

How Fibroblast Growth Factor 23 Works

Shiguang Liu and L. Darryl Quarles

Kidney Institute, University of Kansas Medical Center, Kansas City, Kansas

There is a discontinuum of hereditary and acquired disorders of phosphate homeostasis that are caused by either high or low circulating levels of the novel phosphaturic hormone fibroblastic growth factor 23 (FGF23). Disorders that are caused by high circulating levels of FGF23 are characterized by hypophosphatemia, decreased production of 1,25-dihydroxyvitamin D, and rickets/osteomalacia. On the other end of the spectrum are disorders that are caused by low circulating levels of FGF23, which are characterized by hyperphosphatemia, elevated production of 1,25-dihydroxyvitamin D, soft tissue calcifications, and hyperostosis. Knowledge of the genetic basis of these hereditary disorders of phosphate homeostasis and studies of their mouse homologues have uncovered a bone–kidney axis and new systems biology that govern bone mineralization, vitamin D metabolism, parathyroid gland function, and renal phosphate handling. Further understanding of this primary phosphate homeostatic pathway has the potential to have a significant impact on the diagnosis and treatment of disorders of bone and mineral metabolism.

J Am Soc Nephrol 18: 1637–1647, 2007. doi: 10.1681/ASN.2007010068

Physiology is not only a description of function, it also asks the question[s] ‘why?’ and ‘how?’

—Knut Schmidt-Nielsen (1)

Fibroblast Growth Factor 23 Gene and Protein Structure

The fibroblast growth factor (FGF) family consists of 22 members with varied functions (2). FGF23 is an approximately 32-kD (251 amino acids) protein with an N-terminal region that contains the FGF homology domain and a novel 71–amino acid C-terminus that was originally discovered by homology-based PCR screening of a mouse embryonic cDNA library (3). The FGF23 gene is located on Chr 12p13 and is phylogenetically grouped with FGF19 and 21 gene products (2,3), with which it has approximately 24% and approximately 22% amino acid identities, respectively. FGF23 principally acts as a phosphaturic factor and suppressor of 1 α -hydroxylase activity in the kidney (4,5), making it functionally distinct from other members of this family.

Tissue Expression and Function: Evidence for a Bone–Kidney Axis

FGF23 is predominately expressed in osteocytes in bone (6,7), but it is also expressed in pericyte-like cells that surround the venous sinuses in the bone marrow, in the ventrolateral thalamic nucleus, and in thymus and lymph nodes (Figure 1). The relative contribution of these sites to circulating FGF23 levels is not known, but the high levels of expression in the osteocyte and that osteocytes are the most abundant cell in bone suggest

that the serum levels of FGF23 are derived mainly from bone. In addition, the production of FGF23 by osteocytes, cells that are central to the regulation of osteoblast function and mineralization, likely has functional significance (8). In this regard, osteocytes secrete sclerostin, an inhibitor of bone formation and the phosphaturic factor FGF23, as well as other gene products, such as phosphate-regulating endopeptidase homolog, X-linked (Phex), dentin matrix protein 1 (DMP1), and matrix extracellular phosphoglycoprotein (MEPE), that regulate bone mineralization and FGF23 expression. Phex is a type I cell surface zinc metalloprotease that is involved in the regulation of FGF23 levels and is mutated in X-linked hypophosphatemic rickets (9). Both DMP1 and MEPE are glycoposphoproteins that belong to the small integrin-binding ligand N-linked glycoprotein (SIBLING) family of proteins. MEPE and DMP1 are predominantly expressed in bones and/or teeth, where they respectively inhibit and induce mineralization of extracellular matrix (10). These associations suggest that osteocytes coordinate osteoblast-mediated bone formation with renal regulation of systemic phosphate homeostasis through the regulated expression of sclerostin, FGF23, and possibly other factors. These associations form the basis for the presence of a bone–kidney axis that coordinates bone mineralization and renal handling of phosphate and vitamin D metabolism (Figure 2).

FGF23 Function

FGF Receptor Activation

FGF interact with one of a family of four FGF receptors (FGFR) that belong to type I transmembrane phosphotyrosine kinase receptors. Heparan sulfate proteoglycans (HSPG) act as a co-factor to form a ternary FGF:FGFR:HSPG-signaling complex that is required for receptor activation (11). *In vitro* studies indicate that FGF23 can activate FGFR1c, 3c, and 4c (12,13). The physiologically relevant FGF23 receptor, however, remains unresolved. The prospect of a novel FGF23 receptor is supported

Published online ahead of print. Publication date available at www.jasn.org.

Address correspondence to: Dr. L. Darryl Quarles, 3901 Rainbow Boulevard, MS 3018, Kansas City, KS 66160. Phone: 913-588-9255; Fax: 913-588-9251; E-mail: dquarles@kumc.edu

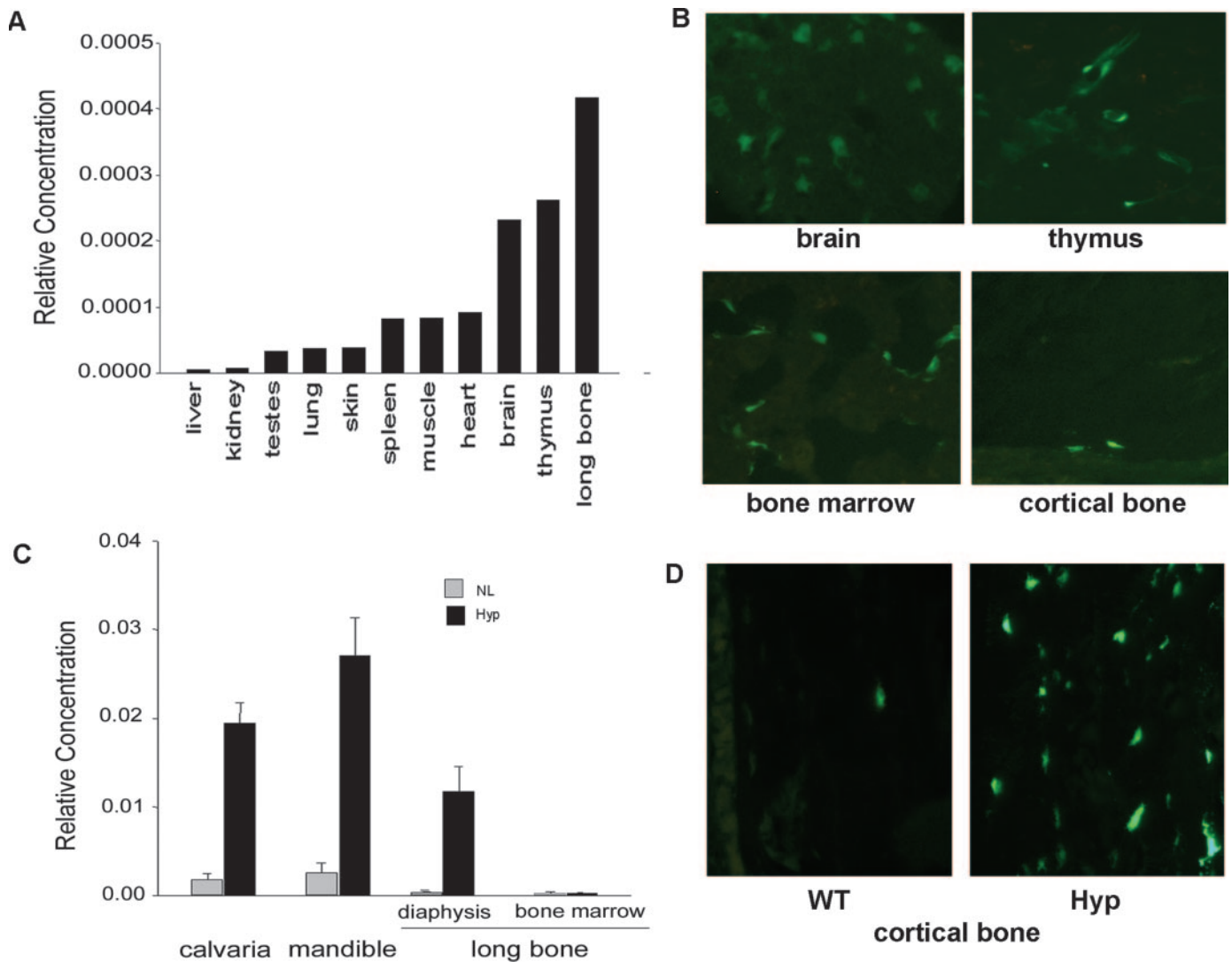


Figure 1. Tissue expression of fibroblast growth factor 23 (FGF23). (A) FGF23 message levels in normal mouse tissues. Expression was assessed by real-time PCR and expressed relative to the level of the cyclophilin control gene. (B) Tissue-specific expression of enhanced green fluorescent protein (eGFP) driven by endogenous FGF23 promoter. FGF23 is expressed in osteocytes in bone, in venous sinusoids in bone marrow and thymus, and in the ventrolateral thalamic nucleus of the brain. (C) FGF23 message levels are increased in association with inactivating mutations of phosphate-regulating endopeptidase homolog, X-linked (PheX) in Hyp mice. Expression was assessed by real-time PCR using RNA that was derived from normal wild-type (NL) and *Hyp* mouse tissues as indicated. (D) Comparison of *FGF23* expression in normal wild-type (WT; left panel) and *Hyp*-derived (right panel) skeletal tissues. *FGF23* was predominantly expressed in bone and markedly increased in Hyp compared with normal bone. Data are derived from previously published studies (6,7).

by the observation that neither activation nor inactivation of known FGFR results in hypo- or hyperphosphatemia, a finding not expected if FGF23 actions are mediated through these receptors.

