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REGULATION OF PHOSPHORUS HOMEOSTASIS BY THE TYPE IIa Na/PHOSPHATE COTRANSPORTER

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■ **Abstract** The type IIa Na/phosphate (Pi) cotransporter (Npt2a) is expressed in the brush border membrane (BBM) of renal proximal tubular cells where the bulk of filtered Pi is reabsorbed. Disruption of the *Npt2a* gene in mice elicits hypophosphatemia, renal Pi wasting, and an 80% decrease in renal BBM Na/Pi cotransport, and led to the demonstration that Npt2a is the target for hormonal and dietary regulation of renal Pi reabsorption. Regulation is achieved by changes in BBM abundance of Npt2a protein and requires the interaction of Npt2a with various scaffolding and regulatory proteins. Molecular studies in patients with renal Pi wasting resulted in the identification of novel regulators of Pi homeostasis: fibroblast growth factor-23 (FGF-23) and a phosphate-regulating gene with homologies to endopeptidases on the X chromosome (PHEX). In mouse models, increased FGF-23 production or loss of Phex function causes hypophosphatemia and decreased renal Pi reabsorption, secondary to decreased BBM Npt2a protein abundance. Thus, Npt2a plays a major role in the maintenance of Pi homeostasis in both health and disease.

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INTRODUCTION

Inorganic phosphate (Pi) is fundamental to cellular function and skeletal mineralization. Pi is sufficiently abundant in the normal diet and Pi deficiency is unlikely to develop except under conditions of extreme starvation or as a consequence of administration of a class of therapeutic agents known as Pi binders. Normal Pi intake in the adult human is in the range of 800 to 1600 mg/day. Approximately 65% to 75% of ingested Pi is absorbed in the small intestine, regardless of the level of Pi intake, and hormonal regulation of this process plays only a minor role in normal Pi homeostasis. Absorbed Pi is eliminated or reabsorbed by the kidney, incorporated into organic forms in proliferating cells, and/or deposited as a component of bone mineral (hydroxyapatite). Bone deposition accounts for a much larger percentage of retained Pi during the growth period. However, even in the growing organism only a small percentage of dietary Pi is retained. Most of the absorbed Pi is excreted in the urine. This means that Pi homeostasis and plasma Pi concentration depend primarily on renal mechanisms that regulate tubular Pi transport. In addition, during times of severe dietary Pi deprivation, the Pi contained in bone provides the only source of Pi for the metabolic needs of the organism.

Pi accounts for approximately 1% of body weight (50). Approximately 85% of body Pi is in the skeleton and teeth, 15% in soft tissue, and the remainder (<1%) in extracellular fluid. Pi exists in the plasma in two forms: an organic form, consisting principally of phospholipids and phosphate esters, and an inorganic form (44). Of the total plasma Pi concentration, approximately 29% is in the inorganic form, and only this form of Pi is routinely measured in clinical settings. Of this, 10% to 15% is protein bound and the remainder, which is freely filtered by the renal glomerulus, exists either as "free" Pi ions or as Pi ions complexed with Na, Ca, or Mg. In principle, four forms of inorganic Pi are present in biological solutions: H_3PO_4, $H_2PO_4^-$, HPO_4^{2-}, PO_4^{3-}. However, only HPO_4^{2-} and $H_2PO_4^-$ are present at significant concentrations at physiological pH. The ratio of divalent to monovalent forms can be determined by the Henderson-Hasselbalch equation, $pH = pKa + \log (HPO_4^{2-}/H_2PO_4^-)$. The dissociation constant, pKa, for Pi is 6.8. Thus, at pH 7.4, the ratio of divalent to monovalent Pi anions is essentially 4:1.

In measuring plasma Pi and relating this value to a clinical situation, it is necessary to take into account the diurnal variation in plasma Pi concentration, with a nadir occurring at 9:30 to 10:00 AM and a peak at 4:00 AM (43). The change in Pi concentration from nadir to peak may be as much as 1 mg/dl, which represents a 25% to 35% change in plasma concentration. Plasma Pi concentration also varies as a function of age, with levels highest in the first three months of life in humans. Mice and rats have higher normal plasma Pi values than humans. This difference correlates with differences in basal metabolic rate, which is higher in both rodent species than in humans.

