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Purine and pyrimidine metabolism: still more to learn

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Mammalian metabolism is heavily dependent on proper functioning of purine and pyrimidine synthesis, interconversion and degradation. Purine- and pyrimidine derived compounds are essential in numerous processes throughout life, including the synthesis of macromolecules, oxidative phosphorylation, signal transduction and high energy transfer. This ubiquitous presence is the reason that disturbances in purine and pyrimidine metabolism can result in life threatening clinical conditions. Understanding of this metabolism under normal and compromised circumstances is essential to diagnose inborn errors of purine and pyrimidine metabolism. The desire to gain more insight in these pathways is the basis of ongoing research covering different aspects of purine and pyrimidine metabolism, with special emphasis on purine biosynthesis, mitochondrial purine and pyrimidine metabolism and the pharmacogenetic aspects of synthetic purine and pyrimidine compounds. Other areas of interest are the role of the enzyme ITPase in mammalian metabolism and the development of diagnostic tools to detect defects in purine and pyrimidine metabolism on the metabolite, protein and molecular level.

Inherited defects in purine and pyrimidine metabolism

The dependence of mammalian life on purines and pyrimidines renders it prone to the effects of disturbances anywhere in the pathways involved in the biosynthesis, interconversion and degradation of these metabolites. Defects can occur in all phases of purine and pyrimidine metabolism, at present a total number of >35 defects are recognized on the basis of the metabolite pattern, enzyme activity or mutations in the genes involved. In table 1 a selection of the most common defects is shown.

The clinical spectrum of defects of purine and pyrimidine metabolism is diverse and even within defects or genotypes various forms of clinical presentation exist. One of the classical disorders in purine metabolism is Lesch-Nyhan syndrome, a deficiency of the enzyme hypoxanthine-guanine phosphoribosyl transferase (HGPRT), a salvage enzyme that recycles the purine bases hypoxanthine and guanine. In boys this X-linked disorder results in a complete or partial deficiency of the enzyme, as can be measured in erythrocytes. As a consequence massive amounts of uric acid are found in the circulation and accumulate in tissues. In most cases a severe clinical picture is associated with this condition, including profound psychomotor retardation, automutilation, movement disorders and nephropathy. One of the consequences of the enzyme defect is a surplus of phosphoribosylpyrophosphate (PRPP), the key compound for purine *de novo* synthesis. The *de novo* synthesis is upregulated and results in increased production of inosinemonophosphate (IMP), subsequently leading to an increased production of xanthine and hypoxanthine, the precursors of uric acid. The clinical picture in females can range from asymptomatic carriers to an attenuated presentation of the disease (1).

So far only 3 defects in the purine *de novo* synthesis pathway have been described, each with a different clinical picture. One of these defects involves the enzyme adenylosuccinate lyase; this bifunctional enzyme is also active in the purine interconversion pathway of IMP to adenosine monophosphate (AMP). A defect in this enzyme causes the accumulation of the substrates from both pathways in body fluids and tissues. Depending on the mutation variations in the excretion pattern of the marker metabolites from both pathways the enzyme plays a role in. We have recently developed a simple method to measure the activity of the interconversion capability of this enzyme in erythrocytes. To measure the activity of the enzyme for the catalysis of the *de novo* reaction is much more complicated, mainly because of the lack of the original substrate (2). Defects in pyrimidine metabolism are as devastating as defects in the purine counterpart. Dihydropyrimidine dehydrogenase (DPD) deficiency is a defect with a clinical picture ranging from asymptomatic homozygous carriers of mutations in the *DPYD* gene, in whom the metabolite pattern in body fluids is clearly aberrant, to patients with motor and mental retardation and convulsions (3, 4). The diagnosis can be made by measuring the excretion of the accumulating metabolites thymine, uracil and 5-hydroxymethyl uracil by UPLC tandem MS or HPLC with UV detection. Confirmation can be done by measurement of DPD

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activity in peripheral lymphocytes and/or by mutation analysis of the *DPYD* gene.
Using sophisticated analytical techniques like UPLC-tandem MS enables detection of most defects in pyrimidine metabolism, making early diagnosis and (eventually) treatment possible, greatly enhancing the quality of patient care.

Mitochondrial diseases and purine/pyrimidine metabolism

A relatively new phenomenon is the patient described in the literature with 'decreased amount of mitochondrial DNA (mtDNA)'. The clinical picture of these patients resembled that of the classical patient with a defect of the oxidative phosphorylation (OXPHOS),

