The Role of Genetics in Calcium Stone Formation

Dr. Benjamin K. Canales
Gainesville, Florida

Familial and twin studies attribute about 50% of patient stone heritability to genetic makeup. However, other than rare hereditary disorders of the kidney (fig. 1), most of my residents are hard-pressed to name a single stone gene that may contribute to idiopathic calcium stone formation. Why is this?

Hypercalciuria and stone disease are complex, polygenic traits that do not fit into classical Mendelian categories and, honestly, this literature is hard to read. In addition, many older genetic studies of stone disease grouped patients generally as stone formers or nonstone formers. Laboratory values, such as urine calcium and stone mineral analysis, and clinical risk factors, such as dehydration and medical comorbidity, were often missing or assumed, leading to a heterogeneous mix of stone formers and, therefore, to confusing results.

In the last decade the quality of genetic studies in stone formers has improved. Now 4 genes have climbed to the top of the stone susceptibility list. With health care technology marching us toward personalized medicine and genomics, it is likely that these candidate genes will have a larger role in the clinical management of stone disease from screening to recurrence prediction and perhaps even gene therapy. What are these genes and what are their putative roles in stone disease?

Calcium-Sensing Receptor

The calcium-sensing receptor (CaSR) is a 1,078 amino acid, G protein-coupled, cell membrane dimer that is predominantly expressed in the parathyroid gland (regulating parathyroid hormone secretion) and distal renal tubules (affecting calcium reabsorption with other secondary effects). Rare autosomal mutations in this gene have long been known to cause hypercalcemia and hyperparathyroidism (loss of function) or hypocalcemia and hypercalciuria (gain of function).

Four genetic association studies, including 2 in idiopathic and 2 in hypercalciuric stone formers, with appropriate controls identified a gain of function missense mutation (Arg^{990}→Gly) on a minor allele in exon 7. Another *CaSR* single nucleotide polymorphism (SNP), intron rs17251221, was identified in a cohort of 189 Taiwanese calcium stone formers compared to 291 controls and linked to stone multiplicity and stone recurrence.[1] Additional SNPs at the 2 regulatory promoter regions of *CaSR* are linked to decreased promoter activity and stones in patients with normal citrate excretion.[2]

Unfortunately, confirmatory linkage analysis between *CaSR* and phenotype stones or hypercalciuria in a large group of brothers was negative, meaning that other factors likely contribute to the stone phenotype in patients with these mutations (fig. 1).

Vitamin D Receptors

Vitamin D receptors (VDRs) are steroid hormone receptors located in the nucleus of almost every cell in the body. When activated by 1,25-dihydroxyvitamin D3 (calcitriol), VDRs can modulate the expression of more than 1,000 genes, impacting processes from calcium absorption in the gut to cellular immunity. When one

▼ Continued on page 24

Genetics and Calcium Stone Formation

▼ Continued from page 23

considers that about 30% of patients with hypercalciuria have increased calcitriol levels, it is extremely plausible that stone formers with increased urinary calcium have some sort of altered response to activated vitamin D.

In the last 15 years more than 25 studies have been published examining the genetic relationship between the *VDR* gene and bone disease, stone formation and hypercalciuria but results remain controversial. The best evidence in stone formers comes not from SNPs in actual coding sequences but from variations in the *VDR* 3' untranslated region (3' UTR). Recall that mRNA, the carrier of the final DNA message out of the nucleus, is highly unstable and requires chaperone proteins to bind it, typically in the 3' UTR region. If these proteins are unable to bind or bind incorrectly (fig. 2), mRNA becomes unstable and less (or no) protein is made.

Since 1994, restriction enzymes (a simpler and less expensive way than sequencing to genotype DNA) have been used to identify 3 SNPs at the 3' UTR region and 1 SNP at the translation start codon. These SNPs were recently confirmed by polymerase chain reaction based restriction analysis in a case-control series of more than 900 patients.[3] Although their exact function is unknown, these *VDR* variations in vitro appear to increase tubular reabsorption of citrate and may enhance stone disease severity through hypocitraturia.

