

Idiopathic Calcium Nephrolithiasis: A Review of Pathogenic Mechanisms in the Light of Genetic Studies

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Key Words

Calcium kidney stones · Calcium excretion · Phosphate excretion · Genetic polymorphism · Randall's plaque

Abstract

Background: Calcium nephrolithiasis is a multifactorial disease with a polygenic milieu. Association studies identified genetic polymorphisms potentially implicated in the pathogenesis of calcium nephrolithiasis. The present article reviews the mechanisms of calcium stone formation and the potential contribution of gene polymorphisms to lithogenic mechanisms. **Summary:** Endoscopy observations suggested that precipitation of calcium-oxalate on the Randall's plaque at the papilla surface may cause idiopathic calcium-oxalate stones. The Randall's plaque is a hydroxyapatite deposit in the interstitium of the kidney medulla, which resembles a soft tissue calcification. Conversely, calcium-phosphate stones may develop from crystalline deposits located at the tip of the Bellini duct. Polymorphisms of eleven genes have been associated with stones in genome-wide association studies and replicated candidate-gene association studies: *VDR*, *SLC34A1*, *SLC34A4*, *CLDN14*, and *CaSR* genes coding for proteins regulating tubular phosphate and calcium reabsorption; *CaSR*, *MGP*, *OPN*, *PLAU*, and *UMOD* genes cod-

ing for proteins preventing calcium salt precipitation; *AQP1* gene coding for a water channel in the proximal tubule. The renal activity of the last gene, *DGKH*, is unknown. Polymorphisms in these genes may predispose to calcium-oxalate and -phosphate stones by increasing the risk of calcium-phosphate precipitation in the tubular fluid. **Key Messages:** Genetic findings suggest that tubular fluid supersaturation with respect to calcium and phosphate predisposes to calcium-oxalate stones by triggering cellular mechanisms that lead to the Randall's plaque formation.

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Introduction

Nephrolithiasis is one of the most frequent disorders in western countries. Its prevalence is between 5 and 10% and calcium nephrolithiasis represents 70–85% of cases [1]. Studies in families and twin pairs showed that this disease is inherited with a non-mendelian transmission pattern involving multiple genes that explain a half of stone determinants [2, 3].

Polymorphisms (SNPs) of eleven genes were associated with calcium kidney stones in case-control studies. Five genes were recognized in replicated studies of candi-

Table 1. Genes and SNPs associated to calcium nephrolithiasis identified in studies then replicated in different populations

Gene	Coded protein	Locus	Origin	Cases/controls, n/n	SNP	Ref.
CaSR	calcium-sensing receptor	3q21.1	Italy	167/214	rs6776158 in the promoter 1 rs7652589 in 5'-UTR rs1501899 in intron 1	[26] [27] [28]
VDR	vitamin D receptor	12q12-14	Italy	110/127	rs10735810 of start codon rs1544410 and rs7975232 in intron 8	[43]
			India	138/166	rs731236 in exon 9	[44]
spp1	osteopontin	4q22.1	Japan	45/54	-145 and -144 in promoter	[55]
			Japan	249/247	-156 in promoter	[56]
MGP	matrix-gla protein	12p12.3	Japan	122/125	rs4236 in the exon 4	[63]
			China	374/354		[64]
PLAU	urokinase	10q24	Taiwan	153/105	rs4065 in 3'-UTR	[58]
			Turkey	80/40		[59]

Table 2. Genes associated with calcium nephrolithiasis identified by GWASs

Gene	Protein	Locus	Origin	Cases/controls, n/n	Markers, n	Associated SNPs	Ref.
SLC34A3	Phosphate carrier NPT2c	9q33.2-34.2	Spain	10/8 in the same family	380	rs111875450	[39]
CLDN14	Claudin-14	21q22.3	Iceland, The Netherlands	3,773/42,510 in 3 steps	303,120	rs219781 rs219778 rs219780 rs219779	[19]
SLC34A1	Phosphate carrier NPT2a	5q35.3	Japan	5,892/17,809 in 3 steps	712,726	rs11746443	[34]
AQP1	Aquaporin 1	7p14.3	Japan	5,892/17,809 in 3 steps	712,726	rs1000597	[34]
DGKH	Diacyl glycerol kinase	13q14.1	Japan	5,892/17,809 in 3 steps	712,726	rs4142110	[34]
UMOD	Uromodulin	16p12.3	Iceland, The Netherlands	3,617/43,201 in 3 steps	302,379	rs4293393	[51]

date-genes (table 1). The other six loci were identified in genome-wide association studies (GWASs) mapping the whole genome (table 2). All these genes are expressed in the kidney.

