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METABOLIC DISORDERS AND MOLECULAR BACKGROUND OF UROLITHIASIS IN CHILDHOOD

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Abstract

Urolithiasis in childhood is less frequently observed than in adults, but it still has a considerable morbidity. In contrast to the situation in adults, an infectious or metabolic cause for stone formation is detected in the majority of pediatric patients. The underlying molecular mechanism of urolithiasis has been shown in a number of conditions, and some of them have been discovered in pediatric patients. Mutations of the AGXT-gene (2q37.3) have been found to be responsible for the enzyme defect in primary hyperoxaluria type I, and two of the genes provoking cystinuria have been identified (type I: 2p21, type III: 19q13.1). In both xanthinuria and 2,8 dihydroxyadeninuria mutations of the responsible gene have been discovered. It is very likely that a molecular basis for the different types of hypercalciuria will also be found, like in X-linked hypercalciuric nephropathy with tubular proteinuria (Dent's disease), or in X-linked recessive nephrolithiasis (Xp11.22). However, the molecular defect does not necessarily predict the clinical course, even in monogenic diseases. Yet in patients with the same disease genotype extreme differences in the disease phenotype have been observed. This review provides current understanding of the metabolic disorders and molecular mechanisms of urolithiasis in childhood.

Key Words: Urinary calculi, urinary metabolism, genes, molecular defect, calcium, oxalate, purine, cystine.

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Introduction

Interesting advances in the understanding of the pathophysiology and the molecular mechanisms of urolithiasis have been made during the last few years. Although some of the molecular mechanisms found, have come from studies in pediatric patients, they turn out to be important for all groups of patients. Due to our better knowledge of both the metabolic basis and the molecular mechanism of stone disease, the management and treatment has been greatly improved.

Urolithiasis in children is far less common than in adults, but still has a considerable morbidity [13]. The incidence of urolithiasis in adults increases with age affecting up to 12% of men and 5% of women by the age of 70 years. In contrast, the incidence is considerably lower in children but differs between countries [7, 13, 81]. Within the United States, urolithiasis is more common in the southeastern regions, where it may account for 1 in 1,000 to 1 in 7,600 hospital discharges [115].

In the majority of pediatric patients with urolithiasis, a metabolic cause for stone formation can be identified and several metabolic disorders are well-defined (e.g., cystinuria or primary hyperoxaluria type 1). However, there is a large subgroup of patients showing a subtle increase of urinary lithogenic factors (calcium, oxalate), or a reduction of stone-inhibitory substances, like urinary citrate [82]. If these latter abnormalities are also classified as metabolic disorders, as it is often done [86, 101, 143], then urolithiasis of metabolic origin constitutes the largest etiologic group. Accordingly, less than one third of all patients will have idiopathic urolithiasis. It is therefore, essential to perform metabolic examinations in all patients and to screen all family members if a metabolic disorder has been diagnosed.

Unlike the situation in adults, a metabolic cause for stone formation is found in the majority of pediatric patients. All children with urolithiasis should, therefore, undergo thorough investigation. The underlying molecular mechanism has been determined in a number of diseases and it is very likely that more common conditions, such as certain forms of hypercalciuria, will also be shown to have a molecular basis.

Calcium Stones

Hypercalciuria

Hypercalciuria and stones composed of calcium-oxalate are the most frequent conditions in both adult and pediatric patients with urolithiasis (Table 1, [13]). There is no sharp differentiation between normal (up to 0.1 mmol (4 mg)/kg per day [36, 45]) or abnormal levels of urinary calcium excretion, so diagnosis of hypercalciuria is sometimes vague except for very high excretions (> 0.2 mmol/kg per day). Whether such children will form stones or develop nephrocalcinosis also depends on additional factors (urine volume, pH) and the concentration of other urinary lithogenic and stone-inhibitory substances, primarily of oxalate and citrate [56, 69, 82].

