Inching toward a Greater Understanding of Genetic Hypercalciuria

The Role of Claudins

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Nephrolithiasis is both common (affecting up to 10% of the Western population over a lifetime) (1) and ancient (first reported over 2000 years ago in Egypt) (2). Not only do stones cause great pain and morbidity, but their treatment also results in significant burden to the health care system, accounting for over $10 billion of cost in the United States alone (1). Up to 80% of human urinary stones are composed of calcium oxalate or phosphate (2–4), and strong evidence suggests underlying genetic risk factors for these most common types (1). There is particularly robust evidence for heritability of urinary calcium excretion (3), and hypercalciuria is a key driver of urinary supersaturation, a well established nephrolithiasis risk factor. Thus, increased knowledge regarding the regulation of calcium excretion is vital for understanding stone pathogenesis and identifying new treatment targets (3,4), recognizing that biologic processes and superimposed environmental factors are also involved in stone development and growth (2).

Ionized calcium is freely filtered at the glomerulus, but over 99% is subsequently reclaimed by the nephron *via* two pathways: transcellular (through epithelial cells) and paracellular (between epithelial cells) (3,5). Approximately 65% of filtered calcium is reclaimed in the proximal tubule, and an additional 25% is reclaimed in the thick limb (6). Much of the bulk reabsorption at these sites is paracellular rather than transcellular (3). Proximal tubular calcium reabsorption does not seem to be regulated, and it is thought to largely occur as a part of bulk paracellular ion and water flows (5,7). The specifics of calcium reabsorption in the thick limb (6) are more complicated (Figure 1). The remaining 10% of filtered calcium is reabsorbed transcellularly in the distal nephron *via* apical calcium channels (TRPV5), intracellular calbindin-28K, a basolateral sodium-calcium exchanger (NCX1), and calcium-ATPase (PMCA1b) (6). Calcium reabsorption in the distal nephron is against an electrochemical gradient under the combined regulation of parathyroid hormone and 1,25-vitamin D, which in turn, are ultimately responsive to peripheral ionized blood calcium concentrations (6,7).

Although it was long assumed that paracellular movements were the passive result of transcellular electrochemical gradients, it is now apparent that they are often highly selective on the basis of the relative expression of the tight junction claudin proteins initially described by Furuse and Tsukita (8). Currently, 27 claudins are known to impart selective permeabilities along the nephron (5,9,10). In the proximal tubule, claudin-2 is essential for maintaining paracellular water, sodium, and calcium permeability (5). In the thick limb, claudin-14, claudin-16, and claudin-19 control calcium and magnesium paracellular movement, whereas claudin-10b is important for paracellular sodium transport in the inner stripe of the outer medulla (7). Claudin-4 seems to be important for sodium movement in the distal tubule and collecting duct (5). The major treatment for hypercalciuria and nephrolithiasis to date has been a low-sodium diet and/or thiazide diuretics that seem to nonspecifically increase calcium reabsorption in the proximal tubule and thick limb as a response to the resulting and subtle extracellular volume depletion (1,2).

Recent observations from human and animal studies together with an increased understanding of paracellular calcium movement provide important new clues regarding the molecular basis for hypercalciuria and its relationship to the human phenotype of nephrocalcinosis and nephrolithiasis, and they suggest additional possible treatment targets. Genome-wide association studies have associated a gain-of-function mutation of *CLDN14*, the gene that encodes claudin-14, with reduced bone mineral density and risk of nephrolithiasis (3,4). Conversely, genetic point mutations of *CLDN16* and *CLDN19* are associated with familial hypomagnesemia with hypercalciuria and nephrocalcinosis, an autosomal recessive syndrome commonly associated with nephrolithiasis (5) and ESKD (7). Human patients with known *CLDN10* mutations are rare and have a variable phenotype. One family manifested anhidrosis and mild kidney failure, whereas another showed a hypokalemic and alkalotic salt-losing tubulopathy (7). A new clinical syndrome composed of hypohidrosis, electrolyte imbalance, lacrimal gland dysfunction, ichthyosis, and xerostomia has also recently been associated with recessive mutations in *CLDN10* (9). Affected individuals manifest hyperaldosteronism secondary to urinary loss of sodium chloride, hypermagnesemia

