

23.1 Introduction

■ Purine, Pyrimidine and Related Disorders

Genetic metabolic purine and pyrimidine disorders were first reported in children as the cause of kidney stones and intractable anaemia in 1954 and 1959 respectively [1]. A genetic basis for gout presenting in childhood with severe neurological deficits (Lesch-Nyhan syndrome) was recognised in 1967. The number of enzyme defects now totals 27, but some of these are relatively benign, with no currently apparent clinical sequelae. Only those with defined clinical consequences are described in this text. Any system can be affected – immunological, haematological, neurological, musculoskeletal and, because of the extreme insolubility of purine bases, renal as well. The broad spectrum of presentation underlines the importance of these ‘housekeeping’ enzymes for providing the vital building blocks for DNA, RNA and ATP, as well as the pyrimidine sugars essential to phospho- and glyco-lipid synthesis (Figs. 1 and 2).

These disorders were hitherto considered paediatric problems, but are now being recognised increasingly as the cause of life-threatening symptoms in adults and may present from birth to the 80’s. Some have more than one form of presentation, as in the Lesch-Nyhan syndrome which frequently presents, as acute renal failure, kidney stones (due to the associated uric acid overproduction), or gout in a child institutionalised for cerebral palsy of unknown cause. Because of their relatively recent recognition these disorders are not well known and may be misdiagnosed, or remain undiagnosed, a problem compounded by the broad spectrum of presentation [1, 2]. Both purine and pyrimidine disorders can also be the cause of catastrophic responses to ‘anti-metabolite’ therapy.

An additional diagnostic problem is the considerable phenotypic variation within a single disorder – both between families and within families with that disorder. It is always the most catastrophic form of presentation which is identified first. Milder forms presenting later, or found only during family screening, are now being recognised. The immunodeficiency disorder adenosine deaminase (ADA) deficiency is a good example. Hitherto considered a

disease of early childhood, it has now been diagnosed in patients in their twenties and thirties [3].

The broad spectrum of clinical presentation highlights the importance of particular steps in purine and pyrimidine metabolism to different cells and tissues and should have assisted in the development of appropriate treatment. Unfortunately, only three of the nineteen disorders described can be treated successfully: hereditary orotic aciduria with life-long uridine, 2,8-dihydroxyadenine lithiasis with allopurinol. ADA deficiency is treatable by bone marrow transplantation (BMT), or enzyme replacement with polyethylene glycol (PEG)-ADA, but the cost is prohibitive. Erythrocyte-encapsulated ADA is effective and less expensive. Oral ribose is reportedly beneficial in myoadenylate deaminase deficiency [1, 4] and also in adenylosuccinase deficiency [1, 5]. PNP deficiency is also treatable by BMT.

Laboratory diagnosis is based on the presence of abnormal concentrations of metabolites in urine, plasma or red cells (or the absence of normal metabolites) and/or establishment of the enzyme defect, sometimes using intact as well as disrupted cells, plus the characteristic changes in red cell nucleotide profiles [2–17]. Measurement of uric acid in plasma and urine can lead to suspicion of several defects, but diagnosis may be complicated in renal failure [1, 13]. Sensitive detection methods include MS-MS [14, 15], capillary electrophoresis [16], or anion exchange/reversed phase/ion-pair HPLC, with in-line Photodiode-array or radiodetection [2, 12]. Adequate control ranges for the healthy local population must be established for enzymes as well as metabolites, particularly uric acid, since dietary purine intake varies from country to country. A combination of tests is essential, especially where the clinical condition has necessitated a blood transfusion, or treatment involves the use of UV absorbing drugs which can co-elute with endogenous purines and pyrimidines in HPLC systems, when enzyme peak shift will be necessary for positive identification.

Prenatal diagnosis is available and has been applied to the detection of some of these disorders in the first trimester using chorionic villi, or in the second using amniotic fluid and amniotic fluid cells, or fetal blood obtained by cordocentesis [1, 2, 11].

23.2 Nomenclature

	Abbreviation	Disorder	Tissue relevant to diagnosis	Chromosomal localisation	McKusick
23.1	ADA	Adenosine deaminase deficiency	RBC, WBC, Fib	20q13.2-qter	102700
23.2	PNP	Purine nucleoside phosphorylase deficiency	RBC, WBC, Fib	14q13.1	164050
23.3a	XDH	Xanthine oxidase/dehydrogenase deficiency	Liver/IM	2p22	278300
23.3b	XDH/SO	Combined XOD/sulphite oxidase deficiency	Liver/IM/Fib	6p21.3	252150
23.3c	XDH/AO	Combined XDH/aldehyde oxidase deficiency	Liver/IM	?	?
23.4	HPRT	Hypoxanthine phosphoribosyltransferase deficiency	RBC, WBC, Fib	Xq26-q27.2	308000
23.4a		a) complete: Lesch-Nyhan syndrome	As above	Xq26-q27.2	
23.4b		b) partial: Kelley-Seegmiller syndrome	As above	Xq26-q27.2	308000
23.5	APRT	Adenine phosphoribosyltransferase deficiency	RBC, WBC, Fib	16q.24	102600
23.6	ADSL	Adenylosuccinate lyase deficiency	RBC, WBC, Fib	22	103050
23.7	MAD	Myoadenylate deaminase deficiency			254750
		AMPD1	Muscle	1p13-p21	102770
23.8	PRPS	Phosphoribosylpyrophosphatesynthetase superactivity	RBC, WBC, Fib	Xq22-q24	311850
23.9	TPMT	Thiopurine methyltransferase deficiency	RBC	6p22.3	187680
23.10	UMPS	UMP synthase deficiency	RBC, WBC, Fib	3q13	258900
23.11a	UMPH1	UMP hydrolase deficiency	RBC	7	266120
23.11b	UMPHS	UMP hydrolase superactivity	Fib	?	?
23.12	TP	Thymidine phosphorylase deficiency			
23.13	DPD	Dihydropyrimidine dehydrogenase deficiency	WBC/Fib	1p22	274270
23.14	DHP	Dihydropyrimidinase deficiency	Liver	8q22	222748
23.15	UP	Ureidopropionase deficiency	Liver	2q11.2	210100
			WBC	22q13	550900

