REVIEW: GENETICS OF UROLITHIASIS

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ABSTRACT

Urolithiasis constitutes a worldwide health problem with a frequency of up to 15% in the general population. The causes are diverse; exogenous influences and genetic factors act in complex interaction and thereby impede research on stone formation. In recent years, important advances on the elucidation of molecular and genetic bases have been made. Numerous genes for urolithiasis and syndromes associated with stone formation have been identified; however, these account only for a minority of all patients. In spite of a frequency of 75% among urinary calculi, the genetic causes of calcium oxalate stones are unknown except for relatively rare entities such as hyperoxaluria or Dent disease. For patients presenting with uric acid stones, metabolic defects can only be detected in rare instances, but in these cases the molecular bases have been widely resolved. In contrast, the genetic basis of cystinuria has mainly been determined with the identification of two genes encoding subunits of a cystine transporter in the proximal renal tubule. In conclusion, the currently applied biochemical and physical methods of kidney stone diagnosis continue to be the basis of both diagnostics and therapy of stone disease, but they are increasingly supplemented by molecular genetic analysis. Though the basic genetic defects in inborn urolithiasis cannot be cured, their identification considerably contributes to an earlier diagnosis and treatment. Genetic tests will not only make an individual therapy possible, but will also allow a better delineation of entities and thereby well-directed genetic counselling.

Keywords: Urolithiasis, genetics, mutations, epigenetics.

INTRODUCTION

Urolithiasis constitutes as a worldwide health problem with a frequency of up to 5% in the general population,[1] but prevalence is even greater in specific geographic regions (e.g. Turkey: ~15%). Its causes are heterogeneous: both environmental factors (e.g. diet, climate, amount of fluid intake) and predisposing genes have been identified. In children, the incidence of urolithiasis is approximately 10% of that in adults,[2] but in paediatric patients the proportion of well-defined underlying metabolic conditions, infections, or urinary tract anomalies is preponderant.

From the genetic point of view, two groups of inborn urolithiasis disorders can be distinguished: diseases with urolithiasis as the cardinal symptom (in the following paper called 'urolithiasis disorders'), and syndromes with stone formation as one feature among others. However, the transitions are fluent, and this separation is rather artificial.

GENETICS AND EPIGENETICS

The search for the genetic causes of congenital disorders has been mainly focused on protein-coding DNA sequences, i.e. those sequences transcribed into RNA and translated into protein. As a result, they represent the most widely studied component of the human genome, and >3,000 genes with phenotype-causing mutations (http://omim.org/statistics/geneMap) have been identified. However, they constitute only a small fraction of the genome (<2%), and only 21,000 protein-coding genes have been estimated in the human genome. In contrast to this relatively small number of predicted genes, the number of proteins is much higher due to the post-transcriptional processing (e.g. alternative mRNA splicing). The non-coding DNA (98% of the genome) includes all those sequences that do not encode proteins. In addition to its function in chromatin-structuring, noncoding DNA also includes non-protein coding genes for RNA molecules with biological functions in

transcription and translation, such as ribosomal RNA (rRNA) or transfer RNA (tRNA). Another group of non-coding RNAs (ncRNA) includes microRNA and long ncRNA with a major impact on transcription and post-transcriptional regulation of gene expression. As a result of this complex interaction between DNA, RNA, and proteins (e.g. transcription factors) the same phenotype can be caused by mutations in different genes belonging to the same network. On the other hand, different mutations in the same gene can produce variable clinical outcomes due to their localisation in the gene and differential expression of its transcripts.

The phenotype of an individual is not only determined by the nuclear DNA sequence itself, but also by epigenetic modifications of the DNA and the chromatin structure. These epigenetic marks do not affect the DNA sequence itself, but consist of DNA methylation (i.e. the covalent modification of cytosine), ncRNAs, and posttranslational modifications of histones such as methylation, acetylation, phosphorylation, and ubiquitinilation. Functionally, epigenetic marks influence the regulation of gene expression.

