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Recent Advances in Renal Phosphate Handling

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Abstract

Phosphate is critical for the maintenance of skeletal integrity, is a necessary component of important biomolecules, and is central to signal transduction and cell metabolism. It is becoming clear that endocrine communication occurs between the skeleton, kidney, and the intestine to maintain proper serum phosphate concentrations, with the kidney being the primary site for minute-to-minute regulation. Identification of genetic alterations in Mendelian disorders of hypo- and hyperphosphatemia has led to the isolation of novel genes and new roles for existing proteins in the control of renal phosphate handling, such as Fibroblast growth factor-23 (FGF23) and its processing systems, the co-receptor α -Klotho (KL), and phosphate transporters. Recent findings also indicate that FGF23 has feedback mechanisms intertwined with parathyroid hormone (PTH) and vitamin D that control phosphate homeostasis. This review will highlight genetic, and *in vitro* and *in vivo* findings, and discuss how these clinical and experimental discoveries have uncovered novel aspects of renal phosphate handling, as well as opened new research and therapeutic avenues.

Keywords

hypophosphatemia; tumoral calcinosis; Klotho; tumor induced osteomalacia; ADHR; XLH; ARHR; hyperphosphatemia; Fibroblast growth factor-23; FGF23; PTH; vitamin D

Introduction

Serum phosphate levels are regulated by complex processes involving the intestine, skeleton, and the kidneys. Maintaining serum phosphate levels is critical for proper bone development and for skeletal integrity. Additionally, phosphorus is a necessary component of DNA and RNA, and is essential for cellular metabolism as an energy source in the form of ATP¹. Phosphate is abundant in the diet, and intestinal absorption is efficiently regulated¹. However, the mechanisms of renal phosphate regulation, the most critical organ system for maintaining short-term serum phosphate concentrations, are incompletely understood. Investigating the molecular etiology of Mendelian disorders characterized by disturbed renal ion homeostasis has been instrumental in identifying new regulators which guide kidney phosphate handling.

This review will address major factors in renal phosphate metabolism, including Fibroblast growth factor-23 (FGF23), parathyroid hormone (PTH), and vitamin D, as well as disorders of hypo- and hyperphosphatemia that have identified novel systems important for phosphate

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balance. Recent advances in a novel intra-renal signaling axis upstream of phosphate reabsorption will also be discussed.

Phosphate Regulation

Phosphate, which composes approximately 1% of total body weight, is widely distributed in the soft tissues of the body, both in inorganic form and as a component of organic molecules, including nucleic acids and membrane phospholipids, as well as other phosphoproteins. However, these non-osseous phosphates comprise less than 20% of the total body content. The remainder is stored in the bone matrix. In humans, the average phosphate intake in an adult is 800–1600 mg/day², and this large range is primarily due to the high phosphate levels in Western diets. The normal range for serum phosphate in adults is 2.5–4.5 mg/dl³, and in children is higher and varies with age (the younger the child, the higher the phosphate)⁴.

Phosphate taken in through the diet is initially absorbed by the small intestine through an active, sodium-dependent process and a passive, diffusional process that is load-dependent⁵. The Type II sodium-phosphate co-transporters are responsible for the majority of physiological phosphate transport. There are three members of this family, NPT2a (SLC34A1), NPT2b (SLC34A2), and NPT2c (SLC34A3). NPT2a is primarily expressed in the apical brush border membrane of the kidney proximal tubule and is central to renal phosphate reabsorption. NPT2b is primarily expressed in the small intestine and is regulated by vitamin D. NPT2b has a low affinity for phosphate, and humans with NPT2b inactivating mutations do not have a phosphate phenotype⁶. However, a conditional-null Npt2b mouse model demonstrated this transporter has a primary role in active intestinal phosphate absorption⁷. Interestingly, deletion of Npt2b resulted in an increase in renal proximal tubule Npt2a expression, and the animals showed normal serum phosphate concentrations, most likely due to compensatory renal reabsorption. Thus changes in intestinal phosphate absorption may affect renal phosphate handling, perhaps indirectly through alterations in serum phosphate concentrations, or potentially through the production of intestinally-derived circulating peptides⁸. These findings indicate that intestinal phosphate absorption may have important dynamics that are yet to be uncovered.

The skeleton represents the largest reservoir of phosphate, primarily complexed with calcium in the form of hydroxyapatite crystals, which constitute the main inorganic component of the mineralized bone matrix. The majority of phosphate retained in the body is deposited in bone as both crystalline hydroxyapatite, which constitutes 85% of bone, and amorphous calcium phosphate that makes up the remaining 15%⁹. As serum phosphate decreases, it is resorbed from bone through the activity of PTH and vitamin D¹⁰.