23.3 Metabolic Pathways

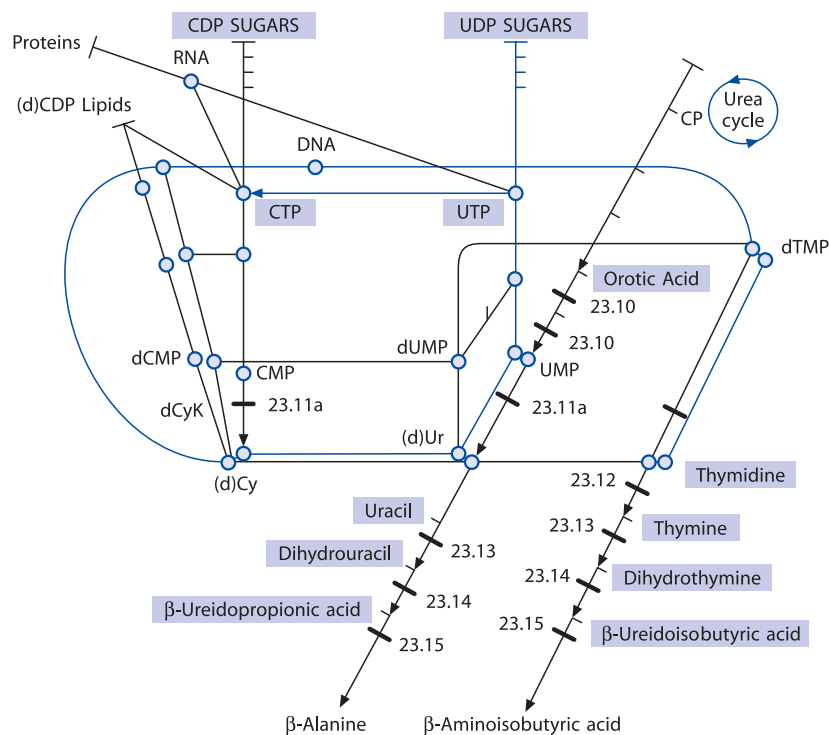


Fig. 23.1. Pyrimidine pathways: Pathways for the de novo synthesis, interconversion, and breakdown of pyrimidine ribonucleotides, indicating their metabolic importance as the essential precursors of the pyrimidine sugars and, with purines, of DNA and RNA. Note that in contrast to purines salvage takes place at the nucleoside not the base level in human cells and pyrimidine metabolism normally lacks any detectable end-product. The importance of this network is highlighted by the variety of clinical symptoms associated with the possible enzyme defects indicated. 23.10, Uridine monophosphate synthase (UMPS), 23.11a, uridine monophosphate hydrolase 1 (UMPH1), 23.12, thymidine phosphorylase (TP), 23.13, dihydropyrimidine dehydrogenase (DPD), 23.14, dihydropyrimidine amidohydrolase (DHP), 23.15, β -ureidopropionase (UP) (23.11b, UMPH superactivity specific to fibroblasts is not shown). CP, carbamoyl phosphate. The pathological metabolites used as specific markers in differential diagnosis are *highlighted*

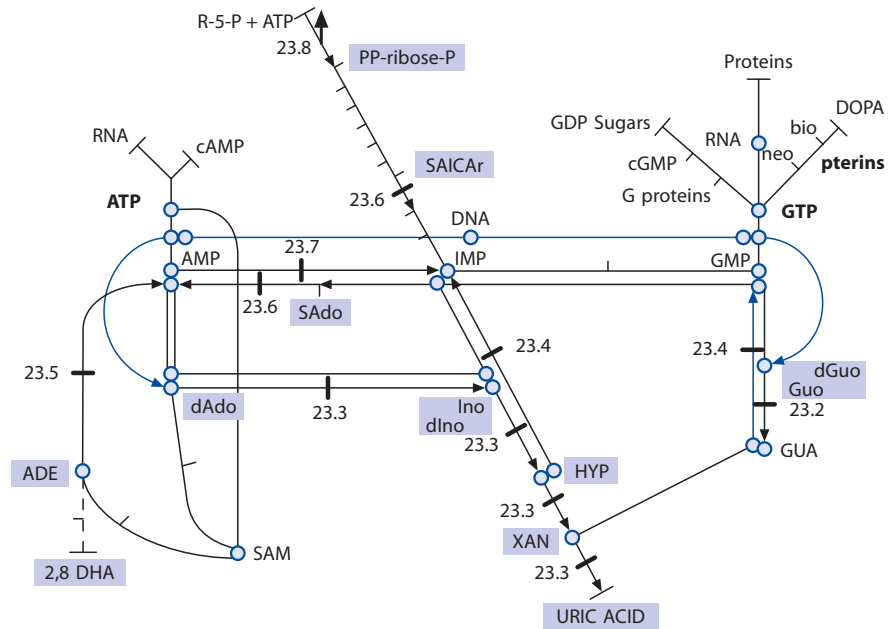


Fig. 23.2. Purine pathways: pathways for the de novo synthesis, interconversion, and breakdown of purine ribonucleotides, indicating their metabolic importance in their own right and as the essential precursors of DNA, RNA, cyclic nucleotides, purine sugars and pterins. The importance of the salvage route for the recycling of bases derived during muscle work, red cell senescence, etc., is illustrated by the variety of clinical symptoms associated with the possible metabolic defects indicated. 23.1, Adenosine deaminase (ADA), 23.2, purine nucleoside phosphorylase (PNP), 23.3, xanthine dehydrogenase (XDH), 23.4, hypoxanthine phosphoribosyltransferase (HPRT), 23.5, adenine phosphoribosyltransferase (APRT), 23.6, adenylosuccinate lyase (ADSL), 23.7, myoadenylate deaminase (MDA), 23.8, phosphoribosylpyrophosphate synthetase superactivity (PRPS), 23.9, thiopurine methyltransferase which catalyses the conversion of thioIMP to methylthioIMP (not shown). Pathological metabolites used as specific markers in differential diagnosis are *highlighted*