Current pathogenetic hypotheses explain idiopathic calcium-phosphate and -oxalate stones with two different pathways [4]. Calcium-phosphate stone may develop from crystal aggregates deposited at the tip of the Bellini duct [4, 5]. Calcium-oxalate stones may result from urinary calcium-oxalate precipitation on the Randall's plaque, which is a hydroxyapatite deposit in the interstitium of the kidney medulla [4, 5].

The present article reviews current pathogenetic hypotheses explaining idiopathic calcium kidney stones. It also reviews SNPs associated with stones in GWASs or

replicated candidate-gene studies and considers the effect of their alleles on the kidney function and the mechanism by which they could contribute to lithogenesis and predispose individuals to calcium stones.

Lithogenic Mechanisms: The Randall's Plaque

Endoscopy and histology findings indicate that calcium-oxalate stones develop from calcium-oxalate precipitation on portions of the Randall's plaque located at the papilla surface and exposed to the urine flux because of urothelial erosion [4, 5]. Excessive hydroxyapatite accumulation in the interstitium could be responsible for the urothelial damage [4, 6]. Therefore, calcium-oxalate stones

may grow attached to the papilla in the urinary tract as a result of spontaneous calcium-oxalate precipitation on the organic matrix that covers the Randall's plaque [4].

Transmission electron microscopy showed that interstitial deposits of hydroxyapatite consist of multiple spherules with maximal diameter of 10 μm . Their morphology alternated light and electron dense layers formed by organic or mineral matrix respectively [6]. The organic matrix of these spherules included osteopontin [4, 6]. The Randall's plaque area at the papillary surface was positively correlated with calcium excretion and stone rate [7].

Histological observations led to the hypothesis that the Randall's plaque arises from the basal membrane of the thin limb of the Henle's loop [4, 6]. An alternative hypothesis suggested that the Randall's plaque develops from perivascular calcifications of atherosclerotic vasa recta walls [9, 10]. Indeed, morphologic features of the Randall's plaque recall the soft tissue calcification, which requires the osteogenic transformation of local cells for its formation [11]. The osteogenic transformation was mainly studied in vascular smooth muscle cells (VSMC) that were enabled to synthesize bone proteins when cultured in the presence of high concentrations of calcium and/or phosphate [11, 12]. Also calcium-phosphate nanocrystals (diameter of 30–500 nm) were shown to trigger this process [13]. Conversely, agonists of CaSR and VDR prevented hydroxyapatite deposition by rat and human VSMCs [11, 14, 15]. The achievement of an osteogenic phenotype by kidney cells was hypothesized in genetically hypercalciuric stone forming rats (GHS). The kidney of these rats had higher gene and protein expression levels of osteopontin, bone morphogenetic protein 2, and osteoblast transcription factors (Runx2 and os-terix) than the kidney of control rats [16].

Lithogenic Mechanisms: The Intratubular Precipitation

Calcium-phosphate stones may develop from spontaneous calcium-phosphate precipitation in the tubular lumen and crystal deposition within the Bellini duct [7]. Crystal aggregates may block and dilate the Bellini duct, thus giving rise to an intratubular nidus from which a calcium-phosphate stone may grow protruding in the urine tract. Tubular fluid supersaturation with respect to calcium and phosphate is the driving force of this lithogenic mechanism and the alkaline pH of the Henle loop fluid may be crucial to decrease the calcium-phosphate

solubility [5]. Tubular cells may internalize crystals and either break them down in lysosomes or deposit them in the interstitium, where local inflammatory reaction may arise [5, 17]. A deficient inhibition of crystal growth by osteopontin, uromodulin, matrix-gla-protein, and other urine macromolecules may contribute to this mechanism, although in particular conditions they may promote crystal growth [5, 18].