RENAL PHOSPHATE REABSORPTION

The kidney is a major regulator of Pi homeostasis by virtue of its ability to increase or decrease its Pi reabsorptive capacity to accommodate Pi need. Thus, considerable effort has been devoted to the study of Pi transport in the kidney (for reviews, see 12, 46–48, 65, 69).

Physiology and Tubular Localization

The proximal tubule is the major site of Pi reabsorption, with 60% to 80% of the filtered load reclaimed in the proximal convoluted tubule, 15% to 20% in the proximal straight tubule, and a small but variable portion (<10%) in more distal segments of the nephron.

Clearance studies in humans and experimental animals show that when the filtered load of Pi is progressively increased, Pi reabsorption increases until a maximum tubular reabsorptive rate for Pi, or TmP, is reached, after which urinary Pi excretion increases in proportion to the filtered load. The measurement of TmP varies among individuals and within the same individual, due in part to variation in glomerular filtration rate (GFR). Thus, the ratio, TmP/GFR, or maximum tubular reabsorption of Pi per unit of GFR, is the most reliable estimate of the overall tubular Pi reabsorptive capacity (8).

Cellular Aspects

Transepithelial Pi transport across the renal proximal tubular cell is essentially unidirectional and involves uptake across the apical brush border membrane (BBM), translocation across the cell, and efflux at the basolateral membrane. Pi uptake at the apical cell surface is the rate-limiting step in the overall Pi reabsorptive process and the major site of its regulation. It is mediated by Na^+-dependent Pi transporters that depend on the basolateral membrane-associated Na^+/K^+-ATPase to maintain the Na^+-gradient (outside > inside). Na/Pi cotransport is electrogenic and sensitive to changes in pH, with 10- to 20-fold increases documented when the pH is raised from 6 to 8.5. Little is known about the translocation of Pi across the cell or the mechanisms involved in the efflux of Pi at the basolateral cell surface.

Molecular Identification of Na/Phosphate Cotransporters

cDNAs encoding Na/Pi cotransporters, designated type I (Npt1, SLC17A1) and type IIa (Npt2a, SLC34A1; formerly type II, Npt2), were identified in mammalian kidney and share only 20% identity (42, 75). Renal expression of Npt1 and Npt2a is restricted to the proximal tubule where the bulk of filtered Pi is reabsorbed. Although both Npt1 and Npt2a mediate high-affinity Na/Pi cotransport, Npt1, which mediates the flux of Cl and organic anions as well as Pi (9), is not an important player in proximal tubular Pi flux. Npt2a-mediated transport is specific for Pi, is electrogenic, and has a pH profile that bears close resemblance to the pH dependence of Na/Pi cotransport across the renal BBM.

A Na/Pi cotransporter with homology to Npt2a, designated Npt2c, was also identified in mammalian kidney (51, 58). Npt2c is approximately one order of magnitude less abundant than Npt2a at the mRNA level and is expressed exclusively in the BBM of proximal tubular cells (51, 58). The relative abundance of Npt2c protein is significantly higher in kidneys of 22-day-old rats than in those of 60-day-old rats, which suggests that Npt2c is a growth-related renal Na/Pi cotransporter (58).

Two additional Na/Pi cotransporters, designated type III, have been detected in mammalian kidney and account for $<1\%$ of renal Na/Pi cotransporter mRNAs. Both are cell-surface viral receptors [gibbon ape leukemia virus (Glvr-1, Pit-1, SLC20A1) and murine amphotropic virus (Ram-1, Pit-2, SLC20A2)] that mediate high-affinity, electrogenic Na-dependent Pi transport (12, 30). Glvr-1 and Ram-1 are widely expressed in mammalian tissues and may serve as "housekeeping" Na/Pi cotransporters. Their precise membrane localization in kidney is not known.

Structure-Function Studies of Npt2a

There is considerable information on the structure of Npt2a (Figure 1). The analytical approaches used to arrive at this structure are summarized as follows: (*a*) hydrophobicity predictions (42); (*b*) antibody accessibility combined with epitope insertion (38); (*c*) cysteine insertion and accessibility of permeant and impermeant sulfhydryl reagents (36, 37); and (*d*) glycosylation (20). These studies demonstrated several important features of Npt2a (Figure 1). The transporter has a large extracellular loop that separates it into two domains (20, 42). There is intramolecular homology within the ICL-1 and ECL-3 domains (36) and both the NH_2- and COOH-termini are oriented intracellularly (38; Figure 1).