Primary (idiopathic) hypercalciuria, which is supposed to have an autosomal-dominant inheritance, is the most common cause of calcium-containing stones [23]. Idiopathic hypercalciuria was primarily found to consist of both intestinal hyperabsorption and urinary hyperexcretion of calcium [91]. It has traditionally been divided into a renal and an absorptive subtype, when urinary calcium excretion in the fasting state is elevated in the renal but normal in the absorptive type [72, 143]. To differentiate both forms the oral calcium loading (1000 mg) test was introduced [54, 111, 142], but is not always accepted and may be misleading [54]. It was demonstrated, that the subtypes are not distinct entities, but rather two extremes [22], and many pediatric patients cannot easily be classified.

The renal form is supposed to result from reduced tubular calcium reabsorption. Hypocalcemia stimulates PTH secretion, which leads to an increase in bone resorption and intestinal calcium absorption and hence to higher vitamin D synthesis and hypercalciuria [84, 86]. The concept of absorptive hypercalciuria is based on an increased intestinal absorption of calcium leading to elevated serum calcium and hence to a suppression of the PTH secretion [12, 50]. As a result, the tubular reabsorption of calcium is decreased, which leads to hypercalciuria [84]. An increased affinity to vitamin D_3, or an increased production of $1.25(OH)_2D_3$ might explain this condition, as high plasma $1.25(OH)_2D_3$ values have been found in patients with hypercalciuria [84, 103, 120]. Relative hypoparathyroidism due to excess calcitriol production has been demonstrated at least in adult stone formers with idiopathic hypercalciuria [51].

A **familial syndrome of hypocalcemia with hypercalciuria** due to mutations in the calcium-sensing receptor was recently observed. Autosomal dominant hypoparathyroidism with hypocalcemia is suspected to be due to a loss of calcium sensing receptor regulation. Five heterogeneous missense mutations have been found on the calcium sensing receptor gene (CASR), which is located on chromosome 3q13.3-21 (Table 2, [75]). The CASR gene can either activate, as it does in this form of hypoparathyroidism, or inactivate the calcium sensing receptor [1, 38]. Hypocalcemia is associated with hypercalciuria and treatment with vitamin D results in an increase of urinary calcium excretion, nephrocalcinosis and later, possibly, in renal impairment [113].

Table 1. Renal stone analysis in infants and children obtained with infrared-spectroscopy [13]

Stones	Girls n = 350	Boys n = 500
Calcium-oxalate		
Weddellite (CaOx-dihydrate)	35.7%	29.0%
Whewellite (CaOx-monohydrate)	27.7%	29.2%
	63.4%	58.2%
Infectious		
Struvite	12.9%	15.0%
Carbonate-apatite	9.7%	12.8%
Ammoniumhydrogen-urate	2.0%	1.2%
	24.6%	29.0%
Other Phosphate stones		
Brushite	1.7%	3.2%
Uric acid	1.4%	2.2%
Uric acid dihydrate	0.3%	0.6%
Cystine	0.3%	1.2%
Protein	1.4%	1.6%
Artefacts	6.9%	4.0%

A molecular basis was also found in a rare but extremely severe form of **idiopathic hypercalciuria with X-linked recessive nephrolithiasis** (XRN) and renal impairment [42]. The primary defect of this renal tubular disorder is unknown, therefore, the mapping of the mutant gene to chromosome Xp11.22 was important to better define this disease [135]. The mutant gene is close to several eye disease genes, so patients have to be carefully screened opthalmologically [126]. Carrier females were said to be asymptomatic, however, a recent study showed, that they could also have (slight) hypercalciuria, and most of them showed low molecular weight proteinuria [126].

X-linked hypercalciuric nephropathy, also called Dent's disease, is a form of Fanconi syndrome with tubular proteinuria, hypercalciuria, rickets, nephrocalcinosis, urolithiasis and eventual renal failure (Table 2, [93]). A microdeletion of chromosome Xp11.22 was found to be

Table 2. Genetic defects in urolithiasis.

