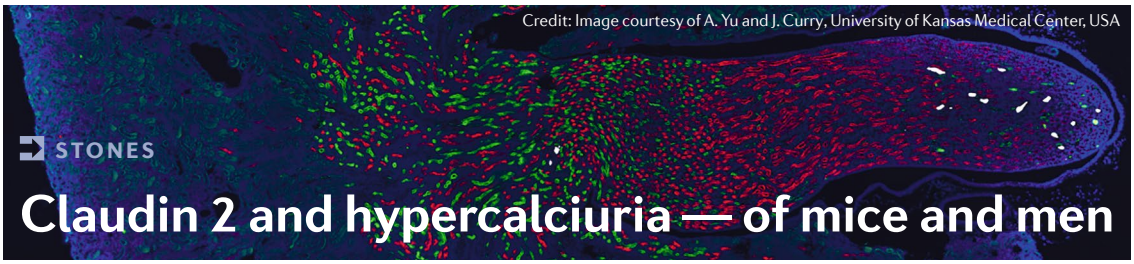

STONES

Claudin 2 and hypercalciuria — of mice and men

> They began by studying claudin 2-knockout (*Cldn2*$^{-}$/*y*) mice … confirming that they are indeed hypercalciuric

Claudin 2 is a regulator of calcium excretion and could be a treatment target for patients with urolithiasis, according to new data published in the *Journal of Clinical Investigation*.

Hypercalciuria is integral to the pathogenesis of stone formation, which is likely to arise via calcium deposition in the renal papilla. The aetiology of hypercalciuria is somewhat uncertain but is thought to include increased bone resorption, calcium hyperabsorption in the intestine and reduced renal reabsorption. Calcium reabsorption occurs largely in the proximal tubule, where ~60% of calcium filtered by the glomerulus is reabsorbed, most likely via paracellular diffusion. This transport is mediated by claudins — a family of membrane proteins found at epithelial tight junctions, which act as charge-selective channels and regulate the movement of solutes across the epithelium.

Claudin 2 is of particular interest as it is permeable to both sodium and calcium and is highly expressed in the proximal tubule and the descending loop of Henle. Furthermore, the proximal tubules of claudin 2-knockout mice exhibit increased fractional excretion of calcium, suggesting that claudin 2 mediates paracellular calcium reabsorption in the proximal tubule.

Thus, Curry and colleagues investigated whether claudin 2 loss predisposes mice to nephrocalcinosis and leads to kidney stone formation in a similar manner to stone formation in humans. They began by studying claudin 2-knockout (*Cldn2*$^{-}$/*y*) mice (*Cldn2* is located on the X chromosome), confirming that they are indeed hypercalciuric. However, serum calcium levels and glomerular calcium filtration were comparable between *Cldn2*$^{-}$/*y* mice and wild-type littermates, suggesting that this difference is due to a decrease in renal calcium reabsorption in *Cldn2*$^{-}$/*y* mice as a result of impaired proximal tubule paracellular calcium transport.

Leading on from the observation of hypercalciuria, the team then investigated whether *Cldn2*$^{-}$/*y* mice were prone to nephrocalcinosis. Accordingly, they observed abundant mineral deposits in the renal papillae of 6-month-old and 1-year-old *Cldn2*$^{-}$/*y* mice. Examination of 5-month-old mice using transmission electron microscopy revealed that these deposits were present in mice before they became easily detected at 6 months and contained cells originating from the ascending and descending limbs of the loops of Henle, indicating that this location was also the origin of the mineral deposits.

This phenotype — hypercalciuria secondary to both proximal tubular calcium wasting and increased intestinal calcium absorption with papillary mineral deposition in the thin limbs of the loops of Henle — is reminiscent of the phenotype of patients with idiopathic hypercalciuria and kidney stone disease. Thus, the group hypothesized that *CLDN2* variants might be involved in the pathogenesis of human kidney stone disease. Previous genome-wide studies did not include the X chromosome in their analysis, so Curry and colleagues studied the association between 12 single-nucleotide polymorphisms (SNPs) in the *CLDN2* locus and stone risk, analysing a total of 11,130 patients with kidney stones and 187,639 controls and identifying 9 SNPs that were significantly associated with risk of nephrocalcinosis ($P < 0.05$). These data were further supported by genome analysis of an Iranian family, who had previously been shown to harbour a rare missense mutation in *CLDN2* that led to obstructive azoospermia. Both male and female members of this family also had marked hypercalciuria.

"Our premise — that defects in proximal tubule calcium handling lead to papillary calcification and, therefore, kidney stone disease — represents a paradigm shift in thinking about tubular calcium handling," corresponding author Alan Yu tells *Nature Reviews Urology*. "We are not the first to postulate the importance of the proximal tubule: Fred Coe, Elaine Worcester and Andrew Evan at Chicago and Indiana were the real pioneers and have been trying to make a case for this in recent years, but I think our study really strengthens this argument."

The data presented suggest a possible new target for clinical kidney stone therapies. "Perhaps interventions to increase proximal tubule calcium reabsorption (and hence reduce calcium delivery to the papilla) might be more effective at reducing stone incidence, than simply reducing overall urine calcium concentration and calcium oxalate supersaturation," comments Yu.

Annette Fenner

ORIGINAL ARTICLE Curry, J. N. et al. Claudin-2 deficiency associates with hypercalciuria in mice and human kidney stone disease. *J. Clin. Invest.* **130**, 1948–1960 (2020)
RELATED ARTICLE Sivaguru, M. et al. GeoBioMed sheds new light on human kidney stone crystallization and dissolution. *Nat. Rev. Urol.* **17**, 1–2 (2020)