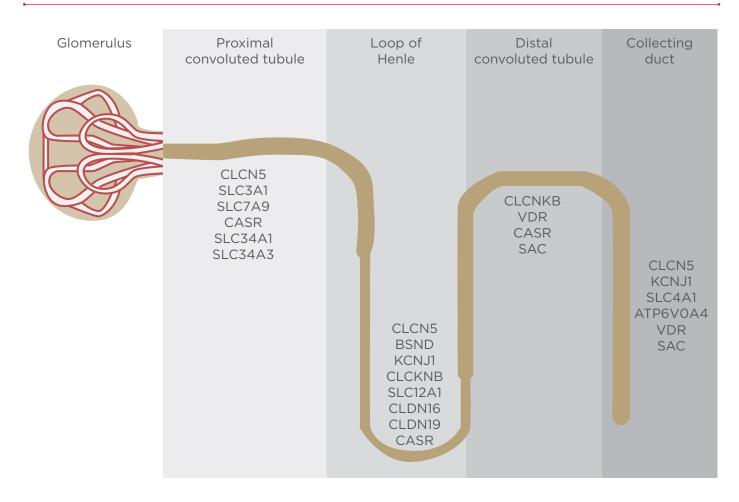## GENETIC CONTRIBUTION TO UROLITHIASIS DISORDERS

Inborn molecular defects comprise different classes of mutations on a genomic, as well as an epigenetic, level. Like other human inherited diseases, genetic forms of urolithiasis are mainly based on alterations (mutations) in the nuclear DNA and are transferred from generation to generation (germline mutations). They can be the direct cause of a pathologic phenotype; alternatively, they can result in an increased susceptibility to disease. With the exception of the X chromosome in males (X-chromosomal inheritance), most alleles exist in duplicate due to the diploid character of the human genome. In diploid organisms, a dominant allele on one chromosome will mask the expression of a recessive gene on the other (autosomal dominant [AD] mode of inheritance). In contrast, a recessive phenotype only appears when the organism carries the recessive allele on both parental chromosomes (autosomal recessive [AR] mode of inheritance).

For a comprehensive genetic diagnostic workup, the knowledge on the whole spectrum of molecular alterations is needed. Dependent on the gene and mode of inheritance, not only point mutations in the coding sequences should be covered, but in specific disorders large genomic rearrangements and epigenetic changes have been reported and, therefore, have to be included.[3,4]

The general contribution of genetic factors to the aetiology of urolithiasis disorders has mainly been estimated from family and twin studies. Based on a large cohort of nearly 38,000 males, an adjusted relative risk of 2.57 in men with a positive family history has been determined.[5] By comparing the incidence of stone formation in twins, a heritability of 52-56% has been estimated.[6] Despite this obvious evidence for genetic causes of urolithiasis, single gene mutations are rarely observed as they account for only 2% of adult and up to 10% of paediatric kidney stone formers.[6] If we split the urolithiasis vitamin D receptor (VDR) disorders according to their stone composition, the molecular basis has been widely resolved for rare entities characterised by cystine or uric acid stones, whereas the aetiology of the frequent calcium stones cannot be ascertained in the majority of patients, despite an extensive evaluation.

Hypercalciuria and Calcium-Containing Stones

Hypercalciuria is the most common metabolic abnormality in urolithiasis. As a result, calcium accounts for >75% of kidney stones, and is therefore the main stone component in both paediatric and adult patients.[7] In the majority of stones, calcium oxalate is the main constituent, whereas calcium phosphate is present in amounts ranging from 1-10%. Many lithogenic and inhibitory factors are involved in the formation of calcium stones, and a broad spectrum of environmental influences, as well as genetic predispositions, contributes to their formation. The family history is often positive in these patients (13-60%), and in these patients stones occur with greater frequencies in male than in female relatives.[8] Thus, the greatest risk factor for calcium urolithiasis after the known dietary determinants is having an affected family member. However, the inheritance of hypercalciuria follows a polygenic quantitative trait, and linkage to specific genes or genetic loci are detectable only in rare cases. Linkage studies in families with idiopathic hypercalciuria revealed an association with a genomic variant of the VDR gene,[9] whereas Scott et al.[10] reported on linkage of calcium-oxalate stones with the soluble adenylyl cyclase (sAC) gene. In these patients, AD, AR, or X-linked modes of transmission have been reported (Table 1).

Figure 1: The renal tubule and localisation of genes involved the aetiology of uroliasis.
Modified from Monico CG et al.[6] with kind permission of Nature Publishing Group.

