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Renal Phosphate–Transporter Regulatory Proteins and Nephrolithiasis

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Nephrolithiasis is a common disorder, and idiopathic hypercalciuria is the most frequent metabolic disorder associated with nephrolithiasis.¹ Several studies have shown that subjects with idiopathic hypercalciuria have phosphaturia or renal phosphate leak. In fact, a study that measured the tubular maximal reabsorption of phosphate (TmP, or maximal renal phosphate threshold) normalized for the glomerular filtration rate (GFR) (the TmP/GFR value) in 207 subjects with calcium nephrolithiasis reported that 20% of persons with normal parathyroid function in whom stones formed have a decreased TmP/GFR value.² The associated mild hypophosphatemia results in increased 1,25-dihydroxyvitamin D production, which causes increased intestinal phosphate and calcium absorption. The combination of hypercalciuria from the increased intestinal calcium absorption and the hyperphosphaturia favors the formation of calcium phosphate complexes that can result in nephrolithiasis (Fig. 1A).

The cause of phosphaturia in subjects with nephrolithiasis has been the subject of several recent investigations, and attention has centered on the potential role of the renal phosphate transporters. Regardless of the serum phosphate concentration, phosphate is freely filtered across the glomerulus and reabsorbed along the renal tubule, mostly the proximal tubule, through two distinct sodium phosphate transporters that are dependent on the sodium gradient: NPT2a (encoded by the *SLC34A1* gene) and NPT2c (encoded by the *SLC34A3* gene).

Studies of Npt2a-knockout mice indicate that NPT2a mediates the reabsorption of 70% of the filtered phosphate. Recently, a missense mutation in Npt2a in mice has been shown to impair renal phosphate transport and result in nephrolithiasis, a result similar to that seen in the Npt2a-knockout mouse.³ Similarly, a report in the *Journal* concerning subjects with nephrolithiasis and osteoporosis associated with hypophosphatemia who had an impaired TmP/GFR value identified heterozygous mutations in the *NPT2a* gene.⁴ Furthermore, follow-up studies indicated that although genetic variants of NPT2a are not rare, they do not seem to be associated with clinically important renal phosphate leak.⁵

Recent studies, however, have definitively identified mutations of NPT2c as the cause of hereditary hypophosphatemic rickets with hypercalciuria, which is an autosomal recessive inherited disorder of mineral and bone metabolism. It is characterized by hypophosphatemia, rickets, and an increased serum 1,25-dihydroxyvitamin D concentration resulting in secondary absorptive hypercalciuria; it is also associated with renal calcification and nephrolithiasis. Another recent study identified new NPT2c mutations that lead to mis-targeting of NPT2c protein and uncoupling of sodium phosphate cotransport as the cause of hereditary hypophosphatemic rickets with hypercalciuria.⁶

The study reported by Karim et al. in this issue of the *Journal* identifies yet another potential new and interesting mechanism of phosphaturia: mutations in the sodium–hydrogen exchanger regulatory factor 1 (NHERF1, also known as EBP50).⁷ NHERF1 is a PSD-95, Discs-large, ZO-1 (PDZ)-domain protein that interacts with the C-terminal tail of NPT2a⁸ and also NPT2c⁹ and plays an important role in the trafficking and transcriptional regulation of NPT2a¹⁰ (Fig. 1B).

In the study by Karim et al., the authors identified three NHERF1 mutations in seven patients who had lower TmP/GFR values, lower serum phosphate concentrations, and higher serum 1,25-dihydroxyvitamin D concentrations than controls. Despite their normal PTH concentrations, the patients had increased urinary cyclic AMP (cAMP) concentrations. The authors then conducted experiments in which wild-type or mutant *NHERF1* complementary DNA (cDNA) was expressed in opossum kidney cells, a model of proximal tubule cells that express NPT2a and the PTH type 1 receptor (PTH1R). Although there were no significant differences in the baseline cAMP concentrations or phosphate uptake between the transfected cells and wild-type cells, a challenge with PTH resulted in greater cAMP stimulation and greater inhibition of phosphate transport in the cells expressing the mutant NHERF1 than in the wild-type cells. Since in addition to its activation of the cAMP–protein kinase A signaling pathway, PTH activates the phospholipase C signaling pathway, the authors then measured cellular calcium and inositol phosphate concentrations and found no alterations in response to PTH.

