X-Linked Hypophosphatemia and FGF23-Related Hypophosphatemic Diseases: Prospect for New Treatment

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ABSTRACT Phosphate plays essential roles in many biological processes, and the serum phosphate level is tightly controlled. Chronic hypophosphatemia causes impaired mineralization of the bone matrix and results in rickets and osteomalacia. Fibroblast growth factor 23 (FGF23) is a bone-derived hormone that regulates phosphate metabolism. FGF23 excess induces hypophosphatemia via impaired phosphate reabsorption in the renal proximal tubules and decreased phosphate absorption in the intestines. There are several types of genetic and acquired FGF23-related hypophosphatemic diseases. Among these diseases, X-linked hypophosphatemia (XLH), which is caused by inactivating mutations in the *phosphate-regulating endopeptidase homolog, X-linked* (*PHEX*) gene, is the most prevalent form of genetic FGF23-related hypophosphatemic rickets. Another clinically relevant form of FGF23-related hypophosphatemic disease is tumor-induced osteomalacia (TIO), a paraneoplastic syndrome associated with FGF23-producing tumors. A combination of active vitamin D and phosphate salts is the current medical therapy used to treat patients with XLH and inoperative TIO. However, this therapy has certain efficacy- and safety-associated limitations. Several measures to inhibit FGF23 activity have been considered as possible new treatments for FGF23-related hypophosphatemic diseases. In particular, a humanized monoclonal antibody for FGF23 (burosumab) is a promising treatment in patients with XLH and TIO. This review will focus on the phosphate metabolism and the pathogenesis and treatment of FGF23-related hypophosphatemic diseases. ***(Endocrine Reviews* 39: 274 – 291, 2018)**

Phosphorus is the second most abundant mineral in the human body, the first being calcium. There are two kinds of phosphorus, organic and inorganic, in our body. Because phosphorus is highly reactive, nearly all of the inorganic phosphorus is present in the form of phosphate in biological systems.

Fibroblast growth factor 23 (FGF23) is a bone-derived hormone that regulates phosphate homeostasis. In a physiological state, FGF23 is primarily secreted from osteocytes/osteoblasts and functions by binding mainly to a complex composed of fibroblast growth factor receptor 1c (FGFR1c) and αKlotho (referred to Klotho in this manuscript), a single-pass transmembrane protein (1, 2). FGF23 restricts phosphate reabsorption in the kidney and also decreases phosphate absorption in the intestines by suppressing serum 1,25-dihydroxyvitamin D [$1,25(OH)_2D$] levels (3). Several types of inherited and acquired forms of hypophosphatemic rickets/osteomalacia are caused by FGF23 excess. X-linked hypophosphatemia [XLH; Online Mendelian Inheritance in Man (OMIM) 307800], which is caused by inactivating mutations in the *phosphate-regulating endopeptidase homolog, X-linked* (*PHEX*) gene, is the most prevalent form of genetic FGF23-related hypophosphatemic rickets (4). Tumor-induced osteomalacia (TIO) is another clinically relevant form of FGF23-related hypophosphatemia (5, 6), caused by tumors that ectopically produce FGF23. It has been shown that FGF23 is physiologically regulated at both the transcriptional and posttranslational levels (7–10), and FGF23 excess occurs when these regulatory networks are disturbed. However, the precise mechanism of FGF23 excess in XLH and TIO is not yet known.

Current medical treatment of patients with FGF23-related hypophosphatemic rickets/osteomalacia is a combination of active vitamin D and oral phosphate salts (11). However, this treatment has several efficacy- and safety-related limitations (11). Several methods that inhibit FGF23 activity have been considered as possible new treatments for FGF23-related

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ESSENTIAL POINTS

- Fibroblast growth factor 23 (FGF23) is a bone-derived hormone that regulates phosphate metabolism
- FGF23 reduces serum phosphate by suppressing proximal tubular phosphate reabsorption and intestinal phosphate absorption
- FGF23 excess causes several hypophosphatemic diseases including X-linked hypophosphatemia and tumor-induced osteomalacia
- The inhibition of FGF23 activity is considered as a unique therapeutic measure for hypophosphatemic diseases caused by FGF23 excess

hypophosphatemic diseases. In particular, clinical trials have demonstrated that a humanized monoclonal antibody to FGF23 (burosumab) is a promising treatment of patients with XLH and TIO (12–14).

In this review, we will focus on the phosphate metabolism, the pathogenesis of FGF23-related hypophosphatemic diseases, and the recent findings regarding the FGF23-targeted therapy for these diseases.

Phosphate Metabolism

Function of phosphate

Phosphate serves many functions in the human body in the form of inorganic and organic substances (Table 1). Approximately 85% of phosphate is stored in bone as a form of hydroxyapatite crystals $[Ca_{10}(PO_4)_6(OH)_2]$ (15). The remaining 15% of phosphate is located either in the cell membrane as phospholipids or in the intracellular space as a constituent of nucleic acids (*e.g.*, DNA and RNA), high-energy nucleotides [*e.g.*, adenosine triphosphate (ATP) and adenosine diphosphate (ADP)], and various signaling molecules. These phosphate compounds are critical for maintaining cell structure and regulating cellular functions such as metabolism, cell signaling, and biosynthesis. Less than 1% of phosphate is present in the extracellular fluid as inorganic phosphate in the form of $H_2PO_4^-$ and HPO_4^{2-}. The serum phosphate concentrations are highest in the neonatal period, when values twofold that of adults are observed, and decline with age (16). There is growing evidence that extracellular phosphate in itself is a signaling molecule regulating the expression of various genes such as *c-fos*, *Jun B*, and *Egr1* (17–19).

Chronic hypophosphatemia leads to complications such as childhood rickets, osteomalacia, and skeletal muscle myopathy (20). Severe hypophosphatemia, often less than 1 mg/dL (0.32 mmol/L), may cause serious conditions such as rhabdomyolysis, acute hemolytic anemia, and arrhythmias due to ATP depletion (21). ATP molecule serves as an energy carrier in cells. The two outermost phosphates in ATP are connected to the rest of the molecule by high-energy phosphoanhydride bonds (22). When ATP is hydrolyzed to ADP and phosphate, large negative free energy is produced. Therefore, many coupled reactions involve the transfer of the terminal phosphate in ATP

Table 1. Functions of Phosphate in the Human Body

Component	Compound	Function
Bone, tooth	Hydroxyapatite	Tissue structure
Cell membrane	Phospholipids	Cell structure
		Cellular signaling
Nucleus	Nucleic acids (DNA, RNA)	Molecular structure
		Genetic information
Intracellular space	High-energy phosphate compounds (*e.g.*, ATP, ADP)	Metabolism
	Coenzymes	Cellular signaling
	Substrate for kinase and phosphatase	Cellular signaling
Extracellular fluid	Phosphate ion (*e.g.*, HPO_4^{2-}, $H_2PO_4^-$)	Buffer
		Regulation of calcification

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to another molecule. Mitochondria maintain a high ATP:ADP ratio in cells by rapidly phosphorylating the ADP to ATP and shuttling ATP out into the cytosol (22).

Chronic hyperphosphatemia, which is most frequently seen in chronic kidney disease (CKD), is related to the pathophysiology of ectopic calcification, secondary hyperparathyroidism, and cardiovascular disease (23, 24). The role of phosphate in vascular calcification has been shown both *in vitro* and *in vivo*. High-phosphate medium induced vascular smooth muscle cell (SMC) calcification (25). In this phosphate-induced vascular calcification, phenotypic change of vascular SMCs into osteochondrogenic-like ones along with increased expression of genes related to osteoblastic differentiation, such as *Runx2/Cbfa1* and *osteocalcin*, was shown (25). Treatment with a competitive inhibitor of the sodium/phosphate cotransporter or knockdown of SLC20A1 (Pit-1), one of type III sodium/phosphate transporters, inhibited phosphate uptake and calcification of vascular SMCs, indicating that phosphate uptake through Pit-1 is essential for the calcification in response to elevated phosphate (25, 26). Another intracellular mechanism of vascular calcification by phosphate is increased production of reactive oxygen species in mitochondria and subsequent activation of the nuclear factor-κB pathway (19). Additionally, in the uremic rats fed with a high-phosphate diet, analysis of the gene expression profile of calcified tissue in the aorta showed suppression of muscle-related genes and overexpression of bone-related genes, such as *secreted frizzled related proteins* (27). Increased expression of *Runx2/Cbfa1* was demonstrated in calcified vessels of dialysis patients, supporting the hypothesis that vascular SMCs transdifferentiate into osteoblastic cells (28). However, an inhibitor of sodium/phosphate cotransporter did not inhibit the upregulation of *Cbfa1* of bovine vascular SMCs by uremic serum (28), suggesting that some other factor than phosphate uptake is involved in the development of vascular calcification in uremia.