Klotho is a single-pass transmembrane protein with homology to β -glucuronidases that has recently been shown to be required for FGF23-mediated receptor activation (12,14). Mice that are deficient in Klotho have a phenotype almost identical to that of FGF23 null mice (15), but additional findings indicate that FGF23-mediated receptor activation requires the co-factor Klotho, which seems to impart tissue specificity to FGF23 actions (12,14). Indeed, Klotho binds to multiple FGFR and in-

creases their affinity to FGF23. Klotho-FGFR coexpression likely defines the tissue specificity of FGF23 effects (12,14). The coexpression of widely expressed FGFR with limited expressed Klotho implicates the kidney, parathyroid gland, pituitary gland, and choroid plexus as possible targets for FGF23. In contrast, the absence of Klotho in bone, lung, liver, skin, spleen, small intestines, and adrenal gland suggests that these tissues are not targets for FGF23 (15). Indeed, recent studies in rodents have demonstrated that functional responses to the intravenous administration of recombinant FGF23 are limited to the kidney, parathyroid gland, and pituitary gland (12).

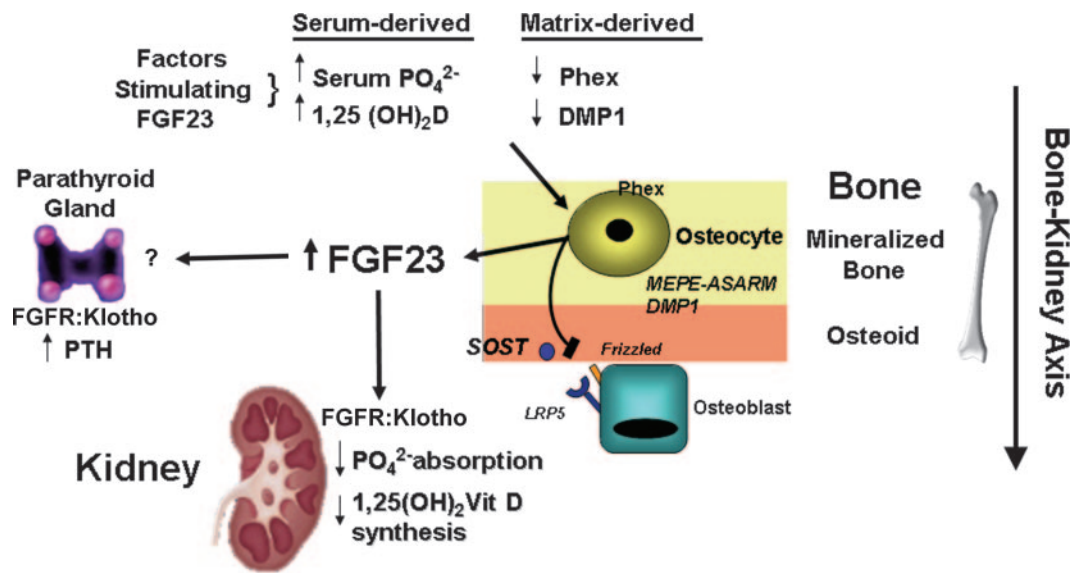


Figure 2. The bone–kidney axis. In this model, osteocytes in bone are the principal cells that coordinate the mineralization process with renal handling of phosphate. Osteocytes secrete FGF23 and sclerostin (SOST), matrix extracellular phosphoglycoprotein (MEPE), and dentin matrix protein 1 (DMP1) and express Phex. FGF23 targets organs, particularly the kidney, via an FGF receptor:Klotho complex that inhibits sodium-dependent phosphate uptake and 1α -hydroxylase activity by a mechanism that remains to be elucidated. In addition, SOST inhibits osteoblastic function by disrupting Wnt and bone morphogenetic protein (BMP) signals that regulate osteoblast-mediated bone formation. Also, the extracellular matrix proteins DMP1 and MEPE respectively positively and negatively regulate the process of extracellular matrix mineralization. Inhibition of bone formation would be associated with decreased phosphate buffering capacity of bone and require increased FGF23-mediated phosphaturia, whereas accelerated bone formation and increased bone phosphate utilization would be associated with suppressed FGF23 and renal phosphate retention to meet the need for bone mineralization. 1,25-dihydroxyvitamin D, $1,25(\text{OH})_2\text{D}$; PTH, parathyroid hormone.

Mouse Models of FGF23 Excess and Deficiency

Knowledge of the function of FGF23 has been derived largely from mouse models with overexpression of FGF23. Either the administration of recombinant FGF23 or implantation of cells that overexpress FGF23 results in phosphaturia and hypophosphatemia (4). Transgenic mice that overexpress either wild-type or a mutant cleavage resistant form of FGF23 displayed decreased urinary phosphate reabsorption, hypophosphatemia, low serum 1,25-dihydroxyvitamin D [$1,25(\text{OH})_2\text{D}$] levels, and rickets and osteomalacia as well as hyperparathyroidism (16,17). In addition, Hyp mice, the mouse homologue of X-linked hypophosphatemic rickets, and DMP1 null mice, a model of autosomal recessive hypophosphatemic rickets, have elevated circulating concentrations of FGF23 and phenotypic features that are similar to those of transgenic mice that overexpress FGF23 (7,18). These models provide important insights into regulation of FGF23 expression by Phex, the disease gene in XLH/Hyp and DMP1, an extracellular matrix protein, both of which are located in bone (see Regulation of FGF23).

Additional information regarding the role of FGF23 in phosphate and vitamin D metabolism has been derived from study of FGF23-deficient mice. Three FGF23 knockout mouse models have been created (7,19,20), all of which are characterized by hyperphosphatemia as a result of increased renal phosphate reabsorption and elevated serum $1,25(\text{OH})_2\text{D}$ levels as a result of enhanced 1α -OHase expression/activity, a phenotype opposite to that of mice that overexpress FGF23. In addition, FGF23

null mice have soft tissue calcifications, severe growth retardation, abnormalities of bone mineralization, a markedly shortened lifespan, and abnormalities of glucose metabolism. The phenotype of FGF23 null mice resembles that of patients with tumoral calcinosis, which is caused either by mutations of FGF23 that lead to decreased circulating FGF23 levels or by mutations in *GALNT3*, which encodes the glycosyltransferase UDP-N-acetyl- α -D-galactosamine:polypeptide N-acetylgalactosaminyl transferase 3 (ppGalNAcT3) that is responsible for initiating O-glycosylation and stability of FGF23 (21). The phenotype of FGF23 null mice also resembles that of Klotho null mice, which also are characterized hyperphosphatemia, elevated $1,25(\text{OH})_2\text{D}$, early mortality, and soft tissue calcifications (15). Klotho null mice, however, have elevated FGF23 levels, and $1,25(\text{OH})_2\text{D}$ induces the expression of Klotho in the kidney (22). This finding, along with *in vitro* data showing that Klotho is an essential co-factor for FGF23 activation of FGFR, suggests that the Klotho null mouse represents, at least in part, a model of end-organ insensitivity to FGF23.