23.4 Signs and Symptoms

Table 23.1. Adenosine deaminase deficiency [Patients: 119 (Europe)]

System	Symptoms/markers	Neonatal	Infancy	Childhood	Adolescence	Adulthood
Characteristic clinical findings	Severe combined immunodeficiency (SCID)	++++	++	+	+	+
	Severe lymphopenia	+++	++	+	+	+
	CD4+, CD8+ CD19 cells, Ig's (bl)	↓↓↓	↓↓	↓	↓	↓
Special laboratory	dAdo (u)	↑↑↑	↑↑	↑↑	↑↑	↑
	dATP (rbc)	↑↑↑↑	↑↑↑	↑↑	↑↑	↑
	X-ray	+	+			
	Enzyme (rbc)	–	–	–	–	–
Other clinical features	Diarrhoea	+	+			
	Oral/vaginal candidiasis	+	+	+	+	+
	Pneumonia	+	+	+	+	+
	Vomiting	+	+			
	Generalised infections	++	+	+	+	+
	Absent lymph nodes	+	+			

Table 23.2. Purine nucleoside phosphorylase deficiency [Patients: >50 (world)]

System	Symptoms/markers	Neonatal	Infancy	Childhood	Adolescence	Adulthood
Characteristic clinical findings	T cell immuno-deficiency	+	+	+		
	CD4+ cells (bl)	↓–N	↓–N	↓–N		
	Most normal Ig's					
	Developmental delay	+	+	+		
	Spastic diplegia or tetraparesis	++	++	++		
Routine laboratory	Hyper-/hypotonia	+	+	+		
	Uric acid ^a (u, p)	↓↓	↓↓	↓↓		
Special laboratory	Ino,Guo,dIno,dGuo (p)	↑↑	↑↑	↑↑		
	Ino,Guo,dIno,dGuo (u)	↑↑↑	↑↑↑	↑↑↑		
	dGTP (rbc)	↑↑↑	↑↑↑	↑↑↑		
	Enzyme (rbc)	–	–	–		
Other clinical features	Recurrent infections (skin,lung, middle ear)	++	++	++		
	Particularly varicella	+++	+++	+++		
	Autoimmune haemolytic anaemia, ITP, SLE	±	±	±		

^a Can be low normal.

Table 23.3. Xanthine dehydrogenase (XDH)/sulphite oxidase (SO)/aldehyde oxidase (AO) deficiencies

Disorder	System	Symptoms/markers	Neonatal	Infancy	Childhood	Adolescence	Adulthood
XDH def. (a)	Characteristic clinical findings	(a) Acute renal failure (ARF),	±	±	±	±	±
Patients: 44 (Europe)		Xanthine lithiasis	±	±	±	±	±
		Convert allopurinol to oxipurinol	+	+	+	+	±
XDH/SO def (b)	(see Chap. 10)	(b) Neonatal fitting, retardation					
Patients: 151 (Europe)		Ocular lens dislocation	++++	+±	±		
XDH/AO def. (c)		(c) ARF, xanthine lithiasis.					
Patients: 9 (Europe)		Cannot convert allopurinol	–	–	–	–	
23.3 a, b, c	Routine laboratory	Uric acid (u, p)	↓↓	↓↓	↓↓	↓↓	↓↓
23.3 a, b, c	Special laboratory	hyp, xan (p)	↑	↑	↑	↑	↑
		hyp, xan (u)	↑↑	↑↑	↑↑	↑↑	↑↑
23.3 b only		Sulphite (u)	↑	↑	↑		
		Thiosulphate (u)	↑	↑	↑		
		s-Sulphocysteine (u, p)	↑	↑	↑		
		Cystine (p)	↓↓	↓↓	↓↓		
23.3 a, c	Other clinical features	Cystine (u)	↓–N	↓–N	↓–N		
		Myopathy				+	+
23.3 b		Dysmorphic features	+	+	+		
		Hypo-/hypertonia	+	+	+		
		Cerebral atrophy	±	±	±		

^a Can be low normal.

Table 23.4. Hypoxanthine-guanine phosphoribosyltransferase deficiency

Disorder	System	Symptoms/markers	Neonatal	Infancy	Childhood	Adolescence	Adulthood
a: Complete (Lesch-Nyhan syndrome: LNS) Patients: 295 (Europe)	Characteristic clinical findings	Cerebral palsy, retardation Self biting, hypertonicity Choreoathetosis Spastic quadriplegia Neurological deficits – mild to none		+	+	+	+
b: Partial (Kelley-Seegmiller syndrome) Patients: 32 (Europe)	Routine laboratory	Uric acid (u) Uric acid (p)	↑↑ N–↑	↑↑ N–↑	↑↑ N–↑	↑↑ ↑↑	↑↑ ↑↑
a, b	Special laboratory	Enzyme (rbc) Nucleotides (rbc) hyp (u)	–/↓↓ ↑ ↑	–/↓↓ ↑ ↑	–/↓↓ ↑ ↑	–/↓↓ ↑ ↑	–/↓↓ ↑ ↑
a, b	Other clinical features	Crystalluria Acute renal failure Lithiasis-uric acid Haematuria, recurrent UTI	++ + +	++ + +	+ + + +	+ + + +	+ + + +

Table 23.5. Adenine phosphoribosyltransferase deficiency (types 1, 11)