Increased excretion	Disease	Defect	Gene	Inheritance*
Calcium	Familial idiopathic hypercalciuria			AD
	Familial hypocalcemia with hypercalciuria	Calcium-sensing receptor (CASR)	3q13.3-21	AD
	X-linked hypercalciuric nephropathy with tubular proteinuria (Dent's disease)	Mutation of CLCN5-gene (renal chloride channel gene)	Xp 11.22	XL
	X-linked recessive nephrolithiasis type I (XRN)	Mutation of CLCN5-gene	Xp 11.22	XL
	X-linked-recessive hypophosphatemic rickets (XLRH)	Mutation of CLCN5-gene	Xp 11.22	XL
	Distal renal tubular acidosis	Mutations of RTA-1 gene ?		AD
	Bartter's syndrome	Na-K-2Cl cotransporter (NKCC2)		
	William's syndrome	Deletion of the elastin gene (ELN) calcitonin receptor gene (?)	7q11.23 7q21.3	
	Wilson's disease	Copper transporting protein	13p14.1-21.1	
Oxalate	Primary hyperoxaluria ..type 1 (PH 1)	Alanine:glyoxylate-aminotransferase	2q37.3	AR
	Primary hyperoxaluria type 2 (PH 2)	Glyoxylate-reductase / D-Glycerate dehydrogenase		AR
Cystine	Cystinuria type I	rBAT / D2H (SLC3A1)	2p21	AR
	Cystinuria type III (type II)		19q13.1	AR
Uric acid	Lesch-Nyhan syndrome	Hypoxanthine-guanine phosphoribosyltransferase	Xq26-27.2	XL
	Phosphoribosyl-pyrophosphate-synthetase superactivity	Phosphoribosyl-pyrophosphate-synthetase	Xq22-24	XL
	Glycogen-storage disease type 1	Glucose-6-phosphatase	17q21	AR
2,8 Dihydroxy-adenine	Dihydroxyadeninuria	Adenine-phosphoribosyltransferase	16q22.2-22.3	AR
Xanthine	Xanthinuria	Xanthin-oxidase	2p23-22	AR

*AD: autosomal-dominant; XL: X-linked; AR: autosomal-recessive.

responsible [116]. Three nonsense, four missense and two donor splice site mutations, together with one intragenic deletion and one microdeletion encompassing the entire gene, have been identified [40].

A candidate gene (CLCN5), which encodes a putative renal chloride channel, has been found in patients with Dent's disease [39, 93]. Chloride channels are important for the control of membrane excitability, transepithelial transport and possibly cell volume regulation [93]. CLCN5 belongs to a family of voltage-gated chloride channel genes (CLCN1-5, CLCNKa and Kb), which encode different proteins (CLC1-5, CLCKa and Kb). In patients with Dent's disease CLC5 functions were specifically hampered, which lead to the characteristic findings [93].

Table 3. Metabolic disturbances associated with urolithiasis.

Metabolic disturbances
Hypercalciuria
Normocalcemic hypercalciuria
Idiopathic hypercalciuria
distal renal tubular acidosis
diuretics (furosemide)
Wilson's disease, Lowe's syndrome
Hypercalcemic hypercalciuria
Primary hyperparathyroidism
immobilisation
hyperthyroidism
hypothyroidism
Cushing-syndrome
adrenal insufficiency
bone metastasis
Bartter's and William's syndrome
Intestinal hyperabsorption
Hypervitaminosis D (A)
idiopathic hypercalcemia of childhood sarcoidosis
Hyperoxaluria
Primary hyperoxaluria type I / II
secondary hyperoxaluria:
⇒ in malabsorption syndromes
⇒ after intestinal resection
⇒ dietary
Hyperuricosuria
Inborn errors of metabolism
Lesch-Nyhan syndrome / gout
glycogen-storage diseases I, III, V, VII
overproduction in leukaemia or non-Hodgkin-lymphoma
high protein diet
Hypocitraturia
d-RTA
idiopathic / dietary

Dent's disease has phenotypic familiarities with XRN and with **X-linked recessive hypophosphatemic rickets (XLRH)**, which has also been mapped to chromosome Xp11.22 ([135], Table 2), but different mutations of the CLCN5 gene were found for these entities [93]. Nevertheless, these findings indicate, that CLCN5 and other renal chloride channels may play an important part in the pathophysiology of urolithiasis.