Additional abnormalities are observed in association with FGF23 excess and deficiency, such as rickets/osteomalacia, abnormalities in glucose homeostasis, growth retardation, abnormalities in thymic function, and age-related changes (7,19,20). It is uncertain whether these changes are due to changes in serum phosphate or alterations in vitamin D metabolism rather than direct effects of FGF23 on additional tissues. At present, how-

ever, it seems that many abnormalities that are associated with FGF23 deficiency, particularly those that are associated with bone, pancreas, and thymus, represent secondary effects of elevated $1,25(\text{OH})_2\text{D}$ as a result of loss of FGF23 effects on the kidney rather than direct effects of FGF23 on these organs. In this regard, hyperphosphatemia, soft tissue calcifications, and survival in FGF23 null mice are improved either by dietary phosphate restriction or by correcting excessive actions of $1,25(\text{OH})_2\text{D}$ either by superimposed nutritional vitamin D deficiency or by ablation of the vitamin D receptor or 1α -hydroxylase gene in FGF23 null mice (23–25). Knowledge of the separate functions of FGF23 is derived from understanding the distribution of the Klotho:FGFR complex (see previous paragraph) and organ-specific actions of FGF23 derived from *in vitro* and *in vivo* studies (12,14).

Tissue-Specific FGF23 Functions

Knowledge of the tissue-specific functions of FGF23 is derived from knowledge of the distribution of the Klotho:FGFR complex (see the previous section) and empiric *in vitro* and *in vivo* studies demonstrating the cell type- and organ-specific actions of FGF23 (12,14). On the basis of these data, FGF23 seems to target the kidney, parathyroid gland, choroid plexus, and possibly the pituitary gland (26).

Kidney

The kidney is the principal target for FGF23, and the major function of this hormone is to regulate phosphate reabsorption and production of $1,25(\text{OH})_2\text{D}$ (4,19). FGF23 inhibits both sodium-dependent phosphate reabsorption and 1α -hydroxylase activity in the proximal tubule, leading to hypophosphatemia and aberrant production and inappropriately low levels of $1,25(\text{OH})_2\text{D}$. In contrast, deficiency of FGF23 in mouse models results in the opposite renal phenotype that consists of hyperphosphatemia and elevated production of $1,25(\text{OH})_2\text{D}$ (7,19,24,27,28). FGF23-induced changes in NaPi2a and $1\alpha\text{OHase}$ expression are independent of the $1,25(\text{OH})_2\text{D}$ /vitamin D receptor system (29). However, the precise segments of the kidney and receptors that mediate the renal response to FGF23 are not entirely clear. *Ex vivo* studies of proximal tubular segments or cell lines derived from the proximal tubule have produced variable results of FGF23-mediated inhibition of sodium-dependent phosphate transport (4,30). The highest expression of Klotho:FGFR complexes is in the distal tubule (26), whereas the major biologic effects of FGF23 are in the proximal tubule. Either low levels of Klotho:FGFR in the proximal tubule are sufficient for FGF23 effects, or FGF23 actions on the proximal tubule may be indirect, possibly through FGF23 stimulation of the distal tubule and release of paracrine factors that regulate proximal tubule function.

Parathyroid Gland

The parathyroid gland is another target for FGF23. Not only are Klotho and FGFR coexpressed in parathyroid glands (15,26), but also mouse models with elevated FGF23 levels consistently manifest elevated circulating levels of PTH. Although FGF23-mediated reductions in $1,25(\text{OH})_2\text{D}$ production

confound interpretation of an association between FGF23 and hyperparathyroidism, additional studies indicate that the acute administration of recombinant FGF23 results in increments in early response gene expression in parathyroid tissue in mice (12). Moreover, there is a strong association between elevated FGF23 levels and the severity of hyperparathyroidism in chronic kidney disease (CKD). It is also tempting to speculate that FGF23 may also mediate some of the effects of hyperphosphatemia on the increase in parathyroid gland function that are observed in patients with CKD. Further studies are needed to define the effects of FGF23 on parathyroid hormone (PTH) production and secretion and parathyroid cell proliferation.

Other Organs

Another potential target of FGF23 is the choroids plexus in the brain. The function of FGF23 is completely unexplored. However, FGF23 is produced in the ventrolateral thalamic nucleus, and the choroids plexus express Klotho and FGFR as well as the sodium-dependent phosphate transporter. Because there is a gradient for phosphate between the cerebral spinal fluid (CSF) and serum (being significantly lower in the CSF than in serum), it is tempting to speculate that FGF23 may also regulate CSF phosphate concentrations.

FGF23 effects on pituitary are less certain. FGF23 seems to target the pituitary gland as evidenced by the upregulation of early response gene expression in response to acute FGF23 administration to mice. However, expression of the Klotho:FGFR complex that is thought to mediate FGF23 tissue-specific effects has not been unequivocally established to be present in the pituitary gland. Also, despite severe growth retardation in FGF23 null mice, specific abnormalities of growth hormone secretion or other abnormalities of pituitary function have not been documented.

Direct action of FGF23 on bone is unsubstantiated. Because Klotho, which is required for FGF23 actions, is not expressed in bone, the observed changes in bone in states of high and low FGF23 seem to be secondary to changes in serum phosphate and $1,25(\text{OH})_2\text{D}$ levels.

Regulation of FGF23

Both transcriptional and posttranslational mechanisms regulate circulating FGF23 levels. FGF23 gene transcription has been studied using the FGF23 promoter *in vitro* and in an FGF23 promoter-reporter mouse model created by substituting green fluorescence protein for exon 1. Both systemic factors and local bone-derived factors seem to regulate FGF23 promoter activity (31). In this regard, circulating FGF23 levels are regulated by phosphate (32) and $1,25(\text{OH})_2\text{D}$ (31) as well as by the Phex endopeptidase located in osteocytes (7) and the SIBLING extracellular matrix proteins DMP1 (18,33) and MEPE (34). Phosphate loading in mice increases FGF23 levels (32), although the data in humans are conflicting (35–37). The administration of $1,25(\text{OH})_2\text{D}$ increases and disruption of the $1,25(\text{OH})_2\text{D}$ pathway reduces circulating FGF23 in mice (31,32). FGF23 levels are also elevated in renal failure (36,38,39), and the degree of elevation correlates with the degree of hyperphosphatemia (39). The targeted deletion of DMP1 increases FGF23 expression in

osteocytes, leading to increased circulating levels (18). In contrast, MEPE administration causes phosphaturia, possibly by increasing FGF23 levels (34,40). Inactivating mutations of *Phex*, an endopeptidase that is located in osteocytes also results in increased transcription of FGF23 and elevated circulating levels (7). Recently, an unidentified chondrocyte-derived factor was shown to inhibit FGF23 transcription (41).

The evaluation of FGF23 promoter-reporter mice has confirmed that the osteocyte is the principal site of FGF23 gene transcription. Regarding systemic factors, only 1,25(OH)₂D has been shown to stimulate directly dosage-dependent increases in FGF23 promoter activity in osteoblasts through a vitamin D-responsive element. Calcium, phosphate, and PTH failed to stimulate FGF23 mRNA levels or FGF23 promoter activity in osteoblast cultures, but these factors have variably been associated with increased circulating FGF23 levels.

Bone-derived factors also regulate FGF23 expression. These include *Phex*, a cell-surface endopeptidase that co-localizes with FGF23 in osteocytes, and the extracellular matrix proteins DMP1 and MEPE. Both mutations in *Phex* and DMP1 increase the transcription of FGF23 in osteocytes, but the mechanism whereby *Phex* and DMP1 regulate FGF23 gene transcription is not clear. It is presumed that an unidentified *Phex* substrate accumulates as the result of inactivating *Phex* mutations and that this substrate stimulates FGF23 promoter activity. However, the precise temporal and spatial expression of *Phex* may be important as evidence by the paradox that deletion of *Phex* from osteocytes causes increased FGF23 levels, but the restoration of *Phex* expression in transgenic mice using heterologous promoters fails to rescue the elevated FGF23 expression (42–44).

Regardless, MEPE also seems to regulate FGF23 indirectly through actions to inhibit *Phex*. More recently, a chondrocyte inhibitor of FGF23 transcription was reported, but the molecular identity of this putative factor has not been defined (41). This local regulation may contribute to the involvement of FGF23 in a bone–kidney axis (see Bone–Kidney Axis).

Proteolytic processing of FGF23 may also play an important role in regulating its biologic activity. FGF23 is cleaved between arg179 and ser180, and this processing abolishes biologic activity. The RXXR is a conserved furin-like cleavage site. The biologic importance of this is demonstrated by point mutations of this site that results in cleavage-resistant FGF23 and elevated circulating levels of FGF23 in autosomal dominant hypophosphatemic rickets (see Diseases of FGF23 Excess). However, the exact member of the furin proprotein convertase that cleaves FGF23 and the physiologic regulation of cleavage have not been evaluated. In addition, there is an O-linked glycosylation site at Thr178 that also regulates FGF23 degradation. Mutations in UDP-N-acetyl- α -D-galactosamine-polypeptide N-acetylgalactosaminyltransferase 3 (*GALNT3*) gene results in reduced FGF23 processing also causes tumoral calcinosis. Consequently, the respective role of local or systemic posttranslational proteolytic processing of FGF23 has not been established.