Disorder	System	Symptoms/markers	Neonatal	Infancy	Childhood	Adolescence	Adulthood
	Characteristic clinical findings	types 1, 11: 2,8-DHA lithiasis, Acute renal failure	±	±	±	±	±
Patients: type 1: >140 (world) type 11: >140 (Japan) types 1, 11	Routine laboratory	'Uric acid' stones	± +	± +	± +	± +	± +
	Special laboratory	Adenine (u) 2,8-DHA (u) 2,8-DHA (stone) Enzyme (rbc)	↑↑ ↑↑ + –/25%N	↑↑ ↑↑ + –/25%N	↑↑ ↑↑ + –/25%N	↑↑ ↑↑ + –/25%N	↑↑ ↑↑ + –/25%N
type 1/type 11	Other clinical features	Loin pain, Haematuria Recurrent UTI Chronic renal failure		+ + + +	+ + + +	+ + + +	+ + + +

Table 23.6. Adenylosuccinatelyase deficiency [Patients: 24 (Europe)]

System	Symptoms/markers	Neonatal	Infancy	Childhood	Adolescence	Adulthood
Characteristic clinical findings	Psychomotor retardation	+	+	+	+	
	Epilepsy, autism	±	±	±	±	
Routine laboratory	Amino acid analysis (u)					
	Aspartate, glycine					
Special laboratory	After acid hydrolysis	↑↑	↑↑	↑↑	↑↑	
	S-Ado (p, u, CSF)	↑↑	↑↑	↑↑	↑↑	
	SAICAR (p, u, CSF)	↑↑	↑↑	↑↑	↑↑	
	Enzyme (liver)	–	–	–	–	
Other clinical features	Cerebellar hypoplasia	±	±	±	±	

Table 23.7. Myoadenylate deaminase deficiency [Patients: >44 (Europe)]

System	Symptoms/markers	Neonatal	Infancy	Childhood	Adolescence	Adulthood
Characteristic clinical findings	Muscle cramps			+	+	+
	Exercise intolerance			+	+	+
Routine laboratory	Elevated CK (p)			+	+	+
Special laboratory	Enzyme (muscle biopsy)			↓	↓	↓
	Ischaemic muscle exercise tolerance test: (p.NH ₃)			↓	↓	↓
Other clinical features	Acquired: associated with neuromuscular rheumatologic disorders					+
						+

Table 23.8. Phosphoribosylpyrophosphate synthetase superactivity [Patients: 24 (world)]

Disorder	System	Symptoms/markers	Neonatal	Infancy	Childhood	Adolescence	Adulthood
Child	Characteristic clinical findings	Developmental delay/ataxia	+	+	+	+	–
		Dysmorphic features	+	+	+	–	–
		Inherited deafness	±	–	–	–	–
Adolescent	Routine laboratory	Gout/uric acid lithiasis	–/–	–/–	–/–	+/+	+/+
		Uric acid (u)	↑↑	↑↑	↑↑	↑↑	↑↑
		Uric acid (p)	N–↑	N–↑	N–↑	↑↑	↑↑
	Special laboratory	hyp (u)	↑	↑	↑	↑	↑
		PPribP content (rbc)	↑	↑	↑	↑	↑
		PRPS superactive (fib)	↑	↑	↑	↑	↑
Other clinical features		Mother-gout/hyperuricaemia	+	+	+	+	+
		±Inherited deafness	±	±	–	–	–

Table 23.9. Thiopurine methyltransferase deficiency [Patients: 27 (Europe)]

System	Symptoms/markers	Neonatal	Infancy	Childhood	Adolescence	Adulthood
Characteristic clinical findings	None unless treated with thiopurines					
Special laboratory	Enzyme (RBC)	↓	↓	↓	↓	↓

Table 23.10. Uridine monophosphate synthase deficiency types 1 and 11

Disorder	System	Symptoms/markers	Neonatal	Infancy	Childhood	Adolescence	Adulthood
Type 1	Characteristic clinical findings	Type I: megaloblastic anaemia	+++	++±	+++		
Hereditary oroticaciduria [Patients: 15 (world)]		Type II: neurological deficits	+	+	+		
		Failure to thrive	+++	+++	+++		
Type 11	Special laboratory	Crystalluria					
Oroticaciduria/orotidinuria [Patients: 4 (world)]		Orotic acid (OA) (p)	↑↑	↑↑	↑↑		
		Type 1: OA (u)	↑↑↑↑	↑↑↑↑	↑↑↑↑		
		Type 11: OA + orotidine (u)	↑↑↑↑/↑↑	↑↑↑↑/↑↑	↑↑↑↑/↑↑		
	Other clinical features	Enzyme (rbc)	-/↓	-/↓	-/↓		
		Strabismus	+	+	+		
		Diarrhoea	+	+	+		
		Obstructive uropathy	±	±	±		
		T cell immunodeficiency	±	±	±	-	-

Table 23.11 a. UMP hydrolase 1 deficiency

Disorder	System	Symptoms/markers	Neonatal	Infancy	Childhood	Adolescence	Adulthood
Pyrimidine 5'-nucleotidase deficiency	Characteristic clinical findings	Non-spherocytic haemolytic anaemia with basophilic stippling	+	+	+	+	+
	Routine laboratory	Reduced glutathione (rbc)	±	±	±	±	±
	Special laboratory	Nucleotides UV (rbc)	+	+	+	+	+
		Nucleotides HPLC (rbc)	Py↑↑↑↑	Py↑↑↑↑	Py↑↑↑↑	Py↑↑↑↑	Py↑↑↑↑
	Other clinical features	Enzyme (rbc)	–	–	–	–	–
		Haemoglobinuria	±	±	±	±	±
		Splenomegaly	?	+	+	+	+
		Acquired deficiency: – lead poisoning		+	+	+	+

^a Similar presentation/laboratory findings, but different derangement in pyrimidine nucleotides found in a putative disorder: CDP-choline phosphotransferase deficiency.