Phosphate homeostasis

Serum phosphate concentrations are mainly regulated by phosphate absorption in the intestine, filtration in the renal glomeruli, and reabsorption in the renal proximal tubular cells. When phosphate balance is maintained, the amount of phosphate absorbed in the intestine is equivalent to that excreted into the urine and feces (29).

Serum phosphate concentrations are also affected by a shift between the phosphate in extracellular fluid and that in bone or the intracellular space. For example, malnourished patients with phosphate depletion often develop severe hypophosphatemia after refeeding. In these patients, an increase in serum insulin following a rise in blood glucose enhances the cellular permeability of phosphate and potassium and causes a shift of these ions into intracellular spaces (30). It is not specified which types of channels and transporters are involved in shifting of these ions.

Regulatory mechanism of serum phosphate

There are two distinct mechanisms of phosphate transport: passive paracellular transport through tight junction complexes and active transport via sodium/phosphate cotransporters (29, 31). Although tight junction proteins, such as occludins and claudins, seem important for ion specificity, the distinct proteins that regulate passive phosphate transport have not been identified. Active inward transport of phosphate across the apical membrane is mediated by two families of sodium/phosphate cotransporters, SLC34 and SLC20 families (32, 33).

SLC34 family includes three types of type II sodium/phosphate cotransporter proteins, SLC34A1 (NaPi-IIa), SLC34A2 (NaPi-IIb), and SLC34A3 (NaPi-IIc), that play essential roles in the kidney and intestines in the regulation of extracellular phosphate levels (32). SLC20 family includes two types of type III sodium/phosphate transporters, Pit-1 and SLC20A2 (Pit-2), that are expressed broadly in a variety of cell types (33)

Extracellular phosphate levels are mainly regulated by the balance between intestinal absorption and urinal excretion of phosphate, which are controlled by hormones, such as FGF23, parathyroid hormone (PTH), and $1{,}25(OH)_2D$ (Fig. 1). In the kidney, phosphate is first filtered in the glomeruli, and then it is reabsorbed in the renal tubules. Phosphate reabsorption is mediated by NaPi-IIa and NaPi-IIc encoded by *SLC34A1* and *SLC34A3*, respectively, in the apical membrane of the renal proximal tubular cells (34). The abundance of these sodium/phosphate cotransporters is regulated by both dietary phosphate intake and hormones such as FGF23 and PTH (3, 35). A high-phosphate diet decreases and dietary phosphate restriction increases the expression of sodium/phosphate cotransporters in the proximal tubules (36–38). PTH and FGF23 reduce the expression of NaPi-IIa and NaPi-IIc. Glucocorticoid therapy and metabolic acidosis also decrease renal phosphate reabsorption through a decrease in renal sodium/phosphate cotransporter expression (39, 40).

In the small intestines, phosphate is absorbed via both sodium-dependent and sodium-independent paracellular transport mechanisms (31). Phosphate absorption in the colon seems to be negligible under physiological conditions and is only relevant when luminal phosphate concentrations are extremely high (41). Sodium-dependent intestinal phosphate absorption is mainly mediated by NaPi-IIb encoded by *SLC34A2* in the apical membrane of the intestinal epithelial cells and type III sodium/phosphate cotransporters Pit-1 and Pit-2 (34, 42). NaPi-IIb and Pit-1 proteins are mostly expressed in the duodenum and jejunum in rats, whereas NaPi-IIb is expressed in jejunum and ileum in mice (43). Expression of NaPi-IIb is regulated by dietary phosphate intake and $1{,}25(OH)_2D$ (44, 45). A low-phosphate diet

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and $1,25(OH)_2D$ enhance the expression of NaPi-IIb. A low-phosphate diet significantly increased NaPi-IIb expression in both wild-type and vitamin D receptor knockout mice, suggesting that phosphate regulates intestinal NaPi-IIb expression independently of $1,25(OH)_2D$ (46). Furthermore, FGF23 reduces $1,25(OH)_2D$ levels and indirectly decreases intestinal absorption of phosphate (3).

The two major causes of chronic hypophosphatemia are a decrease in intestinal phosphate absorption and an increase in renal phosphate loss (Table 2). In addition to malnourishment, underlying gastrointestinal diseases, such as short bowel syndrome, result in impaired intestinal phosphate absorption (47). Hypophosphatemia and bone disease associated with amino acid–based elemental formula use have been reported in infants and children (48). Bioavailability of formula phosphate could be impaired in some children despite adequate formula composition, thus careful monitoring of serum phosphate is necessary when elemental formula products are used as a sole source of nutrition.

Diseases caused by increased renal phosphate loss are largely divided into those that are due to FGF23 excess (FGF23-related hypophosphatemic diseases) and those result from other causes. Hypophosphatemic rickets with hypercalciuria (OMIM #241530), an autosomal recessive disorder due to loss-of-function mutations in *SLC34A3*, is one of the heritable forms of FGF23-independent hypophosphatemia (49). Proximal renal tubular dysfunction also results in FGF23-independent hypophosphatemia. When there is a global dysfunction of renal transport mechanisms in addition to hyperphosphaturia, such as increased excretion of amino acid, glucose, and bicarbonate, it is called Fanconi syndrome. There are various forms of hereditary Fanconi syndrome, such as Lowe syndrome (OMIM #309000) and Dent disease 2 (OMIM #300555) caused by mutations in the *oculocerebral syndrome of Lowe* (*OCRL*) gene (50) and Dent disease 1 (OMIM #300009) caused by mutations in the *chloride channel 5* (*CLCN5*) gene (51, 52). In adult patients, drug toxicity induced by anticancer agents, antivirals, and aminoglycoside antibiotics is the most common cause of proximal tubular dysfunction (53). In systematic diseases such as multiple myeloma and amyloidosis, the deposition of immunoglobulin light chain or amyloid fibrils may cause tubulointerstitial nephritis (54).

FGF23-Related Hypophosphatemic Diseases

Structure and function of FGF23

FGF23 is a member of the FGF19 subfamily of endocrine fibroblast growth factors and is composed of 251 amino acids (55). FGF23 has an N-terminal signal peptide composed of 24 amino acids (5, 56), which acts on distal organs after it is secreted from osteocytes/osteoblasts. Subtilisin-like proprotein convertases cleave the intact FGF23 protein between Arg179 and Ser180 to generate N-terminal and C-terminal FGF23 fragments (Fig. 2). It is believed that only intact full-length FGF23 is able to activate downstream signaling from fibroblast growth factor receptor (FGFR)/Klotho complex (3). Although *in vitro* experiments have shown that the C-terminal FGF23 fragment competes with the full-length FGF23 for binding to an FGFR/Klotho complex and restricts the activity of the intact FGF23 (57), the physiologic significance of this C-terminal fragment of FGF23 *in vivo* is unclear.