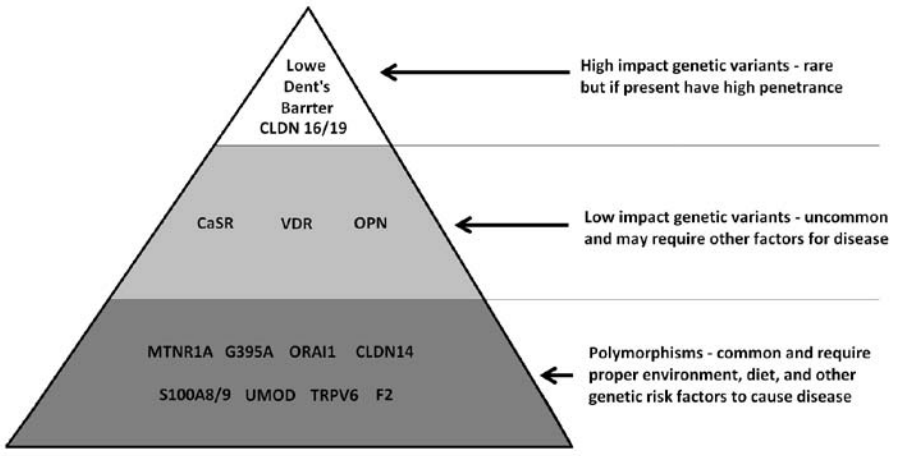

Figure 1. Spectrum of hereditary stone disease. High impact variants include renal tubule mutations affecting ion transport (Bartter's syndrome and Dent's disease) or tight junctions (Lowe syndrome, and *CLDN16* and *19*). Low impact variants in *CaSR*, *OPN* or *VDR* genes are associated with stones and hypercalciuria, and may involve altered gene expression or translation. Common polymorphisms were reported by many groups and require further study, including melatonin (*MTNR1A*), KLOTHO (*G395A*), calcium channel subunit (ORAI1), CLDN14, calgranulin family (*S100A8/9*), uromodulin (*UMOD*), transient calcium receptor (*TRPV6*) and thrombin (*F2*).

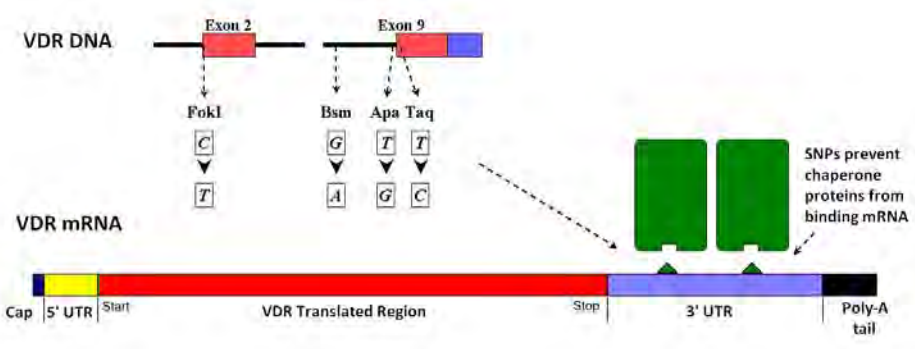

Figure 2. VDR mRNA map. SNPs (C→T, G→A etc) in VDR mRNA 3' UTR were identified through restriction mapping with enzymes FokI, BsmI, ApaI and TaqI. These SNPs may change binding sites in 3' UTR region, preventing chaperone protein binding and leading to unstable, untranslatable VDR protein.

Osteopontin

Osteopontin (OPN) is a highly phosphorylated secretory protein expressed in bone, kidney and lymphocytes. In the kidney OPN is upregulated during times of renal stress or injury and it typically acts to inhibit urinary crystal formation. More than 20 SNPs have been reported in the *OPN* gene in stone formers. Most promising for calcium stone formers are the SNPs –144*G/T*, –145*T/G* and –156*G/T*, located in the gene promoter positions. Three studies with appropriate controls have now shown that patients with these SNPs are at higher calcium stone risk.[4]

Because these 3 SNPs are clustered near each other, it is possible that these loci are associated (termed linkage dysequilibrium) and affect stone disease by lowering the amount of OPN produced by affected individuals.

