Genetics of hypercalciuric stone forming diseases

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With a lifetime incidence of up to 12% in man and 6% in woman, nephrolithiasis is a major health problem worldwide. Approximately, 80% of kidney stones are composed of calcium and hypercalciuria is found in up to 40% of stone-formers. Although the mechanisms resulting in precipitation and growth of calcium crystals in the urinary tract are multiple and not fully understood, hypercalciuria *per se* is recognized as an important and reversible risk factor in stone formation. In this brief review, we summarize the studies assessing the heritability of hypercalciuria and pinpoint recently identified human genetic disorders as well as relevant animal models that provided new insights into the segment-specific tubular handling of calcium and the pathophysiology of renal hypercalciuria and kidney stones. We also discuss novel strategies that may help to unravel the genetic bases of such complex conditions.

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With a lifetime incidence of up to 12% in man and 6% in woman, a constant increase in industrialized countries and a recurrence rate close to 10% per annum, nephrolithiasis is a major health problem worldwide. Lifestyle factors, essentially dietary habits, play an important role in kidney stone formation, as recently reviewed in the Journal.[1] About 80% of kidney stones are composed of calcium (either oxalate or phosphate) and hypercalciuria is found in up to 40% of stone-formers.[2] Although the mechanisms resulting in precipitation and growth of calcium crystals in the urinary tract are multiple and not fully understood, hypercalciuria *per se* is recognized as an important and reversible risk factor in stone formation. Calcium homeostasis reflects the balance between absorption in the intestine, excretion by the kidney, and exchange from bone, the three processes being tightly regulated by endocrine mechanisms. Accordingly, the pathophysiology of hypercalciuria has been classified into (i) primary renal leak; (ii) imbalance between bone resorption and formation; and (iii) gut hyperabsorption. In turn, these primary defects trigger compensatory mechanisms primarily mediated by parathyroid hormone (PTH) and 1,25-dihydroxy-vitamin D3 ($1,25(OH)_2D_3$).[3]

A genetic control of hypercalciuria has been suspected for long, based on epidemiological studies which demonstrated that about half of patients with idiopathic hypercalciuria had a family history of nephrolithiasis, with more frequent stone disease in first-degree relatives (for review, Coe *et al.*[2] and Moe and Bonny[3]). This genetic influence is balanced by the risk profile due to environmental and/or dietary factors: familial aggregation is less prevalent in countries with a high-risk lifestyle (e.g. the United States) than in areas with a protective diet (Mediterranean countries). However, the influence of genetic factors appears to be far greater than diet-related risks.[4] Hypercalciuria is a quantitative trait that cannot be dissected with a single Mendelian approach. Interpretation of hypercalciuria with respect to disease must integrate a series of clinical parameters (such as urine volume or pH, and the excretion of lithogenic vs protective factors), the fact that it may be either the primary event or a compensatory manifestation, and the potential influence of modifier genes involved in multiple pathways (vitamin D pathway; oxalate metabolism; promoters or inhibitors of urine crystallization; bone and intestine calcium handling).[3]

One way to face the complexity of hypercalciuria as a risk factor is to consider the insights gained from the

identification of rare, monogenic diseases associated with hypercalciuria and/or kidney stones in man, and the investigations of animal models. In this brief review, we will summarize the studies assessing the heritability of hypercalciuria, and will discuss recently identified human genetic disorders and animal models relevant for kidney stones and hypercalciuria of renal origin. We will end in considering promising approaches to unravel the genetic bases of this condition.

HERITABILITY OF CALCIURIA

The heritability of urine calcium excretion, that is the proportion of its variation attributable to genetic factors, has been established by a series of family-based studies. The classical twin study is based on the fact that monozygotic (MZ) twins share identical genotypes, whereas dizygotic (DZ) twins share on average 50% of their segregating genes. If MZ twins show a greater resemblance for a specific trait than DZ twins, this is likely to be due to genetic factors. In such a study, performed in 1747 adult female twin pairs (539 MZ and 1208 DZ pairs) from the St Thomas' UK Adult Twin Registry, the intraclass correlation for urinary calcium excretion was higher in MZ twin pairs, and heritability for this parameter was 52%.[5] Using the same approach in 3391 twin pairs (1928 MZ and 1463 DZ pairs) from the VET Registry, Goldfarb *et al.*[6] showed that the proband concordance rate of kidney stones was significantly greater in MZ twins than DZ twins (32.4 vs 17.3%, $P<0.001$), and the heritability of the risk of stones was 56%.