FGF23 activity is regulated by both transcription of *FGF23* and posttranslational cleavage of the FGF23 protein. $1,25(OH)_2D$ is one of the most important stimulators of *FGF23* transcription. $1,25(OH)_2D$ upregulates *FGF23* messenger RNA (mRNA) in osteolineage cells *in vitro* (58, 59). *In vivo*, circulating FGF23 levels are suppressed in *vitamin D receptor* (*VDR*) knockout mice and in patients with vitamin

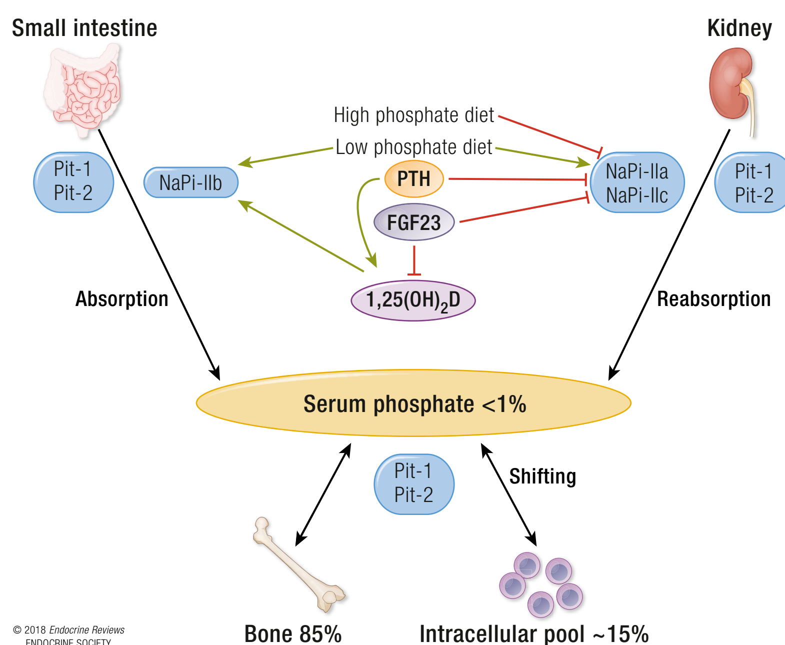

Figure 1. Regulatory mechanism of serum phosphate by PTH, FGF23, and $1,25(OH)_2D$. Approximately 85% of phosphate in the body is stored in bone and 15% in the intracellular space. Less than 1% of total phosphate is present in the extracellular space. Serum phosphate concentrations are regulated by intestinal phosphate absorption, renal phosphate handling, and a shift between the phosphate in the extracellular fluid and that in bone or the intracellular space. PTH and FGF23 suppress proximal tubular phosphate reabsorption by reducing the expression of type IIa and IIc sodium/phosphate cotransporters (NaPi-IIa, NaPi-IIc). PTH increases and FGF23 decreases $1,25(OH)_2D$ levels. $1,25(OH)_2D$ stimulates sodium-dependent intestinal phosphate absorption by enhancing the expression of type IIb sodium/phosphate cotransporter (NaPi-IIb). Dietary phosphate intake regulates the expression of these type II sodium/phosphate cotransporters. Type III sodium/phosphate cotransporters, Pit-1 and Pit-2, are broadly expressed across a variety of cell types and also contribute to phosphate transport.

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Table 2. Major Causes of Chronic Hypophosphatemia

Decreased Intestinal Phosphate Absorption
Malnourishment
Gastrointestinal diseases (*e.g.*, short bowel syndrome)
Increased Renal Phosphate Loss
FGF23-related hypophosphatemic diseases (summarized in Table 3)
Hypophosphatemic rickets with hypercalciuria caused by mutations in the *SLC34A3* gene
Proximal renal tubular dysfunction
Congenital diseases with various responsible genes
Lowe syndrome caused by mutations in the *OCRL* gene
Dent disease 1 caused by mutations in the *CLCN5* gene
Dent disease 2 caused by mutations in the *OCRL* gene
Acquired renal tubular dysfunction
Drug toxicity (*e.g.*, anticancer agents, antivirals, and aminoglycoside antibiotics)
Multiple myeloma
Amyloidosis
Heavy metals

Abbreviations: *CLCN5*, *chloride channel 5*; *OCRL*, *oculocerebral syndrome of Lowe*; *SLC34A3*, *sodium/phosphate cotransporter type IIc*.

D–dependent rickets type 2A caused by inactivating mutations in the *VDR* gene (60, 61). Conversely, administration of active vitamin D to CKD patients with secondary hyperparathyroidism resulted in increased serum FGF23 levels (62, 63). Additionally, extracellular phosphate concentrations seem to regulate *FGF23* transcription. An increase in extracellular phosphate concentration resulted in increased *FGF23* mRNA in UMR-106 cells via enhanced production of reactive oxygen species (64). It has also been shown that dietary phosphate intake can influence serum FGF23 levels in both mice and men (65–67). However, it is not clear whether high luminal phosphate concentrations or subsequent increase in serum phosphate concentrations are necessary to induce osteocyte/osteoblasts to produce FGF23. Because acute changes of serum phosphate levels induced by either phosphate infusion or carbohydrate ingestion did not alter serum FGF23 levels in healthy individuals (68), it is possible that luminal phosphate concentration is involved in FGF23 regulation.

Studies have shown the possibility that both iron status and the treatment modalities for anemia regulate *FGF23* transcription and posttranslational modification of FGF23 protein (69–72). Iron deficiency seems to stimulate *FGF23* transcription (69, 73). Treatment with iron chelator deferoxamine increased *FGF23* mRNA with the activation of the transcription factor hypoxia inducible factor-1α in UMR-106 cells. Additional experiments showed significantly elevated *FGF23* mRNA levels in cells cultured under hypoxic conditions (73). These results suggest that hypoxia inducible factor-1α is involved in the regulation of *FGF23* transcription. In contrast, intravenous administration of certain iron preparations such as saccharated ferric oxide, iron polymaltose, and ferric carboxymaltose causes FGF23-related hypophosphatemia in patients with iron deficiency anemia (72, 74, 75). Study of the biochemical response to intravenous iron in 55 women with iron deficiency anemia has shown that ferric carboxymaltose administration resulted in a decrease in total FGF23 levels along with an increase in intact FGF23 levels (70). Therefore, it is likely that certain iron preparations may inhibit the processing of FGF23 protein and increase intact FGF23 levels. More experiments are needed to prove this theory. In addition, erythropoietin (EPO) may stimulate *FGF23* transcription in osteoblastic cells and hematopoietic progenitor cells (71). In mice injected with recombinant human EPO, upregulation of *FGF23* mRNA was observed in whole-bone and flow cytometry–sorted lineage-c-kit^{+} Sca1^{+} cells along with elevated serum intact FGF23 concentrations (71). Intravenous iron preparations and EPO are often used to treat CKD-associated anemia. Therefore, the impact of these treatments on serum intact FGF23 levels should be considered in CKD patients.

There are two enzymes potentially involved in the posttranslational modification of FGF23. The *polypeptide N-acetylgalactosaminyltransferase 3* (*GALNT3*) gene encodes an enzyme that is responsible for the initiation of mucin-type *O*-linked glycosylation of FGF23. The enzyme is called uridine diphosphate-*N*-acetyl-α-D-galactosamine:polypeptide *N*-acetylgalactosaminyltransferase 3, which attaches *N*-acetylgalactosamine to Ser or Thr residues (76). The *GALNT3* gene product initiates the *O*-linked glycosylation at Thr178 of FGF23 protein and prevents the cleavage of FGF23 between Arg179 and Ser180 (77). It has been shown that a Golgi kinase, referred to as family with sequence similarity 20, member C (FAM20C), phosphorylates FGF23 at Ser180. Phosphorylation of FGF23 by FAM20C has been shown to restrict its *O*-glycosylation by the *GALNT3* gene product and enhances its cleavage by subtilisin-like proprotein convertases (10). Cross-talk between phosphorylation and *O*-glycosylation of FGF23 protein during the secretory process seems to be an important mechanism regulating its function (10).

Several basic studies have shown that PTH stimulates FGF23 production in bone, which is followed by a rapid degradation of intact FGF23 protein (78–80). Treatment with PTH activated the transcription factor Nurr1 and increased *FGF23* transcription in UMR106 cells (78). A single subcutaneous injection of PTH(1-34),

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which corresponds to 34 *N*-terminal amino acids of PTH, in mice resulted in increased serum total FGF23 levels with upregulated bone *FGF23* mRNA but decreased intact FGF23 levels (79). PTH-induced downregulation of *GALNT3* may explain the enhanced proteolytic cleavage of intact FGF23 to FGF23 fragments (80). However, the significance of the effect of PTH on FGF23 production under physiological conditions needs further investigation.