Genes and Lithogenesis

Tables 1 and 2 display genes associated with idiopathic calcium stones in GWASs and replicated candidate-gene studies in European and Asian populations. GWASs selected stone formers without considering stone composition. Candidate-gene studies selected calcium stone formers, but did not consider the differences in calcium stone composition. Due to the structure of these studies, the selection bias cannot be avoided, but our knowledge about stone composition frequency suggests that findings in these studies are supported by patients suffering from pure or mixed calcium-oxalate stones [1, 5].

The CLDN14 Gene

The *CLDN14* gene (21q22.13) was associated with stones in a GWAS including 3,773 stone formers and 42,510 controls from Iceland and the Netherlands. Stones were associated with ancestral alleles at two SNPs located in non-coding regions and two synonymous SNPs, located in the last of the three gene exons. These alleles were also associated with higher calcium excretion and with lower total serum CO_2 and bone mineral density [19]. Therefore, it was hypothesized that *CLDN14* gene alleles having a positive effect on *CLDN14* gene transcription or mRNA processing may predispose to calcium stones.

CLDN14 gene encodes for claudin-14, a 239 amino acid protein [20] expressed in the tight junctions of the thick ascending limb of Henle's loop (TALH), the Corti organ of the inner ear and the liver [21]. Different experiments in lab animals showed that claudin-14 inhibited the paracellular reabsorption of calcium and magnesium in the TALH by closing the channel formed by claudin-16 and -19 in tight junctions (fig. 1) [22, 23]. High dietary calcium intake, high serum calcium, $1,25(\text{OH})_2\text{D}$ administration, and calcimimetic treatment were found to up-regulate claudin-14 expression in the TALH in different mouse strains. This suggested that claudin-14 expression

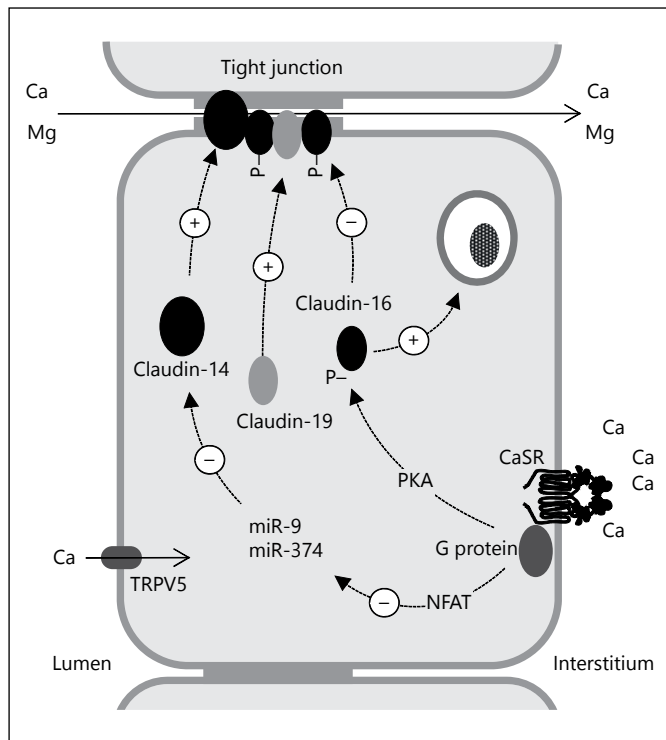


Fig. 1. In TALH cells, claudin-16, -19 and -14 form the tight junction channel for the paracellular passive reabsorption of calcium and magnesium. Claudin 14 inhibits the channel permeability and its expression is enhanced by CaSR through a transcriptional mechanism mediated by the nuclear factor of activated T cell (NFAT) that inhibits the production of two microRNAs, miR-9, and miR-374. CaSR also inhibits claudin-16 phosphorylation and localization in tight junction channels by activating the protein kinase A (PKA). The carrier for calcium ions through the apical cell membrane is the TRPV5 channel. CaSR activity is mediated by G proteins.

in the TALH was under the control of the extracellular calcium through the calcium-sensing receptor (CaSR) [22, 24]. In mice, CaSR activation upregulated claudin-14 expression through a histone deacetylation mechanism mediated by the nuclear factor of activated T cells (NFAT) that inhibited the production of two microRNAs, miR-9, and miR-374, modulating the *CLDN14* transcription [22, 25]. Claudin-14 may thus regulate calcium reabsorption in response to calcium intake.