The studies described above also addressed the functional significance of specific domains of Npt2a (Figure 1). Cysteine-insertion studies suggested that ICL-1 and ECL-3 comprise an important part of a "permeation pore" that participates in both "cotransport" and "Na^+ leak" function (36, 37). Chimera construction—based on different transport properties of Npt2a and NPT2b, which is not expressed in kidney (22, 42)—suggested the involvement of three amino acid residues in determining the pH dependence of Npt2a, namely, increased transport at higher pH

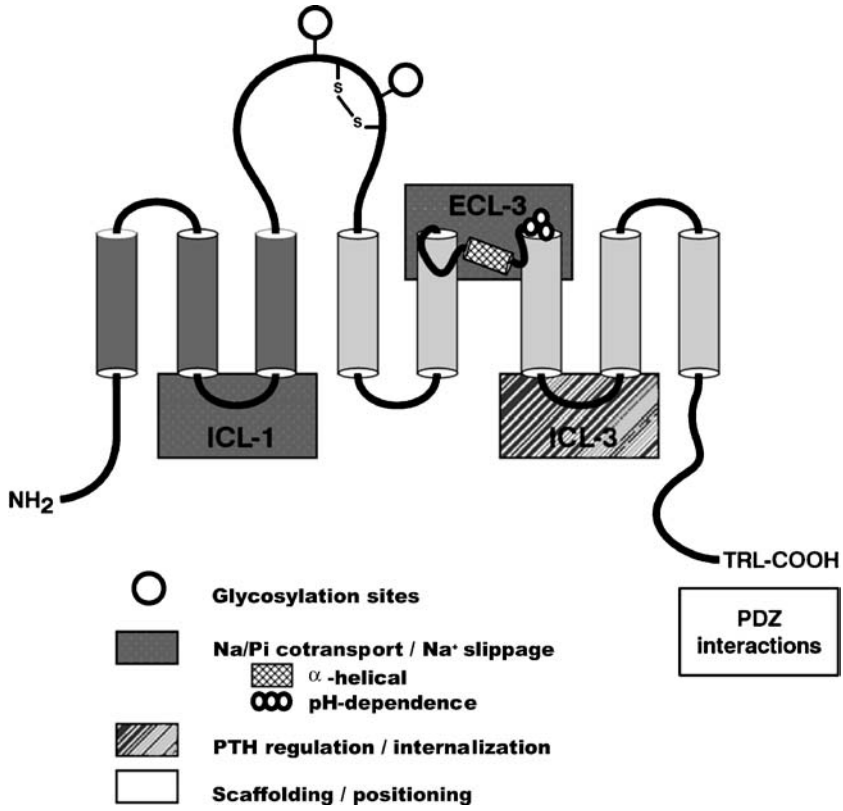

Figure 1 Structure-function relationship of the renal NPT2a Na/Pi cotransporter. See text for details. Reprinted with permission from Reference 69. PDZ, postsynaptic density-95/discs-large/zona occludens-1; PTH, parathyroid hormone.

(13, 14). The chimera approach also suggested the importance of two basic amino acid residues in ICL-3 for parathyroid hormone (PTH)-dependent internalization (28) (see "Parathyroid Hormone" section below). Deletion studies documented that the COOH-terminus contains recognition sequences that play an important role in Npt2a trafficking in response to factors that stimulate or inhibit renal Pi transport (29).

Both the NH_2- and COOH-terminal portions of Npt2a are required for transport activity. However, cleavage of the Npt2a protein backbone, between the two glycosylation sites in the large extracellular loop, does not interfere with transport function (15, 34). It is assumed that under this condition a disulfide bridge within this large extracellular loop stabilizes the transporter (Figure 1). Although Npt2a appears to be part of a multimeric complex (see below), one transporter unit is sufficient to mediate Na/Pi cotransport (35).

REGULATION OF Npt2a AND MOLECULAR MECHANISMS

Regulation of renal Pi reabsorption is achieved primarily by alterations in the abundance of Npt2a protein in the BBM of proximal tubular cells and occurs largely in the absence of changes in Npt2a mRNA (for review, see 47). Variation in apical Npt2a protein amount is accomplished by either membrane insertion or retrieval/lysosomal degradation of the transporter. Although membrane trafficking of Npt2a is a key element in the regulation of renal Pi handling, little is known about the molecular signals that confer specificity to this process, i.e., why other transporters are not inserted into or retrieved from the membrane along with Npt2a. Scaffolding proteins such as PDZK1 (NaPi-Cap1, diphor-1) and NHERF-1 have been shown to interact with Npt2a (16, 17) and play a key role in the maintenance of Npt2a polarity and the formation of regulatory complexes necessary for Npt2a trafficking in response to factors that stimulate or inhibit renal Pi transport (7, 21).