As currently understood, the primary function of FGF23 is to lower serum phosphate concentrations (Fig. 3). FGF23 reduces phosphate reabsorption by downregulating sodium/phosphate cotransporters in the renal proximal tubules (3). FGF23 also suppresses the production of 1,25(OH)$_2$D in the renal proximal tubules by downregulating *CYP27B1*, which encodes 25-hydroxyvitamin D-1α-hydroxylase, and upregulating *CYP24A1*, which encodes 25-hydroxyvitamin D-24-hydroxylase, resulting in decreased phosphate absorption in the intestines (3). In diseases with primary FGF23 excess, inadequately high FGF23 causes chronic hypophosphatemia and subsequent musculoskeletal complications (81). Serum full-length FGF23 levels in subjects with normal kidney function are approximately between 10 to 50 pg/mL (81). FGF23 measured by an enzyme-linked immunosorbent assay that detects only intact FGF23 has been proposed to distinguish FGF23-related hypophosphatemia from hypophosphatemia caused by other etiologies with a cut-off value of 30 pg/mL (81, 82); serum FGF23 levels are 30 pg/mL or more in patients with FGF23-related hypophosphatemic diseases. However, there is an overlap of FGF23 levels between healthy control and patients with FGF23-related hypophosphatemia. This "normal" FGF23 in patients with FGF23-related hypophosphatemia can be interpreted to be inappropriate in the presence of hypophosphatemia. In contrast, FGF23 levels are below 30 pg/mL and sometimes undetectable in patients with chronic hypophosphatemia from other causes. Suppressed FGF23 levels in these patients suggest that FGF23 level is tightly regulated by either the serum phosphate concentration or other metabolic changes associated with chronic hypophosphatemia.

Parathyroid glands express Klotho, and it was reported that FGF23 suppresses PTH production and secretion (83). FGF23 was also reported to be able to suppress PTH secretion in a Klotho-independent manner (84). However, this effect of FGF23 on parathyroid glands is controversial because persistent FGF23 signaling induced parathyroid cell proliferation and PTH secretion (85). It is possible that effects of FGF23 on parathyroid cells are different depending on the duration of FGF23 actions.

There is an argument whether FGF23 has effects on other organs than kidney and parathyroid glands. Recent clinical studies have shown that higher serum FGF23 level is a risk factor for mortality and cardiovascular disease (86–88). The results of experimental studies indicated that FGF23 directly induces left ventricular hypertrophy (LVH) via activation of fibroblast growth factor receptor type 4 (FGFR4) in the absence of Klotho (89). Additionally, there is a possibility that FGF23 stimulates the renin-angiotensin-aldosterone system by suppressing angiotensin-converting enzyme 2 (90). However, considering that children with XLH and adults with FGF23-related hypophosphatemia did not demonstrate LVH (91, 92), conditions other than FGF23 excess may cause LVH in CKD. Although there is growing evidence that extracellular phosphate is involved in the pathogenesis of vascular calcification, the role of FGF23 in vascular calcification is still controversial (93). It has been shown that FGF23 enhances phosphate-induced calcification in the aortas from CKD rats and Klotho-overexpressing vascular SMCs by promoting osteoblastic differentiation (94). In contrast, other reports have shown that FGF23 had either no effect or even a protective effect on calcification of vascular SMCs (95–98).

A receptor for FGF23

Fibroblast growth factor family members bind to FGFRs. There are four *FGFR* genes (*FGFR1* to *FGFR4*), and alternative splicing of these transcripts produces many FGFR subtypes. FGFRs have several immunoglobulin-like loops in the extracellular domains. Alternative splicing produces b (epithelial) and c (mesenchymal) subtypes of FGFR1 to FGFR3 that

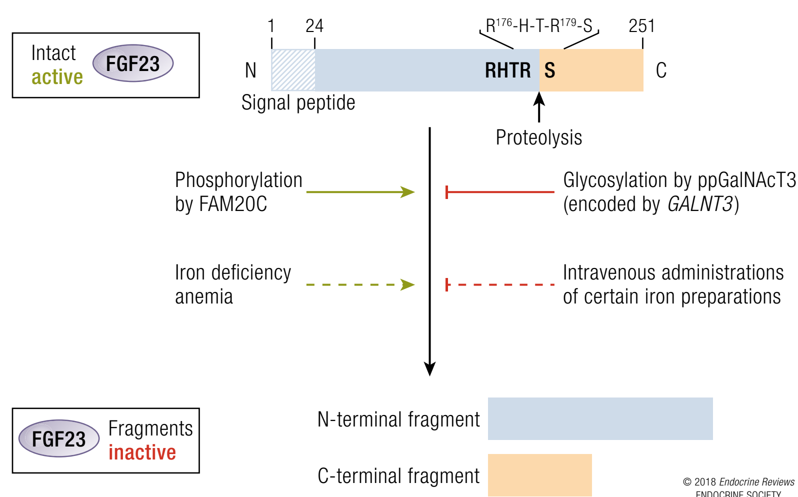

Figure 2. Structure of FGF23 and its posttranslational modification. A signal peptide composed of 24 amino acids present at the N-terminus of FGF23 protein is cleaved before secretion from osteocytes/osteoblasts. Subtilisin-like proprotein convertases cleave the active intact FGF23 between Arg179 and Ser180 to generate inactive fragments. *O*-linked glycosylation at Thr178 by ppGalNAcT3 prevents and phosphorylation at Ser180 by FAM20C enhances proteolytic cleavage of intact FGF23. Iron deficiency anemia and intravenous administration of certain iron preparations seem to regulate FGF23 proteolysis, although the precise mechanism is unknown. C, C-terminal; H, histidine; N, *N*-terminal; R, arginine; S, serine; T, threonine.

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have different immunoglobulin-like loops adjacent to the transmembrane domain (99). *In vitro* and *in vivo* study indicated that FGF23 can bind to FGFR1c-Klotho complex (2, 100, 101). The contribution of FGFR3c and FGFR4 in Klotho-dependent FGF23 actions is controversial. *In vitro* study showed that FGF23 can bind to FGFR3c and FGFR4 in the presence of Klotho (2), whereas another study indicated that FGF23 and Klotho make a complex only with FGFR1c (100). *In vivo* studies also produced conflicting data concerning the importance of FGFR3 and FGFR4 (102, 103). Moreover, recent findings suggested that FGF23 can bind to FGFR4 in the absence of Klotho in cardiac myocytes and activates phospholipase C γ/calcineurin/nuclear factor of activated T cells signaling pathway, which is known to be a potent inducer of LVH (89). It was also reported that FGF23 can promote inflammation by acting on hepatocytes through FGFR4 (104). However, it is not known how FGF23 can activate FGFRs without Klotho. Further studies are necessary to establish the contribution of FGFR3 and FGFR4 in Klotho-dependent FGF23 actions.

Klotho is a type I membrane protein that has a large extracellular domain (105). It has been shown that Klotho is important in the regulation of phosphate homeostasis, whereas βKlotho is required for FGF19 and FGF21 signaling. The Klotho protein is composed of a large extracellular domain, transmembrane domain, and a short intracellular domain (105). The entire extracellular domain can be subjected to ectodomain shedding and is released into the extracellular space as soluble secreted Klotho. Because the expression of most FGFRs are not restricted to specific tissues, membrane-bound Klotho, which has limited expression in tissues such as kidney, placenta, parathyroid glands, and brain, is likely to determine the site of action of FGF23 (2, 100). *Klotho*-deficient mice show features similar to those seen in *FGF23*-null mice, such as ectopic calcifications with elevated serum 1,25(OH)$_2$D, phosphate, and calcium (106). *Klotho*-deficient mice show FGF23 resistance with a 2000-fold increase in serum FGF23 concentrations compared with that of wild-type mice (106). Moreover, *FGF23/Klotho/VDR* triple-knockout mice were indistinguishable from double *FGF23/VDR* and *Klotho/VDR* compound mutants in terms of mineral homeostasis (107). These results suggest that FGF23 and Klotho cooperate in regulating mineral homeostasis.