The CaSR Gene

SNPs of the regulatory region of the calcium-sensing receptor (*CaSR*) gene (3q21.1) were associated with idiopathic calcium stones in two Italian studies [26, 27]. Another study mapping the *CaSR* gene regulatory region ob-

served the association of a SNP (rs6776158) sited within the promoter 1 with calcium stones. The minor allele at this SNP was shown to reduce the promoter 1 transcriptional activity in two renal cell models [28].

The *CaSR* gene (3q13.3–21) codes for a 1,078 amino acid membrane protein ubiquitously expressed as a homodimer. Its molecule includes a large extracellular domain that binds extracellular calcium ions, a seven-membrane spanning domain and a C-terminal intracellular domain interacting with G proteins and filamin-A in order to regulate cell functions [29]. CaSR has its largest renal expression in TALH, where it inhibits paracellular calcium reabsorption by enhancing claudin-14 expression and reducing claudin-16 expression (fig. 1). In the TALH, CaSR also decreases the sodium-potassium-chloride cotransport (NKCC2) activity that sustains the electric gradient driving the paracellular calcium reabsorption [24]. CaSR also inhibits active calcium reabsorption in cortical convoluted tubules, as shown in canine tubular cells [30]. In addition to calcium reabsorption, experiments in lab animals showed that CaSR promoted water excretion and urine acidification in the collecting duct through the inhibition of the tubular response to ADH and the stimulation of proton pump activity, respectively [31, 32]. In the proximal tubule, CaSR antagonizes phosphate reabsorption and promotes proton secretion through the NHE3 sodium-hydrogen countertransport [33]. Therefore, CaSR activity in the proximal tubule and collecting duct may counterbalance the risk of calcium-phosphate precipitation induced by its effect on calcium reabsorption in the TALH and cortical convoluted tubules. A decreased CaSR expression in the kidney might therefore enhance the risk of stone formation by causing an imbalance among its tubular effects.

SLC34A1 and AQP1 Genes

Three loci, 5q35.3, 7p14.3, and 13q14.1, were associated with calcium nephrolithiasis in a Japanese GWAS including 5,892 cases and 17,809 controls [34]. Three genes at these loci are candidates to explain these associations.

DGKH gene is located at 13q14.1 and encodes for the diacylglycerol kinase, a ubiquitously expressed kinase having unknown functions in the kidney.

The *SLC34A1*, located at 5q35.3, codes for the phosphate carrier NPT2a, responsible of the 85% of phosphate reabsorption in the proximal tubule [35]. Knockout-mice for NPT2a developed renal phosphate loss and hypo-

phosphatemia that led to 1,25(OH)D hyperproduction and hypercalciuria. Apatite stones were found in the kidney of these mice [36]. *SLC34A1* gene SNPs causing a loss of the NPT2a function were detected in hypophosphatemic hypercalciuric patients with osteoporosis or kidney stones and could explain patient phenotype with the same mechanism of knockout-mice [37, 38]. Interestingly, also the *SLC34A3* gene (9q33.2–34.2), which codes for the phosphate carrier NPT2c responsible for the 15% of the proximal phosphate reabsorption, was identified as a quantitative trait locus for calcium nephrolithiasis in a small study mapping the whole genome in members of a Spanish family [39].

AQP1, located at 7p14.3, codes for the water channels AQP1 that supports the transcellular water reabsorption in the proximal tubule [40]. Knockout-mice for *AQP1* did not produce stones, but developed dehydration after water deprivation [41]. The *AQP1* gene SNPs could impair water reabsorption in proximal tubules and thus stimulate distal mechanisms of urine concentration.