The Bonnardeaux group in Montreal studied the segregation of hypercalciuria in 567 individuals from 221 French-Canadian nuclear families extracted from 154 pedigrees, some of which were four generations, with at least two siblings having a history of calcium stones.[7] They developed a computer program comparing the likelihood of three models of inheritance of calciuria: a single major gene (dominant, recessive, or codominant); several genes, each with a small effect; and a mixed major gene/polygenic model. The best results were obtained with the single-gene co-dominant and the mixed co-dominant/polygenic models: in both, the heritability attributable to the major gene was estimated to be 58%. Taken together, these studies suggest that more than 50% of the urinary calcium excretion rate is genetically determined and that a major gene may account for it, at least in some stone-formers families.

MONOGENIC DISORDERS LEADING TO HYPERCALCIURIA AND NEPHROLITHIASIS

Dent's disease

Dent's disease belongs to a group of X-linked disorders that are primarily associated with inactivating mutations in the *CLCN5* gene (Table 1).[8] Dent's disease is characterized by proximal tubule (PT) dysfunction and low-molecular-weight proteinuria, associated with hypercalciuria, nephrolithiasis, nephrocalcinosis, and progressive renal failure. Hypercalciuria and nephrocalcinosis are highly prevalent (95 and 75% of patients, respectively), but there is considerable inter- and intra-familial variability in the occurrence of nephrolithiasis (approximately half of the patients). Progression to end-stage renal failure occurs between the third and the fifth decades of life in 30–80% of affected males.[9] *CLCN5* is a member of the CLC gene family of chloride channels and transporters that have been identified by the laboratory of T Jentsch (ref [10] for review). *CLCN5* encodes ClC-5, an isoform that is predominantly expressed in the kidney, where it is located in the PT, the thick ascending limb (TAL) of Henle's loop, and the α-type intercalated cells of the collecting ducts (CD).[10,11] Around 100 families harboring *CLCN5* mutations have been identified thus far. These mutations abolish the currents induced by ClC-5 in heterologous expression systems.[8] Of note, mutations in the *OCRL1* gene encoding a phosphatidylinositol bisphosphate 5-phosphatase have recently been identified in a subset of patients with Dent's disease negative for *CLCN5* mutations.[12] Mutations in *OCRL1* have been previously associated with the oculo-cerebro-renal syndrome of Lowe (OCRL), an X-linked disorder characterized by bilateral congenital cataract, severe mental retardation, renal Fanconi syndrome, and, in some patients, hypercalciuria and nephrolithiasis (Table 1).

Studies in two strains of ClC-5-knockout (KO) mice provided insights into the link between PT dysfunction and hypercalciuria and nephrolithiasis in Dent's disease[13,14] (Figure 1). In PT cells, ClC-5 co-distributes with the vacuolar H^+-ATPase in the early endosomes, which are responsible for the reabsorption and processing of albumin and low-molecular-weight proteins that are filtered by the glomerulus. These vesicles belong to the receptor-mediated endocytic pathway, which involves the multiligand tandem receptors, megalin and cubilin, located at the apical brush border of PT cells.[15] Progression along the endocytic pathway depends on endosomal acidification, driven by the vacuolar H^+-ATPase and requiring a parallel chloride conductance for electroneutrality. Accordingly, the defect in PT endocytosis observed in Dent's disease patients and mice lacking ClC-5 is attributed to a reduced acidification, at least in some endosomes, secondary to the loss of the chloride permeability mediated by ClC-5.[16] The inactivation of ClC-5 is also associated with a severe trafficking defect in PT cells, with loss of megalin and cubilin at the brush border, subsequent loss of their ligands in the urine, and impaired lysosomal processing.[13,14,17] As the megalin/cubilin complex mediates the reabsorption of the vitamin-D-binding protein (DBP) and PTH that are filtered by the glomerulus,[15] the urinary loss of these mediators could potentially lead to opposite effects in PT cells, resulting in variable levels of active $1,25(OH)_2D_3$ levels in the serum.[16] Such variability could explain why renal hypercalciuria and kidney stones are present in one strain of ClC-5 KO mouse[14] but not in the other,[13] reflecting the phenotype variability observed in patients.[9] The potential role(s) of ClC-5 in the TAL (regulated calcium reabsorption) and in the intercalated cells (distal urinary acidification) remain(s) to be defined.[11] Of interest, no *CLCN5* mutations have been detected in patients