Medullary nephrocalcinosis and calcium-phosphate stones are common in patients with **distal renal-tubular acidosis** (d-RTA, [14]). A high urinary pH (> 5.8 in fasting urines), hypercalciuria (secondary to systemic acidosis) and hypocitraturia (due to a tubular defect and the acidosis) are the characteristics [47]. In the complete form of distal RTA the urine pH cannot be lowered to less than 5.4 after an acid loading test, it mostly remains > 6.1. This test should be performed in older children, when diagnosis of distal RTA is suspected [54]. Recently an autosomal dominant form of inheritance was found in a large kindred with a hereditary form of distal RTA. Both, a symptomatic and an asymptomatic form were caused by a single RTA-1 gene and homozygosity was observed to be followed by a severe form of the disease [20].

There are several clinical entities leading to hypercalcemia with secondary hypercalciuria (Table 3, [3, 12, 44]). **Primary hyperparathyroidism**, the most frequent cause of hypercalcemic hypercalciuria in adults, is very rare in children. **Hypervitaminosis D** due to the use of multivitamin preparations including vitamin D, or of vitamin D added to milk preparations, can induce hypercalcemia and hypercalciuria [35, 64, 73, 106]. An excessive daily amount of **vitamin A** (> 10,000 units) may also lead to hypercalcemia and can secondarily induce hypercalciuria [125]. **Immobilization** over only 4 weeks induces a reduction of bone calcium and bone mass of about 15-20% accompanied by hypercalciuria [3]. Long term administration of either furosemide or dexamethasone can lead to hypercalciuria, nephrocalcinosis, or stone disease [2, 71, 78].

Hypercalciuria is also found in several syndromes, either linked to the pathogenesis (**Bartter's and William's syndrome** [26, 44]) or due to renal tubular damage (**Wilson's disease and Lowe's syndrome** [66, 141]). Patients with Bartter's syndrome develop nephrocalcinosis but no stones. The characteristic findings are due to mutations in the Na-K-2Cl cotransporter NKCC2 [139]. William's syndrome (WS), which is characterized by hypercalcemia with aortic stenosis and mental retardation, is sometimes accompanied by hypercalciuria and nephrocalcinosis. It is caused by a deletion of the elastin gene (ELN, [97], which is found on chromosome 7q11.23 (Table 2, [107]). A dysfunction of the human calcitonin receptor (CTR) could lead to disorders of calcium metabolism associated with hypercalcemia, such as the William's syndrome, but the CTR gene is different from the WS gene and located on chromosome 7q21.3 [114]. Wilson's disease, which can be complicated by hypercalciuria and nephrolithiasis even as the first symptom [66], is caused by the Wilson's disease gene (WND), which is located on chromosome 13p14.1-21.1 [41]. It encodes a putative copper transporting protein, that is exclusively expressed in the liver and which is impaired in patients with Wilson's disease [27].

Further conditions leading to hypercalciuria include

both hyper- and hypothyroidism, Cushing syndrome, adrenal insufficiency and metastatic malignant bone disease [23, 86], long term assisted ventilation (acid base changes) and long term parenteral nutrition [65, 138].

Hyperoxaluria

Hyperoxaluria is probably still being underestimated as a cause of calcium-stone formation, although oxalate is a more important risk factor than calcium [61, 151]. Therefore, even slightly elevated values are relevant [6, 69, 90]. Urinary oxalate is mostly of endogenous origin, only 5-10% derive from the daily nutritional intake. Oxalate absorption tests showed however, that oxalate absorption in calcium-oxalate stone formers is twofold increased when compared to normal controls [55, 92]. Glyoxylate, arising from the metabolism of glycine, hydroxyproline and glycolate, and ascorbic acid are the main precursors of oxalate [151].

Primary hyperoxaluria type I (PH 1) is a rare, autosomal recessive inherited disease [85] caused by a defect in glyoxylate metabolism with low or absent activity of liver-specific peroxisomal alanine:glyoxylate aminotransferase (AGT, [28, 31]). The disease prevalence is 2 patients per million population in Europe [83].