The VDR Gene

The association of *VDR* gene (12q12–14) with nephrolithiasis was evidenced in a linkage study in 359 French-Canadian sib-pairs [42] and case-control studies in European and Asian populations. Three *VDR* gene SNPs were identified at the 3'-untranslated end region (UTR) using *BsmI* (*B/b* alleles), *ApaI* (*A/a*), *TaqI* (*T/t*) restriction enzymes. The first two SNPs were within the intron 8; the third was within the exon 9 and was synonymous. The translation start codon SNP was identified using the *FokI* (*F/f*) restriction enzyme [43, 44]. The in-vitro functional study of the 3'-UTR SNPs observed that the *baT* haplotype diminished *VDR* gene expression [45]. Unexpectedly, alleles decreasing the *VDR* expression (*b* and *T*) or activity (*f*) were associated with stone risk [43, 46, 47]. Homozygous patients for the *bT* haplotype showed earlier age of onset, higher stone rate, and lower urinary citrate excretion [43]. Experiments in genetically hypercalciuric stone-forming rats (GHS) showed that selective silencing of the *VDR* gene in the kidney increased calcium excretion and upregulated the expression of the membrane calcium channels, TRPV5 and TRPV6 [48, 49]. *TRPV6* gene SNPs (7q34) were associated with stones in a non-replicated study [50]. In addition to *TRPV5/6* genes, the *VDR*-vitamin D complex may modulate the expression of *CaSR*, *SLC34A1*, *SLC34A3*, *OPN*, and *MGP* [43].

UMOD, MGP, OPN, and PLAUGenes

A SNP next to *UMOD* gene (16p12.3) was associated with nephrolithiasis in a GWAS in Icelandic and Dutch stone-formers [51]. *UMOD* gene codes for uromodulin, an inhibitor of calcium-phosphate precipitation. It is mainly synthesized in TALH where it is anchored to plasma membrane glycosylphosphatidylinositols and secreted into the urine after cleavage of its C terminus [52]. In cultured embryonic renal cells cotransfected with *UMOD* and *TRPV5* genes, uromodulin impaired caveolin-mediated endocytosis. Through this mechanism it could up-regulate the expression of the TRPV5 calcium channels and NKCC2 sodium carrier in the apical membrane of distal tubular cells. As a result, it could enhance calcium reabsorption [53, 54].

Two SNPs of the *SSP1* gene (4q22.1) coding for osteopontin were associated with stones in stone formers of Japan origin. Both SNPs are located in the gene promoter and may modify gene expression [55]. Another study in a Japanese sample associated stones with SNP sited at -156 (delG/G) in the promoter region [56]. Osteopontin belongs to the family of secreted acidic proteins and binds calcium ions with its negatively charged amino acids. As a consequence, it is a natural inhibitor of soft tissue calcification in humans [57] that may be detected in the organic matrix of calcium stones and hydroxyapatite spherules of the Randall's plaque [4, 5].

The SNP rs4065 (C>T), located in the 3'-UTR of the *PLAU* gene (10q24), was associated with calcium nephrolithiasis in two studies performed in Taiwan and Turkey [58, 59]. *PLAU* gene codes for urokinase, a proteolytic enzyme of the serine protease superfamily that cleaves plasminogen to plasmin and stimulates fibrinolysis. In the kidney it may prevent stone formation by cleaving stone matrix proteins [60]. It is likely that the minor allele at rs4065 decreases *PLAU* gene mRNA stability and expression, causing a decrease of urokinase levels in urine [61].

The *MGP* gene (12p13.1–p12.3) codes for matrix-gla-protein, a circulating protein containing five vitamin K-dependent carboxylated residues. It inhibits nucleation and growth of hydroxyapatite and calcium-oxalate crystals and may prevent soft tissue calcification and kidney stone formation [62]. Two studies in Japanese and Chinese calcium stone formers found out that an SNP in the exon 4 (rs4236) halved the stone risk [63, 64]. Matrix-gla-protein production is stimulated by CaSR-activating drugs and exposure of tubular cells to calcium-oxalate [65, 66].