Table 23.11 b. UMPH superactivity [Patients: >4 (world)]

System	Symptoms/markers	Neonatal	Infancy	Childhood	Adolescence	Adulthood
Characteristic clinical findings	Developmental delay, fits	+	+			
	Seizures, hyperactivity	+	+			
	Short attention span	+	+			
	Recurrent infections	+	+			
Routine laboratory	Uric acid (u)	↓	↓			
Special laboratory	PPRP (fib)	↓	↓			
	Enzyme (fib)	↑↑	↑↑			

Table 23.12. Thymidine phosphorylase deficiency (Patients: ?)

System	Symptoms/markers	Neonatal	Infancy	Childhood	Adolescence	Adulthood
Characteristic clinical findings	Mitochondrial, neuro-gastrointestinal myopathy (MINGIE)	+	+	+	+	+
		+	+	+	+	+
Special laboratory	Lactate (b, u)	↑	↑	↑	↑	↑
	Thymidine (p, u)	↑↑	↑↑	↑↑	↑↑	↑↑
	Enzyme (wbc)	↓	↓	↓	↓	↓

Table 23.13. Dihydropyrimidine dehydrogenase deficiency [Patients: 83 (Europe)]

System	Symptoms/markers	Neonatal	Infancy	Childhood	Adolescence	Adulthood
Characteristic clinical findings	Epilepsy, retardation		±	±	±	
	Microcephaly		±	±	±	
	Feeding difficulties	±				
Special laboratory	Uracil, thymine (u, p, CSF)	↑↑	↑↑	↑↑	↑↑	↑↑
	Enzyme (fib, wbc)	↓	↓	↓	↓	↓
Other clinical features	Autistic features			±	±	
	Hypertonia	±				
	Severe toxicity 5-FU					+

Table 23.14. Dihydropyrimidinase deficiency [Patients: 14 (world)]

System	Symptoms/markers	Neonatal	Infancy	Childhood	Adolescence	Adulthood
Characteristic clinical findings	Feeding difficulties		±	?		
	Seizures					
	Epilepsy, mental/motor retardation		±	±		
Special laboratory	Uracil, thymine (u, p, CSF)		↑↑	↑↑		
	Dihydrouracil/thymine (u)		↑↑	↑↑		
	Enzyme (lymph, fib)		–	–	–	–
Other clinical features	Microcephaly, spastic Quadriplegia, developmental		+	+		
	Retardation, congenital		+	+		
	Microvillus atrophy		+	+		

Table 23.15. Ureidopropionase deficiency [Patients: 5 (Europe)]

System	Symptoms/markers	Neonatal	Infancy	Childhood	Adolescence	Adulthood
Characteristic clinical findings	Muscular hypotonia, severe		+			
	Developmental delay, Dystonic movements		+			
	β-Ureidopropionate (u)		↑↑			
Special laboratory	β-Ureidoisobutyrate (u)		↑↑			
	By NMR, amino acid analysis		↑			
	Pre/post hydrolysis		↑			
Other clinical features	Optic atrophy, scoliosis		+			
	Enzyme (liver)		–			

23.5 Reference and Pathological Values

Urine purines

Reference values (mmol/24 h) ^a	Uric acid	Hyp	Xan	dAdo	Ino	dIno	Guo	dGuo	Ade	2,8-DHA	S-Ado	SAI-CAr
Adult (f)	2.7 ^a ±0.5 (= 0.36)	0.05 ^a ±0.02	0.05 ^a ±0.02	–	<0.01	–	–	–	<0.01	–	–	–
Adult (m)	3 ^a ±0.5 (= 0.34)	0.07 ^a ±0.02	0.05 ^a ±0.02	–	<0.01	–	–	–	<0.01	–	–	–
Child (m, f)	1.5 ^a ±0.3 (= 1.2)	0.03 ^a ±0.01	0.03 ^a ±0.01	–	<0.01	–	–	–	<0.01	–	–	–
Variant												
23.1 ADA def.												
23.1 Infant				0.13–0.19								
23.1 Adult				0.01–0.26								
23.2 PNP def.												
23.2 Severe	<0.1	n.d.	n.d.		1.35– 1.9	0.3– 0.65	0.57– 1.0	0.2– 0.41				
23.2 Mild	0.1–0.97	0.03–0.32	0.01–0.12		0.5–0.9	0.1– 0.34	0.3–0.4	0.1– 0.25				
23.3a XDH def.												
23.3a	<0.01	0.3–0.66	1.12–2.9									
23.3b XDH/SO												
23.3b	<0.005– 0.02	0.01–0.13	0.21–1.21									
23.4 HPRT def.												
a: complete LNS	1.8–4.4	0.09–0.27	0.025–0.1									
b: partial	0.6–5.0	0.02–0.08	0.005–0.025									
23.5 APRT def.												
Type 1 or type 2									0.01– 0.04	0.02– 0.08		
23.6 ADSL def.												
											0.17– 0.50	0.04– 0.37
23.8 PRPS sup.												
Child	1.91–2.33	0.13–0.38	0.1–0.12									
Adolescent	5.0–8.3	?	?									
23.9 TPMT def.												

Reference ranges for healthy subjects are listed at the top of the table.

^a indicates excretion in mmol/24 h.

All pathological values are given in mmol/mmol creatinine.

n.d., Not detectable.

Urine pyrimidines

	OA	OR	U	DiHyU	Th	DiHyTh	Thym	β UP	β UIB
Reference values (mmol/mol creatinine)									
Adult (f)	<0.01	<0.01	0.04–0.1	–	–				
Adult (m)	<0.01	<0.01	0.04–0.1	–	–				
Child (m, f)	<0.01	<0.01	0.01–0.05	–	–	<0.01	<0.01	<0.01	<0.01
Variant									
<u>23.10 UMPS def. type 1</u>									
Child	1.0–9.6								
<u>23.10 UMPS def. type 11</u>									
Child	0.6–1.0	0.3–0.5							
<u>21.11a UMPH1 def.</u>									
<u>21.11b UMPH sup.</u>									
<u>Child</u>									
<u>23.12 TP def.</u>									
<u>23.13 DPD def.</u>									
Child			0.06–0.68		0.09–0.44			0.15–0.37	
<u>23.14 DPA def.</u>									
Child			0.01–0.14	0.15–0.63	0.09–0.11	0.01–0.23			
<u>23.15 UP def.</u>									
Child								0.78	0.72

Reference ranges for healthy subjects are listed at the top of the table.

