

Calcium stones are the most prevalent type of nephrolithiasis. They result from a combination of metabolic defects, of which hypercalciuria is the most frequent, being present in 40% of stone formers compared with 5% in the general population. Hypercalciuria is thought to arise from different causes, mainly primary intestinal hyperabsorption, but occasionally from a renal calcium or phosphate leak, or primary bone resorption. There is familial aggregation to calcium oxalate stone formation and hypercalciuria (1), but genetic factors are unknown.

In this issue of *The Journal*, Lloyd et al. (2) extend their molecular genetics analysis of a rare form of familial hypercalciuric Fanconi syndrome with nephrocalcinosis and renal failure to Japanese kindreds with an apparently milder presentation of the disease, low molecular weight (LMW) proteinuria. Dent's disease, as it is now known, is an X-linked trait that results from loss of function mutations in the *CLCN5* gene that encodes a renal chloride channel (3). Such mutations also cause two closely related traits, X-linked recessive nephrolithiasis (XRN) and X-linked recessive hypophosphatemic rickets.

Chloride channels are, most likely, part of the solute reabsorption system of the kidney that involves transporters, ATPases and channels, and whose primary driving force is provided by ionic gradients created directly or indirectly by the energy-dependent Na/K-ATPase. This complex machinery is required to reclaim more than 99% of the 180 liters of tubular fluid and its dissolved molecules that are filtered out of the glomeruli each day. Chloride channels (Fig. 1) first received widespread attention when cystic fibrosis was shown to result from mutations in the cystic fibrosis transmembrane regulator (CFTR). CFTR was later demonstrated to act as a cAMP-activated chloride channel of low conductance (7–9 pS) expressed in a variety of tissues including airway epithelium, sweat glands and kidney tubules. In contrast, chloride channels of the CLC family are voltage-gated channels of various conductances and are involved in a wide variety of functions that include electrical excitability and regulation of cell volume (4). Nine different mammalian CLC channels are known, of which CLC-2, CLC-3, CLC-4, as well as the kidney-specific transcripts CLC-Ka and CLC-Kb located in the basolateral membrane of the thick ascending limb of Henle's loop (5), are expressed in the kidney (reviewed in reference 4). CLC-5 transcripts (*CLCN5* unfortunately designates the gene) are expressed in all tubule segments, predominantly in the S3 segment of the proximal tubule and in the ascending limb of Henle's loop (6), but refined localization has yet to be performed.

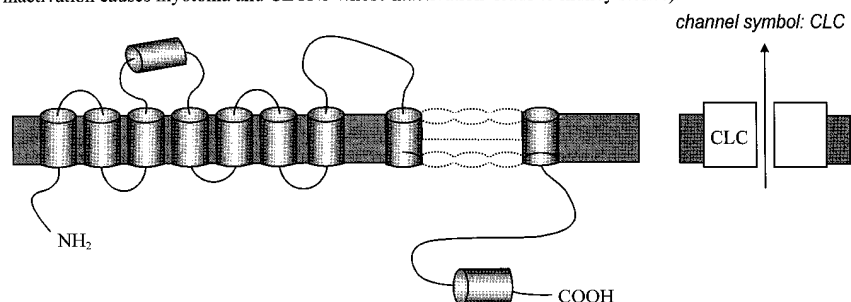
How do mutations in a renal chloride channel result in low-molecular-weight proteinuria, hypercalciuria, nephrocalcinosis, and a Fanconi-like syndrome? The answers are not known, but several interesting hypotheses have been raised previously (7). The presence of low-molecular weight proteins in the urine suggests that at least the proximal tubule is involved.  $\beta$ 2-

microglobulin and other LMW proteins are filtered by the kidney and are reabsorbed in the proximal tubule by endocytosis. Endosomes are intracellular organelles that participate in the degradation of absorbed proteins. Their luminal compartment is acidic and positively charged with respect to the cytosol due to the action of a vacuolar proton ATPase. Chloride channels within the endosomal membrane are necessary to keep the proton pump active by allowing an anionic influx which reduces the positive membrane potential generated by proton influx. Inactivation of this channel would impair the endocytotic process and decrease absorption of LMW proteins. To date, there is no data demonstrating that CLC-5 is targeted to the endosome, and several questions remain unanswered regarding the properties of this channel. After expression in *X. laevis* oocytes or in CHO cells, CLC-5 appears targeted to the plasma membrane where it generates a strong outwardly-rectifying current (Cl influx) which takes off at positive membrane potentials. The expected orientation of this channel within an endosomal membrane (the inside of the vesicle corresponds topologically to the extracellular space) would make it better suited for Cl efflux rather than influx, and would require the presence of a negative luminal potential to be fully active. Unless local conditions change drastically rectification and gating mechanisms, CLC-5 does not appear to present the characteristics required to play a central role in endosomal ionic transport.