Table 1 | Monogenic human disorders associated with renal hypercalciuria and nephrolithiasis

Mode of inheritance disease	MIM	*Gene*, protein, function	Segment	Pathophysiology, renal manifestations	Extra-renal manifestations
X-linked					
Dent's disease (1) (X-linked recessive nephrolithiasis; X-linked hypophosphatemic rickets; idiopathic low-molecular-weight proteinuria)	300009	*CLCN5*, ClC-5 Cl^-/H^+ exchanger	PT, TAL, ICα	Trafficking defect, defective endocytosis PT dysfunction, renal Fanconi syndrome Nephrocalcinosis, stones Impaired urine acidification Renal failure	Rickets
Lowe's syndrome (oculo-cerebro-renal syndrome) Dent's disease (2)	309000 300555	*OCRL*, OCRL1 Phosphatidylinositol 4,5-bisphosphate-5-phosphatase	PT	PT dysfunction, renal Fanconi syndrome Proximal RTA, metabolic acidosis Nephrocalcinosis, stones	Mental retardation, cataract, rickets, cryptorchidism, neuromuscular and behavioral abnormalities, growth delay
Autosomal dominant					
Autosomal dominant hypocalcemia (ADH) (Bartter-like syndrome type V)	146200	*CASR*, CaSR Calcium-sensing receptor	PT, TAL, DCT, CD	Gain-of-function mutations Low serum PTH Hypocalcemia, hypercalciuria Nephrocalcinosis, stones (vitamin D) Salt-losing nephropathy, hypokalemia	Neuromuscular manifestations of hypocalcemia, seizures, basal ganglia calcifications
Distal renal tubular acidosis (dRTA, RTA type I)	179800	*SLC4A1*, AE1 Cl^-/HCO_3^- exchanger	ICα	Impaired anion exchange in ICα Metabolic acidosis, hypercalciuria Nephrocalcinosis, stones	Osteomalacia, rickets, growth retardation
Autosomal recessive					
Antenatal Bartter's syndrome type I (hyperprostaglandin E syndrome)	601678	*SLC12A1*, NKCC2 Na^+-K^+-$2Cl^-$ co-transporter 2	TAL	Profound salt-wasting, polyuria Metabolic alkalosis, hypokalemia Hyperprostaglandinuria Hypercalciuria, nephrocalcinosis	Polyhydramnios, prematurity, fever, vomiting, diarrhea, osteopenia
Antenatal Bartter's syndrome type II (hyperprostaglandin E syndrome 2)	241200	*KCNJ1*, ROMK K^+ channel	TAL, CD	Profound salt-wasting, polyuria Transient (neonatal) hyperkalemia Metabolic alkalosis, hypokalemia Hyperprostaglandinuria Hypercalciuria, nephrocalcinosis	Polyhydramnios, prematurity, Fever, vomiting, diarrhea, osteopenia
Bartter's syndrome type III (classic Bartter's syndrome)	607364	*CLCNKB*, ClC-Kb Cl^- channel	TAL, DCT	Highly variable phenotype Variable calciuria levels, stones	Variable (polyhydramnios)
Familial hypomagnesemia with hypercalciuria and nephrocalcinosis (FHHNC)	248250	*CLDN16 (PCLN1)*, Claudin-16, paracellin-1 Tight junction protein	TAL, DCT	Impaired paracellular transport of calcium and magnesium Calcium and magnesium wasting, renal failure, nephrocalcinosis, stones Incomplete distal tubular acidosis	Convulsions, tetany, chondrocalcinosis, neuromuscular manifestations
Distal renal tubular acidosis (dRTA with progressive nerve deafness)	267300	*ATP6V1B1*, V1 subunit B1 Subunit of V-ATPase	ICα	Impaired urine acidification Metabolic acidosis, kaliuresis Hypercalciuria, nephrocalcinosis, stones	Sensorineural hearing loss, hypokalemic paralysis, rickets, growth impairment
Distal renal tubular acidosis (dRTA with preserved hearing or late onset hearing loss)	602722	*ATPV0A4*, V0 subunit a4 Subunit of V-ATPase	ICα		Normal hearing or late onset sensorineural hearing loss, paralysis, rickets, growth impairment

CD, collecting duct; DCT, distal convoluted tubule; dRTA, distal renal tubular acidosis; ICα, intercalated cell (α-type); RTA, renal tubular acidosis; PT, proximal tubule; TAL, thick ascending limb of Henle's loop; V-ATPase, vacuolar H^+-ATPase.

with idiopathic hypercalciuria[4] and in the genetic hypercalciuric stone-forming (GHS) rat strain (see below).