Although the initial study suggested that *Phex* processes FGF23 (45), subsequent studies have failed to prove that *Phex* mediates the processing of FGF23 (6,46,47). Rather, *Phex* defi-

ciency leads to increased transcription of FGF23 in osteocytes. In addition, recent studies suggest that MEPE may also increase FGF23 expression through binding to and inhibiting *Phex* (34).

Systems Biology

Understanding the function and regulation of FGF23 is leading to new insights into bone and kidney physiology. From a systems biology perspective, FGF23 seems to function as a vitamin D counterregulatory hormone, the primary “phosphaturic” hormone, and in a bone–kidney axis to coordinate the renal phosphate handling and bone mineralization.

Vitamin D Counterregulatory Hormone

Increased 1,25(OH)₂D targets the gastrointestinal tract to increase calcium and phosphate absorption. Increments in calcium along with 1,25(OH)₂D target the parathyroid gland to suppress PTH, which in turn targets the kidney to increase urinary calcium excretion to maintain neutral calcium balance. However, lowering of PTH levels decreases phosphate excretion and would potentially result in positive phosphate balance from vitamin D-mediated increase in gastrointestinal phosphate absorption if not for compensatory elevations of the phosphaturic hormone FGF23, which also suppresses 1,25(OH)₂D to counter the increase in vitamin D (31).

Role in Maintenance of Serum Phosphate Levels

The PTH–vitamin D axis is a well understood hormonal system that is designed to maintain serum calcium. In this axis, PTH is the principal calcemic hormone that targets the kidney to increase renal conservation of calcium and to increase 1,25(OH)₂D production that in turn targets the small intestines to increase gastrointestinal calcium as well as phosphate absorption. PTH has a secondary phosphaturic effect to excrete the additional phosphate absorbed from the gastrointestinal tract. Extracellular calcium acts through the calcium receptor in the parathyroid gland to control PTH secretion and production, thereby creating a mechanism to control PTH levels in response to changes in serum calcium. The essential function of PTH to regulate serum calcium is evident by the fact that PTH deficiency causes hypocalcemia [as well as hyperphosphatemia and decreased 1,25(OH)₂D], whereas PTH excess causes hypercalcemia [along with, hypophosphatemia and increased 1,25(OH)₂D].

FGF23 seems to be the principal “phosphaturic” hormone that maintains serum phosphate levels in the normal range. FGF23 excess causes hypophosphatemia and impaired 1,25(OH)₂D production, whereas FGF23 deficiency causes hyperphosphatemia and elevated 1,25(OH)₂D production. FGF23 may directly regulate parathyroid gland function as evidenced by the presence of *Klotho*:FGFR complexes in the parathyroid gland and effects of FGF23 administration to regulate parathyroid gland expression (48). Also, elevations of FGF23 in *Hyp* (6,7,49) and DMP1 null mice (18) and ablation of FGF23 in FGF23 null mice (7) are respectively associated with increased and decreased PTH levels, although these observations are confounded by concomitant changes in serum 1,25(OH)₂D levels, which can also regulate parathyroid gland function (50).

Further studies will be needed to confirm the importance of potential direct effects of FGF23 on parathyroid gland function.

The mechanism whereby phosphate regulates FGF23 production has not been defined. A phosphate sensor that controls FGF23 production has not been identified, and extracellular phosphate has not been shown to regulate FGF23 gene transcription in osteoblast cultures, raising the possibility that phosphate effects on FGF23 production might be indirect. In addition to its role in normal phosphate homeostasis, FGF23 seems to be important in maintenance of serum phosphate levels in advancing renal failure. Circulating FGF23 increases as a function of decreased GFR and would be expected to work in concert with elevations of PTH to promote phosphaturia. It is interesting that FGF23 would oppose PTH stimulation of 1α -hydroxylase activity during progression of renal failure. The consequent reduction in $1,25(\text{OH})_2\text{D}$ levels, in turn, would protect against hyperphosphatemia by limiting gastrointestinal phosphate absorption. In addition, phosphate regulation of FGF23 might explain the observation that dietary phosphate restriction increases $1,25(\text{OH})_2\text{D}$ levels in CKD. The ability of FGF23 to act as a phosphaturic hormone, suppressor of $1,25(\text{OH})_2\text{D}$ production, and a stimulator of PTH production defines a new hormonal cascade that coordinates various organ systems (*e.g.*, kidney and parathyroid gland) to protect the body from hyperphosphatemia (31,32,51–54).

Bone–Kidney Axis

A very interesting feature of FGF23 is its production by osteocytes in bone and its regulation by Phex, which is coexpressed in osteocytes, and extracellular matrix proteins DMP1 and MEPE, which are important regulators of bone mineralization. The presence of a phosphaturic factor in bone and the importance of phosphate in the mineralization process suggest

that FGF23 may also function to coordinate renal phosphate conservation to meet the need for bone mineralization (31,55,56). In this regard, MEPE or its acidic serine-aspartate-rich MEPE-associated motif (ASARM) proteolytic fragment inhibits mineralization (34,40) and stimulates FGF23 production through actions of MEPE to inhibit Phex (34). The resulting phosphaturic effect increases phosphate excretion in proportion to the decrease in mineralized bone formation. In contrast, DMP1 deficiency results in impaired bone mineralization, as a result of the loss of the mineralization-promoting effects of this extracellular matrix protein (18). The increase in FGF23 production by osteocytes in DMP1 deficiency increases phosphate excretion by the kidneys to match the decrease in mineralization of bone. Because osteocytes also regulate osteoblast-mediated bone formation through production of sclerostin (57), these cells are optimal for coexpressing the phosphaturic hormone FGF23 to coordinate renal phosphate handling to match osteoblast-mediated bone formation. There is little evidence for a direct effect of FGF23 on bone itself. The rickets and osteomalacia that are observed in states of FGF23 excess seem to be due to the hypophosphatemia, whereas the abnormalities in bone described in the FGF23 null mouse model was recently attributed to the excess $1,25(\text{OH})_2\text{D}$ levels (24). The absence of Klotho expression in bone also is consistent with the lack of direct effects of FGF23 on bone mineralization (15). Unequivocal proof of this bone–kidney axis will require further studies.

Diseases of FGF23 Excess

Hereditary Disorders

Several hereditary hypophosphatemic disorders are caused by excessive circulating levels of FGF23 (Table 1). These include autosomal dominant (ADHR; MIM193100), autosomal recessive (ARHR; MIM241520), and X-linked (XLH; MIM307800)

Table 1. Classification of FGF23-dependent disorders^a

Disorder	Disease Gene	Mouse Model
Hypophosphatemic disorders		
hereditary		
X-linked hypophosphatemic rickets	Phex	Hyp, Gy, Ska1, Hyp-2J, Hyp-Duk, Phex-knockout and conditional Phex-knockout
autosomal dominant hypophosphatemic rickets	FGF23	Transgenic overexpression of cleavage-resistant mutant FGF23 cDNA
autosomal recessive hypophosphatemic rickets	DMP1	DMP1-knockout mouse
acquired/sporadic		
McCune-Albright syndrome, polyostotic fibrous dysplasia	GNAS1, FGF23	None
tumor-induced osteomalacia	MEPE, FGF23	FGF23-expressing cell explants
epidermal nevus syndrome	FGF23?	None
Hyperphosphatemic disorders		
hereditary		
tumoral calcinosis	GALNT3, FGF23	FGF23 and Klotho null mice

DMP1, dentin matrix protein 1; FGF23, fibroblast growth factor 23; MEPE, matrix extracellular phosphoglycoprotein.

hypophosphatemic rickets. All have overlapping clinical characteristics, including hypophosphatemia as a result of renal phosphate wasting and inappropriately normal $1,25(\text{OH})_2\text{D}$ levels for the degree of hypophosphatemia and rickets/osteomalacia. ADHR, however, is reported to have incomplete penetrance, delayed onset, and occasional resolution of phosphate wasting. The clinical features are caused by increased circulating FGF23 levels. The mechanisms whereby FGF23 levels are increased differ among the three disorders. ADHR is caused by mutations (R176Q and R179W) in the RXXR furin-like cleavage domain of FGF23 that impairs proteolytic inactivation of FGF23. ARHR is caused by inactivating mutations in dentin matrix acidic phosphoprotein (DMP1), a member of the SIBLING family of extracellular matrix protein that augments mineralization (18,33). Loss of DMP1 results in increased transcription of FGF23 by osteocytes. XLH is caused by inactivating mutations of Phex (9), a cell surface endopeptidase that also is located in osteocytes. Loss of Phex also results in increased expression of FGF23 in osteocytes. The mechanism whereby loss of DMP1 and Phex upregulates FGF23 gene transcription is not known.