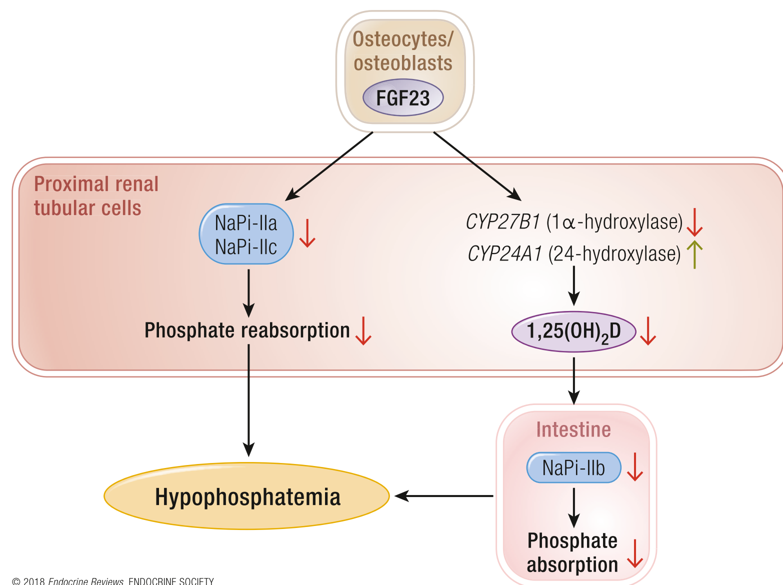

Figure 3. Phosphate regulation by FGF23. FGF23 reduces phosphate reabsorption by downregulating sodium/phosphate cotransporters in the renal proximal tubules. FGF23 also suppresses the production of 1,25(OH)$_2$D in the proximal renal tubules by downregulating *CYP27B1* (1α-hydroxylase) and upregulating *CYP24A1* (24-hydroxylase), leading to decreased phosphate absorption in the intestine.

Studies have shown that Klotho is expressed primarily in the renal distal tubules with much lower abundance in the proximal tubules (108, 109). Systemic or whole-nephron deletion of *Klotho* in mice resulted in hyperphosphatemia with elevated 1,25(OH)$_2$D levels by FGF23 resistance (106, 110). Therefore, kidney is the principal organ for Klotho to mediate its effects in mineral homeostasis. To investigate the segment-specific roles of Klotho in renal tubules, mice with targeted deletion of *Klotho* have been generated (111, 112). Mice with a partial deletion of Klotho in the distal tubules showed hyperphosphatemia with elevated serum FGF23 levels and increased NaPi-IIa protein in kidney brush border membrane. Serum calcium and 1,25(OH)$_2$D levels did not change significantly, but mild increase in urinary calcium with decreased TRPV5 protein was observed (112). At the same time, mice with *Klotho* specifically ablated in the proximal tubules displayed mild hyperphosphatemia, hypophosphaturia, and increased NaPi-IIa protein in the proximal tubules. Changes in serum 1,25(OH)$_2$D levels were modest (111). From these results, it is possible that Klotho in both proximal and distal tubules are contributing to FGF23-mediated regulation of phosphate and vitamin D metabolism. However, further studies are necessary to establish the role of Klotho in different parts of renal tubules.

FGF23 has been shown to bind to FGFR/Klotho complex and activate ERK1/2 and serum/glucocorticoid-regulated kinase 1, which leads to sodium/hydrogen exchanger regulatory factor 1 phosphorylation and subsequent downregulation of the apical membrane expression of NaPi-IIa and NaPi-IIc (113). In the distal tubules, FGF23 has been shown to enhance calcium reabsorption via FGFR/Klotho complex by activating a signaling cascade involving ERK1/2, SGK, and WNK4 and increases intracellular transport of calcium channel TRPV5 from the Golgi apparatus to the apical membrane (114).

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The significance of soluble Klotho in phosphate metabolism has been explored. Low serum Klotho levels have been reported to be associated with poor skeletal muscle strength, high prevalence of cardiovascular disease, and all-cause mortality in community-dwelling adults (115). Decreases in serum soluble Klotho levels are associated with signs of vascular dysfunction such as arterial stiffness in patients with CKD (116). Additionally, the involvement of soluble Klotho in FGF23 production has been indicated (117, 118). A patient with increased plasma soluble Klotho as the result of a chromosomal translocation involving the *Klotho* gene presented with FGF23-related hypophosphatemic rickets (117). Additionally, mice treated with an adenovirus producing soluble Klotho showed FGF23-related hypophosphatemic osteomalacia with upregulated *FGF23* mRNA in bone (118). In these situations, soluble Klotho seems to regulate FGF23 production through soluble Klotho-FGF23-FGFR interactions. However, if soluble Klotho makes a receptor complex with FGFR, tissue-specific actions of FGF23 cannot be explained. Therefore, the membrane-bound Klotho/FGFR1c complex seems to be the primary site of action of FGF23 at least in mineral metabolism.

Table 3. FGF23-Related Hypophosphatemic Diseases

Disease With Known Genetic Causes	Responsible Gene
XLH	*PHEX*
ADHR	*FGF23*
ARHR1	*DMP1*
ARHR2	*ENPP1*
Osteoglophonic dysplasia	*FGFR1*
Jansen-type metaphyseal chondrodysplasia	*PTH1R*
Raine syndrome	*FAM20C*
McCune-Albright syndrome	*GNAS1*
Epidermal nevus syndrome	*HRAS, NRAS*
Acquired Disease	
TIO	
Hypophosphatemia after intravenous infusion of certain iron preparations	
Biliary atresia	

Abbreviations: ARHR1, autosomal recessive hypophosphatemic rickets type 1; ARHR2, autosomal recessive hypophosphatemic rickets type 2; *DMP1, dentin matrix protein; ENPP1, ectonucleotide pyrophosphatase/phosphodiesterase 1; FGFR1, fibroblast growth factor receptor 1; GNAS1, guanine nucleotide binding protein, alpha-stimulating activity polypeptide 1; HRAS, V-HA-RAS Harvey rat sarcoma viral oncogene homolog; NRAS, neuroblastoma RAS viral oncogene; PTH1R, parathyroid hormone 1 receptor.*

FGF23-related hypophosphatemic rickets/osteomalacia

There are several types of FGF23-related hypophosphatemic rickets/osteomalacia (Table 3). Inactivating mutations in the *PHEX* gene result in the most prevalent form of genetic FGF23-related rickets, XLH (4). Mutations in the *FGF23* gene that cause resistance to proteolytic cleavage between Arg179 and Ser180 result in autosomal dominant hypophosphatemic rickets (ADHR; OMIM #193100) (56). Inactivating mutations in the *dentin matrix protein 1* (*DMP1*) and the *ectonucleotide pyrophosphatase/phosphodiesterase 1* (*ENPP1*) genes are responsible for autosomal recessive hypophosphatemic rickets type 1 (OMIM #241520) and type 2 (OMIM #613312), respectively (119–122).

In patients with ADHR, mutations that change the amino acid Arg176 or Arg179, present in the consensus recognition site of Arg-X-X-Arg motif by subtilisin-like proprotein convertases, result in resistance to proteolytic processing (9). Because FGF23 works as a hormone, circulating intact FGF23 levels should be tightly controlled. However, there seems to be a positive correlation between serum intact FGF23 concentrations and the degree of hypophosphatemia in patients with ADHR (123). Additionally, disease severity is variable among patients with ADHR, and clinical manifestation can change over the course of the patient's lifetime (123). Based on these observations, certain environmental factors seem to stimulate FGF23 production in bone and induce hypophosphatemia in patients with ADHR. For example, both the transcription of *FGF23* and cleavage of the FGF23 protein seem to be enhanced by iron deficiency (8). In healthy individuals, enhanced cleavage of FGF23 protein is able to keep serum intact FGF23 and phosphate levels within a normal range. In contrast, because of the resistance to proteolytic cleavage of mutant FGF23, upregulated transcription of *FGF23* directly leads to increased intact FGF23 levels in patients with ADHR. Therefore, it seems likely that any factors that stimulate *FGF23* transcription in bone can theoretically exacerbate hypophosphatemia in patients with ADHR. Further investigations are needed to prove this theory.