The major organ regulating minute-to-minute phosphate homeostasis is the kidney, and approximately 70% of filtered phosphate is reabsorbed within the proximal tubule where NPT2a and NPT2c are localized². Phosphate transport across the renal proximal tubular cell is largely unidirectional and involves uptake across the brush border membrane, translocation across the cell, and efflux at the basolateral membrane. The rate-limiting step in the overall reabsorptive process is phosphate uptake at the cell surface, and consequently is the major site of its regulation¹. Npt2a, and to a lesser extent, Npt2c, actively transport phosphate from the phosphate-rich nephron lumen into proximal tubule cells. In mice, Npt2a is responsible for approximately 70% of active phosphate transport¹¹ with Npt2c likely compromising the remaining 30%. Npt2c is expressed in the renal proximal tubule brush border membrane, with a more restricted expressional pattern than Npt2a, and this transporter was originally thought to primarily play a role in neonatal phosphate balance in

mice¹². In humans, NPT2c may play a larger role in kidney phosphate reabsorption as inactivating mutations in this gene lead to a hypophosphatemic syndrome^{13, 14} (see below).

Control of Renal Phosphate Reabsorption: PTH

The primary function of PTH is to regulate serum calcium concentrations. In this regard, hypocalcemia stimulates the parathyroid glands to produce and release PTH. PTH increases the expression of the proximal tubule 25(OH) vitamin D 1- α hydroxylase¹⁵, the enzyme that produces the active form of vitamin D, 1,25(OH)₂ vitamin D (1,25(OH)₂D), and increases calcium reabsorption in the renal distal convoluted tubule (DCT). PTH also stimulates the release of calcium from bones into the extracellular fluid by increasing osteoclastic bone resorption¹⁶. In addition to its effects on calcium, PTH is one of the best characterized hormonal regulators of serum phosphate concentrations. Following PTH delivery *in vitro* or *in vivo*, NPT2a protein expression in the proximal tubule apical membrane is reduced (Figure 1)¹⁷. This effect results from relatively rapid internalization of Npt2a and Npt2c proteins and to subsequent degradation^{18, 19}, independent of transcriptional control of the co-transporters²⁰. These effects are mirrored in patients with defects in PTH regulation, as patients with hyperparathyroidism develop renal phosphate wasting, and those with hypoparathyroidism have increased renal phosphate reabsorption. To regulate sodium-phosphate co-transporter expression in the apical membrane of proximal tubule cells, PTH signals through the type 1 PTH receptor (PTHr1) via PKA and PKC, as well as through the MAPK pathway^{21, 22} to control internalization and degradation.

1,25(OH)₂D regulates serum phosphate concentrations by increasing intestinal calcium and phosphate absorption, and at high concentrations, increasing phosphate mobilization from bone through increased osteoclast activity²³. The opposing effects of PTH and vitamin D on the kidney and the intestine, respectively, keep phosphate levels in balance while preserving calcium ion homeostasis²⁴. Recent findings indicate that both PTH and vitamin D production could be influenced by FGF23 in negative feedback loops (Figure 1), adding further complexity to this regulatory system (see below).

Fibroblast growth factor-23

FGF23, identified as the causative gene in autosomal dominant hypophosphatemic rickets (ADHR)²⁵, plays a central role in phosphate regulation²⁶. The *FGF23* gene, composed of three exons encoding a 251 residue polypeptide, is located on the human chromosome 12p13²⁵. Although FGF23 mRNA can be detected at low levels in many tissues including heart, liver, thyroid/parathyroid, and small intestine, it is predominantly expressed in bone²⁵ by osteoblasts, osteocytes, flattened bone lining cells, and osteoprogenitor cells²⁷.

Full-length FGF23 (32 kD) is the biologically active form of the protein which can be cleaved into 20 and 12 kDa fragments. The N-terminal region of FGF23 contains a conserved FGF-homology domain, whereas the C-terminus comprises a unique 72-amino acid tail²⁸. Intracellular proteolysis and inactivation of FGF23 occurs at the subtilisin-like proprotein convertase (SPC) site R₁₇₆HTR₁₇₉/S₁₈₀, (RXXR/S motif) that separates the FGF-like domain from the C-terminal tail^{25, 28}. The FGF23 ADHR mutations, R176Q, R179Q, and R179W, destroy this site^{25, 29} and stabilize the full-length active form of the protein. In support of these genetic observations, when full-length FGF23, the N-terminal fragment, or the C-terminal fragment is delivered *in vivo*, only the intact hormone causes a reduction in serum phosphate concentrations²⁸. Interestingly, recent evidence supports that the 72-residue C-terminal fragment of FGF23 is able to bind to an FGFR1-KL signaling complex *in vitro*, and competitively decrease FGF23 bioactivity *in vitro* and *in vivo*³⁰.

Regulation of FGF23 and *in vivo* renal bioactivity

In mice, increased dietary phosphate increases serum phosphate concentrations and circulating FGF23, and the reciprocal relationship is present for low phosphate diets³¹. These findings have also been reported in some human studies, but the effect of phosphate on FGF23 appears to be less robust than in mice^{32–34}. Whether phosphate has direct effects on the skeleton to control FGF23 production and secretion is currently unknown.