So far eleven PH I specific mutations and a variety of normal polymorphisms (C154T) have been found (Table 4) of which the G630A mutation, responsible for a peroxisomal to mitochondrial AGT mistargeting, is the most common [30, 32, 33, 34, 102, 108, 121, 122, 124, 149]. Recently three PH I specific microsatellites (D2S125, D2S140 and D2S895) have been linked to the single AGXT-gene, which is located on chromosome 2q37.3 (Table 2, [32, 123, 148]). The AGXT-gene is distributed over 11 exons, and as the intron sequences flanking each exon have also been determined, the entire coding region of the AGXT-gene can be amplified of genomic DNA by polymerase chain reaction [123]. Two different AGT alleles have been identified in normal individuals: a major allele is differentiated from a minor allele [121, 122, 146]. They differ in at least three positions, two of which lead to single amino acid alterations (e.g., Pro11⇒Leu and Ile340⇒Met substitutions, Table 4, [121]). The third difference is that the minor allele contains a 74-bp duplication within intron 1 [122]. The frequency of the minor allele is said to be within 10-20% [121, 122]. Table 5 shows the variation of clinical expression dependent either on the specific mutations or the major/minor alleles.

A functional deficiency of AGT allows glyoxylate to be oxidized to oxalate and reduced to glycolate, instead of being transaminated to glycine. This leads to the charcteristic highly elevated urinary excretion of oxalate and glycolate (> 0.5 mmol/1.73 m^2 BSA/day). The urine is supersaturated with respect to calcium-oxalate, which can lead to renal calculi, medullary nephrocalcinosis, or both. With disease progression and declining renal function, calcium-oxalate crystals are deposited in the parenchyma of other organs, as well as in bones and retina [85].

Table 4. Mutations and polymorphisms of the AGXT gene [33, 34, 102, 108, 121, 122, 124, 149]

	Location	Amino-acid substitution
PH I specific mutations		
$G_{243}A$	Exon 1	$Gly_{41}Arg$
$C_{320}G$	Exon 2	$Tyr_{66}Ter$
$G_{367}A$	Exon 2	$Gly_{82}Glu$
$T_{576}A$	Exon 4	$Phe_{152}Ile$
$G_{630}A$	Exon 4	$Gly_{170}Arg$
$C_{682}T$	Exon 5	$Ser_{187}Phe$
$T_{735}C$	Exon 6	$Ser_{205}Pro$
$T_{853}C$	Exon 7	$Ile_{244}Thr$
$C_{819}T$	Exon 7	$Arg_{233}Cys$
$G_{820}A$	Exon 7	$Arg_{233}His$
$G_{860}A$	Exon 7	$Trp_{246}Stop$
Normal polymorphisms		
$C_{154}T$	Exon 1	$Pro_{11}Leu$
74bp duplication	Intron 1	-
29/32bp VNTR	Intron 4	-
$A_{1142}G$	Exon 10	$Ile_{340}Met$

Although PH I is a monogenic disease, its clinical severity is only partly correlated with its degree of AGT deficiency [29]. There is a very large clinical, biochemical and genetic heterogeneity with some patients presenting in early renal failure due to nephrocalcinosis and others who only have occasional passage of stones in adult life with preserved renal function [89, 105]. Renal stones or medullary nephrocalcinosis are usually the first signs of PH I. However, diagnosis of PH 1 is often delayed for many years [83]. Thus, it is important to exclude the diagnosis of PH I in all calcium-oxalate stone formers.

As expected for an autosomal-recessive disease, PH I has a horizontal pattern of inheritance in the vast majority of affected families, but it can sometimes show a vertical kind of inheritance [68]. This is mostly due to the segregation of three rather than two mutant AGXT-alleles within these families [68]. Affected members of such families can manifest very different clinical phenotypes, both within and between generations. This can be seen in patients within the same generation and with the same disease genotype, which led to the finding, that patients with a PH I specific AGXT-genotype might remain asymptomatic and undiagnosed for many years [68]. This has to be clearly considered, when genetic counseling is offered in affected families.

