HEREDITARY HYPOPHOSPHATEMIC rickets with hypercalciuria (HHRH) is an autosomal recessive inherited disorder of mineral and bone metabolism. It is characterized by hypophosphatemia, rickets, and increased serum 1,25-dihydroxyvitamin D concentration, resulting in secondary absorptive hypercalciuria and is also associated with renal calcification and renal stone disease (4, 8, 10, 11, 12, 14). HHRH is different than X-linked hypophosphatemia (XLH) and tumor-induced hypophosphatemia because of the increased serum 1,25-dihydroxyvitamin D levels and normal or low parathyroid hormone (PTH) and fibroblast growth factor 23 (FGF-23) levels (1, 5).

In 2006 three publications reported mutations in the gene encoding the renal proximal tubular sodium-phosphate cotransporter NaPi-2c (SLC34A3) as the genetic cause of HHRH (3, 5, 9). However, the molecular mechanisms explaining how these mutations resulted in impaired renal tubular NaPi transport remained largely unknown until the study by Jaureguiberry et al. (6).

NaPi-2c along with NaPi-2a (SLC34A1) belong to the type II class of renal proximal tubular sodium-phosphate cotransporters. However, the expression profile of NaPi-2c (S1 segment) is quite different than NaPi-2a (S1–S3 segments of the renal proximal tubule). In addition, the regulation of NaPi-2c by dietary phosphate (Pi) and PTH follows a different pattern and time course than NaPi-2a. There may well be species differences in the expression level and regulation of NaPi-2a and NaPi-2c; however, it is clear that both transporters are necessary for normal and full Pi reabsorptive capacity of the renal proximal tubule (2, 7).

The study by Jaureguiberry et al. (6) describes two novel compound heterozygous mutations, c.410C > T (p.T137M) (T137M) on the maternal and g.4225_50del on the paternal allele of SLC34A3, in a previously reported male with HHRH and recurrent kidney stones. For functional analysis in vitro, the investigators generated expression plasmids encoding enhanced green fluorescence protein (EGFP) tagged to the NH2-terminus of wild-type or mutant human NaPi-IIc: 1) EGFP-hNaPi-IIc; 2) EGFP-(M137)hNaPi-IIc, representing the maternal mutation; and 3) EGFP-(Stop446)hNaPi-IIc, representing the paternal mutation (the V446Stop mutation truncates NaPi-IIc).

The V446Stop mutant showed complete loss of expression and function when assayed for apical membrane expression in opossum kidney (OK) cells and sodium-dependent phosphate uptake into Xenopus laevis oocytes. The cause of the impaired apical membrane expression is not known but may include dysfunctional interactions with sodium-hydrogen exchanger regulator 1 and/or sodium-hydrogen exchanger regulator 3, which recently were shown to be important for NaPi-2c apical membrane expression (13), and perhaps other apical membrane-targeting proteins.

In comparison, the apical membrane expression of the T137M mutant EGFP-(M137)hNaPi-IIc in OK cells was reduced by 60%, and after correction for surface expression, the rate of sodium-dependent phosphate uptake by oocytes was decreased by an additional 60%.

The resulting overall highly significant reduction in function of the T137M NaPi-IIc mutation together with complete loss of expression and function of the g.4225_50del (V446Stop) mutation thus appear to be sufficient to explain the hypophosphatemic phenotype in the patient. Interestingly, additional dual isotope sodium-phosphate uptake experiments in oocytes indicated that the stoichiometric ratio of 22Na and 33P uptake was increased to 7.1 ± 3.65 for EGFP-(M137)hNaPi-IIc compared with wild-type. Two-electrode studies indicated that EGFP-(M137)hNaPi-IIc is nonelectrogenic, but displayed a significant phosphate-independent inward-rectified sodium current, which appeared to be insensitive to phosphonoformic acid, an inhibitor of sodium gradient-dependent phosphate transport.

M137 may therefore impair sodium-phosphate cotransport by decreasing sodium uptake which may 1) decrease the sodium gradient that is needed for sodium gradient-dependent phosphate uptake, 2) decrease internalization of NaPi-2c cotransporters from the apical membrane, or 3) decrease exocytosis of the NaPi-2c cotransporters to the apical membrane. In addition, it is also possible that these mutations may be associated with alterations in the posttranslational modification of the NaPi-2c protein, including glycosylation, phosphorylation, and ubiquitinylation.

Furthermore, this mutation may also alter the function of other proximal tubular apical membrane cotransporters such as the sodium-hydrogen exchanger (NHE3), the sodium-sulfate cotransporter (Na-Si), and the sodium-citrate cotransporter (Na-citrate), which could also impair urinary acidification and citrate excretion, and therefore play an important role in urinary stone formation.

The study by Jaureguiberry et al. (6) therefore provides novel insights into how mutations in NaPi-2c may result in hypophosphatemia in HHRH. The rare but highly novel and important HHRH mutations may teach us about the importance of NaPi-2c in the regulation of human renal phosphate transport and also possibly novel causative factors for renal stone formation and renal calcification.

REFERENCES