Sporadic/Acquired Disorders

McCune-Albright syndrome (MIM174800), also called polyostotic fibrous dysplasia, is caused by a mosaicism for postzygotic activating mutations in the GNAS1 gene that leads to variable clinical characteristics, including polyostotic fibrous dysplasia; pigment patches of the skin; and endocrinologic abnormalities, including precocious puberty, thyrotoxicosis, pituitary gigantism, and Cushing syndrome, that depend on the tissue location of the mutation. Approximately 50% of these patients have hypophosphatemia as a result of renal phosphate wasting and rickets/osteomalacia. In these patients, the fibrodysplastic tissues express FGF23, and the appearance of hypophosphatemia and elevated circulating FGF23 levels depends on the disease burden (58–60).

Tumor-induced osteomalacia (TIO), or oncogenic osteomalacia, is a paraneoplastic syndrome of renal phosphate wasting, aberrant vitamin D metabolism, and osteomalacia. Because elevated FGF23 levels are associated with TIO and removal of the tumor results in normalization of FGF23 levels and because the inhibition of renal phosphate transport and 1α -hydroxylase activities are known biologic effects of FGF23, it is likely that TIO is caused by production of FGF23 by the tumor. These tumors tend to be of mesenchymal origin and have a propensity for localization in the craniofacial region and long bones. $^{111}\text{-Pentetreotide}$ scintigraphy (octreotide scanning) may be useful in detecting tumors that are positive for somatostatin receptors. Mixed connective tissue-type mesenchymal tumors that are characterized by an admixture of spindle cells, osteoclast-like giant cells, prominent blood vessels, cartilage-like matrix, and metaplastic bone are the most common histologic type. Other genes have been implicated in TIO, including MEPE, secreted frizzled-related protein 4 (sFRP4) (61), and FGF7 (62). Whereas MEPE is an extracellular matrix protein that inhibits mineralization, loss of MEPE does not affect serum phosphate levels. Rather, MEPE was recently shown to interact

with and inhibit Phex and could indirectly cause phosphaturia by increasing FGF23 levels (34). The roles of sFRP4 and FGF7 remain to be elucidated.

Linear sebaceous, or epidermal nevus, syndrome is a rare a group of congenital syndromes that comprise epidermal nevi in conjunction with central nervous system, ocular, musculoskeletal, and other organ anomalies. Although the cause of epidermal nevus syndrome is unknown, recent studies have revealed a mosaicism of activating FGFR3 mutations in the human epidermis of approximately one third of patients with epidermal nevi. A subset of these patients have ipsilateral focal bone disease associated with hypophosphatemic rickets, elevated circulating FGF23 levels, and aberrant $1,25(\text{OH})_2\text{D}$ levels, similar to other syndromes that are caused by elevated FGF23. Treatment with the somatostatin agonist octreotide and excision of the nevus have been reported to normalize FGF23 (63–65).

Circulating levels of FGF23 are increased in end-stage renal failure and correlate with the degree of hyperphosphatemia (39). The precise mechanism and site of increased FGF23 levels have not been evaluated in renal failure models. It is possible that both increased production and decreased metabolism or clearance of FGF23 contributes to elevated levels of FGF23 in CKD. Although no studies have yet documented increased production of FGF23, the correlation of FGF23 levels with decreasing GFR and the rapid decrease in FGF23 levels in some patients after renal transplantation suggest that FGF23 is cleared by the kidney (36,66). FGF23 levels increase early in the development of CKD (67); in the setting of residual renal function, this likely contributes to maintenance of normal serum phosphate levels by working in concert with increased PTH to decrease renal phosphate reabsorption. In ESRD, in which the principal target of FGF23, the kidney, is no longer responsive to FGF23, the function of elevated FGF23 levels is not known. In light of the fact that vitamin D is a potent regulator of FGF23 gene transcription, the use of high-dosage vitamin D analogues to treat hyperparathyroidism in CKD might be expected to increase FGF23 levels further. At sites where Klotho:FGFR are coexpressed, such as the parathyroid and choroids plexus and possibly pituitary glands, effects of elevated FGF23 might be expected in ESRD. With regard to the parathyroid gland, elevated FGF23 levels have been associated with refractory hyperparathyroidism. There are no data regarding FGF23 effects on the choroids plexus or pituitary gland in ESRD, although future studies to investigate the effect of FGF23 on cognitive dysfunction and growth retardation in ESRD seem warranted. Finally, increased FGF23 levels can persist after restoration of kidney function by renal transplantation. Elevated FGF23 may explain some instances of associated hypophosphatemia after kidney transplantation, especially in the absence of elevated PTH and suppression of $1,25(\text{OH})_2\text{D}$ levels (66,68,69).

Diseases of FGF23 Deficiency

FGF23 can be detected in the circulation of normal individuals, suggesting that this hormone is necessary to maintain phosphate homeostasis. Diseases that are caused by reductions in FGF23

levels further support the essential role of FGF23 in regulation of serum phosphate and circulating 1,25(OH)₂D levels.

Hereditary Disorders

Hyperphosphatemic familial tumoral calcinosis (HFTC; MIM211900) is a rare autosomal recessive disorder that is characterized by low bioactive circulating FGF23 levels that lead to hyperphosphatemia, normal or elevated 1,25(OH)₂D levels, and soft tissue calcifications, typically massive lobulated peri-articular calcifications levels (21,28,70–74). Tumoral calcinosis is caused by biallelic mutations of the genes that encode either FGF23 (28,70) or GalNAc transferase 3 (GALNT3) (21,71–74). These mutations alter FGF23 metabolism. Missense mutations in FGF23 have been shown to result in failure to secrete the intact protein that seems to be retained in the Golgi complex. Both null mutations of GALNT3 and missense mutations in the glycosyl transferase domain of the GALNT3 gene also cause tumoral calcinosis. These mutations destabilize FGF23 and/or impair its secretion, resulting in low-normal intact serum FGF23 levels but high levels of biologically inactive C-terminal FGF23 fragments.

Acquired Disorders

Uremic tumoral calcinosis is an acquired disorder that is characterized by ectopic calcification especially around large joints, hyperphosphatemia, and elevated serum 1,25(OH)₂D level. The coexistence of granulomatous disease with the ectopic production of 1,25(OH)₂D levels and consequent hyperphosphatemia may explain some of the cases of uremic tumoral calcinosis (75,76). In contrast to hereditary tumoral calcinosis, elevated FGF23 levels are present in ESRD. The increased FGF23 cannot function on the kidney to regulate either serum phosphate or 1,25(OH)₂D production. Because Klotho production by the kidney is also reduced in ESRD, Klotho deficiency might also contribute to development of uremic tumoral calcinosis by inducing end-organ resistance to FGF23. Low serum levels of Klotho cannot be demonstrated in ESRD because of technical problems related to measurements. Severe elevations of PTH that lead to increased Ca × P are also associated with uremic tumoral calcinosis, and marked improvement has been reported after parathyroidectomy and lowering for calcium-phosphate product (77).

Diagnosis and Treatment of Hypophosphatemic and Hyperphosphatemic Disorders: Implications of FGF23

There are three ELISA assays for measuring circulating FGF23, one C-terminal assay (Immunotopics C-terminal assay, Carlsbad, CA) and two intact (Immunotopics Intact and Kainos Intact assays) (78). In patients with TIO and documented tumors, the Kainos intact assay (Kainos Laboratories Inc., Tokyo, Japan) has greater sensitivity compared with the Immunotopics C-terminal and intact assays (100 *versus* 92 and 38%, respectively).

The availability of assays for assessing intact FGF23 should be useful in diagnosing hypophosphatemic disorders, whereas the C-terminal assay in combination with the intact assay may

be useful in diagnosis of tumoral calcinosis. Indeed, the biochemical profile of low serum phosphate, low or inappropriately normal serum 1,25(OH)₂D levels, mild elevations of PTH, and marked elevations of FGF23 represents a primary effect of excessive circulation FGF23 and should allow the diagnosis of FGF23-mediated hypophosphatemia. However, high serum phosphate, elevated 1,25(OH)₂D, increased calcium, and suppressed PTH along with low intact FGF23 and high C-terminal values for FGF23 should define tumoral calcinosis. The utility of measuring FGF23 levels in patients with CKD stages III through V is not certain. Greater knowledge regarding the biologic effects of elevated FGF23 and whether elevated levels result in toxic or salutary effects is necessary to understand better whether efforts to normalize FGF23 levels are desirable. The exception to this may be in patients with hypophosphatemia after renal transplantation, in whom measurement of intact PTH and FGF23 levels should be able to discriminate between PTH- and FGF23-mediated hypophosphatemia (66,68,69).