These monogenic forms have been discussed to be associated either with defective intestinal calcium absorption, bone calcium resorption, or renal calcium reabsorption (Figure 1).

The broad range of clinical consequences of mutations in the same gene is impressively illustrated by genomic variants *CASR* gene.[11] Loss-of-function mutations in this G protein-coupled receptor gene cause specific types of hypercalcaemia and hyperthyroidism, whereas gain-of-function mutations result in both AD hypocalcaemia with hypercalciuria and Bartter's syndrome Type 5 (Table 1). Another example is the mutations in the *CLCN5* gene, an X-chromosomally encoded factor that is typically mutated in Dent's disease and in Bartter's syndrome Type 6. The broad spectrum of genetic factors involved in stone formation could recently be illustrated by the identification of loss-of-function mutations in *CYP24A1*, the vitamin D 24-hydroxylase gene, causing hypercalciuric nephrolithiasis and nephrocalcinosis.[12,13]

Hyperoxaluria

Hyperoxaluria occurs due to an increased urine calcium concentration, and can result in the formation of calcium oxalate stones. Whereas dietary or enteric hyperoxaluria can occur in later life, primary hyperoxaluria Types 1 and 2 (PH1, PH2) are congenital errors of metabolism and are present at birth. For both PH1 and PH2, causative genomic mutations can be identified (Table 1).[14] PH1 is caused by the deficiency of the glyoxylate aminotransferase (AGXT) which normally catalyses the conversion of glyoxylate to glycine. In case of mutations in the PH1 causing the AGXT gene, glyoxylate is oxidised to oxalate and reduced to gycolate. Clinically, persistent and marked hyperoxaluria are observed from early infancy, whereas hyperglycolic aciduria is variable. For PH2, mutations in the *GRHPR* gene could be identified. *GRHPR* catalyses the reduction of glyoxylate to glycolate and of hydroxypyruvate to D-glycerate. The clinical features of PH1 and PH2 are similar as are their urinary oxalate concentrations, but in PH2 L-glyceric aciduria is often present.
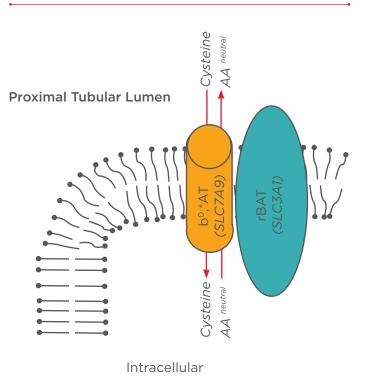
Table 1: Genes and chromosomal loci associated with metabolic disturbances resulting in kidney stones.

Metabolic disturbance	Disease	Mode of inheritance	Chromosomal localisation	Gene	Ref
Hypercalciuria: calcium-containing stones	Idiopathic hypercalciuria	AD	Linkage with 1q23.3q24	Association with *SAC* polymorphisms	21
			Linkage with 9q33.2q34.2	Gene unknown	22
	CaOx stone formation	AD	Linkage with 12q12q14	Association with *VDR* polymorphisms	10
	ADHH	AD	3q21.1	*CASR*	11
	BS Type 1	AR	15q21.1	*SLC12A1*	23
	BS Type 2	AR	11q24	*KCNJ1*	24
	BS Type 3	AR	1q36	*CLCNKB*	25
	BS Type 4	AR	1q31	*BSND*	26
	BS Type 5	AD	3q21.1	*CASR*	27
	BS Type 6	X chrom	Xp11.22	*CLCN5*	28
	Dent's disease	X chrom	Xp11.22	*CLCN5*	29
	Lowe's syndrome	X chrom	Xq25	*OCRL1*	30
	HHRH	AR	9q34	*SLC34A3 (NPT2c)*	31
	Nephrolithiasis, osteoporosis, and HP	AD	5q35	*SLC34A1 (NPT2a)*	32
	FHHNC	AR	3q28	*CLDN16 (PCLN1)*	33
	FHHNC with ocular abnormalities	AR	1q32.2	*CLDN19*	34
	dRTA	AD	17q21	*SLC4A1*	35
	dRTA with sensorineural deafness	AR	2p13	*ATP6B1*	36
	dRTA with preserved hearing	AR	7q34	*ATP6N1B*	37
	CYP21A1 deficiency	AR	20q13.2-q13.3	*CYP24A1*	12, 13
Hyperoxaluria	PH Type 1	AR	2q37.3	*AGXT*	14*
	PH Type 2	AR	9p13.2	*GRHPR*	38*
Cystinuria	Cystinuria Type A**	AR, AD	2p21	*SLC3A1*	16*
	Cystinuria Type B**	AD	19q13	*SLC7A9*	16*
	Hypotonia-cystinuria syndrome	AR	2p21	Microdeletions affecting both *SLC3A1* + *PREPL*	17*
Purine metabolism	Lesch-Nyhan syndrome	X chrom	Xq26	*HPRT1*	19*
	APRT deficiency	AR	16q24	*APRT*	18*
	XO deficiency	AR, AD	2p23	*XDH*	4*