Dietary Phosphate Intake

Dietary Pi intake is a key determinant of renal Pi handling. Both acute and chronic Pi deprivation induce increases in BBM Na/Pi cotransport V_{max} and Npt2a protein abundance but not in Npt2a mRNA (53). The response to Pi deprivation is mediated by microtubule-dependent recruitment of existing Npt2a protein to the apical membrane (41). In contrast, exposure to high dietary Pi intake leads to the internalization of cell surface Npt2a protein into the endosomal compartment by a microtubule-independent mechanism (41). Internalized Npt2a protein is then delivered to the lysosome, by a microtubule-dependent process, for degradation (53).

Parathyroid Hormone

PTH acts directly on proximal tubular cells and inhibits both apical and basolateral Na/Pi cotransport by mechanisms that involve the internalization of cell surface Npt2a protein (31) and its subsequent lysosomal degradation (54). PTH binding to receptors on the basolateral membrane activates protein kinase A (PKA) and/or protein kinase C (PKC) signaling pathways, whereas PTH binding to apical receptors activates PKC (73). The PKA and PKC signaling pathways converge on the extracellular signal-regulated kinase/mitogen-activated protein kinase (ERK/MAPK) pathway to internalize Npt2a protein (3). Although the downstream targets for ERK/MAPK-mediated phosphorylation remain unknown, changes in the phosphorylation state of Npt2a are not associated with its PTH-induced internalization (25). Rather, regulation of Na/Pi cotransport by PTH may be achieved by the phosphorylation of proteins that associate with Npt2a.

AKAP79, an A kinase anchoring protein (33), and RAP, a receptor-associated protein (2), participate in the PTH-mediated retrieval of Npt2a from the plasma

membrane of proximal tubular cells. In opossum kidney (OK) cells, AKAP79 associates with Npt2a and the regulatory and catalytic subunits of PKA, and this process is necessary for PKA-dependent inhibition of Na/Pi cotransport (33). In RAP-deficient mice, PTH-induced internalization of Npt2a is significantly delayed (2).

Other Hormonal and Novel Regulators

Other hormones also contribute to the regulation of proximal tubular Pi transport. Growth hormone, insulin-like growth factor-I, insulin, 1,25-dihydroxyvitamin D_3 [$1,25(OH)_2D$], thyroid hormone, and stanniocalcin all stimulate Pi reabsorption, whereas PTH-related peptide, calcitonin, atrial natriuretic factor, epidermal growth factor, transforming growth factor-α, and glucocorticoids inhibit Pi reclamation (for review, see 47). Although there is some evidence that the actions of $1,25(OH)_2D$, thyroid hormone, and dexamethasone may occur via alterations in *Npt2a* gene transcription, hormonal regulation of renal Pi reabsorption is accomplished for the most part by changes in Npt2a protein abundance without corresponding changes in Npt2a mRNA.

Fibroblast growth factor (FGF)-23 is a novel secreted peptide that regulates renal Pi handling. It is produced by tumors from patients with oncogenic hypophosphatemic osteomalacia (OHO, or tumor-induced osteomalacia) (61), and mutations in the corresponding gene were identified in patients with autosomal dominant hypophosphatemic rickets (ADHR) (1) (see "Disorders of FGF-23 Production and Processing" section below). Administration of FGF-23 to mice elicits significant decreases in serum Pi, renal Pi reabsorption (61), and renal BBM Npt2a protein abundance (59). The hypophosphatemic effect of FGF-23 is independent of PTH (59) and robust in transgenic mice expressing the human *FGF23* gene (39). Targeted disruption of the *Fgf23* gene in mice leads to increases in serum Pi, TmP/GFR, and renal BBM Npt2a protein abundance (60). Thus, FGF-23 is an important regulator of Pi homeostasis.

Secreted frizzled protein-4 (sFRP-4), like FGF-23, is highly expressed in OHO tumors and, when infused in rats, induces an increase in urinary Pi excretion and hypophosphatemia (6). sFRP-4 also inhibits Na/Pi cotransport in OK cell cultures (6).