Primary hyperoxaluria type II (PH 2) is less frequently observed than PH 1. It is characterized by increased urinary excretion of oxalate and L-glyceric acid due to a

Table 5. Mutations of the AGT gene and phenotypical expression [28, 30, 31]

Phenotypical expression (% occurence)	Mutation / Polymorphism	minor / major Allele
Peroxisome to mitochondrion AGT-mistargeting (41%)	$G_{630}A$	minor allele
	$C_{154}T$	homozygous $\Rightarrow$ AGT activity $\Uparrow$
Partial peroxisome to mitochondrion AGT-mistargeting and intraperoxisomal AGT aggregation (3%)	$G_{243}A$	
	$T_{576}A$	minor allele (homozygous)
	($G_{630}A$)	
Normal localization of catalytically inactive AGT (16%)	$G_{367}A$	major allele (homozygous)
No catalytic AGT-activity and AGT-immunoreactivity (40%)	$C_{682}T$	major allele (homozygous)
	$T_{735}C$	
Miscellaneous	$C_{320}G$	heterozygous / normal (minor) allele probably not expressed

defect in both liver specific D-glycerate dehydrogenase and glyoxylate reductase ([104, 137, 150], Table 2). Urinary glycolate excretion is normal. The clinical course of PH 2 is much milder than in PH 1, although its clinical characteristics are comparable [62, 79, 80, 94]. End stage renal failure is rather the exception (in 5-10% of patients, [95]).

Mild metabolic hyperoxaluria is a term referring to patients with slightly elevated urinary oxalate excretion and plasma oxalate levels [129], but is not well defined and needs to be differentiated from PH 1. As glycolate excretion is also increased, hyperoxaluria due to overingestion or overabsorption of dietary oxalate is ruled out [129]. Patients might have severe recurrent urolithiasis [46].

Secondary (enteric) hyperoxaluria is a complication in patients with fat malabsorption, e.g., cystic fibrosis and chronic inflammatory bowel diseases (Crohn's disease) or in patients with intestinal resections (short bowel syndrome [67, 98, 110]). Normally, oxalate is intestinally bound to calcium to form insoluble calcium-oxalate, which is not absorbed. In patients with enteric hyperoxaluria, calcium instead binds to fatty acids, thus the soluble oxalate is better absorbed. Additionally, malabsorbed bile salts increase the permeability for oxalate of the distal colon [151]. Enteric hyperoxaluria may also lead to progressive nephrocalcinosis and renal failure if not treated adequately.

Cystine Stones

Cystinuria is an autosomal recessive inherited disorder, which is caused by the defective transport of cystine and the dibasic amino acids lysine, ornithine and arginine through the epithelial cells of the renal tubule and the intestinal tract. It is one of the most frequent genetic disorders with an overall prevalence of 1:7000 [13]. Cystine stones occur at any age but are infrequently seen in infants. Whether stones are formed depends not only on the cystine excretion, but also on the urine volume and on a (low) urinary pH. A high dietary sodium intake may increase the urinary cystine excretion [74, 109]. It is necessary to pay special attention to the method used for analyzing urinary cystine excretion: differentiation between cysteine and cystine excretion is important, as only cystine is relatively insoluble at normal urinary pH, but not cysteine [8].

Three different phenotypes have been distinguished based on the urinary excretion in obligate heterozygotes [130]. Cystinuria type I includes the heterozygotes, type II is characterized by a moderate elevation of cystine excretion and type III shows a mild elevation of cystine excretion. Whereas the intestinal transport is disturbed in type II and III, there is no uptake at all in type I.

Recently, one of the genes responsible for cystinuria type I named rBAT or D2H (genome data base nomenclature: SLC3A1, [16]) has been identified, mapping it to the subregion of chromosome 2p21 [117, 153]. This gene may encode an activator of the renal/intestinal basic amino acid transporter. Several mutations have been found (Table 6), all of which were associated with the type I phenotype [17, 70]. The most common mutation involves the substitution of threonine for methionine at codon 467 (Met467Thr, Table 6, [9, 118]). Whether all type I patients have mutations in the rBAT / D2H gene remains to be determined. Other genes are expected to be responsible for the other subtypes, as the cystinuria type III gene was localized to chromosome 19q13.1 [10].

Purine Stones

Uric acid stones are frequent in the adult population (5-25% of all stones, particularly in men and at an older age), but are rarely found in children in Western Europe (1%). They, however, are more frequent (5-10%) in Eastern Europe and the near East [7, 81]. Uric acid has a pK of 5.35

and is poorly soluble in acidic urine, but well soluble at pH 6.5. Therefore, a low urine pH is next to a low urine volume and an increased excretion of uric acid, the most important risk factor for uric acid stone formation. Hyperuricosuria may result from high-purine diets, myeloproliferative disorders, tumor lysis syndrome, enzyme defects etc. (Table 3). Some drugs (e.g., probenecid, high dose salicylates, contrast media) can increase uric acid excretion.