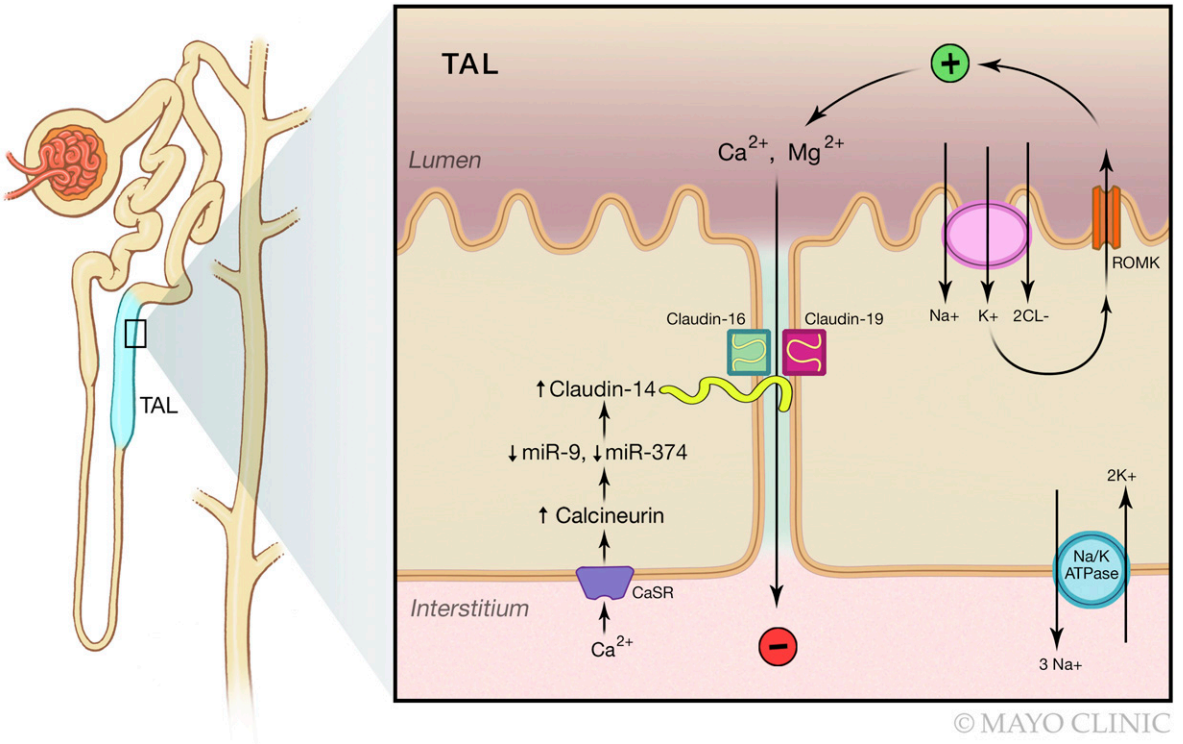

Figure 1. | Claudins play a critical role controlling divalent cation reabsorption in the thick ascending limb (TAL). About 25% of filtered calcium is reabsorbed in the TAL, mainly by a paracellular route. The basolateral sodium-potassium (Na^+-K^+) ATPase creates gradients that ultimately drive passive ion movement *via* the sodium-potassium-2 chloride (Na^+-K^+-$2Cl^-$) cotransporter on the luminal surface. The luminal renal outer medullary potassium (ROMK) channel allows back leak of potassium, which is, in turn crucial, for generating a net positive electrochemical gradient for paracellular calcium (and magnesium) reabsorption. Claudin-16 and claudin-19 seem to form a functional pore in the paracellular space crucial for the selective movement of these divalent cations. Claudin-14 can interact with claudin-16 to functionally decrease movement through this pore. The calcium-sensing receptor (CaSR) is a dimer-forming G protein–coupled receptor consisting of three protein domains. Calcium stimulation of the basolateral CaSR (as well as parathyroid hormone receptor) seems to decrease paracellular calcium movement. CaSR receptor stimulation sets into motion a cascade of signals that include intracellular calcineurins to ultimately downregulate expression of the microRNAs (miRs) miR-9 and miR-374. Decreased intracellular miR-9 and miR-374 concentration, in turn, allows increased *CLDN14* gene and protein expression, which in turn, decreases functional activity of the claudin-16/claudin-19 complex and reduces paracellular reabsorption of calcium. The net effect is to increase urinary calcium excretion (and decrease serum calcium concentration). Consistent with this model, increased dietary calcium inhibits production of both miR-9 and miR-374 to suppress CLDN14 gene transcription. In addition, in cell culture models, overexpression of claudin-14 decreases paracellular calcium permeability, whereas treatment with the CaSR agonist cincacalcet increases CLDN14 mRNA expression.