Hereditary hypophosphatemic rickets with hypercalciuria

Hereditary hypophosphatemic rickets with hypercalciuria (HHRH) is an autosomal recessive disorder characterized by renal phosphate wasting, with hypophosphatemia and rickets, resulting in a compensatory increase in $1,25(OH)_2D_3$ levels favoring intestinal absorption of phosphate and calcium, and hypercalciuria. The combination of hypercalciuria and phosphaturia, similar to Dent's disease, is associated with nephrocalcinosis and kidney stones.[18] The

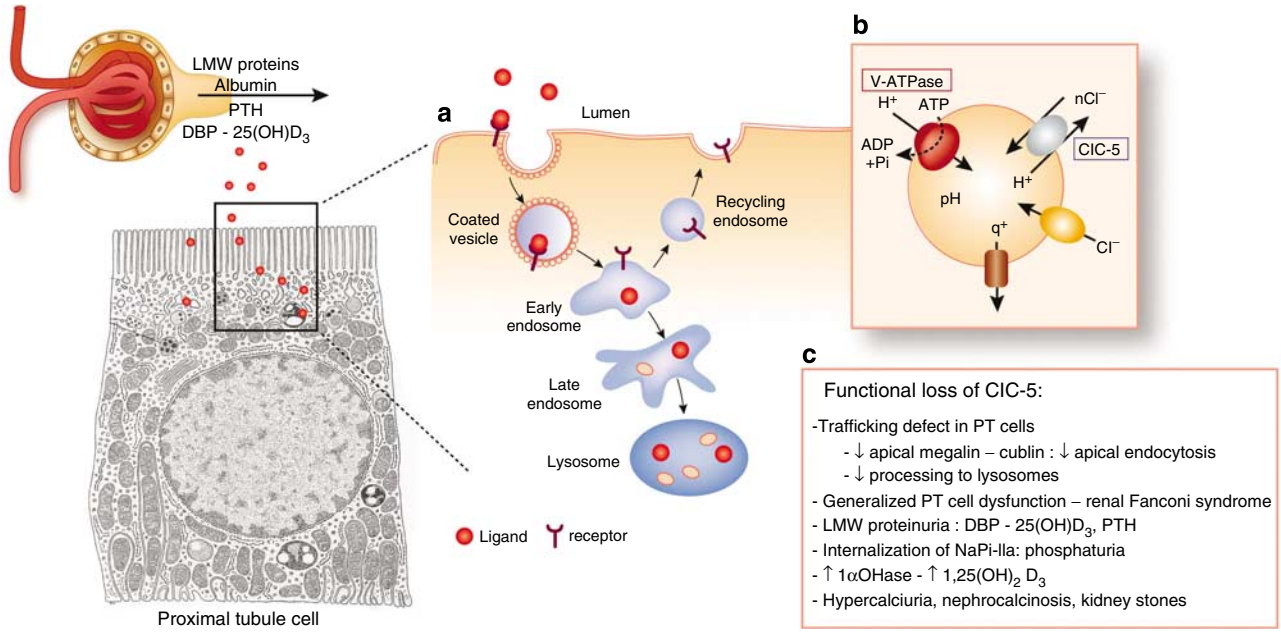

Figure 1 | Schematic model linking defective endocytosis in the PT and nephrolithiasis in Dent's disease. Albumin and low-molecular-weight proteins, including PTH and DBP with $25(OH)D_3$, are filtered into the primary urine and endocytosed by PT cells via the megalin-cubilin receptor pathway. Following internalization in coated vesicles (**a**), the receptor-ligand complexes progress along the endocytic pathway. The endosomes undergo a progressive, ATP-dependent acidification that results in the dissociation of the receptor–ligand complexes, with megalin and cubilin being recycled in the apical membrane, whereas the ligand is directed to lysosomes for degradation. In the case of $25(OH)D_3$-DBP, DBP is degraded in lysosomes, whereas $25(OH)D_3$ is released in the cytosol and metabolized to active $1,25(OH)_2D_3$ in mitochondria before being released into the circulation. Vesicular acidification (**b**) is mediated by the vacuolar H^+-ATPase, which requires a net Cl^- conductance to dissipate the positive charge gradient. ClC-5 and other members of the CLC family are probably involved in that Cl^- conductance. ClC-5 functions as an electrogenic nCl^-/H^+ exchanger, which is predicted to facilitate acidification (at the expense of more energy) and to play a role in keeping high vesicular Cl^- concentration. The functional loss of ClC-5 (**c**) in patients with Dent's disease and ClC-5-null mice is reflected by multiple trafficking defects in PT cells, with (i) loss of megalin and cubilin in the apical brush border and their accumulation in sub-apical vesicles; (ii) defective apical endocytosis and low-molecular-weight proteinuria; and (iii) impaired progression toward lysosomes. These modifications result in generalized dysfunction of PT cells and renal Fanconi syndrome. As DBP carries most of the circulating vitamin D in the plasma, its urinary loss may lead to bone defects, and loss of $25(OH)D_3$ substrate. The increased amount of bioactive PTH in the PT lumen may interact with apical PTH receptors and stimulate internalization and degradation of the apical NaPi-IIa co-transporter, leading to phosphaturia. In turn, the phosphate loss could stimulate the 1α-hydroxylase activity in PT cells, leading to increased $1,25(OH)_2D_3$ synthesis depending on substrate availability. The balance between these effects may result in phenotype variations observed in man and mouse (see text).