DMP1 is a glycoprotein that is highly expressed in bone and teeth and controls mineralization (124). Patients with FGF23-related hypophosphatemia caused by mutations in the *DMP1* gene show phenotypes of rickets, such as leg deformities and short stature in childhood (119, 122). However, some of the hypophosphatemic patients by a mutation in *DMP1* develop an unexpected increase in bone density and bone overgrowth of the long bones, the spine, and the skull with advancing age, which resembled osteopetrosis (125). Therefore, the main function of DMP1 seems to maintain phosphate homeostasis by regulating FGF23 transcription rather than to directly promote mineralization. The mechanisms regarding how mutations in *DMP1* result in FGF23-related hypophosphatemia remain unknown.

ENPP1 is a plasma membrane protein that generates extracellular inorganic pyrophosphate (PPi) by hydrolyzing extracellular ATP to adenosine

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5′-monophosphate (126). PPi is a physiologic inhibitor of hydroxyapatite formation, and PPi deficiency caused by loss-of-function mutations in the *ENPP1* gene results in ectopic calcification (127). Mutations in *ENPP1* were identified as a cause of generalized arterial calcification of infancy 1 (OMIM #208000) (128). Generalized arterial calcification of infancy 1 is usually a fatal disease with severe arterial calcification during infancy. Mutations in *ENPP1* were also identified in families with autosomal recessive hypophosphatemic rickets with elevated serum FGF23 levels (120, 121). Tissue-nonspecific alkaline phosphatase (TNAP), which is also referred to as alkaline phosphatase, liver/bone/kidney type, hydrolyzes PPi into phosphate. Double-knockout mice null for both the *Akp2* (which encodes TNAP) and the *Enpp1* genes have shown normal bone mineralization with normal PPi concentrations in osteoblast, suggesting that ENPP1 and TNAP cooperate to regulate the extracellular PPi concentrations (129). The tip-toe walking ($Enpp1^{ttw/ttw}$) mouse is a mouse model of ossification of the posterior longitudinal ligament of the spine caused by a nonsense mutation in *Enpp1* (127). Treatment with the alkaline phosphatase inhibitor levamisole in combination with exogenous PPi increased serum PPi levels and slowed the progression of ossification of spinal ligament in $Enpp1^{ttw/ttw}$ mice (130). Moreover, it has been shown that $Enpp1^{ttw/ttw}$ mice fed a high-phosphate diet exhibited phenotypes resembling *Klotho* mutant mice, such as short life span, arteriosclerosis, and osteoporosis (131). These aging-like phenotypes were rescued by *Klotho* overexpression, indicating that *Enpp1* loss leads to downregulation of renal *Klotho* expression under phosphate overload conditions and resistance to FGF23 (131). However, further studies are needed to clarify the precise mechanism of how loss-of-function mutations in the *ENPP1* gene result in FGF23-related hypophosphatemic rickets.

Exome sequencing has revealed that adult patients with Raine syndrome (OMIM #259775) caused by inactivating mutations in the *FAM20C* gene develop FGF23-related hypophosphatemia (132, 133). Raine syndrome is an autosomal recessive disorder characterized by generalized osteosclerosis with periosteal bone formation and a distinctive facial phenotype. Raine syndrome was first reported as a lethal osteosclerotic bone dysplasia, and nonlethal cases with FGF23-related hypophosphatemia were subsequently reported (132, 134, 135). FAM20C is one of the Golgi kinases that is expressed in a wide variety of tissues (136). In the skeletal and dental tissues, FAM20C was detected in the osteoblasts, odontoblasts, and ameloblasts and was proposed to act as an inhibitor for mineralization during osteogenesis (137). The small integrin-binding ligand, *N*-linked glycoproteins, which include DMP1, dentin sialophosphoprotein, osteopontin, matrix extracellular phosphoglycoprotein, and bone sialoprotein, have multiple *S*-x-E/pS motifs that are phosphorylated by FAM20C (138). Small integrin-binding ligand, *N*-linked glycoproteins may regulate proper growth and formation of hydroxyapatite crystals, and abnormal phosphorylation of these proteins has been shown to be responsible for the deranged mineralization in Raine syndrome (139, 140). In the calvaria of *Fam20c* conditional knockout mice, downregulation of *Dmp1* mRNA was observed along with the upregulation of *FGF23* mRNA (141), suggesting that loss of Dmp1 activity leads to FGF23 elevation in *Fam20c* knockout mice. It was also reported that FAM20C directly phosphorylates FGF23 protein at Ser180 and promotes FGF23 cleavage and that decreased kinase activity of FAM20C leads to elevated intact FGF23 (10). Therefore, changes of both *FGF23* transcription and posttranslational modification of FGF23 protein may explain high serum intact FGF23 concentrations in patients with mutations in *FAM20C*. Additionally, patients with osteoglophonic dysplasia (OMIM #166250) with activating mutations in the *FGFR1* gene (142), Jansen-type metaphyseal chondrodysplasia (OMIM #156400) with mutations in the *PTH1 receptor* gene (143), McCune-Albright syndrome/fibrous dysplasia (OMIM #174800) with mutations in the *guanine nucleotide binding protein, alpha-stimulating activity polypeptide 1* gene (144), and epidermal nevus syndrome (OMIM #162900), also known as linear nevus sebaceous syndrome, with mutations in the *Harvey rat sarcoma viral oncogene homolog* or the *neuroblastoma RAS viral oncogene homolog* (*NRAS*) gene can develop FGF23-related hypophosphatemia (145, 146). It has been shown that extracellular phosphate induces FGFR1 and NRAS activation (17), suggesting the presence of a lower set point of extracellular phosphate for activation of intracellular signaling in certain types of inherited FGF23-related hypophosphatemia.

TIO and hypophosphatemia following intravenous iron infusion are the major causes of the acquired form of FGF23-related hypophosphatemia (5, 72, 147, 148). As the name suggests, TIO is commonly diagnosed in adult patients. However, there are reports of children affected with TIO who develop rickets (149–151). In TIO, FGF23 is usually secreted by phosphaturic mesenchymal tumors located in bone or soft tissues (152). The tumors are usually benign and are found in various sites, though most often in the lower extremities and in the head and neck regions (153). However, several malignancies, such as those located in the colon, ovaries, and prostate, can also cause TIO (154–156). In addition to fluorodeoxyglucose–positron emission tomography/computed tomography, imaging studies that target somatostatin receptors are used to localize FGF23-producing tumors. Combination of imaging techniques and selective venous sampling of FGF23 has been shown to be useful in identifying tumors in some cases (157). Selective venous sampling of FGF23 is a technique often

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performed by a radiologist. A catheter was inserted through the femoral vein, and blood samples were collected at 10 to 30 different body sites to measure serum FGF23 levels. Venous sampling of FGF23 is recommended when multiple suspicious lesions are identified by imaging studies or when greater certainty is demanded because of high surgical morbidity in resecting the lesion (6, 158). Although complete resection of tumors can relieve hypophosphatemia and skeletal complications, medical treatment with active vitamin D and phosphate salts is necessary for patients with inoperative or residual tumors.

Intravenous administration of certain iron preparations such as saccharated ferric oxide, iron polymaltose, and ferric carboxymaltose causes FGF23-related hypophosphatemia in patients with iron deficiency anemia as mentioned previously (72, 74, 75). Additionally, FGF23-related hypophosphatemic disease has been reported in patients with biliary atresia, a condition in which FGF23 seems to be produced in the liver.

A mirror image of FGF23-related hypophosphatemic disease is an inherited hyperphosphatemic disease caused by FGF23 insufficiency, called hyperphosphatemic familial tumoral calcinosis (HFTC; OMIM #211900). The known responsible genes for HFTC are *GALNT3*, *FGF23*, and *Klotho* (7, 159–161). HFTC is an autosomal recessive disorder characterized by ectopic calcified masses, hyperphosphatemia, and inappropriately elevated $1,25(OH)_2D$ concentrations, resembling features found in *FGF23*-null and *Klotho* mice (106, 162).