Knowledge of the regulation of FGF23 by phosphate and vitamin D and its mechanism of action to inhibit phosphate reabsorption and suppress 1,25(OH)₂D levels in the proximal tubule could also have an impact on treatment of hypophosphatemic disorders. The current treatments for XLH, ADHR, and ARHR are based on empiric observations that combined administration of phosphate and calcitriol results in healing of the rickets/osteomalacia and increments in serum phosphate compared with the administration of phosphate alone. Despite oral phosphate and 1,25(OH)₂D treatment, many patients have suboptimal growth and bone healing, and this treatment also seems to result in secondary hyperparathyroidism and soft tissue calcifications, particularly nephrocalcinosis (79,80). Given what we now know about regulation of FGF23, the current treatment of XLH may need to be reexamined. In particular, there is the potential for phosphate and vitamin D therapy to increase FGF23 levels further in these hereditary hypophosphatemic disorders (81). In support of this, preliminary studies suggest that FGF23 levels are higher in treated compared with nontreated patients with XLH (81). Regardless, new therapeutic agents that target FGF23 transcription and/or degradation to either raise or lower circulating FGF23 levels, as well as recombinant FGF23 or FGF23 blocking antibodies, might have utility in managing disorders with low and elevated FGF23 levels.

Acknowledgments

This work was supported by National Institutes of Health grant RO1-AR45955 from the National Institute of Arthritis and Musculoskeletal and Skin Diseases and National Institutes of Health grant P20 RR-17708 from the National Center for Research Resources.

We thank Steffani Webb for editorial assistance.

Disclosures

None.

References

- Schmidt-Nielsen K: *Animal Physiology Adaptation and Environment*, Cambridge University Press, New York, 1975
- Itoh N, Ornitz DM: Evolution of the Fgf and Fgfr gene families. *Trends Genet* 20: 563–569, 2004
- Yamashita T, Yoshioka M, Itoh N: Identification of a novel fibroblast growth factor, FGF-23, preferentially expressed in the ventrolateral thalamic nucleus of the brain. *Biochem Biophys Res Commun* 277: 494–498, 2000
- Shimada T, Mizutani S, Muto T, Yoneya T, Hino R, Takeda S, Takeuchi Y, Fujita T, Fukumoto S, Yamashita T: Cloning and characterization of FGF23 as a causative factor of tumor-induced osteomalacia. *Proc Natl Acad Sci U S A* 98: 6500–6505, 2001
- White KE, Carn G, Lorenz-Depiereux B, Benet-Pages A, Strom TM, Econs MJ: Autosomal-dominant hypophosphatemic rickets (ADHR) mutations stabilize FGF-23. *Kidney Int* 60: 2079–2086, 2001
- Liu S, Guo R, Simpson LG, Xiao ZS, Burnham CE, Quarles LD: Regulation of fibroblastic growth factor 23 expression but not degradation by PHEX. *J Biol Chem* 278: 37419–37426, 2003
- Liu S, Zhou J, Tang W, Jiang X, Rowe DW, Quarles LD: Pathogenic role of Fgf23 in Hyp mice. *Am J Physiol Endocrinol Metab* 291: E38–E49, 2006
- Poole KE, van Bezooijen RL, Loveridge N, Hamersma H, Papapoulos SE, Lowik CW, Reeve J: Sclerostin is a delayed secreted product of osteocytes that inhibits bone formation. *FASEB J* 19: 1842–1844, 2005
- The HYP Consortium: A gene (PEX) with homologies to endopeptidases is mutated in patients with X-linked hypophosphatemic rickets. *Nat Genet* 11: 130–136, 1995
- Fisher LW, Fedarko NS: Six genes expressed in bones and teeth encode the current members of the SIBLING family of proteins. *Connect Tissue Res* 44[Suppl 1]: 33–40, 2003
- Ornitz DM: FGFs, heparan sulfate and FGFRs: Complex interactions essential for development. *Bioessays* 22: 108–112, 2000
- Urakawa I, Yamazaki Y, Shimada T, Iijima K, Hasegawa H, Okawa K, Fujita T, Fukumoto S, Yamashita T: Klotho converts canonical FGF receptor into a specific receptor for FGF23. *Nature* 444: 770–774, 2006
- Yu X, Ibrahim OA, Goetz R, Zhang F, Davis SI, Garringer HJ, Linhardt RJ, Ornitz DM, Mohammadi M, White KE: Analysis of the biochemical mechanisms for the endocrine actions of fibroblast growth factor-23. *Endocrinology* 146: 4647–4656, 2005
- Kurosu H, Ogawa Y, Miyoshi M, Yamamoto M, Nandi A, Rosenblatt KP, Baum MG, Schiavi S, Hu MC, Moe OW, Kuro-o M: Regulation of fibroblast growth factor-23 signaling by klotho. *J Biol Chem* 281: 6120–6123, 2006
- Kuro-o M, Matsumura Y, Aizawa H, Kawaguchi H, Suga T, Utsugi T, Ohyama Y, Kurabayashi M, Kaname T, Kume E, Iwasaki H, Iida A, Shiraki-Iida T, Nishikawa S, Nagai R, Nabeshima YI: Mutation of the mouse klotho gene leads to a syndrome resembling ageing. *Nature* 390: 45–51, 1997
- Larsson T, Marsell R, Schipani E, Ohlsson C, Ljunggren O, Tenenhouse HS, Juppner H, Jonsson KB: Transgenic mice expressing fibroblast growth factor 23 under the control of the alpha1(I) collagen promoter exhibit growth retardation, osteomalacia, and disturbed phosphate homeostasis. *Endocrinology* 145: 3087–3094, 2004
- Bai X, Miao D, Li J, Goltzman D, Karaplis AC: Transgenic mice overexpressing human fibroblast growth factor 23 (R176Q) delineate a putative role for parathyroid hormone in renal phosphate wasting disorders. *Endocrinology* 145: 5269–5279, 2004
- Feng JQ, Ward LM, Liu S, Lu Y, Xie Y, Yuan B, Yu X, Rauch F, Davis SI, Zhang S, Rios H, Drezner MK, Quarles LD, Bonewald LF, White KE: Loss of DMP1 causes rickets and osteomalacia and identifies a role for osteocytes in mineral metabolism. *Nat Genet* 38: 1310–1315, 2006
- Shimada T, Kakitani M, Yamazaki Y, Hasegawa H, Takeuchi Y, Fujita T, Fukumoto S, Tomizuka K, Yamashita T: Targeted ablation of Fgf23 demonstrates an essential physiological role of FGF23 in phosphate and vitamin D metabolism. *J Clin Invest* 113: 561–568, 2004
- Sitara D, Razzaque MS, Hesse M, Yoganathan S, Taguchi T, Erben RG, Juppner H, Lanske B: Homozygous ablation of fibroblast growth factor-23 results in hyperphosphatemia and impaired skeletogenesis, and reverses hypophosphatemia in PheX-deficient mice. *Matrix Biol* 23: 421–432, 2004
- Kato K, Jeanneau C, Tarp MA, Benet-Pages A, Lorenz-Depiereux B, Bennett EP, Mandel U, Strom TM, Clausen H: Polypeptide GalNAc-transferase T3 and familial tumoral calcinosis. Secretion of fibroblast growth factor 23 requires O-glycosylation. *J Biol Chem* 281: 18370–18377, 2006
- Segawa H, Yamanaka S, Ohno Y, Onitsuka A, Shiozawa K, Aranami F, Furutani J, Tomoe Y, Ito M, Kuwahata M, Imura A, Nabeshima Y, Miyamoto K: Correlation between hyperphosphatemia and type II Na-Pi cotransporter activity in klotho mice. *Am J Physiol Renal Physiol* 292: F769–F779, 2007
- Hesse M, Frohlich LF, Zeitz U, Lanske B, Erben RG: Ablation of vitamin D signaling rescues bone, mineral, and glucose homeostasis in Fgf-23 deficient mice. *Matrix Biol* 26: 75–84, 2007
- Sitara D, Razzaque MS, St-Arnaud R, Huang W, Taguchi T, Erben RG, Lanske B: Genetic ablation of vitamin d activation pathway reverses biochemical and skeletal anomalies in fgf-23-null animals. *Am J Pathol* 169: 2161–2170, 2006
- Stubbs J, Liu S, Tang W, Quarles LD: Role of hyperphosphatemia and 1,25(OH)2D3 in vascular calcifications and mortality in FGF23 null mice [Abstract]. *J Am Soc Nephrol* 17: 689A, 2006
- Li SA, Watanabe M, Yamada H, Nagai A, Kinuta M, Takei K: Immunohistochemical localization of Klotho protein in brain, kidney, and reproductive organs of mice. *Cell Struct Funct* 29: 91–99, 2004
- Benet-Pages A, Orlik P, Strom TM, Lorenz-Depiereux B: An FGF23 missense mutation causes familial tumoral calcinosis with hyperphosphatemia. *Hum Mol Genet* 14: 385–390, 2005
- Larsson T, Davis SI, Garringer HJ, Mooney SD, Draman MS, Cullen MJ, White KE: Fibroblast growth factor-23 mutants causing familial tumoral calcinosis are differentially processed. *Endocrinology* 146: 3883–3891, 2005
- Shimada T, Yamazaki Y, Takahashi M, Hasegawa H, Urakawa I, Oshima T, Ono K, Kakitani M, Tomizuka K, Fujita T, Fukumoto S, Yamashita T: Vitamin D receptor-independent FGF23 actions in regulating phosphate and