Primary purine overproduction occurs in some rare inherited deficiencies of the purine salvage enzymes hypoxanthine-phosphoribosyltransferase (HPRT) and adenine-PRT (APRT, Table 2). Partial deficiency of HPRT can result in urolithiasis and renal failure [21, 136]. Complete deficiency of HPRT leads to the **Lesch-Nyhan syndrome**, which is a severe X-linked recessive neurological disorder, characterized by mental retardation, automutilation, choreoathetosis, gout and uric acid nephrolithiasis [18, 37]. It is due to a defect of the hypoxanthine-phosphoribosyltransferase gene (HPRT-gene), which is located on chromosome Xq26-27.2 and consists of nine exons and eight introns totaling 57 kb [76]. Mutations affecting the splicing of exons 1, 2 and 9 and two missense mutations in exons 3 and 8 [11, 96] and a deletion of the HPRT gene locus [127] lead to a profound enzyme deficiency. A 5 kb DNA sequence deletion was found to have its endpoints in the first and third introns of the HPRT-gene [127].

Primary uric acid overproduction is also found in X-linked inherited superactivity of phosphoribosylpyrophosphate synthetase 1 (PPS-1). The PPS-1 gene is located on chromosome Xq22-24 [147].

Gout and nephrolithiasis have also been reported in **glycogen storage disease type 1** [128], which is due to a mutation of the glucose-6-phosphate gene on chromosome 17q21 (G6P-gene, [87, 88]). In Japanese patients an exon redefinition by point mutation within exon 5 of the G6P gene was found to be the major cause of glycogen storage disease type 1a [77].

Deficiency of adenine-phosphoribosyltransferase (APRT) results in the autosomal recessive inherited **2,8-dihydroxyadeninuria** (Table 2, [19]). Several missense mutations and a 7-bp deletion were found in the adenine-phosphoribosyltransferase gene on chromosome 16q22.2-22.3 [15, 133]. Serum uric acid levels are normal, the stones are radiolucent and may be confused with uric acid stones. Due to APRT-deficiency renal deposition of 2,8 dihydroxyadenine takes place leading to nephrolithiasis and chronic renal failure [43]. The urine contains characteristic brownish round crystals. Diagnosis is confirmed from APRT activity in red blood cells or from excretion of 2,8 dihydroxyadenine in the urine [58, 59].

The xanthine-dehydrogenase-gene (XDH) codes for the last enzyme of the purine catabolic pathway and mutations in this gene cause the autosomal-recessive disease **xanthinuria**. The human gene for xanthine-dehydrogenase has been localized by *in situ* hybridization to chromosome 2p22.3⇒22.2 (Table 2, [132, 152]). In xanthinuria, serum uric acid concentration is very low due to deficiency of xanthine-oxidase which converts xanthine to uric acid [5]. Characteristic findings of xanthinuria are an orange-brown urinary sediment or orange-stained diapers [5] and later xanthine stones [145].

Table 6. Mutations and polymorphisms in the rBAT / D2H gene [9, 16, 70, 118].

Name	Effect on coding sequence	Nucleotide change
Missense		
R365W	$Arg_{365}Trp$	$C_{1093}T$
P128Q	$Pro_{128}Gln$	$C_{383}A$
Y151N	$Tyr_{215}Asn$	$T_{451}A$
R181Q	$Arg_{181}Gln$	$G_{542}A$
T216M	$Thr_{216}Met$	$C_{647}T$
E268K	$Glu_{216}Lys$	$G_{802}A$
T341A	$Thr_{341}Ala$	$A_{1021}G$
R362C	$Arg_{362}Cys$	$C_{1084}T$
M467K	$Met_{467}Lys$	$T_{1400}A$
M467T	$Met_{467}Thr$	$T_{1400}A$
P615T	$Pro_{615}Thr$	$C_{1843}A$
Y582H	$Tyr_{582}His$	$T_{1744}C$
T652R	$Thr_{652}Arg$	$C_{1932}G$
L678P	$Leu_{678}Pro$	$T_{2030}C$
F648S	$Phe_{648}Ser$	$T_{1943}C$
Stop codon		
R270X	Arg Stop at 270	$C_{808}T$
E483X	Glu Stop at 483	$G_{1447}T$
Frameshift, deletion, insertion		
1749delA		del of A at 1749
1306insC		insC at 1306
5'del1192		del1192pb
3'del ?		del
Polymorphism		
114A/C	no aa change	A or C at 114
231 T/A	no aa change	T or A at 231
1136+3delT	5' intron 6	T or G at 1136+3
1398C/T	no aa change	C or T at 1398
1473C/T	no aa change	C or T at 1473
1854A/G	M or I at 618	A or G at 1854
2189C/T	3'-UTR	C or T at 2189