Tabel 1

Defect	Index metabolites	Clinical presentation (main symptoms)
PRPPS deficiency	urate ↓ orotate ↑	MR + convulsions
PRPPS superactivity	urate ↑ sensorineuronal deafness (±),	PMR/ataxia, congenital dysmorphic features, gout, urolithiasis
Adenylosuccinate lyase deficiency	succinyladenosine ↑ SAICAR ↑	PMR, convulsions, autism, hypotonia, periferal hypertonia, feeding difficulties
AICAR transformylase/ IMP cyclohydrolase deficiency	AICAR ↑	MR, hypotonia, blindness, dysmorphic features, convulsions
AMP-Deaminase-deficiency	NH3 ↓ CK ↑ (only after strenuous exercise)	Myalgia, exercise intolerance
HGPRT-deficiency	hypoxanthine, xanthine, urate ↑	PMR, hypotonia, automutilation Lesch-Nyhan and Kelley-Seegmiller syndromes
APRT-deficiency	2,8-dihydroxyadenine	Urolithiasis, (acute) renal failure
ADA-deficiency	deoxyadenosine, adenosine ↑	SCID
PNP-deficiency	(deoxy)inosine, (deoxy) guanosine ↑	T-Cell immune deficiency
XDH (isol) XDH/AO	urate ↓, hypoxanthine, xanthine ↑	XDH deficiency: often asymptomatic; sometimes xanthine lithiasis, acute renal failure, myopathy; able to convert allopurinol + AO deficiency: same, unable to convert allopurinol
XDH/SO (Molybdenum cofactor defiency)	additional: S-sulphocysteine, sulphite, thiosulphate ↑, cystine ↓	Molybdenum cofactor deficiency: same, + neonatal convulsions, PMR, lens dislocation, dysmorphic features, hypo/hypertonia, cerebral atrophy
UMPS deficiency (OPRT-deficiency)	orotate	Megaloblastic anaemia, ftt, retardation
Deoxyguanosine kinase deficiency	None specific	Hepatocerebellar mtDNA-depletion
TK-2 deficiency	None specific	Muscular mtDNA-depletion
TP-deficiency	thymidine, deoxyuridine	MNGIE-syndrome
DPD-deficiency	thymine, uracil	PMR, convulsions, autism, growth retardation, 5FU-toxicity
Dihydropyrimidinase-deficiency	dihydrothymine/uracil	PMR, convulsions, 5FU-toxicity
ß-Ureidopropionase-deficiency	NC-BALA, NC-BAIBA ↑	Muscular hypotonia, developmental delay, dystonia
UMPH superactivity (pyrimidine 5'-nucleotidase)	urate ↓	Developmental delay, convulsions, recurrent infections
UMPH deficiency (pyrimidine 5'-nucleotidase)	erythrocyte pyrimidine nucleotides ↑	Haemolytic anaemia
TPMT deficiency	Intra cellular thioguanine nucleotides ↑	Pancytopenia
ITPase-deficiency	Intracellular (d)ITP ↑	Pharmacogenetic defect
IMPDH deficiency	Unknown	Decreased thiopurine efficacy

showing an impaired overall ATP generating capacity; however the specific activity of the individual enzymes of the respiratory chain appeared to be normal or (only slightly) decreased. Further evaluation of these patients showed a decreased amount of mtDNA, suggesting a reduced number of mtDNA copies inside the cell and, in some cases, multiple deletions in the mtDNA.

Since the first description of mtDNA depletion in a patient mimicking classical mitochondrial disease, a number of genetic defects that cause mtDNA depletion syndrome have been elucidated. These inherited traits are autosomal defects, in contrast to the 'true' mitochondrial defects which are caused by mtDNA mutations. Depletion of mtDNA can be caused by defects throughout the cascade of mtDNA formation, including disturbances in mtDNA processing (POLG, TWINKLE), defects in nucleotide synthesizing enzymes (TP, TK-2, dGuoK), nucleotide transport (ANT1, DNC) and structural proteins (TFAM, MPV17, Succ-CoA synthase).

Deoxynucleotides used to synthesize mtDNA originate from either *de novo* synthesis or through the salvage pathways; one of the essential factors in this pathway is DNA polymerase γ (POLG). This DNA polymerase is solely present in mitochondria and responsible for mtDNA replication. Defects in POLG result in an imbalanced intramitochondrial nucleotide pool and the clinical picture is consistent with a mitochondrial disorder, hypotonia, liver disease, epilepsy, and other characteristic symptoms (5).

Through reverse genetics, deficiency of thymidine phosphorylase (TP) was identified as the cause of the clinical syndrome called MNGIE, mitochondrial neurogastrointestinal encephalomyopathy. As is denoted by its name, TP catalyzes the conversion of thymidine to thymine and 2-deoxyribose-1-phosphate and has a regulatory role in the intramitochondrial homeostasis of thymidinetriphosphate (dTTP). In resting cells the remaining thymidine is shuttled into the mitochondrion through the equilibrative nucleoside transporter (ENT1) and is phosphorylated to dTMP by the mitochondrial thymidine kinase-2 (TK-2). (figure 1).

