Inching toward a Greater Understanding of Genetic Hypercalciuria
The Role of Claudins

Ronak Jagdeep Shah and John C. Lieske

Nephrolithiasis is both common (affecting up to 10% of the Western population over a lifetime) and ancient (first reported over 2000 years ago in Egypt) (2). Not only do stones cause great pain and morbidity, 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...
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.

Acknowledgments
The authors acknowledge research support of grant U54-DK100227 from the O’Brien Urology Research Center, Rare Kidney Stone Consortium grant U54DK083908-01 (part of the Rare Diseases Clinical Research Network, an initiative of the Office of Rare Diseases Research, the National Center for Advancing Translational Sciences, and the National Institute of Diabetes and Digestive and Kidney Diseases), and the Mayo Foundation.

Disclosures
None.

References

Published online ahead of print. Publication date available at www.cjasn.org.