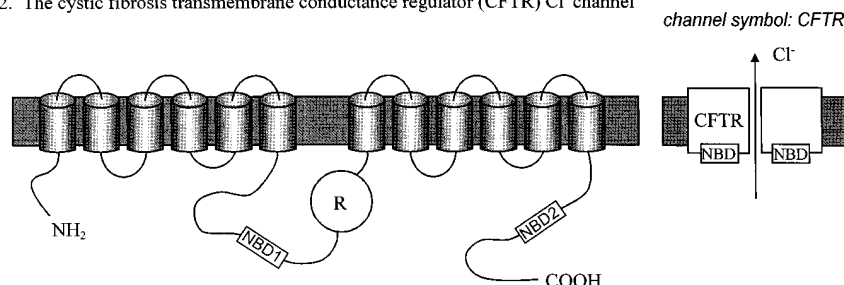
The presence of nephrocalcinosis and hypercalciuria is also unexplained. Although calcium excretion may be high in patients with proximal tubular defects, nephrocalcinosis is seldom noted, perhaps because of increased excretion of urinary citrate. In the kidney, 70% of the filtered calcium load is reabsorbed by the proximal tubule, largely through a passive, paracellular pathway. About 10–15% is reabsorbed paracellularly by the ascending limb of Henle's loop, using the favorable transepithelial potential provided by the secretion of potassium through apical K channels. Regulated transcellular calcium reabsorption occurs in the distal tubule through the conjunction of apical calcium channels on one hand, and basolateral calcium ATPases and sodium-calcium exchangers on the other hand. In tissue culture cells originating from the distal tubule, basolateral Cl channels were thought to regulate apical calcium entry through a mechanism that remains to be completely elucidated. Finally, hypercalciuria could also be secondary to phosphate depletion per se which can result in increased urinary calcium excretion from decreased calcium reabsorption in the proximal and distal tubules (8) and increased vitamin D synthesis.

Phenotypic variability (allelic heterogeneity) appears to be a feature of the mutations in the *CLCN5* gene, as X-linked recessive nephrolithiasis (XRN), X-linked hypophosphatemic rickets and Dent's disease share a common loss-of-function defect in channel activity. Expression of the mutated channels in *X. laevis* oocytes has not revealed the mechanism, although expression in mammalian cell lines might have identified more subtle differences. However, careful analysis of the pedigrees suggests that these diseases are in fact more similar than would appear at first glance, and probably represent different manifestations of a clinical spectrum. For example, the pedigree used for the linkage analysis of XRN reveals that affected

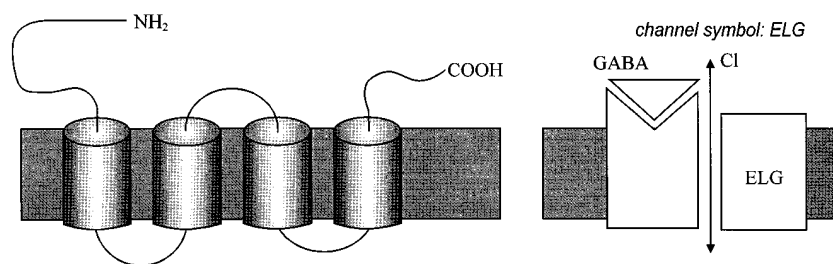
1. The CLC family of  $\text{Cl}^-$  channels with nine different CLC genes currently known including CLC-1 whose inactivation causes myotonia and CLCN5 whose inactivation leads to kidney stones).



2. The cystic fibrosis transmembrane conductance regulator (CFTR)  $\text{Cl}^-$  channel



3. Extracellular ligand-gated (ELG), post-synaptic  $\text{Cl}^-$  channels (e.g. gamma-amino-butyric acid - GABA - and glycine receptors).



**Figure 1.** Three classes of chloride channels with their topology models (*left*) and schematic functional representation (*right*). CLC  $\text{Cl}^-$  channels have about 12 transmembrane domains. CFTR has two blocks of six transmembrane domains followed by the nucleotide binding domains 1 and 2. GABA and glycine receptors have four transmembrane domains and may function as pentamers. Schematic representation redrawn from reference 4.

members shared most of the features of Dent's disease (9), but did not systematically present with calcium stones. Similarly, the assertion made by Lloyd et al. in this issue that their study "expands" the clinical spectrum of CLCN5 mutations to idiopathic low-molecular weight proteinuria is an oversimplification. All subjects studied were children (14 or younger), and surprisingly, maternal relatives were not ascertained to yield a more complete picture of the disease in Japan. Even in the original description of the Japanese families (10), decreased renal function, nephrocalcinosis and small stature were present in several patients, although overt rickets was not, and the authors themselves concluded that idiopathic low molecular weight proteinuria was inappropriate to describe the features of the disease. It is also possible that the recruitment mode of this study (children of school age) has resulted in ascertainment bias, leaving out severe cases (previously identified), and selecting milder ones, possibly because of "milder" mutations, but most likely because of genetic and/or environmental modifiers. Indeed, intrafamilial heterogeneity seems to be a feature in the Caucasian kindreds previously described (11).

To resolve some of these issues, additional studies will be needed, in particular to define the structure, function and localization of CLC-5. Until then, we will be left with several stones unturned.

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