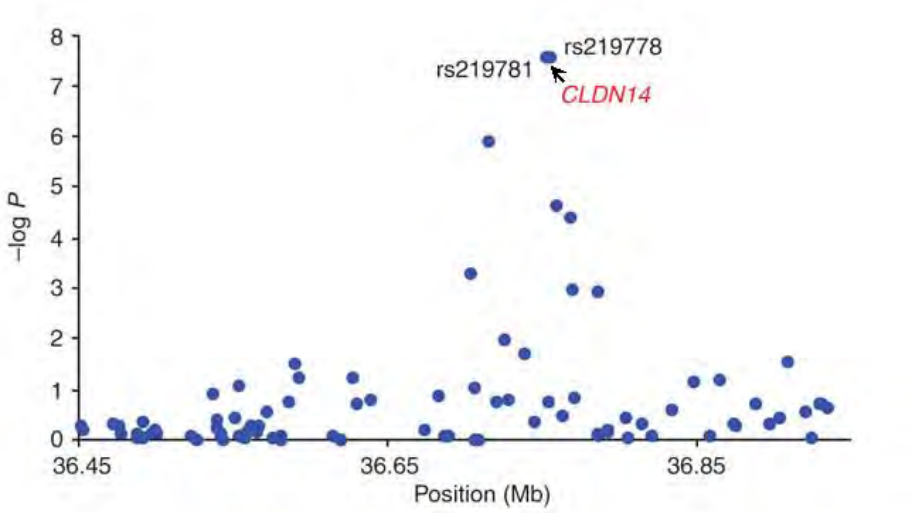

Figure 3. Regional association plot from chromosome 21 q22 section from more than 45,000 Icelandic and Dutch patients in GWAS. Blue circles represent all markers tested. Higher –log P location (y axis) indicates higher association with kidney stone phenotype. Two blue circles indicate *CLDN14* with high –log P, and association with kidney stones and decreased bone mineral density. Reprinted with permission.[5]

Claudin 14

Claudin (CLDN) 14 is a transmembrane tight junction protein in the CLDN family that regulates distal renal tubular cell polarity and water permeability. In 2009 several SNP variants of the *CLDN14* gene were found to be associated with radiopaque kidney stones, higher urinary calcium excretion, decreased bone mineral density in females and lower total plasma CO_2 in a large genome wide association study (GWAS) (fig. 3).[5] This GWAS, the largest of its kind in nephrolithiasis, used a high throughput search of whole genomic DNA from 3,773 stone formers and 42,510 control patients of Icelandic or Dutch descent. Although the exact role of these *CLDN14* SNPs in stone formation is unknown, gene mutations in its close relatives *CLDN16* and *CLDN19* can cause familial hypomagnesemia, hypercalciuria and nephrocalcinosis (fig. 1).

Conclusions

The future of personalized health care lies in being able to predict the disease susceptibility and response to treatment of an individual. Although we are far from this in nephrolithiasis, the genes *CaSR*, *VDR*, *OPN* and *CLDN14* may someday be used in the clinical setting to better stratify patient risk and guide preventive stone therapy. For this to become a reality, high quality studies using modern technology, such as GWASs, must be combined with careful, standardized patient phenotyping, as determined by the experienced clinical urologist. ◆

1. Chou YH, Woon PY, Chen WC et al: A genetic polymorphism (rs17251221) in the calcium-sensing receptor gene (CASR) is associated with stone multiplicity in calcium nephrolithiasis. PLoS One 2011; **6**: e25227.
2. Vezzoli G, Terranegra A, Arcidiacono T et al: Calcium kidney stones are associated with a haplotype of the calcium-sensing receptor gene regulatory region. Nephrol Dial Transplant 2010; **25**: 2245.
3. Wang S, Wang X, Wu J et al: Association of vitamin D receptor gene polymorphism and calcium urolithiasis in the Chinese Han population. Urol Res 2012; **40**: 277.
4. Liu CC, Huang SP, Tsai LY et al: The impact of osteopontin promoter polymorphisms on the risk of calcium urolithiasis. Clin Chim Acta 2010; **411**: 739.
5. Thorleifsson G, Holm H, Edvardsson V et al: Sequence variants in the CLDN14 gene associate with kidney stones and bone mineral density. Nat Genet 2009; **41**: 926.