

Idiopathic Calcium Nephrolithiasis And Hypercalciuria: The Role Of Genes

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Abstract. Idiopathic calcium nephrolithiasis and hypercalciuria are multifactorial disease conditions, the pathogenesis of which involves the interaction of environmental and individual factors. Data support a strong role of genes in the pathogenesis of these two conditions. Findings obtained in monogenic disorders characterized by renal calcium stones, and/or hypercalciuria, and/or nephrocalcinosis have proposed a number of genes as candidate genes in the pathogenesis of the common idiopathic calcium nephrolithiasis and hypercalciuria. The physiological role of these genes, and findings in monogenic disorders and idiopathic, multifactorial disorders will be presented.

Keywords: calcium sensing receptor, chloride channel 5, anion exchanger 1, soluble adenylyl cyclase, hypercalciuria, calcium nephrolithiasis.

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INTRODUCTION

Idiopathic calcium nephrolithiasis (ICN) and hypercalciuria (IHC) are multifactorial disease conditions, the pathogenesis of which involves the interaction of environmental and individual factors. The importance of hereditary factors in ICN and IHC has emerged from a number of investigations in families of patients with renal stones and/or hypercalciuria.

A study in monozygotic and dizygotic twins have shown that genetic background explain 56% of the risk of stones, larger than dietary and environmental influences on nephrolithiasis [1].

The excretion in urine of lithogenic substances seems to be under the influence of genetic factors, as Goodman et al [2] observed that in stone formers the relative risks of an abnormal excretion of calcium and citrate, which are partly genetically driven, was 9.18 and 3.79 respectively, far higher than the relative risks for abnormal nutritional intakes [3]. This suggests that the contribution of genetic factors on the risk of stones is much larger than that associated with nutritional exposures.

The analysis of calciuria in families supports the importance of genes since calcium excretion is positively correlated between parents and their progeny and between siblings, but not between spouses [4]. According to findings obtained in parents and offspring, half of the variance of calciuria is due to genetic factors [4]. This conclusion has been recently confirmed in families studied for nephrolithiasis. Evidence for a

major gene explaining 58% of the segregation of hypercalciuria, with an 11% additive polygenic effect has been shown [5].

However, the specific genes involved in ICN and IHC have yet to be identified. Candidate genes have been proposed according to their identification as responsible for Mendelian disorders characterized by calcium stones or allied phenotypes (i.e., hypercalciuria, nephrocalcinosis), or as involved in calcium handling in kidney, bone and intestine. Some are or have been actively investigated and are discussed here, while others have not yet been thoroughly investigated (Table 1).

Calcium-sensing receptor

The calcium-sensing receptor (CaSR) gene, located on chromosome 3q13.3-q21, encodes for a plasma membrane G-protein-coupled receptor that is activated by binding calcium ions to its extracellular domain. It is present in all tissues involved in extracellular calcium regulation, where it controls cellular activity in relation with the plasma calcium concentration [6]. CaSR regulates PTH secretion by parathyroid cells, calcium reabsorption by kidney tubular cells, and influences intestinal calcium absorption and bone remodeling.

Activating mutations of the gene for the CaSR extracellular domain leads to autosomal dominant condition known as familial hypocalcemia with hypercalciuria [7]. Patients belonging to these kindreds have a clinical syndrome mirroring hypoparathyroidism: They have hypocalcemia and normal (i.e. relatively low) serum parathyroid hormone levels. Activating mutations in the CaSR gene causes hypercalciuria and hypocalcemia, because activation of basolateral CaSR in cells of the thick ascending limb of Henle's loop inhibits active and passive calcium reabsorption, and because inhibition of PTH secretion further down-regulates calcium reabsorption in the distal tubule.

The hypothesis for a role of CaSR in hypercalciuria and nephrolithiasis arises from the idea that a mild activation of the CaSR causes the increased intestinal calcium absorption and calcium renal loss observed in IHC with the autosomal inheritance generally observed. The alternative hypothesis is that inactivating mutations of the CaSR cytoplasmic domain cause IHC & ICN in association with border-line/mild hypercalcemia as shown in the very rare familial hypercalcemia with hypercalciuria [8]. However, two studies carried out in subjects with idiopathic stone disease did not support a pathogenetic role of CaSR. In 359 French-Canadian sib pairs of stone formers the CaSR locus was shown by linkage analysis not to be associated with IHC & ICN [9]. In addition, in 9 French families with idiopathic hypercalciuria, no CaSR mutation was discovered [10]. It is not possible to rule out that mutations of CaSR could be found in some other families or other populations, or that mutations of regulatory regions of the gene exist; nevertheless these findings suggest that CaSR mutations do not play a major role in IHC.