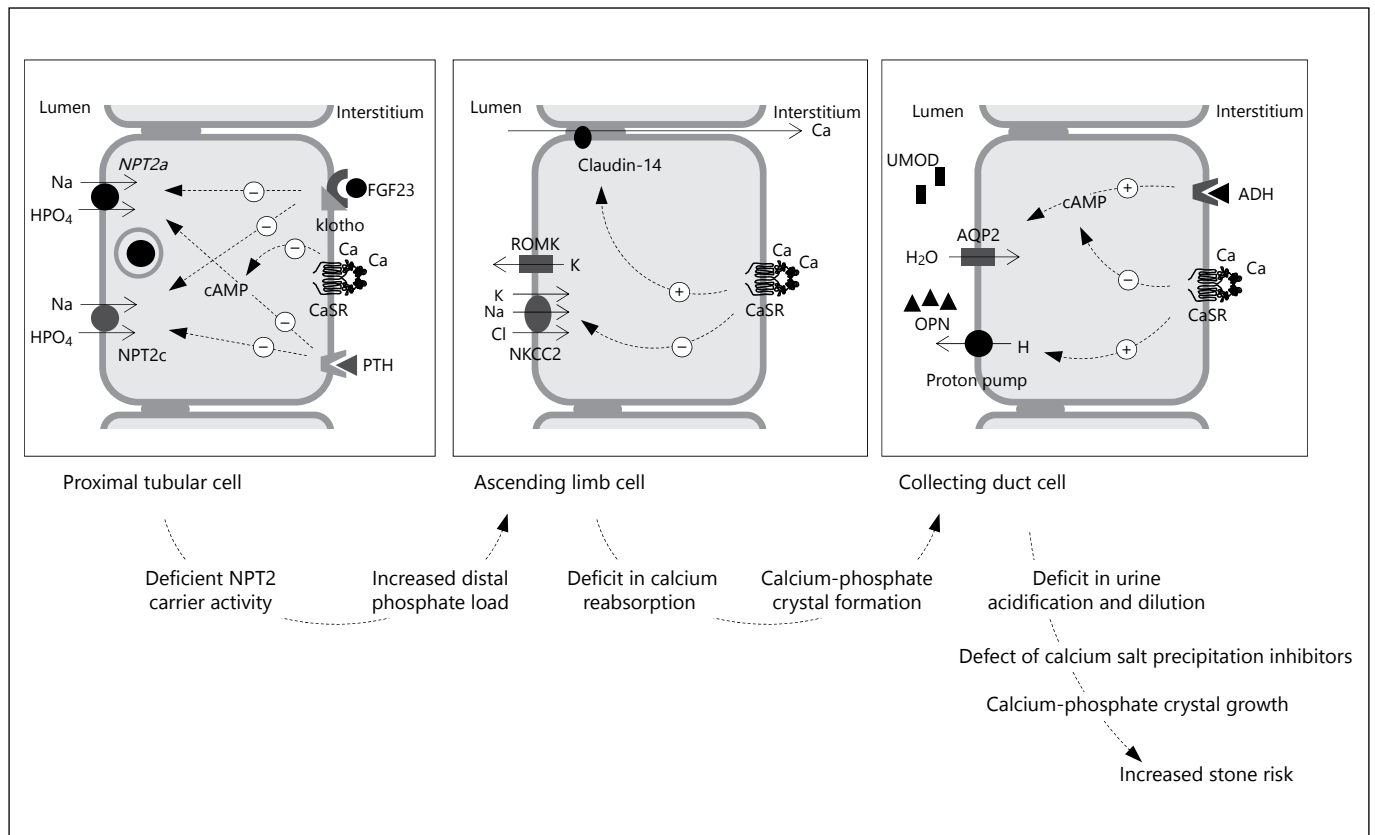


Fig. 2. Genetic studies suggest that stone risk is enhanced by the combination of different modification of tubular functions. In the proximal tubule, a deficient activity of phosphate sodium-phosphate cotransporters (NPT2a and NPT2c) may increase phosphate release to the distal tubule. These carriers are inhibited by fibroblast-growth factor 23 (FGF23) and parathyroid hormone (PTH). CaSR decreases the cAMP-mediated phosphaturic effect of PTH and exerts an antiphosphaturic effect. In the TALH, claudin-14 decreases the paracellular calcium reabsorption. CaSR up-regulates claudin-14 expression and inhibits sodium-potassium-