Similar to PTH, FGF23 functions to reduce phosphate reabsorption in the proximal tubule. However, in the converse manner to PTH, FGF23 reduces expression of the renal vitamin D 1- α -hydroxylase and increases expression of the catabolic 25(OH)D-24-hydroxylase, thus decreasing circulating 1,25(OH)₂D concentrations (Figure 1)³⁵. The transgenic over-expression of FGF23 results in marked hypophosphatemia^{36, 37} due to renal phosphate wasting through the down-regulation of Npt2a and Npt2c^{36, 37}. As expected, the FGF23 transgenic mice have rickets/osteomalacia^{36, 37}, similar to patients with ADHR, X-linked hypophosphatemia (XLH), and tumor-induced osteomalacia (TIO). In disorders of elevated FGF23 such as ADHR and XLH, the serum vitamin D concentrations are referred to as ‘inappropriately low or normal,’ as the physiological response to the prevailing hypophosphatemia should be an increase in 1,25(OH)₂D. Collectively, these observations are consistent with a negative feedback process between kidney and bone as 1,25(OH)₂D stimulates FGF23 promoter activity *in vitro*^{38, 39} and production *in vivo*⁴⁰ (Figure 1).

The *Fgf23*-null mouse has the reciprocal phosphate phenotype to the FGF23 transgenic mice, with severe hyperphosphatemia and elevated 1,25(OH)₂D resulting in soft tissue calcifications, growth retardation, and abnormal bone mineralization^{41, 42}. A reduction in FGF23 bioactivity leads to increased circulating 1,25(OH)₂D (Figure 1) most likely through release of 1- α -hydroxylase suppression⁴³ (Figure 1), as observed in both *Fgf23*-null mice, mice null for the FGF23 co-receptor α -*Klotho* (*KL*)^{41, 42, 44}, and patients with hyperphosphatemic tumoral calcinosis (TC)^{45–47}. Hyperphosphatemia is also observed in the *Galnt3*-null mouse, a model of familial TC⁴⁸, which arises due to altered glycosylation and increased degradation of FGF23⁴⁹. Confirming the relationship between FGF23 and vitamin D, mating the *Fgf23*-null or *KL*-null to the 1- α -hydroxylase (*Cyp27B1*)-null or *VDR*-null mice results in a reversal of the hyperphosphatemia associated with genomic loss of FGF23 and *KL*^{43, 50}. Thus, the pathogenesis observed in the *Fgf23*- and *KL*-null mice may largely be due to the elevated 1,25(OH)₂D concentrations.

Recently, other minerals, such as iron, have been implicated to alter FGF23 expression in humans, which then results in osteomalacia⁵¹. It was reported that some patients receiving parenteral iron infusions became hypophosphatemic with decreased 1,25(OH)₂D^{52, 53}. Case reports have since demonstrated that these patients significantly increased serum FGF23 in response to this treatment^{54, 55}. Although a striking relationship between iron infusion and elevated FGF23 is present, the mechanisms underlying these observations are currently unknown.

Box 1. Mediators of FGF23 expression

- High serum/dietary phosphate: increase
- 1,25(OH)₂D: increase
- PTH: increase
- Iron infusion: increase
- FGFs/Wnt activity: increase and/or decrease?

Renal FGF23 bioactivity and associations with α -Klotho

FGF23 binds to a signaling complex composed of the co-receptor α -Klotho (KL) and a fibroblast growth factor receptor (FGFR) for bioactivity⁵⁶. KL is expressed in limited tissues including choroid plexus, parathyroid glands, and the kidney⁴⁴, which provides for tissue-specific effects of circulating FGF23. *In vitro* evidence supports associations between FGFR1c and KL as part of a receptor complex to elicit FGF23 signaling through the mitogen activated protein kinase (MAPK) cascade and phospho-ERK1/2 (p-ERK1/2)⁵⁶. Interactions between multiple FGFRs and KL have also been identified *in vitro*⁵⁷. Underscoring the importance of the formation of a KL-FGFR receptor complex, high levels of FGF23 signaling *in vitro* occur when KL and FGFR1c are co-expressed⁵⁶, and this activity can be blocked by anti-FGF23 antibodies that disrupt FGFR-FGF23-KL associations⁵⁸. In further support of direct FGF23-KL interactions, the *Fgf23*- and *KL*-null animals have identical hyperphosphatemic phenotypes^{41, 42, 44, 59}. Additionally, a recessive, loss of function mutation in the human *KL* gene resulted in impaired KL expression and activity *in vitro*, and to a severe tumoral calcinosis (TC) phenotype, most likely due to end-organ resistance to FGF23⁴⁵. Interestingly, in the converse situation, a case has been reported with a novel balanced translocation that increases the expression of α -KL in the circulation. This genetic rearrangement was associated with elevated FGF23 and PTH, and hypophosphatemic rickets⁶⁰, however the molecular mechanisms underlying this phenotype are currently unknown.