AD: autosomal dominant; CaOx: calcium oxalate; ADHH: autosomal dominant hypocalcaemia with hypercalciuria; BS: Bartter's syndrome; AR: autosomal recessive; X chrom: X chromosome; HHRH: hereditary hypophosphataemic rickets with hypercalciuria; HP: hypophosphataemia; FHHNC: familial hypomagnesaemia with hypercalciuria and nephrocalcinosis; dRTA: distal renal tubular acidosis; PH: primary hyperoxaluria; APRT: adenine phosphoribosyltransferase; XO: xanthine oxidase.
* For review.
** For cystinuria, patients carrying mutations in both genes have been reported.

Proximal Tubular Lumen

Cysteine
AA ^{neutral}

b$^{0,+}$AT
(*SLC7A9*)

rBAT
(*SLC3A1*)

Cysteine
AA ^{neutral}

Intracellular

Figure 2: Cystinuria - cellular localisation and function of rBAT (*SLC3A1*) and b$^{0,+}$AT (*SLC7A9*).

Cystinuria

Cystinuria accounts for 1-5% of kidney stones.[15] It is an inborn congenital disorder characterised by a defective cystine metabolism resulting in the formation of cystine stones. In contrast to other kidney stone diseases, cystinuria is exclusively caused by gene mutations, whereas the clinical course is influenced by endogenic and environmental factors. So far, two genes responsible for cystinuria have been identified: *SLC3A1* encodes the heavy subunit rBAT of a renal b$^{0,+}$ transporter while SLC7A9 encodes its interacting light subunit b$^{0,+}$AT (Figure 2).[16] Mutations in *SLC3A1* are generally associated with an AR mode of inheritance, whereas *SLC7A9* variants result in a broad clinical variability even within the same family. The detection rate for mutations in these genes is >85% but it is influenced by the ethnic origin of a patient and the pathophysiological significance of the mutations. In addition to isolated cystinuria, patients suffering from the hypotonia-cystinuria syndrome have been reported as carrying deletions including at least the *SLC3A1* and the *PREPL* genes in 2p21.[17]
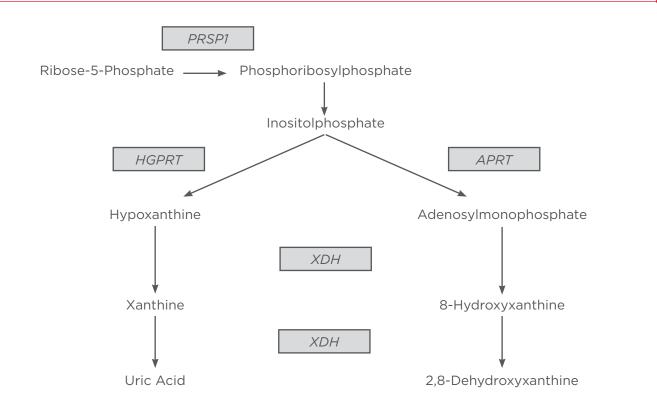
By extensive molecular screening studies in a large cohort of patients, a wide range of mutations has been identified. Several of these variants were functionally analysed and thereby allowed insights into the pathology of the disease as well as in the renal trafficking of cystine and the dibasic amino acids.