Extrinsic factors

Primary bladder stones used to be very frequent but have almost disappeared in the Western world, this trend away from bladder calculi to upper urinary tract stones

is seen in association with industrialization and increasing affluence. Bladder stones are often composed of concentric layers and contain calcium-oxalate and/or uric acid. Dietary factors are mainly incriminated in their formation [13, 81]. Once removed, they do no tend to recur. Some primary bladder stones are of infectious origin. Bladder stones are sometimes found in association with foreign bodies or after surgical procedures, where sutures or metallic staples give basis for crystal deposition and agglomeration [25].

Anatomical anomalies like uretero-pelvic junction obstruction, primary ureter or neurogenic bladder, are often found to be the reason for stone disease in pediatric patients. Renal calculi then develop due to disturbances in urine transport, due to urine stasis or changes in urinary flow (Table 2, [13]).

Urolithiasis may occur from crystallization of several drugs, e.g., after high dose sulfonamide or ceftriaxon therapy (Table 3, [52, 86, 100]). Finally, stone analysis with infrared spectroscopy will occasionally reveal an artefact, e.g., gypsum (Table 1, [53]).

Modifiers of Crystallization

Hypocitraturia

A low citrate excretion is not always adequately mentioned as a risk factor for renal stone disease [99]. Citric acid, a tricarboxylic acid, is a very potent inhibitor of calcium-oxalate and calcium-phosphate crystallization. Ten to 35% of the glomerular filtered citrate is excreted in the urine. In alkalosis citrate excretion increases, when less citrate is reabsorbed in the proximal tubule [140]. Depending on the urinary pH, a stable calcium-citrate complex is formed and calcium ions remain soluble, as less ions are bound to oxalate. The "activity" product for calcium-oxalate will therefore improve. Only 16% of calcium is bound to citrate in an acidic urine, but more than 45% will be bound to citrate at pH 8 [112].

Low urinary citrate excretion is characteristic of the complete form of **d-RTA** [119]. Hypocitraturia is also observed in persistent mild or latent metabolic acidosis, in hypokalemia and in patients with malabsorption syndromes [110]. **Idiopathic hypocitraturia** may be secondary to low intestinal alkali absorption [134].

Other inhibitors

Glycosaminoglycans (heparan-sulfate), Tamm-Horsfall protein, nephrocalcin, uropontin and prothrombin fragment 1 are other potent inhibitors of the CaOx crystallization process [48, 49, 57, 60, 63, 131, 144]. However, their physiological role has not yet been fully elucidated [4, 24, 49, 63].

Outlook

The molecular mechanism of some underlying metabolic conditions for the development of urolithiasis has been recently determined. Whether every single urinary excretion parameter has its own genetic "basis" is of great interest, but can currently not be answered. Hopefully, further work will reveal the molecular basis of other inherited metabolic disorders leading to urolithiasis, like most of the hypercalciurias, of primary hyperoxaluria type II, and of stone-inhibitory substances, e.g., citrate excretion.

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Discussion with Reviewer

B. Hess: How frequent is the incomplete form of d-RTA in children?

Authors: During recent years we did not see a case of incomplete d-RTA in our patient population (children). We also could not find any informative data regarding the incidence of incomplete RTA in childhood in the literature. Additionally, the incomplete form of d-RTA is not that easy to diagnose. An acid loading test has to be carefully considered, especially in small infants and children and is therefore not very often performed.