X-Linked Hypophosphatemic Rickets

Cause and pathophysiology

XLH is a rare disorder with an incidence of approximately 1 in 20,000 births (163). The loss-of-function mutations in the *PHEX* gene result in FGF23-related hypophosphatemia (164). More than 300 kinds of mutations of the *PHEX* gene have been reported in patients with XLH and are assembled in a database (www.phexdb.mcgill.ca/). Mutations in some patients with XLH can be *de novo*.

Hyp mouse is a murine homolog of XLH and has a deletion in the 3′ region of the *Phex* gene (165). Because *FGF23* mRNA in bone tissue from *Hyp* mice is elevated compared with that of control mice, *PHEX* mutations that are found in patients with XLH are likely to cause enhanced production of FGF23 in bones of patients with XLH (166). Both global *Phex* knockout mice and mice with conditional *Phex* inactivation in osteoblasts/osteocytes have exhibited hypophosphatemic rickets/osteomalacia with elevated serum FGF23 levels and *FGF23* mRNA in femurs, suggesting that aberrant function of *Phex* in osteoblasts/osteocytes alone can explain *Hyp* mice phenotypes (167).

PHEX is a member of the M13 family of neutral endopeptidases and is expressed on the cell surface of bones and teeth (168). Although PHEX is presumed to be a protease based on its structure, its substrate remains unknown. *In vitro* study showed that FGF23 is not cleaved by PHEX (166). Therefore, there is no established functional analysis of PHEX proteins to determine whether mutations that are found in patients with XLH are truly pathogenic. To clarify the functions of PHEX protein in phosphate homeostasis, *in vivo* experiments using double-mutant mice have been performed (Table 4). Studies using combined *FGF23*- and *Phex*-deficient mice have shown that the deletion of *FGF23* from *Hyp* mice reversed hypophosphatemia and decreased $1,25(OH)_2D$ levels and rickets/osteomalacia that are associated with Phex deficiency (169). Compound *Phex* and *Dmp1* mutant mice did not display worsening of serum FGF23 elevation, hypophosphatemia, and severity of rickets/osteomalacia, suggesting that Phex and Dmp1 share a common pathway regulating FGF23 production (170). *Galnt3*/*Phex* double-mutant mice showed an intermediate biochemical phenotype compared with *Phex* mutant mice (171). However, *FGF23* mRNA expression in bone in *Galnt3*/*Phex* double-mutant mice was as much as 24-fold higher compared with that in *Phex* mutant, which is accompanied by accelerated proteolysis of intact FGF23, suggesting that *Phex* mutations make FGF23-producing osteoblasts/osteocytes more sensitive to phosphate levels (171).

“Although PHEX is presumed to be a protease based on its structure, its substrate remains unknown.”

Furthermore, a study of *Hyp* mice has shown that the decreased expression of *Sgne1* (7B2) mRNA and 7B2 protein in bone might explain the mechanism of how Phex deficiency causes FGF23 excess (172). 7B2 is a chaperon protein of proprotein convertase 2 (PC2), which seems to promote FGF23 cleavage, and decreased 7B2 leads to diminished PC2 activity. Further experiments using mouse osteoblast TMob cells have shown that transfection of *Pcsk2* (PC2) and *Sgne1* (7B2) resulted in FGF23 cleavage by enhanced PC2 activity, and transfection of *Sgne1* (7B2) resulted in significantly decreased *FGF23* mRNA and increased Dmp1 proteolysis (172). However, the mechanism by which *Phex* mutation causes downregulation of 7B2 expression in *Hyp* mice remains unknown. Further work is required to understand how loss of function of PHEX leads to aberrant production and post-translational modification of FGF23.

Clinical features

Although the clinical features are variable, most patients with XLH are diagnosed with rickets during childhood (11). Hypophosphatemia, renal phosphate wasting, and elevated serum alkaline phosphatase levels are the most common biochemical findings of XLH. Skeletal deformities such as bowed legs and short

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Table 4. Effect of Genetic Modulation of Phosphate-Regulating Genes

		Serum Biochemistry				Skeletal Phenotype	FGF23 Regulation			
Reference	Genotype	Ca	P	1,25(OH)$_2$D	PTH	Rickets/ Osteomalacia	Serum Total FGF23	Serum Intact FGF23	Bone FGF23 mRNA	Remarks
169	*Hyp*	→	↓	↓	↑	Yes	N.E.	↑	N.E.	Superimposing FGF23 deficiency onto *Hyp* mice corrected the abnormalities in serum phosphate and 1,25(OH)$_2$D levels.
	Hyp/Fgf23$^{+/-}$	→	↓	→	↑	Yes	N.E.	↑, but less than *Hyp*	N.E.	
	Hyp/Fgf23$^{-/-}$	↑	↑	↑	↓	No, but BMD is low	N.E.	Undetectable	N.E.	
170	*Hyp*	→	↓	→	↑	Yes	N.E.	↑	↑	Compound *Phex* and *Dmp1* mutant mice did not display worsening of serum FGF23 elevations, hypophosphatemia, and severity of rickets.
	Dmp1$^{-/-}$	→	↓	→	↑	Yes	N.E.	↑	↑	
	Hyp/Dmp1$^{-/-}$	→	↓	→	↑	Yes	N.E.	↑	↑	
171	*Phex* mutant (K496X)	↓	↓	→		Yes	↑	↑↑	↑	In double-mutant mice, significantly upregulated bone *FGF23* mRNA with increased proteolytic cleavage of FGF23 protein was observed.
	Galnt3$^{-/-}$	→	↑	→		No	↑	↓	↑	
	Phex/Galnt3$^{-/-}$ double mutant	↓	↓	→		Yes, but less severe	↑↑	↑	↑↑	

Abbreviations: BMD, bone mineral density; N.E., not examined.

stature are common features of patients with childhood-onset XLH (Table 5). In some patients, incidentally found hypophosphatemia in adulthood leads to a diagnosis of XLH. Adult patients can show symptoms of osteomalacia such as bone pain, insufficiency fractures, myopathy, and neurologic complications due to enthesopathy and ectopic calcification (Table 5). Along with skeletal abnormalities, dental diseases, such as root abscesses, often develop because of the dentin defect and microdefects in the enamel (173). Mineralizing enthesopathy, such as mineralization of the insertion sites of tendon and ligament, and osteoarthropathy frequently develop in adulthood (174). Similarly, ossification of spinal ligaments can be found (175). Enthesopathy is also found in other FGF23-related hypophosphatemic diseases, such as autosomal recessive hypophosphatemic rickets type 1 and type 2, regardless of treatment history (176, 177). A study with *Hyp* mice has suggested that enthesopathy is not a result of osteogenic bone formation but is caused by a substantial expansion of mineralizing fibrochondrocytes that develop to protect entheses from excessive compressive forces (178).

Diagnosis

The differential diagnosis of XLH includes nutritional rickets, metaphyseal dysplasia, physiological bowing, and other forms of renal phosphate wasting disorders. If a patient with rickets shows chronic hypophosphatemia and inappropriately elevated serum FGF23 levels, the diagnosis of FGF23-related hypophosphatemia is probable. Absence of a family history of rickets does not rule out the possibility of XLH, because *de novo* mutations in the *PHEX* gene are possible. Although several methods have been used to detect mutations in the *PHEX* gene, such as direct sequencing of genomic polymerase chain reaction products, multiplex ligation-dependent probe amplification, and *PHEX* mRNA analysis (179–181), these all have their limitations. Genetic testing is not always necessary to make a clinical diagnosis of XLH.