^a indicates excretion in mmol/24 h.

All pathological values are given in mmol/mmol creatinine.

n.d., Not detectable.

Blood purines

	Erythrocyte		Plasma ($\mu\text{mol/l}$)									
	Enzyme (normal ranges in italics)	Nucleotide (normal values in italics)	Uric acid	Hyp	Xan	dAdo	Ino	dIno	Guo	dGuo	S-Ado	SAI- CAr
Reference values												
Child (m, f)			170±35	<5	<1	–	<1	–	–	–	–	–
Adult (f)			222±42	<5	<1	–	<1	–	–	–	–	–
Adult (m)			261±41	<5	<1	–	<1	–	–	–	–	–
Variant (nmol/h/mgHb, protein) ($\mu\text{mol/l}$)												
<u>23.1 ADA def.</u>	ADA (72±17)	dATP (<1)										
23.1 Infant	n.d.	1121–2478		<5	<1	1–5						
23.1 Adult	n.d.	105–234		<5	<1	<1						
<u>23.2 PNP def.</u>	PNP	dGTP (<1)										
	(4724±787)											
23.2 Severe	n.d.	4–7	<4	<1	<1		40–71	5–19	8–23	5–14		
23.2 Mild	44–388	<1	109	<5	<1		9	<1	<1	<1		
<u>23.3 a, c XDH def.</u>	–	Normal	<5	<5	14–39							
23.3 b XDH/SO	–	Normal	<5–100	<5	15–32							
<u>23.4 HPRT def.</u>	HPRT	NAD										
	(115±19)	(69±15)										
a: complete LNS	n.d.	177–367	270–860									
b: partial	n.d.–6	151–228	450–610									
<u>23.5 APRT def.</u>	APRT (24±4)											
type 1	n.d.	Normal	Normal	<5	<1							
<u>23.6 ADSL def.</u>	–	Normal	Normal	<5	<1						–	–
<u>23.8 PRPS supa</u>	PRPS	NAD										
	(16–37)	(69±15)										
Child	ud	14–44	250–500									
Adolescent	45–74	?	370–850									
<u>23.9 TPMT def.</u>	8–14.5	?	?									
	(nmol/h/ml RBC)											
Adult	<8.0	?	?	<5	<1							

–, Normally not detectable.

n.d., Not detectable by the assay used.

?, No values for this age group.

Reference ranges for

a) Blood enzymes and nucleotides are listed in parenthesis for each disorder in the 2 left columns.

b) Plasma metabolite concentrations for healthy subjects are listed at the top right of the table

Blood pyrimidines

	Erythrocyte		Plasma (μmol/l)			
	Enzyme (normal ranges in italics)	Nucleotide (normal values in italics)	OA	U	Thy	Thym
Reference values						
Child (m, f)			<0.5	<0.2	–	<0.1
Adult (f)			<0.5	<0.2	–	<0.1
Adult (m)			<0.5	<0.2	–	<0.1
Variant (nmol/h/mg Hb or protein) (μmol/l)						
23.10 UMPS def.	OPRT ODC	UTP CTP CDPC				
Child	<i>0.26±0.16 0.18±0.15</i>	– – <3				
Types 1, 11	n.d.-trace	4 107	29–68	–	–	–
23.11a UMPH1 def.	UMPH1 (9–20)	UTP CTP CDPC				
Child/adult	n.d.	600 >1–2 μmol/l	–	–	–	–
23.11b UMPH sup	UMPH (<i>92.4±51.6:Fib</i>)	?				
Child	488–565	?				
23.12 TP def.	TP (<i>670±210:WBC</i>)	?				
Child/adult	9±21	?				14–42
23.13 DPD def.	–	normal		9–30	13–17	
23.14 DHP def.	–	normal				
23.15 UP def.	–	?				

–, Normally not detectable.

n.d., Not detectable by the assay used.

?, No values for this age group.

Reference ranges for

a) Blood enzymes and nucleotides are listed in parenthesis for each disorder in the 2 left columns.

b) Plasma metabolite concentrations for healthy subjects are listed at the top right of the table.

23.6 Loading Tests

Loading tests, based on the increment in the urinary excretion of different pyrimidines have been used to confirm DPD, DHP or UP deficiency before methods for determination of the activity of the relative enzymes were available. Moreover, they still are important to determine the *in vivo* status, and to determine the carrier status for urea cycle disorders, but are not helpful in any other disorders in this group [17].

■ DPD, DHP and UP Deficiency

Loading tests with uracil and thymine (1 mmol/kg) and their dihydroderivatives have been given to children suspected to have one of the above two defects, from the elevated urine thymine and uracil using GC, or GC-MS methods predominantly developed for organic acids [1, 14, 15]. However, one has to be aware of the highly variable matrix-dependent extraction

yields of the bases, as well as their dihydroderivatives. In addition, the presence of elevated concentrations of uracil alone in the urine of a retarded child requires detailed investigation. It is generally indicative of a genetic urea cycle disorder (see below). In extremely rare instances uracil can arise from the degradation of pseudouridine [2]. Consequently pseudouridine must always be measured as well.

■ Pyrimidinuria as a Marker of Carrier Status for Urea Cycle Disorders

Elevated concentrations of orotic acid and uracil may be found in the urine of heterozygote carriers for urea cycle disorders, most commonly ornithine carbamoyltransferase (OCT) deficiency. This is due to the increased flux through the pyrimidine pathway which occurs, especially where the relevant enzymes are expressed only in liver tissue, as is the case when urea cycle enzymes are defective. A protein load was used previously to stress this route and the elevation in orotic acid excretion used as a diagnostic marker. However, the test frequently failed to detect known carriers. The allopurinol loading test (measurement of the increment in urinary orotic acid and orotidine in three separate 8 hour urine collections over the 24 hours following a 300 mg allopurinol tablet) is the most reliable test so far for carrier detection for such disorders [17].