with normal/low fractional excretion of magnesium, and hypocalciuria, all pointing toward a complex dysfunction of the thick limb. Mice lacking claudin-16 display divalent cation wasting similar to familial hypomagnesemia with hypercalciuria and nephrocalcinosis, whereas claudin-10 knockout mice manifest hypermagnesemia, hypocalciuria, polyuria, and interstitial nephrocalcinosis (7). The differences between the human and mouse phenotypes associated with claudin-10 genetic variation remain to be determined. In particular, the nephrocalcinosis observed in the claudin-10 knockout mice would not necessarily be expected given the known physiology. Nevertheless, in total, these observations point toward an important role of the thick limb and the claudins in this segment for regulation of calcium reabsorption. In particular, the combined group of claudin-14, claudin-16, and claudin-19 seems to play a critical role in many human diseases that manifest with hypercalciuria, nephrocalcinosis, or nephrolithiasis (4).

The report by Arcidiacono *et al.* (10) in this issue of the *Clinical Journal of the American Society of Nephrology* adds to our overall understanding of the regulation of calcium reabsorption in the thick limb, including the interaction between claudin genotypes and sodium loading. In this study, a total of 31 single-nucleotide polymorphisms (SNPs) in the 3′ region of *CLDN14* were associated with baseline calcium excretion in a cohort of subjects who were hypertensive. Furthermore, calcium excretion increased in all genotype groups after saline infusion but remained highest in the group with the SNPs associated with higher baseline calcium excretion. Polymorphisms in *CLDN16* and *CLDN19* were not associated with calcium excretion before or after saline. On the basis of *in silico* analyses, the SNP in the 3′ region of *CLDN14* that most associated with calcium excretion was predicted to influence splicing of its transcript, which in turn, could potentially alter protein-protein interactions of claudin-14 and claudin-16. A previous study found that the 62% of the general Icelandic population homozygous for the major allele of a *CLDN14* SNP (rs219780) (4), implicated by Arcidiacono *et al.* (10) in calcium excretion, had a 1.64-fold increased risk of nephrolithiasis (4). A

previous study in large Icelandic and Danish cohorts (4) also identified a lower serum parathyroid hormone, lower calcium excretion and higher bone mineral density amongst carriers of the minor allele of *CLDN14* SNP rs219780, consistent with physiologic predictions.

Although this study (10) was not designed or powered to detect an association of the measured claudin genotypes with nephrolithiasis, it still provides important insights regarding the potential contribution of claudin-14 to the human phenotype of hypercalciuria. Indeed, the body of evidence in the literature together with this study suggest that claudin-14 plays an important role regulating urinary calcium regulation in the general population and that genetic variation of this claudin modifies nephrolithiasis and osteoporosis risk. Dietary sodium seems to increase calcium excretion, regardless of *CLDN14* genotype. Certainly questions remain. Will other genes be identified that contribute to urinary calcium excretion in specific individuals or groups? If so, do they affect these pathways or others? It is also unclear what percentage of hypercalciuric stone formers might have genetic variants of *CLDN14*. Finally, it remains likely that additional roles for claudins in the kidney disease pathogenesis will emerge (5).

In conclusion, our knowledge regarding the key role that claudins play to regulate paracellular movements in the kidney continues to expand. Perhaps not unexpectedly, genetic variability in "regulatory" protein claudin-14 seems to contribute to the common phenotype of hypercalciuria and nephrolithiasis, whereas genetic alterations in the "structural" proteins claudin-16 and claudin-19 associate with more severe phenotypes (nephrocalcinosis, severe electrolyte imbalances, and ESKD). Thus, claudin-14 has emerged as an interesting potential therapeutic target.

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Disclosures

None.

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