- vitamin D metabolism. *Am J Physiol Renal Physiol* 289: F1088–F1095, 2005
30. Saito H, Kusano K, Kinoshita M, Ito H, Hirata M, Segawa H, Miyamoto K, Fukushima N: Human fibroblast growth factor-23 mutants suppress Na⁺-dependent phosphate co-transport activity and 1 α ,25-dihydroxyvitamin D₃ production. *J Biol Chem* 278: 2206–2211, 2003
 31. Liu S, Tang W, Zhou J, Stubbs JR, Luo Q, Pi M, Quarles LD: Fibroblast growth factor 23 is a counter-regulatory phosphaturic hormone for vitamin D. *J Am Soc Nephrol* 17: 1305–1315, 2006
 32. Saito H, Maeda A, Ohtomo S, Hirata M, Kusano K, Kato S, Ogata E, Segawa H, Miyamoto K, Fukushima N: Circulating FGF-23 is regulated by 1 α ,25-dihydroxyvitamin D₃ and phosphorus in vivo. *J Biol Chem* 280: 2543–2549, 2005
 33. Lorenz-Depiereux B, Bastepe M, Benet-Pages A, Amyere M, Wagenstaller J, Muller-Barth U, Badenhop K, Kaiser SM, Rittmaster RS, Shlossberg AH, Olivares JL, Loris C, Ramos FJ, Glorieux F, Vikkula M, Juppner H, Strom TM: DMP1 mutations in autosomal recessive hypophosphatemia implicate a bone matrix protein in the regulation of phosphate homeostasis. *Nat Genet* 38: 1248–1250, 2006
 34. Liu S, Rowe PS, Vierthaler L, Zhou J, Quarles LD: Phosphorylated acidic serine-aspartate-rich MEPE-associated motif peptide from matrix extracellular phosphoglycoprotein inhibits phosphate regulating gene with homologies to endopeptidases on the X-chromosome enzyme activity. *J Endocrinol* 192: 261–267, 2007
 35. Burnett SA, Gunawardene SC, Bringham FR, Juppner H, Lee H, Finkelstein JS: Regulation of C-terminal and intact FGF-23 by dietary phosphate in men and women. *J Bone Miner Res* 21: 1187–1196, 2006
 36. Larsson T, Nisbeth U, Ljunggren O, Juppner H, Jonsson KB: Circulating concentration of FGF-23 increases as renal function declines in patients with chronic kidney disease, but does not change in response to variation in phosphate intake in healthy volunteers. *Kidney Int* 64: 2272–2279, 2003
 37. Nishida Y, Taketani Y, Yamanaka-Okumura H, Imamura F, Taniguchi A, Sato T, Shuto E, Nashiki K, Arai H, Yamamoto H, Takeda E: Acute effect of oral phosphate loading on serum fibroblast growth factor 23 levels in healthy men. *Kidney Int* 70: 2141–2147, 2006
 38. Imanishi Y, Inaba M, Nakatsuka K, Nagasue K, Okuno S, Yoshihara A, Miura M, Miyauchi A, Kobayashi K, Miki T, Shoji T, Ishimura E, Nishizawa Y: FGF-23 in patients with end-stage renal disease on hemodialysis. *Kidney Int* 65: 1943–1946, 2004
 39. Weber TJ, Liu S, Indridason OS, Quarles LD: Serum FGF23 levels in normal and disordered phosphorus homeostasis. *J Bone Miner Res* 18: 1227–1234, 2003
 40. Rowe PS, Kumagai Y, Gutierrez G, Garrett IR, Blacher R, Rosen D, Cundy J, Navvab S, Chen D, Drezner MK, Quarles LD, Mundy GR: MEPE has the properties of an osteoblastic phosphatonin and minihibin. *Bone* 34: 303–319, 2004
 41. Masuyama R, Stockmans I, Torrekens S, Van Looveren R, Maes C, Carmeliet P, Bouillon R, Carmeliet G: Vitamin D receptor in chondrocytes promotes osteoclastogenesis and regulates FGF23 production in osteoblasts. *J Clin Invest* 116: 3150–3159, 2006
 42. Bai X, Miao D, Panda D, Grady S, McKee MD, Goltzman D, Karaplis AC: Partial rescue of the Hyp phenotype by osteoblast-targeted PHEX (phosphate-regulating gene with homologies to endopeptidases on the X chromosome) expression. *Mol Endocrinol* 16: 2913–2925, 2002
 43. Erben RG, Mayer D, Weber K, Jonsson K, Juppner H, Lanske B: Overexpression of human PHEX under the human beta-actin promoter does not fully rescue the Hyp mouse phenotype. *J Bone Miner Res* 20: 1149–1160, 2005
 44. Liu S, Guo R, Tu Q, Quarles LD: Overexpression of Phex in osteoblasts fails to rescue the Hyp mouse phenotype. *J Biol Chem* 277: 3686–3697, 2002
 45. Bowe AE, Finnegan R, Jan de Beur SM, Cho J, Levine MA, Kumar R, Schiavi SC: FGF-23 inhibits renal tubular phosphate transport and is a PHEX substrate. *Biochem Biophys Res Commun* 284: 977–981, 2001
 46. Benet-Pages A, Lorenz-Depiereux B, Zischka H, White KE, Econs MJ, Strom TM: FGF23 is processed by proprotein convertases but not by PHEX. *Bone* 35: 455–462, 2004
 47. Guo R, Liu S, Spurney RF, Quarles LD: Analysis of recombinant Phex: An endopeptidase in search of a substrate. *Am J Physiol Endocrinol Metab* 281: E837–E847, 2001
 48. Zhang F, Zhai G, Kato BS, Hart DJ, Hunter D, Spector TD, Ahmadi KR: Association between KLOTHO gene and hand osteoarthritis in a female Caucasian population. *Osteoarthritis Cartilage* January 29, 2007 [epub ahead of print]
 49. Liu S, Brown TA, Zhou J, Xiao ZS, Awad H, Guilak F, Quarles LD: Role of matrix extracellular phosphoglycoprotein in the pathogenesis of X-linked hypophosphatemia. *J Am Soc Nephrol* 16: 1645–1653, 2005
 50. Li YC, Pirro AE, Amling M, Delling G, Baron R, Bronson R, Demay MB: Targeted ablation of the vitamin D receptor: An animal model of vitamin D-dependent rickets type II with alopecia. *Proc Natl Acad Sci U S A* 94: 9831–9835, 1997
 51. Inoue Y, Segawa H, Kaneko I, Yamanaka S, Kusano K, Kawakami E, Furutani J, Ito M, Kuwahata M, Saito H, Fukushima N, Kato S, Kanayama HO, Miyamoto K: Role of the vitamin D receptor in FGF23 action on phosphate metabolism. *Biochem J* 390: 325–331, 2005
 52. Ito M, Sakai Y, Furumoto M, Segawa H, Haito S, Yamanaka S, Nakamura R, Kuwahata M, Miyamoto K: Vitamin D and phosphate regulate fibroblast growth factor-23 in K-562 cells. *Am J Physiol Endocrinol Metab* 288: E1101–E1109, 2005
 53. Perwad F, Azam N, Zhang MY, Yamashita T, Tenenhouse HS, Portale AA: Dietary and serum phosphorus regulate fibroblast growth factor 23 expression and 1,25-dihydroxyvitamin D metabolism in mice. *Endocrinology* 146: 5358–5364, 2005
 54. Yu X, Sabbagh Y, Davis SI, Demay MB, White KE: Genetic dissection of phosphate- and vitamin D-mediated regulation of circulating Fgf23 concentrations. *Bone* 36: 971–977, 2005
 55. Quarles LD: Evidence for a bone-kidney axis regulating phosphate homeostasis. *J Clin Invest* 112: 642–646, 2003
 56. Kolek OI, Hines ER, Jones MD, LeSueur LK, Lipko MA, Kiela PR, Collins JF, Haussler MR, Ghishan FK: 1 α ,25-Dihydroxyvitamin D₃ upregulates FGF23 gene expression in bone: The final link in a renal-gastrointestinal-skeletal axis that controls phosphate transport. *Am J Physiol Gastrointest Liver Physiol* 289: G1036–G1042, 2005
 57. Winkler DG, Sutherland MK, Geoghegan JC, Yu C, Hayes T, Skonier JE, Shpektor D, Jonas M, Kovacevich BR, Staehling-Hampton K, Appleby M, Brunkow ME, Latham JA:

- Osteocyte control of bone formation via sclerostin, a novel BMP antagonist. *EMBO J* 22: 6267–6276, 2003
58. Kobayashi K, Imanishi Y, Koshiyama H, Miyauchi A, Wakasa K, Kawata T, Goto H, Ohashi H, Koyano HM, Mochizuki R, Miki T, Inaba M, Nishizawa Y: Expression of FGF23 is correlated with serum phosphate level in isolated fibrous dysplasia. *Life Sci* 78: 2295–2301, 2006
 59. Riminucci M, Collins MT, Fedarko NS, Cherman N, Corsi A, White KE, Waguespack S, Gupta A, Hannon T, Econs MJ, Bianco P, Gehron Robey P: FGF-23 in fibrous dysplasia of bone and its relationship to renal phosphate wasting. *J Clin Invest* 112: 683–692, 2003
 60. Shore EM, Ahn J, Jan de Beur S, Li M, Xu M, Gardner RJ, Zasloff MA, Whyte MP, Levine MA, Kaplan FS: Paternally inherited inactivating mutations of the GNAS1 gene in progressive osseous heteroplasia. *N Engl J Med* 346: 99–106, 2002
 61. Berndt T, Craig TA, Bowe AE, Vassiliadis J, Reczek D, Finnegan R, Jan De Beur SM, Schiavi SC, Kumar R: Secreted frizzled-related protein 4 is a potent tumor-derived phosphaturic agent. *J Clin Invest* 112: 785–794, 2003
 62. Carpenter TO, Ellis BK, Insogna KL, Philbrick WM, Sterpka J, Shimkets R: FGF7: An inhibitor of phosphate transport derived from oncogenic osteomalacia-causing tumors. *J Clin Endocrinol Metab* 90: 1012–1020, 2005
 63. Carey DE, Drezner MK, Hamdan JA, Mange M, Ahmad MS, Mubarak S, Nyhan WL: Hypophosphatemic rickets/osteomalacia in linear sebaceous nevus syndrome: A variant of tumor-induced osteomalacia. *J Pediatr* 109: 994–1000, 1986
 64. Heike CL, Cunningham ML, Steiner RD, Wenkert D, Horning RL, Gruss JS, Gannon FH, McAlister WH, Mumm S, Whyte MP: Skeletal changes in epidermal nevus syndrome: Does focal bone disease harbor clues concerning pathogenesis? *Am J Med Genet A* 139: 67–77, 2005
 65. Hoffman WH, Jueppner HW, Deyoung BR, O'Dorisio MS, Given KS: Elevated fibroblast growth factor-23 in hypophosphatemic linear nevus sebaceous syndrome. *Am J Med Genet A* 134: 233–236, 2005
 66. Pande S, Ritter CS, Rothstein M, Wiesen K, Vassiliadis J, Kumar R, Schiavi SC, Slatapolsky E, Brown AJ: FGF-23 and sFRP-4 in chronic kidney disease and post-renal transplantation. *Nephron Physiol* 104: p23–p32, 2006
 67. Gutierrez O, Isakova T, Rhee E, Shah A, Holmes J, Colerone G, Juppner H, Wolf M: Fibroblast growth factor-23 mitigates hyperphosphatemia but accentuates calcitriol deficiency in chronic kidney disease. *J Am Soc Nephrol* 16: 2205–2215, 2005
 68. Bhan I, Shah A, Holmes J, Isakova T, Gutierrez O, Burnett SA, Juppner H, Wolf M: Post-transplant hypophosphatemia: Tertiary 'hyper-phosphatoninism'? *Kidney Int* 70: 1486–1494, 2006
 69. Evenepoel P, Naesens M, Claes K, Kuypers D, Vanrenterghem Y: Tertiary 'hyperphosphatoninism' accentuates hypophosphatemia and suppresses calcitriol levels in renal transplant recipients. *Am J Transplant* March 19, 2007 [epub ahead of print]
 70. Araya K, Fukumoto S, Backenroth R, Takeuchi Y, Nakayama K, Ito N, Yoshii N, Yamazaki Y, Yamashita T, Silver J, Igarashi T, Fujita T: A novel mutation in fibroblast growth factor 23 gene as a cause of tumoral calcinosis. *J Clin Endocrinol Metab* 90: 5523–5527, 2005
 71. Campagnoli MF, Pucci A, Garelli E, Carando A, Defilippi C, Lala R, Ingrosso G, Dianzani I, Forni M, Ramenghi U: Familial tumoral calcinosis and testicular microlithiasis associated with a new mutation of GALNT3 in a white family. *J Clin Pathol* 59: 440–442, 2006
 72. Garringer HJ, Fisher C, Larsson TE, Davis SI, Koller DL, Cullen MJ, Draman MS, Conlon N, Jain A, Fedarko NS, Dasgupta B, White KE: The role of mutant UDP-N-acetyl-alpha-D-galactosamine-polypeptide N-acetylgalactosaminyltransferase 3 in regulating serum intact fibroblast growth factor 23 and matrix extracellular phosphoglycoprotein in heritable tumoral calcinosis. *J Clin Endocrinol Metab* 91: 4037–4042, 2006
 73. Ichikawa S, Imel EA, Sorenson AH, Severe R, Knudson P, Harris GJ, Shaker JL, Econs MJ: Tumoral calcinosis presenting with eyelid calcifications due to novel missense mutations in the glycosyl transferase domain of the GALNT3 gene. *J Clin Endocrinol Metab* 91: 4472–4475, 2006
 74. Spektor P, Cooper JG, Indelman M, Sprecher E: Hyperphosphatemic familial tumoral calcinosis caused by a mutation in GALNT3 in a European kindred. *J Hum Genet* 51: 487–490, 2006
 75. Hamada J, Tamai K, Ono W, Saotome K: Uremic tumoral calcinosis in hemodialysis patients: Clinicopathological findings and identification of calcific deposits. *J Rheumatol* 33: 119–126, 2006
 76. Quarles LD, Murphy G, Econs MJ, Martinez S, Lobaugh B, Lyles KW: Uremic tumoral calcinosis: Preliminary observations suggesting an association with aberrant vitamin D homeostasis. *Am J Kidney Dis* 18: 706–710, 1991
 77. Sabeel A, Al-Homrany M: Complete resorption of massive soft tissue calcification in a hemodialysis patient after parathyroidectomy. *Am J Nephrol* 20: 421–424, 2000
 78. Imel EA, Peacock M, Pitukcheewanont P, Heller HJ, Ward LM, Shulman D, Kassem M, Rackoff P, Zimering M, Dalkin A, Drobny E, Colussi G, Shaker JL, Hoogendoorn EH, Hui SL, Econs MJ: Sensitivity of fibroblast growth factor 23 measurements in tumor-induced osteomalacia. *J Clin Endocrinol Metab* 91: 2055–2061, 2006
 79. Makitie O, Kooh SW, Sochett E: Prolonged high-dose phosphate treatment: A risk factor for tertiary hyperparathyroidism in X-linked hypophosphatemic rickets. *Clin Endocrinol (Oxf)* 58: 163–168, 2003
 80. Verge CF, Lam A, Simpson JM, Cowell CT, Howard NJ, Silink M: Effects of therapy in X-linked hypophosphatemic rickets. *N Engl J Med* 325: 1843–1848, 1991
 81. Levy-Olomucki R, Moore WV, Stubbs J, Liu S, Quarles LD, Alon US: FGF-23 and 1,25D in children with hypophosphatemic rickets: Effect of treatment with phosphate. *J Bone Miner Res* 21: S318, 2006