23.7 Specimen Collection

Test	Preconditions	Material	Handling	Pitfalls
Enzymes (RBC)	Caffeine-free or unrestricted diet	Erythrocytes from heparinised /EDTA blood	Room temperature NOT on ice	a) Blood transfusion b) ADA unstable at -20°C c) some enzymes very labile
Nucleotides (RBC)	Caffeine-free or unrestricted diet	Guthrie card for some Erythrocytes from heparinised/EDTA blood	Room temperature as soon as possible; NOT on ice	Blood transfusion; spuriously low in old blood due to rapid breakdown
Purines (P)	Caffeine free diet preferable	Plasma from heparinised/EDTA blood	Centrifuged and separated immediately	Spuriously elevated in old blood due to nucleotide breakdown
Pyrimidines (P)				
Purines (U); Pyrimidines (U)	Caffeine-free diet preferable	Urine preferably 24 h collection	Toluene or thymol preservative NOT acid Can be shipped on dry ice or lyophilised If with blood, send both at room temperature	a) Diurnal variation in excretion b) Bacterial contamination c) Deoxynucleosides degraded if collected in acid e) Drugs with similar HPLC f) Must be shaken and warmed to ensure complete solution of uric acid

23.8 Prenatal Diagnosis

Disorder	Tissue or specimen	Timing, Trimester	Pitfalls/comments
23.1 ADA def.	CV	I	Separation of maternal and fetal cells is vital for
	AF AFC	II	both DNA analysis and enzyme assay
23.2 PNP def.	CV	I	As for 23.1
	AFC FB	II	
23.3b XDH/SO	CV	I	Test available only in special centre
	AF AFC	II	
23.4 HPRT def.	CV	I	As for 23.1. DNA analysis only has led to
a:complete LNS	AFC FB	II	missed diagnosis. Must use enzyme assay also
23.6 ADSL def.	CV	I	As for 23.1
23.8 PRPS sup ^a	(child ?FB) (?II)		
23.10. UMPS def.	CV	I	As for 23.1
	AF AFC	II	
23.12 DPD def.	AF	II	?

^a Characteristic nucleotide pattern in infancy may allow diagnosis from FB.

23.9 DNA Analysis

Disorder	Tissue or specimen	Methodology
23.1 ADA def.	CV, AFC, FeBL, BL, LB, FB	cDNA and genomic sequencing
23.2 PNP def.	CV, AFC, FeBL, BL, LB, FB	cDNA and genomic sequencing
23.3b XDH/SO	CV, AFC, FB	cDNA and genomic sequencing
23.4 HPRT def.		
a:complete LNS	CV, AFC, FeBL, BL, LB, FB	cDNA and genomic sequencing
b: partial	BL, LB, FB	
23.5 APRT def.	BL, LB, FB	cDNA and genomic sequencing
23.6 ADSL def.	CV, AFC, FeBL, BL, LB, FB	cDNA and genomic sequencing
23.7 MAD def.	BL, LB, FB	restriction endonuclease digestion
23.8 PRPS sup.	BL, LB, FB	cDNA and genomic sequencing
23.9 TPMT def.	BL	genomic sequencing
23.10 UMPS def.	CV	cDNA and genomic sequencing
23.11a UMPH 1 def.	BL, FB	cDNA and genomic sequencing
23.11b UMPH sup.	?	?
23.12 TP def.	BL	nDNA
23.13 DPD def.	AFC, BL, FB	cDNA and genomic sequencing
23.14 DHP def.	BL, FB	genomic sequencing
23.15 UP def.	BL, FB	genomic sequencing

23.10 Initial Treatment

In an emergency clinicians can consult the appropriate reference guide to laboratories diagnosing purine and pyrimidine disorders locally.

To obtain information regarding the nearest hospital group providing advice on appropriate clinical treatment, or where such advice may be obtained, for all EU countries see ref. [12].

The two severe immunodeficiency disorders (ADA and PNP deficiency) will invariably require referral to a specialist centre for initial assessment, decision on, availability and implementation of the treatment required.

If blood or platelet transfusion are necessary for the above immunodeficiency disorders they must always be irradiated to prevent the risk of life-threatening Graft versus host disease (GVHD).

In both these two immunodeficiency and haematological disorders particularly, but also in all other disorders diagnosed by enzyme assay in erythrocytes, if possible transfusion should be delayed until the confirmation (or elimination) of a diagnosis is made. Donor enzyme activity will take 6 months to disappear.

23.11 Summary/Comments

It is impossible to provide an adequate coverage of disorders with the wide clinical spectrum of presentation exemplified by the purine and pyrimidine disorders listed here in a book aiming at providing a summary to assist clinicians in the rapid diagnosis of all genetic metabolic disorders. The reader is referred to specific and comprehensive reviews (referenced in [1], which include references to earlier work in the particular disorder of interest).

■ Aids to Clinical Diagnosis and Management in Problem Cases

This is discussed in ref. [1], but the following points are important:

a) It is unwise to eliminate a purine disorder normally associated with the absence, or elevated concentrations of uric acid in plasma, from the finding of a normal uric acid alone – the full clinical picture must be considered. Patients with Molybdenum Cofactor deficiency and PNP deficiency are now being recognised with relatively normal uric acid levels, due to tissue-specific variation in enzyme expression, and children with Lesch-Nyhan syndrome are frequently misdiagnosed because the high renal clearance of uric acid in children can result in a normal plasma urate [2, 13].

b) The four disorders associated with the overproduction of the insoluble purine bases, uric acid, xanthine and 2,8-dihydroxyadenine can all be

the unsuspected cause of the symptoms in a child presenting in coma or acute renal failure. In many instances renal ultrasound has given the first clue to the underlying crystal nephropathy [10]. A number of such cases have presented following a severe infection (infectious mononucleosis), or bout of diarrhoea, when excessive cell breakdown and/or dehydration have been exacerbating factors [13]. Crystals on the diaper are a very early warning sign.