The role of CaSR polymorphisms has also been investigated in the Italian population using a case-control design with the hypothesis that polymorphisms may not be neutral in regulating the CaSR gene product activity or its transcription levels, and thus they may increase the risk for hypercalciuria [11]. The association of calciuria

with CaSR exon 7 SNPs (G/T at codon 986, G/A at codon 990, and C/G at codon 1011) in normocalciuric control subjects, and in both hypercalciuric and normocalciuric calcium nephrolithiasic patients was evaluated. Although 90% of the hypercalciuric stone formers did not present the 990Gly SNP, it explained a small but significant variance of calciuria. Interestingly, in human embryonic kidney cells transfected with a plasmid containing the CaSR gene, spikes of intracellular calcium concentration induced by increasing the extracellular calcium were much higher in cells with the polymorphic 990Gly SNP receptor than in those with the wild type CaSR [12]. Thus, the 990Gly allele corresponds to an activating SNP and may increase CaSR sensitivity or its response to calcium ions, increasing inhibition of renal calcium reabsorption and eventually increasing calciuria.

Recent findings in over 600 Italian ICN patients indicate that the genotypes of the 5'UTR tract of the CaSR gene and the haplotype of the first block of the CaSR gene characterized by the sequence CATTCA are associated with kidney stone disease [13]. No relationship was observed between the haplotype and measured phenotypes (urinary excretion of calcium, plasma levels of PTH and calcium). Since this sequence comprises the region that regulates CaSR gene transcription, different renal CaSR expression might be expected in stone formers which would favor lithogenesis through an influence on water and/or calcium metabolism in distal and collecting tubule within the papilla.

Chloride channel 5

Nine different chloride channel (ClC) proteins have been described, some of which are plasma membrane channels and others, including the ClC-5, which reside mostly in intracellular membranes.

ClC-5, located in subapical endosomes of kidney tubular cells, contributes to the acidification of the organelles involved in the endocytosis of low molecular-weight proteins by proximal tubules [14]. The channel CLCN5 gene [15] is located on the short arm of the X chromosome (Xp11.22) [16], and encodes a 746-amino acid protein expressed in the renal proximal tubules, the thick ascending loop of Henle, and the α -intercalated cells of the collecting ducts [14]. Mutations of this gene are responsible for Dent's disease, which includes a group of inherited disorders of the renal tubule: X-linked recessive nephrolithiasis with renal failure, X-linked recessive hypophosphatemic rickets and idiopathic low molecular weight proteinuria [17]. These very rare conditions are characterized by medullary nephrocalcinosis, nephrolithiasis, hypercalciuria, low molecular-weight proteinuria and other tubular dysfunctions, renal failure and rickets in variable combinations. CLC5 knockout mice demonstrate severe impairment of receptor-mediated endocytosis, including the endocytotic retrieval of the plasma membrane multiligand megalin and cubilin receptors [18]. Similarly to humans, knockout mice have low molecular weight proteinuria, but do not develop hypercalciuria and nephrolithiasis. So far, the relationship between impaired endocytosis and renal calcium handling remains unclear. It has been proposed that hypercalciuria results from secondary changes in the regulation of calciotropic hormone caused by urinary loss of key hormone-binding proteins.

To date, more than 70 different nonsense or missense mutations, insertions or deletions in the CLCN5 have been reported in the literature [19].

It has been suggested that the disease may occur more commonly than believed, in less overt forms and perhaps without apparent family histories. This hypothesis arose from the observation that in the large pedigree described by Frymoyer et al [20], a man was identified with an inactivating mutation in the CLCN5 gene who did not have low molecular weight proteinuria (together with the other features of the Dent's disease) and in whom the only biochemical abnormality was hypercalciuria [21]. This suggested the theory that mutations in CLCN5 might contribute to the phenotype in patients with the diagnosis of IHC and/or ICN.

Scheinman et al looked for CLCN5 gene mutations in a group of 32 adults and children with idiopathic hypercalciuria, but no CLCN5 gene mutations were observed [21]. An alternative approach to this problem is to search for CLCN5 mutations in groups of patients who may have a high prevalence of mutations, i.e. ESRD patients with previous stones. We have addressed the problem of possible missed diagnosis of Dent's disease by looking for CLCN5 mutations in 24 ESRD patients who had renal stones and found no mutations in the CLCN5 gene, confirming that Dent's disease is really quite rare even in a pool of patients in which it might have been over-represented [22].

To our knowledge, no linkage or polymorphism association study has been performed in IHC and/or ICN looking at the CLCN5 gene.

Anion Exchanger(s)

Several transporters in type α intercalated cells play a role in the transepithelial acid secretion and bicarbonate reabsorption. Among these are the multiple gene products encompassing the vacuolar H^+ -ATPase, band 3-AE1, the plasma membrane Cl/HCO_3^- exchanger of erythrocytes (SLC4A1), and carbonic anhydrase II [23]. Mutations in one of these components could be responsible for primary distal renal tubular acidosis (dRTA), a hereditary renal tubular disorder, which is characterized by impaired renal acid secretion resulting in metabolic acidosis. Clinical symptoms are nephrocalcinosis, nephrolithiasis, osteomalacia, and growth retardation. Biochemical alterations consist of hyperchloremic metabolic acidosis, hypokalemia with muscle weakness, hypercalciuria, and inappropriately raised urinary pH. dRTA is genetically heterogeneous; however, most frequently the anion exchanger AE1 gene is involved. Mutations in the AE1 gene (on chromosome 17) have been found in both autosomal dominant and recessive dRTA cases [24]. Since it is known that subtle forms of dRTA may present only with hypercalciuria and calcium renal stones, it has been suggested that the dRTA molecular defects may also cause IHC & ICN. We have previously looked for a linkage between the AE1 locus and familial ICN [25]. The association of an anomalous erythrocyte oxalate self-exchange with ICN, which is dependent on an abnormal phosphorylation of the band 3-AE1 exchanger, has been described [26,27]. Physiological and clinical studies support the importance of this defect in ICN; interestingly, partial acidification defects were disclosed in stone formers with the erythrocyte anomaly [28]. In a family study, the abnormal erythrocyte oxalate self-