chloride cotransport (NKCC) activity that maintains the electric driving force for the calcium reabsorption. In the collecting duct, CaSR stimulates proton-pump activity and antagonizes the cAMP-mediated effect of the antidiuretic hormone (ADH) on the aquaporin-2 water channel (AQP2). A deficient CaSR expression may therefore decrease tubular cell capability to acidify and dilute urine that predispose to calcium-phosphate precipitation. A deficient activity of uromodulin (UMOD), osteopontin (OPN) and other precipitation inhibitors may contribute to calcium salt precipitation.

Genetic Polymorphisms and Lithogenic Mechanisms

Genes associated with calcium kidney stones encode for proteins implicated in the reabsorption of calcium, phosphate, and water and in the activity of precipitation inhibitors (fig. 2). Variant alleles at *SLC34A1/3* and *CaSR* genes may cause phosphate overload in the distal tubule, while *CLDN14* gene SNPs may cause calcium overload in the connecting duct. Variant alleles at *MGP*, *SSP1*, *PLAU*, *UMOD*, *CaSR*, and *VDR* genes may decrease the protection against calcium salt precipitation. *AQP1* gene variants may require urine concentration. It is noteworthy that no substantial associations have so far been reported among stones and SNPs at genes regulating oxalate or ci-

trate metabolism. Genetic findings, instead, indicate that SNPs influencing calcium and phosphate reabsorption were involved in calcium-oxalate stone risk. This leads to the conclusion that calcium-phosphate supersaturation in the tubular fluid may predispose to the production of calcium-oxalate stones.

Likely, the Randall's plaque is the factor linking calcium-phosphate supersaturation with calcium-oxalate precipitation in urine [4–8]. The Randall's plaque is a hydroxyapatite deposit in renal interstitium and may be considered a soft tissue calcification; this implies that its formation needs a cell-mediated mechanism [6–10]. The study of vascular calcification in lab animals or cultured VSMCs identified at least three stimuli that may trigger

cell osteogenic transformation in the kidney: high calcium and phosphate concentrations in the tubular fluid [11, 12]; a deficient expression of CaSR and VDR in tubular cells [11, 15]; and the presence of calcium-phosphate nanocrystals in the tubular fluid [13]. Genetic polymorphisms might amplify these osteogenic stimuli in the kidney and enable interstitial or tubular cells to achieve an osteogenic phenotype and deposit hydroxyapatite in the kidney interstitium. In this way SNPs associated with stones could predispose individuals to the formation of the Randall's plaque and calcium-oxalate stones [16].

Physical chemical causes may instead underlie calcium-phosphate stones. SNPs at genes associated with stones may predispose to the intratubular calcium-phosphate precipitation by increasing urine saturation with respect to calcium and phosphate. However, calcium-phosphate salts are more often mixed with calcium-oxalate in stones because of their precipitation on calcium-oxalate aggregates or Randall's plaque fragments joined to oxalate stones [5, 7].

In addition to genes, individual susceptibility to calcium stones may be influenced by dietary habits. Nutrient intake may change urine composition, but may also influence gene expression with epigenetic mechanisms [67]. Interestingly, the larger part of SNPs associated with stones may change gene expression and could consequently modify nutrient effect on gene expression. An example is the epigenetic activation of claudin-14 expression by calcium intake mediated by microRNAs [25], but other studies are needed to explore the interaction between genes and nutrients and to understand better gene involvement in kidney stone production.

In conclusion, available genetic findings emphasize the relevance of renal calcium and phosphate in calcium stone formation and support current pathogenetic hypotheses on calcium stone formation.

Disclosure Statement

The authors declare no conflict of interest.

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