In studies towards identifying the renal *Fgfr* that mediates FGF23 bioactivity, the *Fgfr3*- and *Fgfr4*-null mice were mated to the *Hyp* mouse model of XLH. Genetic removal of these receptors did not reverse the hypophosphatemic phenotype observed in the *Hyp* mouse⁶¹, indicating that these receptors individually may not be the primary FGFRs that transduce FGF23 signaling with KL *in vivo*. Thus, whether FGFR1 and/or FGFR2 are responsible for FGF23-dependent signaling, or whether KL can partner with multiple FGFRs *in vivo* is currently unknown.

KL is produced as at least two isoforms due to alternative splicing of the same gene. Membrane bound KL (mKL) is a 130 kD single-pass transmembrane protein characterized by a large extracellular region (KL1 and KL2 domains) and a very short (10 residue) intracellular domain that does not possess signaling capabilities⁶². The secreted form of KL (sKL) is approximately 80 kD and is spliced within exon 3 to result in a KL protein that does not contain the transmembrane region, and is thus secreted into the circulation⁶². A third isoform of KL ('cut KL', or cKL), also found in the circulation, can be derived by the proteolytic processing of KL near the extracellular membrane surface⁶³.

Although KL permits FGF23 signaling when co-expressed with FGFRs *in vitro*, the mechanisms underlying FGF23 bioactivity in the kidney *in vivo* are unclear. In this regard, KL protein and mRNA have been mapped to the distal convoluted tubule (DCT)^{44, 64}, whereas FGF23 mediates effects on *Npt2a*, *Npt2c*, and the vitamin D metabolizing enzymes within the proximal tubule^{35, 36} (Figure 2). Further, *in vitro* experiments support that the mKL isoform is capable of initiating FGF23-dependent MAPK bioactivity⁶⁴. In contrast to PTH, which has direct effects on the proximal tubule to regulate *Npt2a* expression¹⁷, it was recently demonstrated that following FGF23 injection, initial FGF23 activity (5–10 min) as tested through detection of phospho-ERK1/2, occurs in the renal DCT, and was not localized with *Npt2a* in the proximal tubule⁶⁴. Therefore, there is likely a spatial separation of initial FGF23 bioactivity in the DCT from *NPT2a*, however at this time it cannot be ruled out that a novel, direct signaling process occurs in the proximal tubule following FGF23 delivery. It has also been proposed that soluble KL, produced in the DCT, could have direct effects in the proximal tubule as a mediator of FGF23 activity⁶⁵. Additional mechanistic studies are

needed to clarify the role of the soluble KL isoforms in phosphate handling, but increased activity of circulating soluble KL could potentially explain the phenotype of the hypophosphatemic patient with the balanced translocation described above⁶⁰. KL is an attractive therapeutic target for disorders involving increased FGF23 bioactivity, although it will be necessary to first determine which KL isoforms are responsible for FGF23-mediated phosphate handling, and whether soluble forms of KL regulate FGF23 activity within, as well as outside of the kidney.

FGF23 and PTH

Recent evidence points to a role for FGF23 in directly regulating PTH production, as the FGF23 co-receptor KL is highly expressed in the parathyroid glands⁴⁴. Independent, parallel studies demonstrated that FGF23 delivery to bovine parathyroid cells *in vitro*, or to rats *in vivo*, increased p-ERK1/2 activity and reduced PTH mRNA and circulating concentrations, respectively^{66, 67} (Figure 1). In further support of a PTH-FGF23 axis, a patient with Jansen's metaphyseal chondrodysplasia, caused by activating mutations in the Type 1 PTH/PTHrP receptor (PTHR1), had significantly elevated serum FGF23 levels, despite persistent hypophosphatemia⁶⁸, suggesting that PTH could potentially increase FGF23 production in bone. In support of this hypothesis, *in vitro* experiments have shown a direct relationship between PTH and FGF23. In a study reported in preliminary form, treatment of primary cultures of differentiated calvarial osteoblasts/osteocytes with PTH, PTHrP, and cAMP analogs resulted in dose- and time-dependent increases in Fgf23 mRNA⁶⁹. Moreover, a transgenic mouse model carrying the same Jansen's PTHR1 mutation (H223R) specifically in osteocytes showed elevated serum Fgf23^{68, 70}. These studies support the idea that PTH may increase FGF23 in bone, and subsequently FGF23 may activate KL and an FGFR in the parathyroids to reduce PTH production (Figure 1). Certainly, these systems are more complex than realized, as patients with primary hyperparathyroidism have been reported to have variable serum FGF23⁷¹, and the fact that PTH could potentially regulate FGF23 indirectly through 1,25(OH)₂D. These findings also indicate that additional regulatory loops may be involved in FGF23 production, and speculatively, that these loops may be physiologically 'prioritized'. In this regard, the hypophosphatemia in primary hyperparathyroidism may strongly suppress FGF23 production at the genomic level and not permit PTH-dependent increases in FGF23. Collectively, these findings underscore the complexity of phosphate homeostasis, and further study is needed to resolve potentially new regulatory pathways between FGF23, PTH, and vitamin D.