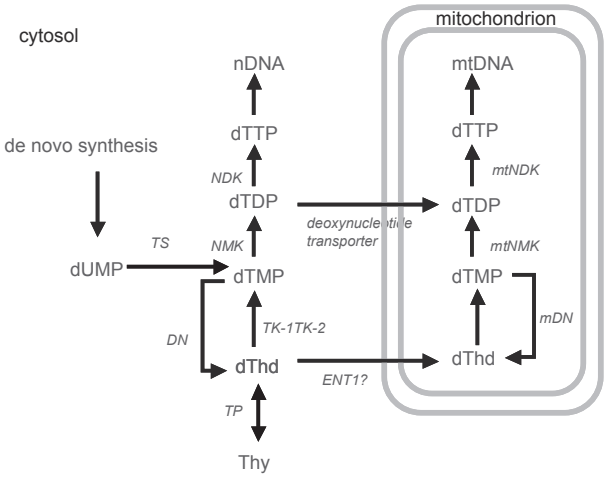

Figure 1. Thymidine metabolism in the cytosol and mitochondrion

Although TP deficiency is a multisystem disease, the gastrointestinal problems are the most prominent and in most cases the first presenting symptoms of the disease. Visceral manifestations are due to mitochondrial dysfunction of the intestinal smooth muscle. Blondon et al. reported these mitochondrial abnormalities in small intestine biopsies to be identical to the aberrations observed in skeletal muscle of patients with mitochondrial disease (6). The clinical picture develops gradually in the course of life and cases can be misdiagnosed easily, especially when the 'mild' symptoms are not recognized as a part of the MNGIE picture, although brain MRI often shows a leukoencephalopathy. As a consequence of the mtDNA depletion, mitochondrial function is affected and there is a reduction in overall OXPHOS capacity as well as a decreased activity of the enzymes of the respiratory chain with mtDNA encoded subunits. More in-depth laboratory investigations show elevated concentrations of thymidine and deoxyuridine in all body fluids. TP activity in peripheral leucocytes is nearly undetectable in homozygous patients, while in heterozygotes the residual TP activity is about 30%. Mutation analysis of the gene encoding TP, *ECGF1*, revealed about 20 different mutations in the TP patients diagnosed so far. Molecular genetic studies of mtDNA in patients with TP deficiency showed a depletion of the amount of mtDNA in cells, as well as multiple deletions (7). It is obvious that in case of a patient with neurological dysfunction and gastrointestinal symptoms TP deficiency has to be ruled out as the cause of the disease.

Pharmacogenetic aspects of purine and pyrimidine metabolism

The key function of purine and pyrimidine metabolites in cellular processes, especially proliferation, has served as a template for the invention of synthetic purine and pyrimidine analogues used in the treatment of cancer and viral infections. These non-natural compounds are metabolised by the same pathways as natural occurring purines and pyrimidines and the activated metabolites will interfere with normal metabolites, hereby affecting normal cell metabolism and proliferation.

The enzyme dihydropyrimidine dehydrogenase (DPD) is involved in the degradation of 5-fluoro-uracil (5-FU), a potent cytostatic drug, used in breast and colon cancer therapy. Mutations in the *DPYD* gene, either in the heterozygous or homozygous state, cause an enhanced intra-cellular concentration of the toxic metabolites, leading to severe adverse drug reactions (ADRs), potentially causing death if not recognized. We advocate precautionary advance-testing of all patients who are candidates for treatment with 5-FU (or its derivatives) by measuring enzyme activity level in mononuclear cells. These results can be available within 2-3 working days, and, if applicable, conformational molecular analysis results are available within a reasonable timeframe

Efficacy of thiopurine therapy is dependent on proper functioning of purine degradation and interconversion pathways (8). One of the most important enzymes in this respect is thiopurine-S-methyltransferase

(TPMT), an enzyme with an unknown biological function under normal circumstances, but it is absolutely essential in the deactivation of thiopurine compounds. Polymorphisms in the *TPMT* gene, leading to impaired enzyme activity, are responsible for the accumulation of unwanted intracellular concentrations of the active thioguanine nucleotides, ultimately resulting in ADRs like pancytopenia and pneumonitis (9, 10). Co-medication can also be responsible for changes in therapeutic efficacy of thiopurines, e.g. the effect of mesalazine is a well known example (11).
Recently the role of inosine triphosphatase (ITPase) in thiopurine metabolism was recognized (12). Although some debate is still ongoing on the clinical relevance of ITPase in thiopurine therapy, our group has shown that thioinosine triphosphate is a substrate for ITPase, and therefore activity lowering polymorphisms in the *ITPA* gene can in potential be responsible for ADRs under thiopurine therapy (13).
New insights in the function of ITPase and/or *ITPA* are suggestive for a role in immune mediated disease (14). In this respect Fellay et al. described an association between polymorphisms and the occurrence of ribavirin induced anaemia in patients with chronic Hepatitis C infection (15). Future research is directed to gain more insights in the exact role of this house keeping gene in mammalian metabolism, both under normal and immune compromised situations.

Conclusion

The central and omnipresent role purine and pyrimidine metabolism plays in human life, and how much there still remains to be unravelled is our inspiration and motivation for continuing our investigations. Increasing the diagnostic scope in order to identify patients with known and yet unknown inherited and acquired defects in purine and pyrimidine metabolism will contribute to the elucidation of new defects, new effects of 'old' defects and the interaction between purine- and pyrimidine-analogue drugs and human metabolism. These, our main topics of research, allow us to interact with expert colleagues sharing interest in purines and pyrimidines and help us to elucidate more facets of this very complicated, but also very intriguing, aspect of metabolism.

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