transport of phosphate across the apical membrane of PT cells is mediated by two sodium-coupled transporters of the SLC34 family located in the brush border. The type 2a sodium-phosphate co-transporter NaPi-IIa (encoded by *SLC34A1* or *NPT2a*) is responsible for the bulk of phosphate reabsorption, whereas its homologue NaPi-IIc (encoded by *SLC34A*, or *NPT2c*) is thought to play a minor role (at least in mouse).[19] As the *Slc34a1*-null mice show renal phosphate wasting and hypercalciuria,[20] *SLC34A1* was considered as a candidate gene for hereditary hypophosphatemic rickets with hypercalciuria. A first study of 20 patients with persistent hypophosphatemia due to phosphaturia, complicated with urolithiasis or bone demineralization, identified two heterozygous missense mutations in *SLC34A1* in two patients. Functional studies in *Xenopus laevis* oocytes suggested that the mutant NaPi-IIa had a decreased affinity for phosphate and defective membrane expression, with a dominant-negative effect causing the disorder.[21] It must be pointed, however, that the functional defect associated with these *SLC34A1* variants has not been confirmed by additional analyses.[19] A recent study in a cohort of 98 pedigrees with hypercalciuric stone-formers showed no difference in serum and urinary phosphate levels or urinary calcium levels between carriers and non-carriers of *SLC34A1* polymorphisms, of which three were non-synonymous variants. The phosphate transport activity of these mutants was either normal or with minor kinetic changes and lower expression levels than the wild type.[22] Thus, the potential role of *SLC34A1* (NaPi-IIa) variants in hereditary hypophosphatemic rickets with hypercalciuria or, more generally, in renal phosphate wasting with nephrolithiasis remains to be confirmed.

Recently, homozygous and compound heterozygous mutations in *SLC34A3* encoding NaPi-IIc have been identified in consanguineous kindreds and additional families with hereditary hypophosphatemic rickets with hypercalciuria.[23] Furthermore, individuals of the affected kindreds who were heterozygous for a *SLC34A3* mutation

frequently showed hypercalciuria.[23] These data suggest that NaPi-IIc, which was initially considered less important than NaPi-IIa for renal phosphate reabsorption, could play an important role in the human kidney.[19]