DISRUPTION OF THE *Npt2a* GENE IN MICE

Given that Npt2a mediates Na/Pi cotransport that is regulated by dietary Pi and PTH, major regulators of renal Pi handling, and is abundantly expressed in the nephron segment where the bulk of filtered Pi is reabsorbed, it was of interest to define its precise contribution to the overall maintenance of Pi homeostasis. To accomplish this goal and determine the impact of Npt2a on skeletal mineralization, the *Npt2a* gene was cloned and characterized (19), and inactivated in mice by targeted mutagenesis (4).

Effect of *Npt2a* Gene Disruption on Clinical and Biochemical Phenotype

Mice homozygous for the disrupted *Npt2a* gene ($Npt2a^{-/-}$) are viable, fertile, and do not display any gross physical or behavioral abnormalities. The mutants exhibit increased urinary Pi excretion, an ~80% decrease in renal BBM Na/Pi cotransport, and hypophosphatemia (4). Npt2c is upregulated in $Npt2a^{-/-}$ mice and likely accounts for residual renal BBM Na/Pi cotransport in the mutants (64).

$Npt2a^{-/-}$ Mice Fail to Respond to Major Regulators of Renal Pi Transport

PTH had no effect on serum Pi concentration, fractional Pi excretion, and BBM Na/Pi cotransport in $Npt2a^{-/-}$ mice, whereas the expected changes in these parameters were elicited in wild-type littermates (78). PTH did elicit a significant increase in urine cAMP in $Npt2a^{-/-}$ mice, which indicates that the absence of the PTH response could not be attributed to generalized PTH resistance (78). Moreover, appropriate PTH responses were elicited in Pi-depleted wild-type mice, a finding which suggests that Pi deficiency per se was not responsible for PTH resistance in the mutants (78). $Npt2a^{-/-}$ mice also failed to exhibit an adaptive increase in BBM Na/Pi cotransport in response to Pi restriction and to show an age-related decrease in BBM Na/Pi cotransport (23). These findings provide compelling evidence that Npt2a is the target for regulation of renal Pi reabsorption by PTH and dietary Pi and is responsible for the ontogenic pattern of renal Pi handling.

Npt2a Heterozygotes

We reported that urine Pi/creatinine and fractional Pi excretion are modestly increased in mice heterozygous for the disrupted *Npt2a* gene ($Npt2a^{+/-}$), relative to wild-type littermates (4). However, these differences were not apparent in mice backcrossed to C57Bl/6J mice for nine generations (Table 1). $Npt2a^{+/-}$ mice exhibit a 50% decrease in renal *Npt2a* mRNA abundance, relative to wild-type littermates, whereas both renal BBM Npt2a protein abundance and Na/Pi cotransport are normal in heterozygotes (Table 1), as reported previously (4). These findings indicate that $Npt2a^{+/-}$ mice exhibit appropriate adaptive upregulation of Npt2a protein in response to the loss of one *Npt2a* allele. In light of these results, the effects of Pi deprivation were compared in wild-type and $Npt2a^{+/-}$ mice. A low-Pi diet elicits significant decreases in serum Pi, urine Pi/creatinine, and fractional Pi excretion, and significant increases in BBM Na/Pi cotransport and Npt2a protein abundance in both groups of mice (Table 1). However, the adaptive BBM responses to Pi restriction are blunted in heterozygotes relative to wild-type littermates (Table 1), consistent with the loss of one *Npt2a* allele. Npt2a mRNA is not increased by Pi deprivation in either wild-type or $Npt2a^{+/-}$ mice, confirming that post-transcriptional mechanisms mediate the adaptive response.

TABLE 1 Effect of one copy of the disrupted *Npt2a* gene and dietary Pi intake on serum and urine Pi and renal Na/Pi cotransport and *Npt2a* gene expression

	Wild-type mice		$Npt2a^{+/-}$ mice	
Genotype diet	**1% Pi**	**0.02% Pi**	**1% Pi**	**0.02% Pi**
Serum Pi (mM)	2.5 ± 1.3	1.3 ± 0.2[a]	2.6 ± 0.3	1.4 ± 0.3[a]
Urine Pi/Cr	34 ± 7	0.5 ± 0.2[a]	36 ± 4	0.7 ± 0.7[a]
FEI-Pi	15 ± 3	0.4 ± 0.2[a]	15 ± 4	0.5 ± 0.2[a]
Npt2a mRNA (%)	100 ± 13	76 ± 23	47 ± 5[b]	59 ± 8
Npt2a protein (%)	100 ± 16	1727 ± 226[a]	108 ± 17	808 ± 76[a,b]
Na/Pi cotransport (%)	100 ± 6	412 ± 36[a]	102 ± 19	274 ± 43[a,b]

Wild-type (+/+) and $Npt2a^{+/-}$ (+/−) mice (70 ± 10 days of age, n = 5–8 mice/group) were fed diets containing 1% and 0.02% Pi for five days and serum, urine, and renal parameters determined as described in text (4).