Disorders of reduced renal phosphate reabsorption

As described above, ADHR is caused by gain of function mutations in FGF23 (Figure 2; Table 1). ADHR is a rare renal phosphate wasting disorder characterized by hypophosphatemia, elevated FGF23 serum levels, inappropriately normal 1,25(OH)₂D levels, normal PTH, and normocalcemia⁷² (Table 1). In general, patients with ADHR present with bone pain, osteomalacia, fractures, and tooth abscesses, similar to XLH patients. However, ADHR is unique among the hypophosphatemic syndromes in that patients manifest the disease in two distinct groups either as children, with bone pain, weakness, and insufficiency fractures; or as adults, with low TmP/GFR and no extremity deformities, but potentially with pseudofractures. Additionally, unaffected carriers of ADHR have also been observed⁷². Importantly, the degree of disease manifestation in ADHR is correlated with serum FGF23 concentrations⁷³.

X-linked hypophosphatemic rickets (XLH) is the most common heritable phosphate wasting disorder, occurring in approximately 1/20,000 births. In contrast to ADHR, XLH is a fully penetrant dominant disorder with variable severity⁷⁴. Patients with XLH present with

defects similar to ADHR patients (Table 1), but also manifest enthesopathy (calcification of the tendons and ligaments). XLH is caused by inactivating mutations in *PHEX* (Phosphate-regulating gene with homologies to endopeptidases on the X chromosome)⁷⁵, a member of the M13 family of membrane-bound metalloproteases. Studies show that there is no predominant *PHEX* mutation that leads to XLH⁷⁶.

PHEX is expressed in osteoblasts and osteocytes, and odontoblasts in teeth⁷⁷. Serum FGF23 is elevated in most XLH patients (Table 1)^{78–80}, and is also elevated in *Hyp* mice, which display approximately 10-fold higher concentrations than wild type controls⁸¹. Quantitative real-time RT-PCR (qPCR) analysis examining bone revealed that *Fgf23* mRNA is also elevated⁸². Additionally, renal *Npt2a* expression is reduced by ~50% in this model⁸³. These findings provide support for a physiological connection between *PHEX* activity and FGF23 expression.

A relatively new disorder, autosomal recessive hypophosphatemic rickets (ARHR) in which patients have Dentin matrix protein-1 (*DMP1*) inactivating mutations, has been identified^{84, 85}. ARHR is characterized by a similar biochemical phenotype to that of ADHR and XLH (Table 1), including elevated serum FGF23 concentrations in some patients. Of significance, ARHR patients manifest peri-osteocytic lesions upon bone biopsy, a hallmark of XLH⁸⁴.

DMP1 is a member of the ‘short integrin-binding ligand interacting glycoprotein’ (SIBLING) family of skeletal matrix proteins⁸⁶, and is highly expressed in osteocytes and in the canaliculi within the bone matrix. *DMP1* has been proposed to have roles in canaliculi function and structural support⁸⁷ and regulating hydroxyapatite formation⁸⁸. The *Dmp1*-null mouse has a severe hypophosphatemic rickets phenotype and marked elevation of *Fgf23*⁸⁴. Histomorphometric and EM analyses of *Dmp1*-null bone and ARHR patient iliac crest biopsies demonstrated malformed osteocytes and disorganization of the canicular system⁸⁴. *Fgf23* was shown to be elevated in *Dmp1*-null osteocytes by in situ hybridization, consistent with the elevated serum levels. These findings revealed an important role for skeletal matrix protein in bone cell formation and in the downstream regulation of phosphate handling. Mechanistic studies using the *Dmp1*-null mouse demonstrated that loss of *Dmp1* impairs osteocyte maturation and gene expression, leading not only to elevated *Fgf23* (Figure 2), but also to the inappropriate expression of Type I collagen and alkaline phosphatase in osteocytes, which may indicate a general cell defect⁸⁴. The prevailing hypophosphatemia results in pathological changes in bone mineralization in this model⁸⁴, which can be largely, but not completely abrogated by administering a high phosphate diet. Importantly, *Dmp1*-null mice parallel the phenotypes of *Hyp* mice (and patients with ARHR and XLH), which all share a distinctive bone histology characterized by periosteocytic lesions of non-mineralized bone⁸⁴, abnormal osteocyte morphology⁸⁹, and an ‘intrinsic’ bone defect that cannot be completely resolved by increased delivery of phosphate. Thus, these findings suggest that *PHEX* may also have a role in osteocyte maturation that leads to over expression of FGF23 (Figure 2). More recently, gene array analyses of bone RNA from *Hyp* mice shows alterations in components of *Fgf* and *Wnt* signaling³⁹. Both of these pathways are known to be central to cell differentiation and skeletal development, therefore these autocrine systems may either underlie, or be a response to, the potential differentiation defects observed in XLH/*Hyp* and ARHR/*Dmp1*-null mice.