Autosomal-dominant hypocalcemia

The G protein-coupled, extracellular calcium-sensing receptor (CaSR) is a key regulator of PTH secretion and renal tubular calcium reabsorption in response to blood calcium levels.[24] The CaSR is expressed on the apical membrane of the PT cells and principal cells of the medullary CD and on the basolateral membrane of cells lining the TAL and distal convoluted tubule (DCT).[24] Gain-of-function mutations in the *CASR* gene that encodes the CaSR lead to autosomal-dominant hypocalcemia, a disorder characterized by low serum PTH levels with hypocalcemia and hypercalciuria (reflecting decreased reabsorption of calcium in the TAL and DCT), and in which vitamin D treatment is associated with nephrocalcinosis and lithiasis.[25] A potent gain-of-function mutation of the CaSR (L125P) has been associated with a salt-losing tubulopathy resulting in a secondary aldosteronism, inappropriate kaliuresis, and hypokalemia, associated with hypercalciuria and nephrolithiasis.[26] These manifestations are probably explained by the inhibition of the apical renal outer medullary potassium channel ROMK, secondary to the constitutive activation of the mutated CaSR, leading to decreased paracellular calcium reabsorption in the TAL. The defect has been qualified as 'Bartter-like syndrome type V', in reference with other disorders affecting NaCl transport, and indirectly calcium reabsorption, in that segment. In particular, antenatal Bartter's syndromes type I (mutations in *SLC12A1* coding for the Na^+-K^+-$2Cl^-$ co-transporter NKCC2) and II (mutations in *KCNJ1* coding for the K^+ channel ROMK) are associated with fasting hypercalciuria and nephrocalcinosis, whereas the classical Bartter's syndrome type III (mutations in *CLCNKB*, coding for the Cl^- channel ClC-Kb) is associated with variable calciuria (Table 1). Studies looking for an association between polymorphisms in the *CASR* gene and hypercalciuria among stone-former patients yielded discrepant results,[4] and the role for CaSR in idiopathic hypercalciuria remains to be established.

Familial hypomagnesemia with hypercalciuria and nephrolithiasis

Claudin-16 (or paracellin-1) is a renal tight junction protein that is expressed in the TAL, where it plays a role in the paracellular reabsorption of magnesium and calcium.[27] Over 20 loss-of-function mutations in the *CLDN16* gene that encodes claudin-16 have been identified in kindreds with familial hypomagnesemia with hypercalciuria and nephrocalcinosis. Familial hypomagnesemia with hypercalciuria and nephrocalcinosis is an autosomal-recessive tubular disorder characterized by progressive magnesium and calcium wasting, leading to nephrocalcinosis and nephrolithiasis (with incomplete distal renal tubular acidosis (dRTA) and hypocitraturia), and generally complicated by chronic renal failure in early childhood or adolescence.[28] These mutations can interfere with the intracellular trafficking of claudin-16, or its paracellular magnesium permeability.[29] Interestingly, in 11 of 23 families with familial hypomagnesemia with hypercalciuria and nephrocalcinosis due to a mutation in *CLDN16*, there was at least one heterozygous individual with either hypercalciuria or nephrolithiasis, which led to suggest that heterozygosity for *CLDN16* mutation could account for hypercalciuria and nephrolithiasis.[28] It should be noted, however, that most patients with nephrolithiasis in that cohort had no hypercalciuria and that the prevalence of nephrolithiasis (9/55 patients, ~16%) or hypercalciuria (7/55, ~13%) among heterozygotes was similar to the general population.

These studies highlighted the importance of claudin-16/paracellin-1 for the paracellular transport of magnesium and calcium and, more generally, the functional role of tight junction proteins. Mutations of *CLDN16* are responsible for familial hypomagnesemia with hypercalciuria and nephrocalcinosis, but the potential role for *CLDN16* heterozygous mutations in idiopathic hypercalciuria remains unclear.

Hereditary distal renal tubular acidosis

Primary dRTA is caused by the failure of the CD to acidify urine appropriately in the presence of systemic metabolic acidosis and otherwise normal renal function. The cellular basis of dRTA is a dysfunction of the α-type intercalated cells, which mediate the secretion of protons into the urine via the apical, multisubunit proton ATPase that is functionally coupled to the basolateral anion exchanger AE1.[30] Mutations in three genes involved in the α-type intercalated cells have been associated with the autosomal dominant (*SLC4A1*, coding for AE1) or recessive (*ATP6V1B1* and *ATP6V0A4*, coding for the B1 and a4 subunits of the proton pump, respectively) forms of dRTA (Table 1). The biochemical manifestations of dRTA include hyperchloremic metabolic acidosis and hypokalemia, with inappropriate alkaline urine pH. Clinical manifestations are variable, including growth defects, osteomalacia, and rickets, and are usually milder in the autosomal dominant than in the recessive forms of the disease. The latter are also causing sensorineural hearing loss in some patients. Primary dRTA is frequently complicated by nephrocalcinosis and/or nephrolithiasis, attributed to the combination of hypercalciuria, hypocitraturia (due to upregulated citrate reabsorption in the PT), and alkaline urine. A recent biopsy study showed that stone-forming patients with dRTA have a more diffuse papillary renal disease than common idiopathic calcium oxalate stone formers. The main abnormalities were plugging of inner medullary CD profiles with calcium phosphate deposits in the form of apatite crystals, and epithelial cell injury surrounded by interstitial fibrosis.[31] Interestingly, most of the radiographic calcifications in these patients were surgically removable stones rather than nephrocalcinosis itself.[31]