[a]Effect of diet.

[b]Effect of genotype, $p < 0.05$.

Abbreviations: Pi, inorganic phosphate; Cr, creatinine; FEI, fractional excretion index; NPT2a, type IIa Na/phosphate cotransporter.

CALCIUM HOMEOSTASIS IN $Npt2a^{-/-}$ MICE

The serum concentration of $1,25(OH)_2D$ and the serum and urine concentrations of Ca are significantly increased in $Npt2a^{-/-}$ mice when compared with wild-type littermates (4). To define the mechanisms responsible for these increases, the effects of *Npt2a* gene ablation on intestinal Ca absorption and renal vitamin D metabolism were examined.

Intestinal Calcium Hyperabsorption

The observation that hypercalcemia and hypercalciuria are corrected in $Npt2a^{-/-}$ mice after an overnight fast suggested that dietary Ca intake is necessary for these abnormalities to manifest (63). Subsequent studies revealed that both absorption of ^{45}Ca by isolated duodenal loops and ^{45}Ca appearance in the plasma are increased in $Npt2a^{-/-}$ mice relative to wild-type littermates (63). In addition, the increase in duodenal Ca absorption in $Npt2a^{-/-}$ mice is associated with significant increases in the duodenal expression of ECaC1 (TRPV5) and ECaC2 (TRPV6), thereby implicating both epithelial Ca channels in mediating the intestinal response (63).

Upregulation of Renal 1,25-Dihydroxyvitamin D Synthesis

The elevated serum concentration of $1,25(OH)_2D$ in $Npt2a^{-/-}$ mice suggested that the mutants can increase their renal synthesis of the vitamin D hormone in response to endogenous hypophosphatemia (4). This notion was confirmed by the demonstration of increased activity and mRNA abundance of renal mitochondrial

1α-hydroxylase, the enzyme responsible for the biosynthesis of 1,25$(OH)_2$D (68). In addition, the mutants exhibit a decrease in the mRNA abundance of renal 24-hydroxylase (68), the enzyme responsible for catabolism of 1,25$(OH)_2$D to its final inactivation product. Furthermore, *Npt2a*$^{-/-}$ mice mounted a further increase in renal 1α-hydroxylase activity and mRNA and serum 1,25$(OH)_2$D concentration in response to dietary Pi restriction (68). Therefore, contrary to a previously held notion, normal renal Na/Pi cotransport is not necessary for the regulation of renal 1,25$(OH)_2$D production by Pi.

Thus, hypophosphatemia, secondary to targeted disruption of the *Npt2a* gene, serves as a trigger for increased renal synthesis and decreased renal catabolism of 1,25$(OH)_2$D. This results in increased serum 1,25$(OH)_2$D levels, intestinal Ca hyperabsorption, increased intestinal expression of ECaC1 and ECaC2, and the development of hypercalcemia and hypercalciuria in the mutant strain. Reduced serum PTH levels, secondary to increased serum 1,25$(OH)_2$D, also contribute to hypercalciuria in *Npt2a*$^{-/-}$ mice (4).

Renal Calcification

Because hypercalciuria is a risk factor for the development of nephrocalcinosis, kidneys from wild-type and knockout mice were examined for renal calcification (11). Von Kossa staining of renal sections and microcomputed tomography of intact kidneys revealed mineral deposits, composed of Ca and Pi, in kidneys of *Npt2a*$^{-/-}$ mice but not in wild-type littermates (11) or in *Npt2a* heterozygotes (unpublished findings). Pi supplementation or 1α-hydroxylase gene ablation reduced urine Ca/creatinine and renal calcification in *Npt2a*$^{-/-}$ mice (67). Furthermore, both treatments were associated with significant decreases in serum 1,25$(OH)_2$D levels, attesting to a key role of the vitamin D hormone in the development of nephrocalcinosis in *Npt2a*$^{-/-}$ mice (67).