Tumor induced osteomalacia (TIO) is an acquired disorder of renal phosphate wasting associated with tumors, typically arising from a mesenchymal origin⁹⁰. Patients with TIO share similar biochemical and skeletal phenotypes with patients with ADHR, XLH, and ARHR, and may also report weakness. The study of this disorder introduced the idea for circulating factors produced by the tumor, referred to as “phosphatonins,” that act upon the kidney to reduce phosphate reabsorption, due to the fact that surgical removal of the tumor

leads to a reversal of the hypophosphatemia and metabolic bone disease^{91, 92}. FGF23 mRNA is highly expressed in TIO tumors^{35, 93, 94} (Figure 2), and patients can have markedly elevated serum FGF23, that rapidly returns to control levels after tumor resection^{78, 95}. As support for the phosphatonin theory, transplant of tumor tissue into mice resulted in mirroring the original hypophosphatemic syndrome in these animals⁹⁶.

Hereditary hypophosphatemic rickets with hypercalciuria (HHRH) was first described in a consanguineous Bedouin tribe⁹⁷. Similar to the disorders described above, HHRH is characterized by hypophosphatemia with rickets and short stature, however, the distinguishing biochemical characteristic of this disorder when compared to the FGF23-related diseases such as ADHR and XLH is the notable increase in serum 1,25(OH)₂D as a compensatory response to the prevailing hypophosphatemia⁹⁷. Affected individuals also have markedly elevated urine calcium excretion with increased intestinal absorption of phosphorus and calcium, which can lead to nephrolithiasis.

To identify the genetic defect responsible for HHRH, homozygosity mapping was undertaken. With this approach, the disease locus was narrowed to the portion of chromosome 9q34 containing the *SLC34A3* gene, which encodes NPT2c (Table 1)^{13, 14}. Following direct DNA sequencing of NPT2c in unrelated HHRH kindreds, recessive, single-nucleotide deletions, compound heterozygous deletions and missense mutations have been identified. These changes are predicted to be loss of function, and most likely reduce renal phosphate absorption through decreasing apical membrane expression of NPT2c or the uncoupling of sodium-phosphate co-transport in the proximal tubule^{98, 99}. An important implication of identifying the HHRH changes in NPT2c is that this transporter appears to have a significant role in renal Pi reabsorption throughout life, and is not limited to a role in phosphate homeostasis early in life, as hypothesized. The fact that NPT2c mutations lead to disease in humans, but not when genetically deleted in mice¹⁰⁰, may also indicate that this transporter plays a more significant role in humans than other species. There have also been reports of mutations in NPT2a and NHERF, which regulates Npt2a expression and activity, leading to hypophosphatemia^{101, 102}. Further in vitro and in vivo studies will be useful in determining the molecular mechanisms underlying these syndromes.

Approximately half of all patients affected by polyostotic fibrous dysplasia associated with McCune-Albright syndrome (MAS/FD) will develop hypophosphatemic rickets/osteomalacia²⁷ (Table 1). MAS/FD is caused by somatic mutations in *GNAS1*, and characterized by benign fibrous tissue that replaces normal bone. Individuals with MAS/FD who develop hypophosphatemia and isolated renal phosphate wasting have elevated serum FGF23, which is produced by osteogenic cells in the fibrous lesions²⁷.

Box 2. Animal models of human disorders involving FGF23

Hypophosphatemia:

XLH: *Hyp* mouse

ARHR: *Dmp1*-null mouse

TIO: FGF23 transgenic mice

Hyperphosphatemia:

TC/HHS: *Galnt3*-null mouse

TC: *Fgf23*-null mouse

TC: *KL*-null mouse

Disorders of increased renal phosphate reabsorption

Tumoral calcinosis (TC) is an autosomal recessive syndrome characterized by hyperphosphatemia, and normal or elevated 1,25(OH)₂D concentrations, often with the development of severe ectopic and vascular calcifications¹⁰³; a phenotype that closely resembles that of the *Fgf23*- and *KL*-null mice^{37, 44}. TC was first determined to be caused by inactivating mutations in the GalNAc transferase 3 (*GALNT3*) gene, which initiates O-linked glycosylation (Table 1)⁴⁷. Mature active FGF23 protein is O-glycosylated in three regions, with the most critical being within the ₁₇₆RH₁₇₇T₁₇₈R₁₇₉/S₁₈₀ SPC-like domain, at position T₁₇₈¹⁰⁴. This key glycosylation site likely prevents intracellular degradation of FGF23 at the RXXR motif by SPC-like enzymes in the trans-Golgi network¹⁰⁴ (Figure 2). When inactivating *GALNT3* mutations are present in TC patients, only minute amounts of intact FGF23 are produced, leading to hyperphosphatemia⁴⁹. In support of this, subsequent analysis of the *Galnt3*-null mouse revealed that this model has reduced circulating intact Fgf23 and increased Fgf23 circulating fragments, but has increased bone Fgf23 mRNA. These studies indicate that the mice display the expected physiological response to increased circulating phosphate and 1,25(OH)₂D by increasing Fgf23 transcription, but the defective O-glycosylation reduces intact, bioactive Fgf23 concentrations⁴⁸.