Thus far, none of the genes responsible for dRTA has been associated with common idiopathic hypercalciuria or idiopathic calcium nephrolithiasis.[4] We should mention here that sequence variations in the *SAC* gene segregate with an autosomal dominant form of absorptive hypercalciuria (HCA2) in some kindreds.[32] Interestingly, *SAC* encodes a bicarbonate-activated, soluble adenylyl cyclase that is expressed in the kidney and other acid-secreting epithelia where it could play a role of pH sensor, mediating bicarbonate-activated cyclic adenosine 3′,5′ monophosphate signalling and vacuolar H^+-ATPase recycling important for luminal acidification.[33]

RODENT MODELS: QUANTITATIVE TRAIT LOCUS MAPPING AND GENE TARGETING

The genetic hypercalciuric stone-forming rat model

The most studied animal model of polygenic hypercalciuria is the GHS rat model developed by Bushinsky *et al.*[34] The GHS rat model was produced by selective inbreeding of hypercalciuric 'normal' Sprague–Dawley rats over 60 generations. Urine calcium excretion in these rats exceeds that of the parental strain by 10-fold, and all of them develop kidney stones. In agreement with a polygenic model, the pathophysiology of hypercalciuria in the GHS rat includes increased intestinal calcium absorption; increased bone reabsorption; and decreased renal tubular calcium reabsorption. Serum levels of calcium are normal, but there are excessive levels of the vitamin D receptor in all target tissues and of the CaSR in kidney. Breeding experiments allowed to identify several quantitative trait loci associated with hypercalciuria in this model. In particular, a calcium excretion quantitative trait loci (hypercalciuria 1, *HC1*) was identified on chromosome 1 and further isolated in a normocalciuric Wistar–Kyoto background by developing congenic strains.[35] Microarray analysis of the gene expression patterns in the hypercalciuric congenic strains revealed a bias in expression change for genes located in the *HC1* quantitative trait loci region. Furthermore, a significant number of genes with altered expression patterns are involved in calcium metabolism at large.[35] Further studies of the polygenic GHS rat model will combine fine mapping and expression profiling, in order to identify candidate genes contributing to hypercalciuria.

Trpv5 KO mice

The epithelial calcium channel TRPV5 is the rate-limiting step for active, transcellular calcium reabsorption in the DCT and CT of the kidney. TRPV5 is a member of the transient receptor potential (TRP) channel family, which includes 28 ion channels that function as cellular sensors and regulate various cell functions.[36] Hoenderop *et al.*[37] generated a *Trpv5* KO mouse characterized by diminished active calcium reabsorption in the DCT despite enhanced $1,25(OH)_2D_3$ levels, with compensatory hyperabsorption of dietary calcium, severe hypercalciuria, and significant bone abnormalities. Despite robust hypercalciuria, mice lacking TRPV5 showed no kidney stones, but a significant polyuria. It has been suggested that hypercalciuria activates the apical CaSR in the principal cells of the CD, which results in the inhibition of aquaporin-2 (AQP2)-mediated water reabsorption, diluting the luminal calcium and reducing the risk of calcium precipitation.[37] No human mutations of *TRPV5* (locus 7q35) have been identified thus far and the importance of this gene in idiopathic hypercalciuria is unknown. It must be noted, however, that the TRPV5-mediated calcium transport could be regulated by other molecules in the DCT, including the serine/threonine kinase WNK4.[38] Mutations of *WNK4*, the gene that encodes WNK4, cause autosomal dominant pseudo-hypoaldosteronism type II (MIM 601844), characterized by hyperkalemic hypertension associated with hypercalciuria. These features were reproduced in transgenic mice bearing a pseudo-hypoaldosteronism type II mutant *Wnk4* allele, and it was demonstrated that they are caused by an increased activity of the sodium-chloride co-transporter NCC in the DCT.[39] These findings reveal that a tight interaction between transporters and their regulators is important for calcium reabsorption in the DCT and that inherited differences in the activity of these molecules could influence calcium handling in the distal nephron.