exchange appeared to be genetically determined, segregating as an autosomal dominant trait. However, the 17q21-qter locus containing the AE1 gene and the abnormal oxalate self-exchanger were not in linkage in 2 three-generation families with renal stones [25]. Therefore, most likely the erythrocyte oxalate self-exchanger is an intermediate phenotype, possibly with polygenic determination.

Recently, the physiological role of the anion exchanger SLC26A6 expressed in the apical membrane of mouse proximal tubule and intestinal epithelium has been investigated [29]. It is capable of mediating the exchange of a number of anions: Cl^- /formate, Cl^-/OH^- , $\text{Cl}^-/\text{HCO}_3^-$, and also $\text{Cl}^-/\text{oxalate}$. Interestingly, knockout mice lacking SLC26A6 develop hyperoxaluria, hyperoxalemia and calcium oxalate renal stones. According to *in vitro* studies, animals lacking SLC26A6 have a defective intestinal secretion of oxalate resulting in enhanced oxalate absorption. This animal model represents a condition of primary intestinal hyperoxaluria, of which the existence in humans is uncertain. Hence, whether such an exchanger (and in particular variants of it) plays a role in human calcium oxalate nephrolithiasis is not known.

TABLE 1. Other Candidate Genes.

Candidate Gene	Abnormality	Disorder	Phenotype Relevant to Stones	Notes
$\text{Na}^+\text{-K}^+\text{-2Cl}^-$ cotransporter (NKCC2) (15q15-q21.1)	Trans epithelial NaCl transport across the medullary thick limb of the Henle's loop	Bartter's syndrome type 1	Hypercalciuria, nephrocalcinosis	No investigation in stone patients and families
Luminal potassium channel, (ROMK) (1p36)	Trans epithelial NaCl transport across the medullary thick limb of the Henle's loop	Bartter's syndrome type 2	Hypercalciuria, nephrocalcinosis	No investigation in stone patients and families
Claudin16/Paracellin-1 (3q27)	Tight junctions of the Henle's thick ascending limb, magnesium-calcium reabsorption	Familial hypomagnesaemia with hypercalciuria and nephrocalcinosis	Hypercalciuria, nephrocalcinosis, incomplete dRTA, hypocitraturia	No investigation in stone patients and families
Sodium-phosphate transported tp. 2a (NPT2a) (5q35)	Proximal phosphate reabsorption	Hypophosphatemic nephrolithiasis and osteoporosis	Nephrolithiasis	Only 2 patients have been described. No investigation in stone patients and families [33]
Epithelial Ca^{2+} channel 1 (ECaC1) (7q35)	Renal and small intestine apical calcium entry	None described	None described	No mutation found in 9 hypercalciuric families [31]

Soluble Adenylyl Cyclase

Intracellular cAMP plays a major role in cell biology because of the modulating activity of a number of activities, including gene transcription. Many different adenylyl cyclase (AC) isoforms have been recognized, but one, the ubiquitously expressed soluble adenylate cyclase (sAC), is particularly interesting in reference to renal stones.

Reed et al [30] have reported linkage of absorptive hypercalciuria to chromosome 1q23-24 in 3 families with familial absorptive hypercalciuric and low vertebral mineral density. Although this region is rather broad, they hypothesized that the sAC gene mutations play a pathogenetic role in this syndrome. Actually sAC gene mutations/variants were more frequently observed in stone forming patients with absorptive hypercalciuria than in controls. Two studies have addressed the role of a gene belonging to the same locus or of the sAC gene with contradictory results. The 1q23-24 locus was not associated with IHC in 9 European hypercalciuric families [31]. On the contrary, the association between polymorphism 923C>T of the sAC and hypercalciuria was reported in ICN Italian patients [32].

Since none of the evidence given by Reed et al [30] irrefutably demonstrates that the sAC gene plays a role in absorptive hypercalciuria, whether sAC has a role in ICN is still an open question.

CONCLUSIONS

While research on the role of most of the candidate genes in the common ICN and IHC has produced negative or only marginal results so far, the recent results obtained in Italy from a very large population of ICN patients suggest a special influence of CaSR in the risk (and possibly in the pathogenesis) of renal stones.

Discovering that the CaSR gene or other genes are involved in IHC and ICN may lead to the discovery of new pharmacological targets or tools for diagnosing the risk of ICN, and last, but not least, to advancement in the understanding of the pathogenesis of ICN and IHC.

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