The TC phenotype is also caused by recessive inactivating mutations in the conserved FGF-like domain of *FGF23* (Figure 2; Table 1), which destabilize the full-length form of the hormone^{105–107}. Strikingly, patients with mutations in *GALNT3* and *FGF23*, both have low intact serum FGF23 levels, but significantly elevated C-terminal fragments of FGF23^{47, 49}, which likely represents an attempt to increase FGF23 production. The biochemical profile of elevated C-terminal serum FGF23 in conjunction with low intact serum FGF23 is specific for *GALNT3*- and *FGF23*-TC. Importantly, it has recently been shown *in vitro*, that recombinant FGF23 carrying FGF23-TC mutations has defective O-glycosylation, thus making this species of FGF23 more prone to intracellular degradation. These observations likely provide clarification for the identical serum FGF23 profile of patients with *FGF23*- and *GALNT3*-mutant TC¹⁰⁸. Hyperostosis-hyperphosphatemia syndrome (HHS) was first described as a separate disorder from TC due to radiological findings of abnormalities involving cortical bone hyperostosis¹⁰⁹. However mutational analysis of patients with HHS revealed identical mutations to those of patients diagnosed with *GALNT3*-TC and the same inappropriate intact/C-terminal FGF23 concentrations^{106, 109}, thus the two syndromes are allelic. The molecular mechanisms underlying the large range of phenotypes between TC and in HHS are currently unknown.

A homozygous mutation in *KL*, H193R, was shown to be a third cause of TC in a single case⁴⁵ (Figure 2). This patient presented with hyperphosphatemia, hypercalcemia, elevated PTH, and ectopic calcifications in the heel and brain⁴⁵. The missense mutation is localized within a highly conserved region in exon 1, present in all isoforms of *KL*. *In vitro* analysis of the mutant protein illustrated decreased mature, glycosylated *KL* expression, as well as reduced FGF23-dependent signaling through MAPK, thus this mutation is assumed to prevent FGF23 bioactivity *in vivo*. In contrast to TC patients with mutations in *GALNT3* and *FGF23*, the *KL*-TC patient had significantly elevated serum intact and C-terminal FGF23 (>150-fold control). This increase is likely a response to the significant hyperphosphatemia and elevated 1,25(OH)₂D, as the *FGF23* gene itself is not altered in this patient. These findings provide important support for direct interactions between FGF23 and *KL*.

CKD and emerging therapeutics

As described herein, a large body of evidence supports that FGF23 and its signaling complexes are central to renal phosphate homeostasis. In clinical practice, the most common

form of pathological change involving serum phosphate is hyperphosphatemia from chronic kidney disease (CKD), which is now estimated to affect 1 in 8 in the US alone¹¹⁰. The increase in serum phosphate balance in these patients results from several different mechanisms, however the most important factor is reduced renal phosphate excretion. A further aggravating factor is secondary hyperparathyroidism, which is manifested by a large proportion of patients with CKD, and may result in increased bone resorption and further elevations in phosphate arising from skeletal stores¹¹¹.

Due to the increase in serum phosphate in CKD patients and the known physiological response of FGF23, it was hypothesized that patients with advanced renal disease may have elevated FGF23¹¹². This has now been demonstrated using multiple FGF23 serum assays^{78, 95}, and importantly, in CKD patients assessed as quartiles with similar serum phosphate concentrations, the groups with elevated FGF23 demonstrated a 5–6 fold increase in the odds for mortality¹¹³. Further, the circulating FGF23 may be in part responsible for the suppressed 1,25(OH)₂D concentrations observed in these patients, as FGF23 is known to reduce the renal 1- α -hydroxylase and stimulate the catabolic 24-hydroxylase⁴⁰. Importantly, this may occur under situations of hyperparathyroidism in CKD, which should have the opposite effects on vitamin D metabolism.

Progress towards potential treatments for the heritable hypophosphatemias was initially tested in model systems, and is underway in clinical trials through the use of inactivating monoclonal antibodies targeting FGF23. In the *Hyp* mouse, the anti-FGF23 completely reversed the hypophosphatemia and associated bone disease, including the osteomalacia and reduced bone length, after four weeks of treatment⁵⁸. Humanized anti-FGF23 therapy ('KRN23') is now undergoing Phase I clinical trial in patients with XLH. It is tempting to speculate that similar classes of therapies could be applied to FGF23 or its mediators of activity to modulate renal phosphate and vitamin D metabolism, or potentially PTH production, in patients with early stage CKD. In patients with partial renal function, suppression of FGF23 activity may elevate 1,25(OH)₂D, however this approach could also increase renal phosphate reabsorption, and further raise serum phosphate concentrations. Certainly, additional studies examining the role of FGF23 in animal models will need to be performed to relate these observations to potential future therapeutics targeting FGF23 in this disorder.