NHERF1 deficiency in mice and NHERF1 inhibition in cells have been definitively shown to impair the transport activity and expression of NPT2a in the apical membrane, and NHERF1 phosphorylation by PTH has been shown to be important in the internalization of NPT2a.¹¹ However, it is not clear how the currently described NHERF1 mutations mediate abnormalities of phosphate transport in humans, since expression of the mutant *NHERF1* cDNA per se had no effect on phosphate transport. More in vivo studies will be needed to clarify this issue. In addition, it will be important to determine whether these mutations directly modulate the transport activity and expression of NPT2c as well as NPT2a and whether there are secondary effects on sodium–potassium–ATPase, which is also regulated by PTH through an NHERF1-dependent pathway and may regulate sodium phosphate cotransport by means of decreased generation of the sodium gradient.

Recent studies also indicate that NHERF1 interacts with mouse urate transporter 1 to regulate uric acid transport in the renal proximal tubule and that NHERF1-knockout mice have increased uric acid excretion.¹² Even more recent data also indicate a potential interaction between NHERF1 and the renal-proximal-tubule sodium sulfate transporter NaSi-1, but it is not known whether NHERF1-knockout mice have increased urinary sulfate excretion.¹³ Obviously, it is important to know whether the subjects with NHERF1 mutations also had abnormalities in uric acid or sulfate excretion, since these are also highly relevant to nephrolithiasis.

Other mutations may be relevant to sodium phosphate transport and nephrolithiasis. Potential mutations of another PDZ protein, PDZ domain containing 1 protein (PDZK1; also called CAP70, PDZD1, and NHERF3) should be considered, since a recent study demonstrated that NPT2c interacts with PDZK1.⁹ In addition, mutations in chloride channel 5 (CLCN5), a member of the CLCN5 family of voltage-gated chloride channels and transporters and the cause of Dent's disease (a nephrolithiasis disorder associated with hypercalciuria and low-molecular-weight proteinuria), can also cause phosphaturia. Clcn5-knockout mice have a defect of proximal tubular endocytosis that results in an increased luminal PTH concentration and stimulation of the apical PTH1R, causing increased endocytosis of NPT2a and activation of 1 α ,25-hydroxyvitamin D hydroxylase, which may result in increased 1,25-

dihydroxyvitamin D concentrations.¹⁴ Furthermore, alterations in fibroblast growth factor 23 (FGF-23) and klotho also regulate the sodium phosphate transporters, and gain-of-function mutations could cause hyperphosphaturia; however, the FGF-23–klotho complex is known to inhibit 1 α ,25-hydroxyvitamin D hydroxylase and, in the absence of an increased 1,25-dihydroxyvitamin D concentration, would not be expected to cause hypercalciuria or nephrolithiasis.¹⁵

Therefore, in addition to documenting the potential role of mutations of the renal sodium phosphate transporters, this study suggests that mutations in proteins interacting with sodium phosphate may also play an important role in renal phosphate leak and nephrolithiasis. This set of findings opens a new area in nephrolithiasis research for further investigations to unravel the causes of this interesting and important disorder.

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Figure 1. Postulated Mechanisms of Nephrolithiasis and Phosphate-Transport Inhibition

Panel A shows postulated mechanisms by which a primary renal phosphate leak could result in hypercalciuria and formation of renal stones. Panel B shows postulated mechanisms of how parathyroid hormone (PTH) signaling through the PTH type 1 receptor (PTH1R) in the apical brush-border membrane and basolateral membrane results in the phosphorylation of NHERF1, which leads to disassociation of NHERF1–NPT2a complexes and endocytosis (internalization) of apical NPT2a protein and inhibition of phosphate transport. The mechanisms of interactions between PTH and PDZ domain containing 1 protein (PDZK1) and of PTH-induced NPT2c endocytosis remain unknown. PKA denotes protein kinase A, and PKC protein kinase C.