c) Treatment of the uric acid-overproduction in HPRT deficiency with allopurinol requires care. Lesch-Nyhan children especially respond much more rapidly than do patients with primary gout and are at risk of replacing the insoluble uric acid with the much more insoluble xanthine, leading to xanthine stones or nephropathy. Alkalinisation of the urine will increase the solubility of uric acid ten-fold, but will not improve that of xanthine. Allopurinol must also be given in reduced dose in renal failure [13].

d) The presence of small peaks, or even the absence of the pyrimidine bases and dihydropyrimidines in the frequently-used GC-MS methods for organic acids does not exclude DPD or DHP deficiency due to variable matrix-dependent extraction fields and derivatisation efficiencies. HPLC with in-line diode-array or HPLC/tandem-MS methods are much more reliable.

e) Amino acid analysis, before and after acid hydrolysis of the urine, provides important information regarding the presence of a pyrimidine degradation defect and also the purine disorder, adenylosuccinase deficiency [1].

f) Bacterial contamination of the urine by insufficient preservation or urinary tract infection may lead to mis- or missed diagnosis of patients.

References

1. Various authors. Purines and pyrimidines (Part 11, Chapters 106–113). In: Scriver CR, Beaudet AL, Sly WS, Valle D, eds. *The Metabolic and Molecular Bases of Inherited Disease*, 8th edition, Volume II. New York: McGraw-Hill, 2001; pp 2512–2702
2. Simmonds HA, Duley JA, Davies PM. Analysis of purines and pyrimidines in blood, urine and other physiological fluids, Chapter 25. In: Hommes F Ed, NY: Wiley-Liss. *Techniques in Diagnostic Human Biochemical Genetics: A laboratory Manual* 1991; p 397–424
3. Hershfield MS, Arrendondo-Vega FX, Santisteban I. Clinical expression, genetics and therapy of adenosine deaminase (ADA) deficiency. *J Inherit Metab Dis* 1997; 20:179–185
4. Zöllner N, Reiter S, Pongratz D, Reimers CD, Gerbitz K, Paetzke I, Deufel T, Hubner G. Myoadenylate deaminase deficiency: successful symptomatic therapy by high dose oral administration of ribose. *Klin Wochenschr* 1986; 64:1281–1290
5. Köhler M, Assmann B, Bräutigam C, Storm W, Marie S, Vincent MF, Van den Berghe G, Simmonds HA, Hoffmann GF. Adenylosuccinase deficiency: possibly underdiagnosed encephalopathy with variable clinical features. *Eur J Paediatr Neurol* 1999; 3:3–6

6. Classen CF, Sigi-Kraetzig M, Hoffmann GF, Simmonds HA, Fairbanks LD, Debatin KM, Friedrich W. Successful HLA-identical bone marrow transplant in a patient with PNP deficiency using bisulfan and fludarabine for conditioning. *Bone Marrow Transplantation* 2001; 28:93–96
7. Simmonds HA, Hoffmann GF, Pérignon JL, Micheli V, Van Gennip AH. Diagnosis of molybdenum cofactor deficiency. *The Lancet* 1999; 353:675
8. Besley GTN, Walter JH, Fairbanks LD, Simmonds HA, Marinaki AM, Van Gennip AH. Hereditary oroticaciduria without megaloblastic anaemia. *J Inher Metab Dis* 2000; 23 (Suppl 1):194
9. Marinaki AM, Escuaredo E, Duley JA, Simmonds HA, Amici A, Napponelli V, Magni G, Seip M, Ben Bassat I, Harley EH, Thain SL, Rees DC. Genetic basis of haemolytic anaemia caused by pyrimidine 5' nucleotidase deficiency. *Blood* 2001; 97:3327–3332
10. Page T, Yu A, Fontanesi J, Nyhan WL. Developmental disorder associated with increased cellular nucleotidase activity. *Proc Natl Acad Sci* 1997; 94:11601–11606
11. Jakobs C, Stellaard F, Smit LM, van Vugt LJM, Duran M, Berger R, Rovers P. First prenatal diagnosis of dihydropyrimidine dehydrogenase deficiency. *Eur J Paediatr* 1991; 150:291
12. Directory of Laboratories Diagnosing Inborn Errors of Purine and Pyrimidine Metabolism in Europe. EC BMH4-CT98-3079 publication; 2001: <http://www.amg.gda.pl/~essppmm/>
13. Maranaki AM, Cameron JS, Simmonds HA. Inherited disorders of purine metabolism and transport, Chapter 16.5.3. In, *Oxford Textbook of Clinical Nephrology* 2002, Vol 3 (in press).
14. Ito T, Van Kuilenberg ABP, Bootsma AH, Haasnoot AJ, van Cruchten A, Wada Y, Van Gennip AH. Rapid screening of high-risk patients for disorders of purine and pyrimidine metabolism using HPLC-electrospray tandem mass spectrometry of liquid urine or urine-soaked filter paper strips. *Clin Chem* 2000; 46:445–452
15. Van Lenthe H, Van Kuilenberg ABP, Ito T, Bootsma AH, van Cruchten A, Wada Y, Van Gennip AH. Defects in pyrimidine degradation identified by HPLC-electrospray tandem mass spectrometry of liquid urine or urine-soaked filter paper strips. *Clin Chem* 2000; 46:1916–1922
16. Adam T, Fairbanks LD, Cevcic J, Bartack P. Capillary electrophoresis for detection of inherited disorders of purine and pyrimidine metabolism. *Clin Chem* 1999; 45: 2086–2093
17. Fairbanks LD, Sebesta I, Simmonds HA, Leonard JV. The allopurinol loading test for evaluation of carriers for ornithine carbamoyltransferase deficiency. *Clin Chim Acta* 1994; 224:45–54