Purine Stones

Uric acid, as the end product of purine metabolism, influences kidney stone formation in two ways: 1) hyperuricaemia, hyperuricosuria, and low pH (<6.0) favour the development of uric acid stones; 2) an increased uric acid excretion promotes calcium oxalate stone formation. As a result, up to 33% of patients with idiopathic hyperuricaemia suffer from stones. Patients with gout also have an increased risk of forming uric acid stones, but in general, congenital disturbances of the purine metabolism are rare. As several enzymes are involved in purine metabolism and have been reported to be affected by genomic mutations, different purine stones can develop (Table 1; Figure 3). Adenine phosphoribosyltransferase (APRT) deficiency results in accumulation of adenine.[18] The excess of adenine is oxidised by xanthine dehydrogenase (XDH) and leads to 2,8-dihydroxyadenine urolithiasis (2,8-DHA). As 2,8-DHA is highly insoluble, 2,8-DHA crystals are formed, which are injurious for renal parenchyma and cause kidney stones. An early identification of carriers of XDH mutations is important for a well-directed therapeutic regimen. The application of allopurinol reduces 2,8-DHA stone formation and thereby avoids renal damage.[18]

The deficiency of hypoxanthine-guanine phosphoribosy-transferase (HPRT) results in hyperuricaemia, hyperuricosuria, and uric acid urolithiasis as well as neurological symptoms. Mutations in the X-linked *HPRT1* gene are associated with the Lesch-Nyhan syndrome; in case of partial enzyme deficiency, a milder phenotype (Kelley-Seegmiller syndrome) can occur.[19] As allopurinol treatment elevates urinary xanthine and hypoxanthine, xanthinuria can occur in the course of HPRT deficiency therapy.

Mutations in the *XDH* gene lead to an increase of xanthine and hypoxanthine in urine, and therefore, to the formation of xanthine stones.[4] The disorder mainly occurs in the Mediterranean area and the Middle East. A careful analysis of the stone composition is therefore the prerequisite of a directed molecular genetic testing.

Figure 3: Simplified illustration of the purine metabolism and the involved enzymes for which mutations have been reported (for further information on the enzymes/genes see Table 1).

Inborn Syndromes and Disorders Associated with Stone Formation

Based on its numerous metabolic causes, stone formation as an attendant symptom, has been reported for several diseases, among them hyperparathyroidism and multiple endocrine neoplasia Type 1 (Table 1).[6] A careful diagnostic workup also for stone predisposing features is indicated in this group of patients despite other features, and their treatment is the focus of therapy. A good example is Beckwith-Wiedemann syndrome (BWS), a congenital disorder characterised by overgrowth, omphalocele, and an increased risk for abdominal tumours. As Goldman and coworkers[3] showed, 22% of BWS patients had hypercalciuria, prompting the authors to claim a general evaluation for hypercalciuria in this group of patients.

Impact of Genetic Diagnosis on Clinical Management

Though the basic genetic defects in inborn urolithiasis cannot be cured, their identification considerably contributes to an earlier diagnosis and treatment. Indeed, delays in diagnosis of urolithiasis are frequent, in many cases after the recurrent occurrence of stones, even after kidney transplantation. For PH1, a delay in diagnosis has been reported for 42% of patients,[20] including 30% diagnosed after end-stage renal disease. A similar delay has been reported for 2,8-DHA urolithiasis.[20] Molecular genetic testing has the potential to identify early carriers of monogenic forms of urolithiasis. The knowledge on the molecular defects contributes to an individualised therapeutic management, both in patients already suffering from urolithiasis, as well as in probands with a positive family history. On the other hand, a careful biochemical workup of urine and stone composition helps to differentiate between the subtypes of the different urolithiasis disorders, allowing a directed genetic testing regiment (e.g. in case of hyperoxaluria or cystinuria). The combined use of genetic and biochemical tests provides us with new insights on the aetiology of urolithiasis. It helps us to develop effective treatment strategies and to predict response to nutrients and drugs.

CONCLUSION

Consistent with other congenital disorders, genetics has become increasingly important in routine diagnostic testing. Thus, it is crucial to increase interdisciplinary efforts for the benefit of each individual patient. In general, genetic testing

should be preceded by genetic counselling of the affected person or family. It assists affected and/or at-risk individuals to understand the nature of the genetic disorder, its transmission, and the options open to them in management and family planning. In the future, discovery of the whole genome by high-throughput technologies (i.e. next generation sequencing) will make the delineation of a genetic profile possible, opening the option of an appropriate therapy and diet with sufficient scientific evidence.

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