Npt2a IN BONE

Npt2a Expression and Protein Interactions in the Osteoclast

Npt2a mRNA and protein were detected in osteoclasts, the cells mediating bone resorption (18, 32), but not in osteoblasts, the cells responsible for bone formation. Furthermore, Npt2a in mouse osteoclasts interacts with NHERF-1, a PDZ protein that is essential for Npt2a trafficking in kidney (see "Regulation of Npt2a and Molecular Mechanisms" section above), and colocalizes with NHERF-1 and actin at the osteoclast plasma membrane (32). These findings suggest that similar mechanisms are involved in Npt2a membrane sorting and regulation in both tissues.

Skeletal Phenotype in *Npt2a*$^{-/-}$ Mice

Npt2a gene disruption is associated with age-dependent skeletal abnormalities (4). At weaning, *Npt2a*$^{-/-}$ mice exhibit retarded secondary ossification, increased

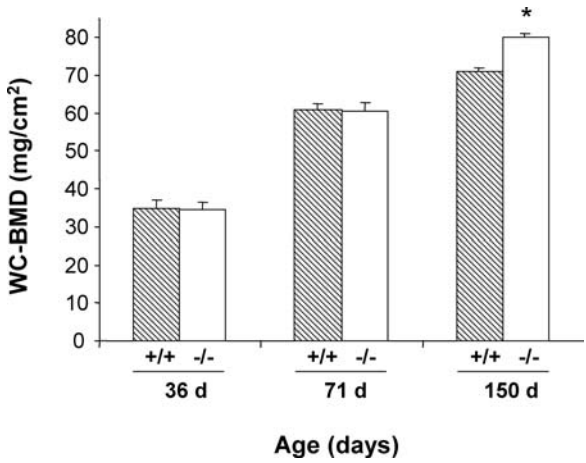

Figure 2 Effect of the *Npt2a* gene knockout on longitudinal bone mineral density. Bone mineral density was determined in vivo on both femora in wild-type (+/+) and *Npt2a*$^{-/-}$ mice at 36, 71, and 150 days of age. Weight-corrected bone mineral density (WC-BMD) was calculated by regression residuals as described by Orwoll et al. (52). Means $\pm$ SD for each 10–12 mice per group are shown. *$P < 0.05$.

trabecular thickness, and a reduction in osteoclast number (4, 18). With increasing age, the decrease in osteoclast number is less pronounced and indices of bone formation are increased in the mutants, relative to wild-type littermates (18). These studies provide evidence for osteoclast dysfunction and osteoblast adaptation in Npt2a-deficient mice, and demonstrate that both the osteoclast and the proximal tubule must be considered in the role of Npt2a on Pi homeostasis.

Based on the above findings (18, 32), we hypothesized that bone resorption is compromised and bone mineral density increased in the mutants. A longitudinal study revealed that bone mineral density is significantly increased in 150-day-old *Npt2a*$^{-/-}$ mice when compared with age-matched wild-type mice (Figure 2, unpublished data, H.S. Tenenhouse). Further work is necessary to establish whether loss of Npt2a function protects the skeleton from age-related bone loss.

Npt2a IN PHOSPHATE-DEFICIENCY DISORDERS

Disorders of FGF-23 Production and Processing

One acquired (OHO) and two Mendelian [ADHR and X-linked hypophosphatemia (XLH)] hypophosphatemic bone disorders are characterized by hypophosphatemia, decreased renal Pi reabsorption, normal-to-low serum $1,25(OH)_2D$ concentrations that are inappropriate for the degree of hypophosphatemia, and rickets and osteomalacia (66). Based on these similarities and the studies described below,

it has been hypothesized that defects in a common metabolic pathway involving FGF-23 can explain the underlying basis for renal Pi wasting in all three disorders.

Excision of tumors from OHO patients corrects the clinical and biochemical abnormalities, which indicates that factors secreted by the tumor are responsible for the phenotypic features that characterize this disorder (for review, see 57). Molecular analysis of OHO tumors led to the demonstration of abundant expression of FGF-23 (61) (see "Other Hormonal and Novel Regulators" section above). In addition, serum FGF-23 concentrations are markedly elevated in OHO patients (27). These findings support the hypothesis that renal Pi wasting in OHO can be attributed to excess tumor production of FGF-23 and is likely associated with decreased renal BBM NPT2a abundance, as reported in mice (39).

