion results in synthesis of an enzyme with reduced stability [73]. Three point mutations have been identified in two Japanese families [74].

Diagnostic Tests

Urinary analysis reveals a massive over excretion of orotic acid, reaching, in infants, 200- to 1000-fold the normal adult value of 1–1.5 mg per 24 h. Occasionally, orotic acid crystalluria is noted, particularly upon dehydration. Enzymatic diagnosis can be performed on red blood cells. In all patients reported hitherto, except one, both OPRT and ODC activities were deficient. This defect is termed type I. In a single patient, referred to as type II, only the activity of ODC was initially deficient, although that of OPRT also subsequently decreased [72].

Treatment and Prognosis

The enzyme defect can be by-passed by the administration of uridine, which is converted into UMP by uridine kinase (◘ Fig. 35.3). An initial dose of 100-150 mg/kg, divided over the day, induces prompt hematologic response and acceleration of growth. Further dosage should be adapted to obtain the lowest possible output of orotic acid. In some cases normal psychomotor development was achieved, but not in others, possibly owing to delayed onset of therapy.

35.2.2 Dihydropyrimidine Dehydrogenase Deficiency

Clinical Picture

Two forms occur. The first is found in children, most of whom display epilepsy, motor and mental retardation, often accompanied by generalized hypertonia, hyperreflexia, growth delay, dysmorphic features including microcephaly, and autistic features [75, 76]. In these patients, the deficiency of dihydropyrimidine dehydrogenase (DPD) is complete or near-complete. Nevertheless, the severity of the disorder is highly variable and even asymptomatic cases have been identified. The second clinical picture is found in adults who receive the pyrimidine analog, 5-fluorouracil, a classic treatment of various cancers including breast, ovary or colon [77, 78]. It is characterised by severe toxicity, manifested by profound neutropenia, stomatitis, diarrhea and neurologic symptoms, including ataxia, paralysis and stupor. In these patients, DPD deficiency is as a rule partial, and only revealed by 5-fluorouracil therapy.

Metabolic Derangement

The deficiency of DPD, which catalyzes the catabolism of uracil and thymine into dihydrouracil and dihydrothymine, respectively (◘ Fig. 35.3), leads to the accumulation of the former compounds [75]. Why a profound DPD deficiency becomes manifest in some pediatric patients, but not in others, is not known. How the defect leads to neurological symptoms also remains elusive, but reduction of the concentration of β-alanine, a neurotransmitter, may play a role. The marked potentiation of the action of the anticancer drug 5-fluorouracil, and henceforth of its toxicity, is explained by a block of the catabolism, via DPD, of this pyrimidine analog.

Genetics

The infantile form of DPD deficiency is inherited as an autosomal recessive trait. The DPD gene is localized on chromosome 1, and about 40 mutations have been identified. Most frequent is a splice site mutation (IVS14+1G>A), which results in skipping of a complete exon [76, 78, 79]. Strikingly, patients who carry the same mutation may display widely variable clinical symptoms. In the adult form of DPD deficiency, characterized by 5′-fluorouracil toxicity, approximately 25% of patients are heterozygotes for the IVS14+1G>A mutation [78].

Diagnostic Tests

Patients excrete high amounts of uracil (56–683 mmol/mol creatinine, as compared to 3–33 in control urine) and of thymine (7–439 mmol/mol creatinine, as compared to 0–4 in control urine). Elevations of uracil and thymine in plasma and cerebrospinal fluid are much less prominent [76]. Excretion of both compounds may also be less elevated in patients with high residual DPD activity. The pyrimidine catabolites can be detected by HPLC, GC-MS, and analysis of amino acids in urine before and after acid hydrolysis [80].

The enzyme defect can be demonstrated in the patients' fibroblasts, liver and blood cells, with the exception of erythrocytes [75, 76, 78]. In the pediatric patients, DPD deficiency is complete or near-complete; in the adult cancer patients experiencing acute 5-fluorouracil toxicity it is partial, with residual enzyme activities ranging from 3 to 30%.

Treatment and Prognosis

No treatment is available for pediatric patients. Symptoms usually remain the same, but death in early infancy of a more severely affected child has been reported. In the adult cancer patients, discontinuation of 5-fluorouracil results in slow resolution of the toxic symptoms [77, 78].