Conclusions

In sum, the regulation of renal phosphate handling is complex and controlled by overlapping endocrine processes. PTH is primarily responsible for calcium balance, but plays significant roles in kidney phosphate homeostasis by directly acting upon the proximal tubule. The molecular discoveries associated with the heritable hypophosphatemias and TC demonstrate that FGF23 is central to phosphate and vitamin D metabolism in humans. Increased FGF23 bioactivity is associated with renal phosphate loss and inappropriately normal 1,25(OH)₂D concentrations, whereas decreased FGF23 bioactivity results in increased renal phosphate reabsorption and elevated 1,25(OH)₂D. Further, the identification of NPT2c inactivating mutations has revealed novel mechanisms guiding the direct control of proximal tubule phosphate handling. Finally, the recently-discovered relationships between the kidney, skeleton, and intestine will provide new and important insight into phosphate homeostasis under normal circumstances and in disease.

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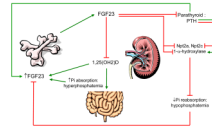


Figure 1. FGF23 regulatory systems in phosphate metabolism

FGF23 is produced in bone and secreted into the circulation, potentially in response to increased phosphate, $1,25(\text{OH})_2\text{D}$, and PTH. FGF23 acts in the kidney to decrease Npt2a and Npt2c expression and decrease $1,25(\text{OH})_2\text{D}$ production, resulting in hypophosphatemia. Potentially, in a novel feedback loop FGF23 may reduce PTH mRNA and protein. (Red arrows indicate suppressive effects; green arrows indicate stimulatory effects).

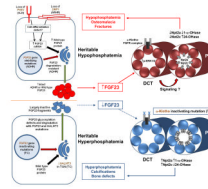


Figure 2. The molecular and physiological consequences of genetic alterations in heritable hypo- and hyperphosphatemia

FGF23 is produced in osteoblasts and osteocytes (left). In hypophosphatemic disorders (outlined in red and red arrows), loss of PHEX and DMP1, in XLH and ARHR, respectively, are associated with a cell differentiation defect that causes elevated FGF23 by unknown mechanisms. The ADHR gain of function alterations in FGF23 result in a more stable full length protein. Circulating FGF23 (wild type or ADHR-mutant) signals through p-ERK1/2 in the renal DCT and down-regulates Npt2a, Npt2c, and the 1- α -hydroxylase, and increases the catabolic 24-hydroxylase in the proximal tubule through unknown mechanisms. This process leads to hypophosphatemia, osteomalacia, and fracture. In the hyperphosphatemic disorder TC (outlined in blue and blue arrows), loss of function mutations in FGF23 and GALNT3 result in incompletely glycosylated FGF23, which increases susceptibility to proteolysis and results in the secretion of inactive FGF23 fragments. Loss of FGF23 bioactivity results in the converse expression of the sodium-phosphate co-transporters and vitamin D metabolizing enzymes. The effect on serum biochemistries is hyperphosphatemia with elevated 1,25(OH)₂D, which leads to ectopic and vascular calcifications.

Table 1

Molecular and physiological manifestations in disorders involving FGF23

Syndrome	OMIM Ref. nos.	Gene	Mutation type	Effect on FGF23-related systems	Effect on serum Pi	Effect on serum 1,25D	Intact FGF23 ELISA	C-terminal FGF23 ELISA
ADHR	193100 (<i>FGF23</i> : 605380)	<i>FGF23</i>	Gain of function	Stabilize full-length FGF23	↓	* ↔	↔ or ↑	↔ or ↑
XLH	307800 (<i>PHEX</i> : 300550)	<i>PHEX</i>	Loss of function	Increased FGF23 production in osteocytes	↓	* ↔ or ↓	↔ or ↑	↔ or ↑
ARHR	241520 (<i>DMP1</i> : 600980)	<i>DMP1</i>	Loss of function	Increased FGF23 production in osteocytes	↓	* ↔	↔ or ↑	↔ or ↑
MAS/FD	174800	<i>GNAS1</i>	Gain of function	Increased FGF23 production in lesions	↓	* ↔	↔ or ↑	↔ or ↑
TIO	605380 (<i>FGF23</i>)	--	--	FGF23 produced by tumor	↓	* ↔ or ↓	↔ or ↑	↔ or ↑
TC	211900	<i>FGF23</i>	Loss of function	Destabilize full-length, active FGF23	↑	↔ or ↑	↓	↑
TC	211900 (<i>GALNT3</i> : 601756)	<i>GALNT3</i>	Loss of function	Destabilize full-length, active FGF23	↑	↔ or ↑	↓	↑
HHS	610233	<i>GALNT3</i>	Loss of function	Destabilize full-length, active FGF23	↑	↔ or ↑	↓	↑
TC	211900 (<i>KL</i> : 604824)	<i>KL</i>	Loss of function	Decreased FGF23-dependent signaling	↑	↔ or ↑	↑	↑

* Inappropriately normal 1,25D