Missense mutations in the *FGF23* gene were identified in patients with ADHR (1). The mutations involve two Arg residues that reside in a consensus proprotein convertase cleavage site, RHTR (ArgHisThrArg), and prevent the processing of the secreted, 227 amino acid FGF-23 peptide to its 155 amino acid N-terminal and 72 amino acid C-terminal fragments (76). The mutant FGF-23 peptide retains full phosphaturic activity, whereas neither the N- nor C-terminal fragments derived from the wild-type peptide exhibit phosphaturic activity (62). Because the arg mutations stabilize the full-length FGF-23 peptide, it is likely that in ADHR the mutant peptide is responsible for Pi wasting by decreasing the BBM abundance of NPT2a protein. Serum concentrations of FGF-23 have not yet been reported in ADHR patients.

XLH is caused by mutations in *PHEX*, a Pi-regulating gene with homology to endopeptidases on the X-chromosome (24), which is expressed in bones and teeth but not in kidney (56). In *Hyp* mice, which harbor a large 3′ deletion in the *Phex* gene (5), renal Pi wasting has been attributed to a 50% decrease in renal BBM Na/Pi cotransport and Npt2a protein abundance (70). Furthermore, parabiosis (45) and renal transplantation (49) studies in *Hyp* mice demonstrated that a circulating factor(s) is responsible for hypophosphatemia and decreased renal Pi reabsorption in the mutants. In this regard, it is of interest that serum FGF-23 levels are more than tenfold higher in *Hyp* mice (77) and significantly elevated in XLH patients (27), relative to normal counterparts. However, the mechanisms whereby loss of Phex/PHEX function leads to FGF-23 accumulation are unknown. Both decreases in FGF-23 degradation (10) and increases in FGF-23 synthesis (40) have been proposed.

Hereditary Hypophosphatemic Rickets with Hypercalciuria

Hereditary hypophosphatemic rickets with hypercalciuria (HHRH) is a Pi wasting disorder first described in a large Bedouin kindred (66, 71, 72). Although the biochemical features of HHRH patients closely resemble those of *Npt2a*$^{-/-}$ mice

and clinical studies suggest that HHRH is a primary renal Pi wasting disorder (71), putative disease-causing mutations in the *NPT2a* gene were not identified in patients with this disorder (26). Furthermore, two single-nucleotide polymorphisms in the *NPT2a* gene failed to segregate with HHRH in the Bedouin kindred (26). These studies excluded *NPT2a* as a candidate gene and suggested that another Na/Pi cotransporter or a regulator thereof is responsible for HHRH.

Heterozygous *Npt2a* Mutations

Heterozygous missense mutations in the *NPT2a* gene were identified in two individuals with renal Pi wasting and hypophosphatemia, associated with either urolithiasis or osteoporosis (55). Although it was suggested that each amino acid substitution was sufficient for the expression of the respective phenotypic abnormalities (55), functional studies of the mutant *NPT2a* cDNAs in Xenopus oocytes failed to provide evidence for altered kinetics or dominant negative effects (74). The latter findings and the demonstration that BBM Na/Pi cotransport and Npt2a protein abundance are normal in mice heterozygous for the disrupted *Npt2a* gene (Table 1) (4) indicate that the heterozygous mutations in *NPT2a* cannot account for renal Pi wasting and hypophosphatemia in the patients studied (55).

SUMMARY AND CONCLUSIONS

Npt2a resides in the BBM of renal proximal tubular cells and plays a crucial role in the maintenance of Pi homeostasis. Npt2a is a target for regulation by PTH and dietary Pi, which elicit changes in Npt2a protein abundance in the BBM and require the interaction of Npt2a with a variety of scaffolding and regulatory proteins essential for its membrane insertion, retrieval, and degradation. Targeted disruption of the *Npt2a* gene in mice underscored the importance of Npt2a in the regulation of renal Pi reabsorption and maintenance of Pi balance, and suggested a role for Npt2a in osteoclast function. Two novel Pi regulating genes, *FGF23* and *PHEX*, which are mutated in patients with Mendelian Pi wasting disorders, have been identified and studies in mouse models demonstrated that increased circulating levels of FGF-23 or loss of Phex function induces hypophosphatemia by decreasing the renal BBM abundance of Npt2a protein. Future studies are necessary to uncover additional renal Na/Pi cotransporters, and regulators thereof, and to define the precise mechanisms whereby FGF-23 and PHEX regulate renal Pi handling.

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