35.2.3 Dihydropyrimidinase Deficiency

Clinical Picture

This disorder was first reported in a single male baby of consanguineous parents, presenting with convulsions and metabolic acidosis [81]. Additional patients have been diagnosed since then [76]. As in DPD deficiency, the clinical picture varies from severe psychomotor retardation with epilepsy, dysmorphic features or microcephaly, to completely asymptomatic.

Metabolic Derangement

Dihydropyrimidinase (DHP) catalyzes the cleavage of dihydrouracil and dihydrothymine into, repectively, β-ureidopropionate and β-ureidoisobutyrate (◘ Fig. 35.3). Consequently, considerable quantities of dihydrouracil and dihydrothymine, which are normally found in small amounts, are excreted in urine [76]. There is also a moderate elevation of uracil and thymine excretion. As in DPD deficiency, the reasons for the appearance and the mechanisms of the symptoms remain unexplained, and reduced concentrations of the neurotransmitter β-alanine may play a role. Increased sensitivity to 5-fluorouracil, leading to severe toxicity has also been reported [82].

Genetics

The defect is inherited as an autosomal recessive trait. Studies of the DHP gene, localized on chromosome 8, have led to the identification of one frameshift and five missense mutations in one symptomatic and five asymptomatic individuals [83]. Enzyme expression showed no significant difference in residual activity between the mutations of the symptomatic and the asymptomatic individuals.

Diagnostic Tests

Elevation of urinary dihydrouracil and dihydrothymine can be detected by the techniques used for measurement of uracil and thymine in DPD deficiency. Enzyme assay requires liver biopsy, since more accessible tissues do not possess DHP activity [81].

Treatment and Prognosis

There is no therapy and prognosis seems unpredictable. The first reported patient recovered completely and apparently displays normal physical and mental development [81]. In contrast, another patient had a progressive neurodegenerative clinical course [84].

35.2.4 Ureidopropionase Deficiency

In a female infant of consanguineous parents, presenting with muscle hypotonia, dystonic movements and severe developmental delay, in vitro H-NMR spectroscopy of urine revealed elevated ureidopropionic acid (also called *N*-carbamyl-β-alanine) and ureidoisobutyric acid (also called *N*-carbamyl-β-aminoisobutyric acid) [85]. These findings led to the identification of ureidopropionase deficiency (also termed β-alanine synthase) in the liver [86].

35.2.5 Pyrimidine 5′-Nucleotidase Deficiency

This defect, restricted to erythrocytes, leads to accumulation of pyrimidine nucleotides resulting in basophilic stippling and chronic hemolytic anemia [87]. The mechanism by which the increased pyrimidine nucleotides cause hemolysis remains unknown.

35.2.6 Cytosolic 5′-Nucleotidase Superactivity

Four unrelated children have been described with a syndrome including developmental delay, growth retardation, seizures, ataxia, recurrent infections, autistic features and hypouricosuria [88]. Studies in the patients' fibroblasts showed 6- to 20-fold elevations of the activity of cytosolic 5′-nucleotidase, measured either with a pyrimidine (UMP) or a purine (AMP) as substrate. Based on the possibility that this increased catabolism might cause a deficiency of pyrimidine nucleotides, the patients were treated with uridine at the dose of 1 g/kg per day. Remarkable developmental improvement, and a decrease in frequency of seizures and infections were recorded.

35.2.7 Thymidine Phosphorylase Deficiency

Patients with mitochondrial neurogastrointestinal encephalomyopathy (MNGIE), an autosomal recessive disease associated with multiple deletions of skeletal muscle mitochondrial DNA (► also Chap. 15), have been shown deficient in thymidine phosphorylase, owing to a variety of mutations [89]. The enzyme deficiency results in marked accumulation of thymidine, which most likely provokes imbalance of the mitochondrial nucleotides, and hence compromises the replication of mitochondrial DNA.

35.2.8 Thymidine Kinase Deficiency

In four independent patients with very severe, isolated myopathy, and depletion of muscular mitochondrial DNA (► also Chap. 15), two mutations of the gene encoding thymidine kinase-2, the mitochondrial form of the thymidine salvage enzyme, have been identified [90]. As in the deficiencies of deoxyguanosine kinase and thymidine phosphorylase, the defect likely produces imbalance of the mitochondrial nucleotides which disturbs the replication of mitochondrial DNA.

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