Caveolin-1 KO mice

Caveolin-1 is the major structural component of caveolae, small invaginations of the plasma membrane that are present in most cell types and play an important role in endocytosis, transcytosis, and signal transduction.[40] Caveolin-1 is predominantly distributed in the basolateral area of DCT cells in the mouse kidney.[41] Of interest, male caveolin-1 KO mice showed hypercalciuria and a higher risk for calcium stones in the urinary bladder, whereas no changes were observed in females. These changes were associated with an aberrant distribution of the basolateral plasma membrane calcium ATPase in DCT cells.[41] Although the reason for the exclusive bladder stone presentation in this strain remains unclear, these studies pointed at caveolin-1 as a potential determinant of the renal handling of calcium – at least in males.

CONCLUSION AND PERSPECTIVES

Our understanding of the pathophysiology of hypercalciuria and nephrolithiasis has been improved by the identification of rare monogenic disorders affecting distinct tubular segments of the kidney, and the development and characterization of mouse models. A case in point is Dent's disease, in which the functional loss of a chloride transporter leads to the unexpected combination of defective PT endocytosis with hypercalciuria and nephrolithiasis. The deciphering of monogenic diseases yielded candidate genes for association studies with idiopathic hypercalciuria or calcium nephrolithiasis. The results of such association studies are thus far disappointing, reflecting methodological or statistical problems as well as limitations of the hypothesis-driven candidate gene selection for complex traits.

Taking into account the different organs and the multiple factors contributing to nephrolithiasis remains essential to get new candidate genes – as illustrated in the following examples. The majority of calcium stones are composed of calcium oxalate. Oxalate transport is mediated by the chloride-oxalate exchanger SLC26A6 expressed in the apical membrane of epithelial cells in the kidney and intestine. Jiang *et al.*[42] generated *Slc26a6*-null mice, which developed a high incidence of calcium oxalate kidney stones and hyperoxaluria. The latter was due to increased plasma oxalate concentration, secondary to a primary defect in intestinal oxalate secretion. This study demonstrated the important role of SLC26A6 in limiting the net intestinal absorption of oxalate, as an oxalate-free diet greatly reduced the urinary and plasma oxalate concentrations in this model. Another example is provided by the inactivation of *Umod* in mouse.[43] The *UMOD* gene encodes uromodulin (Tamm–Horsfall protein), the most abundant protein in the normal urine. Uromodulin is a glycosylphosphatidylinositol (GPI) anchor-linked protein that is synthesized in the cells lining the TAL, where it is released into the urine by proteolytic cleavage.[44] A sizeable fraction ($\sim$15%) of the *Umod* KO mice showed spontaneous formation of calcium oxalate crystals located in the medullary CDs, whereas such crystals were never found in wild-type littermates.[43] The development of crystals was triggered by exposure of KO mice to high calcium/high oxalate conditions. These results demonstrated that uromodulin is a protective factor against nephrolithiasis, which could be due to its affinity to bind calcium (via calcium-binding domains and negatively charged sialylated residues) and a capacity to inhibit calcium crystal formation *in vivo*. A deficiency in uromodulin maturation or function could thus contribute to nephrolithiasis in man. Yet, two genes that may potentially interfere with the maturation of uromodulin (*DPM2* and *DOLPP1*) are located within a new locus for autosomal-dominant nephrolithiasis (*NPL1*) that was identified by a genome-wide search in a Spanish kindred.[45]

The quest for genes involved in hypercalciuria and nephrolithiasis will undoubtly take advantage of high-throughput technologies, such as genome-wide association studies performed in selected kindreds or populations;[45] microarray-based expression studies performed in relevant animal models such as the GHS rat (see above);[35] and large-scale random mutagenesis approaches (such as the *N*-ethyl-*N*-nitrosourea (ENU) or the isopropyl methane sulfonate programs) in which gene discovery is driven by phenotype analysis rather than by hypothesis. The latter approach proved effective in generating mutants relevant for complex human diseases including ectopic tissue calcifications.[46]

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