Current treatment and limitations

A combination of active vitamin D with phosphate salts is the current standard treatment of pediatric patients with XLH. Skeletal deformities and growth retardation may improve with the treatment, but these ailments are not completely resolved in many cases (182). These medications are also used for symptomatic adult patients with XLH to improve impaired bone mineralization (183). An observational study suggested that treatment of adult patients with XLH can reduce pain symptoms and improve fracture healing after orthopedic surgery but does not prevent or reverse enthesopathy (174, 183). A 6-year prospective study that examined the impact of medical treatment in adult XLH patients has shown that spine and hip areal bone mineral density did not change significantly between treated and nontreated groups, despite an increase in bone resorption marker in the treated patients (184).

The current medications can cause several adverse events. Hypercalciuria with or without hypercalcemia may develop from active vitamin D overdose,

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potentially leading to nephrocalcinosis, nephrolithiasis, and impaired renal function. Oral administration of phosphate salts results in just a temporary increase in serum phosphate levels, and multiple dosage regimens are necessary to show efficacy. Moreover, phosphate salts can cause several gastrointestinal symptoms, such as diarrhea and abdominal pain, which may lead to poor medication adherence. Finally, phosphate salts seem to be involved in the pathogenesis of secondary or tertiary hyperparathyroidism in patients with XLH. Cinacalcet, a calcimimetic used for the treatment of primary and secondary hyperparathyroidism, has shown some efficacy in lowering PTH and improving renal phosphate reabsorption in patients with XLH (185, 186).

Unique Treatment of FGF23-Related Hypophosphatemic Diseases

Preclinical studies

Several reports indicated that FGFR is necessary not only for transducing FGF23 signals into cells, but also for the production of FGF23 (187, 188). Therefore, FGFR inhibitors and suppressors of the signaling pathways downstream of FGFR are expected to inhibit both FGF23 production and its functions. FGFR inhibitors, such as PD173074 and NVP-BGJ398, and an inhibitor of the mitogen-activated protein kinase pathway, such as PD0325901, showed the inhibitory effects on FGF23 functions both *in vivo* and *in vitro* (187, 188). However, because FGFR and mitogen-activated protein kinase are widely expressed in various tissues, more selective inhibitors of FGF23 activity are preferable for clinical application. The C-terminal fragment of FGF23 and some small compounds have been shown to prevent the binding of full-length FGF23 to an FGFR/Klotho complex and inhibit FGF23 activity (57, 189–191). Further study is necessary to prove that these peptides or compounds can be used as potential treatments for patients with FGF23-related hypophosphatemic diseases. In addition, two kinds of monoclonal antibodies have been developed that recognize the N-terminal and C-terminal portions of murine FGF23. These anti-FGF23 antibodies successfully inhibited FGF23 activity in both wild-type and *Hyp* mice and increased serum phosphate and $1,25(OH)_2D$ levels and improved rickets in *Hyp* mice (192–194). Furthermore, anti-FGF23 antibodies improved the reduced grip power of *Hyp* mice and increased spontaneous movement (193). From these results, the inhibition of FGF23 actions by anti-FGF23 antibodies is considered to be clinically useful for patients with FGF23-related hypophosphatemic diseases.

Clinical trials

Burosumab is a recombinant human immunoglobulin γ_1 monoclonal antibody that binds to FGF23 and inhibits its activity. In a phase 1 double-blind, placebo-controlled study, a single intravenous or subcutaneous administration of burosumab was tested in 38 adult patients with XLH (12). The antibody temporarily increased the ratio of tubular maximal reabsorption of phosphate to glomerular filtration rate, serum phosphate, and serum $1,25(OH)_2D$. Serum phosphate peaked between days 8 and 15 and returned to baseline within 50 days after subcutaneous injection. Because the effect of subcutaneous injection continued longer than that of intravenous injection, subcutaneous dosing every 4 weeks seemed rational (12). In a multicenter phase 1/2 open-label, dose-escalation study, the safety and efficacy of subcutaneous burosumab administration was evaluated in adults with XLH (13). Burosumab was administered every 28 days in a 4-month dose-escalation study (0.05 to 0.6 mg/kg), followed by a 12-month extension study (0.1 to 1 mg/kg). Monthly burosumab resulted in prolonged improvement in the tubular maximal reabsorption of phosphate to glomerular filtration rate, serum phosphate, and serum $1,25(OH)_2D$ with a favorable safety profile (13). Burosumab was also shown to improve the quality of life of adult patients with XLH (195). However, these published papers did not clarify whether burosumab actually improves rickets and osteomalacia. In addition, the long-term safety of burosumab needs to be tested.

Table 5. **Clinical Manifestations of XLH**

Childhood
Leg deformities (*e.g.*, bowing)
Short stature
Dental diseases
Adulthood
Skeletal pain (*e.g.*, lower extremities, lower back, hip)
Insufficiency fractures
Myopathy
Enthesopathy (*e.g.*, Achilles tendon, patellar tendon, spinal ligament)
Dental diseases
Degenerative joint disease (*e.g.*, knee, hip)
Healing delay after orthopedic surgery
Treatment-Related Complications
Secondary-tertiary hyperparathyroidism
Nephrocalcinosis
Hypercalciuria
Hypercalcemia
Gastrointestinal symptoms

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Currently, there are several ongoing clinical trials using burosumab for patients with XLH and TIO (https://clinicaltrials.gov).

Future Directions

The discovery of FGF23 supports the notion that bone, formerly considered just as a component of the skeletal system, can also work as an endocrine organ (1). Several genes responsible for FGF23-related hypophosphatemic diseases have been identified, but the precise mechanism of how transcription and proteolytic inactivation of FGF23 are regulated remains largely unknown. Moreover, molecular mechanisms of FGF23 overexpression in causative tumors in TIO are not fully understood. Recently, RNA sequencing has revealed the presence of FN1-FGFR1 and FN1-FGF1 fusion genes in some of the causative tumors in TIO (196, 197). Because FGFR not only serves as a receptor for FGF23, but also plays a role in FGF23 expression in bone, the product of these fusion genes may promote FGF23 production from the tumors.

Although FGF23 works as a phosphotropic hormone, it is not clear how phosphate regulates FGF23 activity. Furthermore, the mechanism of how extracellular phosphate is sensed in the human body remains unknown. It is not clear if there is a "phosphate-sensing receptor" in the cell surface to detect extracellular phosphate concentrations similar to calcium-sensing receptors that regulate serum calcium concentration. Because phosphate is a component of high-energy nucleotides such as ATP, intracellular phosphate abundance could be detected by energy sensors such as adenosine 5′-monophosphate–activated protein kinase. Investigating phosphate sensors may clarify the mechanisms underlying FGF23 regulation in human bone and in causative tumors of TIO.

Antihormonal antibody has not been used in routine clinical practice to treat diseases caused by hormone excess. If further trials prove burosumab to be a safe and clinically useful treatment of patients with FGF23-related hypophosphatemic diseases, it may lead to the establishment of unique therapeutic strategies for other endocrine diseases.

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Abbreviations

1,25(OH)$_2$D, 1,25-dihydroxyvitamin D; ADHR, autosomal dominant hypophosphatemic rickets; ADP, adenosine diphosphate; ATP, adenosine triphosphate; CKD, chronic kidney disease; EPO, erythropoietin; FAM20C, family with sequence similarity 20, member C; FGF23, fibroblast growth factor 23; FGFR, fibroblast growth factor receptor; FGFR1c, fibroblast growth factor receptor 1c; FGFR4, fibroblast growth factor receptor type 4; *GALNT3*, *polypeptide N-acetylgalactosaminyltransferase 3*; HFTC, hyperphosphatemic familial tumoral calcinosis; LVH, left ventricular hypertrophy; mRNA, messenger RNA; *NRAS*, *neuroblastoma RAS viral oncogene homolog*; *OCRL*, *oculocerebral syndrome of Lowe*; OMIM, Online Mendelian Inheritance in Man; PC2, proprotein convertase 2; *PHEX*, *phosphate-regulating endopeptidase homolog, X-linked*; PPi, inorganic pyrophosphate; PTH, parathyroid hormone; SMC, smooth muscle cell; TNAP, tissue-nonspecific alkaline phosphatase; TIO, tumor-induced osteomalacia; *VDR*, *vitamin D receptor*; XLH, X-linked hypophosphatemia.

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