

REVIEW

FGF23 and Phosphate Wasting Disorders

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A decade ago, only two hormones, parathyroid hormone and 1,25(OH)₂D, were widely recognized to directly affect phosphate homeostasis. Since the discovery of fibroblast growth factor 23 (FGF23) in 2000 (1), our understanding of the mechanisms of phosphate homeostasis and of bone mineralization has grown exponentially. FGF23 is the link between intestine, bone, and kidney together in phosphate regulation. However, we still do not know the complex mechanism of phosphate homeostasis and bone mineralization. The physiological role of FGF23 is to regulate serum phosphate. Secreted mainly by osteocytes and osteoblasts in the skeleton (2-3), it modulates kidney handling of phosphate reabsorption and calcitriol production. Genetic and acquired abnormalities in FGF23 structure and metabolism cause conditions of either hyper-FGF23 or hypo-FGF23. Hyper-FGF23 is related to hypophosphatemia, while hypo-FGF23 is related to hyperphosphatemia. Both hyper-FGF23 and hypo-FGF23 are detrimental to humans. In this review, we will discuss the pathophysiology of FGF23 and hyper-FGF23 related renal phosphate wasting disorders (4).

Keywords: FGF23; Klotho; hypophosphatemic rickets; XLH; ADHR; ARHR; ENS; OGD; NF; McCune Albright syndrome; DMP-1; PHEX

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Introduction

According to pathogenesis, rickets classified into calicopenic rickets, phosphopenic rickets, and a miscellaneous group associated with direct inhibitors of mineralization (5). In general, most instances of nutritional rickets are calicopenic, whereas heritable causes are usually phosphopenic. In this review we focus on hypophosphatemic rickets or osteomalacia. The causes of hypophosphatemia are various, of which the most prominent one is decreased reabsorption of phosphate in the proximal tubule. Although renal tubular disease can result in excessive renal phosphate wasting, in most hypophosphatemic disorders no abnormalities are found in the proximal tubule (6-7). It is speculated that an unknown factor is responsible for this phenomenon. The discovery of fibroblast growth factor 23 (FGF23), a member of the

FGF family, which mediates renal tubular defects in phosphate reabsorption, has given new light to understanding hypophosphatemic disorders. FGF23 was identified by positional cloning in 2000 as the gene responsible for autosomal dominant hypophosphatemic rickets (ADHR) (1). Subsequent analyses indicate that several kinds of hypophosphatemic rickets are associated with high circulatory levels of FGF23. Thus, hypophosphatemic rickets can now be divided into types that are FGF23-mediated and those that are not (4). Table 1 lists FGF23 related and unrelated hypophosphatemic rickets/osteomalacia.

Phosphate homeostasis

Phosphate comprises about 1% of total body weight. About 85% of total body phosphate resides in the bone, 14% in the cells, and only 1% in the serum and extracellular fluids. Maintenance of serum phosphate within its normal range allows for optimal mineralization of bone without deposition in vascular and other soft tissues. Serum phosphate concentration is determined

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by the balance among intestinal absorption of phosphate from the diet, its storage in bone, and its excretion in the urine. The proximal tubule is responsible for the reabsorption of phosphate filtered at the glomerulus and is the primary regulator of phosphate balance in the body. Transportation in the proximal tubule is driven primarily by sodium–potassium ATPase, which is located in the baso-lateral membrane of the cell (8-11). Under normal conditions, about 85% of the filtered phosphate is reabsorbed via the sodium–phosphate co-transporter (NaPi2a and NaPi2c) in the proximal tubule (9, 11). Parathyroid hormone (PTH) is one of the most potent hormonal regulators of phosphate transport and promotes renal excretion of phosphate. It has now become clear that the mechanism of action of PTH is to stimulate endocytosis of the NaPi2a co-transporters from the

apical membrane of the proximal tubule cells (9, 12). FGF23 is also an important factor resulting in renal phosphate wasting. FGF23 acts in conjunction with PTH to decrease phosphate reabsorption by down-regulating NaPi2a and NaPi2c expression in the brush border of the proximal tubule (13-16). This, in turn, results in hyperphosphaturia and hypophosphatemia. FGF23 is a counter-regulatory hormone for 1,25(OH)₂D in the bone–kidney feedback loop. 1,25(OH)₂D stimulates FGF23 production, resulting in increased circulating FGF23, which in turn suppresses 1, 25(OH)₂D concentrations. The mechanism will be discussed later. Thus, conditions associated with FGF23 excess characteristically have suppressed or inappropriately normal circulating 1,25(OH)₂D concentrations in the face of hypophosphatemia.

Table 1 Types of hypophosphatemic rickets/osteomalacia

| FGF 23 related | Types |
|----------------|--|
| related | X linked hypophosphatemic rickets (XLH) |
| | Autosomal dominant hypophosphatemic rickets (ADHR) |
| | Autosomal recessive hypophosphatemic rickets (ARHR) |
| | Tumor induced osteomalacia (TIO) |
| | Fibrous dysplasia (FD)/McCune Albright Syndrome (MAS) |
| | Neurofibromatosis (NF) |
| | Hypophosphatemic rickets and hyperparathyroidism (HRHPT) |
| | Osteoglophonic dysplasia (OGD) |
| | Linear nevus sebaceous syndrome (LNSS) |
| | unrelated |

Long term hypophosphatemia can result in rickets in children, while it can result in osteomalacia in adults. The clinical signs of hypophosphatemic rickets include squared skull and bowing of the legs, while adults typically present with bone pain and pathologic fracture. In the growth plate, hypophosphatemia causes arrest of apoptosis in the hypertrophic chondrocytes leading to rickets, while in the osteoblasts, hypophosphatemia inhibits maturation and mineralization, leading to osteomalacia (17).

FGF23 and phosphate regulation

The discovery of FGF23

Prader was the first to propose the idea of a circulating factor that could cause phosphate wasting (18). The first evidence that a circulating factor was responsible for the hypophosphatemia of phosphaturic disorders was demonstrated by Meyer *et al* and Nesbitt *et al* (19-20). The first to support this concept in humans were the findings from Miyauchi *et al* (21). This phosphaturic substance was termed ‘phosphatonin’ by Econs and Drez-

ner (22) because of its ability to lower blood phosphorus levels. The first identification of FGF23 as the putative phosphatonin was when mutations in FGF23 were identified as the cause of autosomal dominant hypophosphatemic rickets (ADHR) (1). Since then, FGF23 has been found to be related to numerous hypophosphatemic disorders.

The structure of FGF23

FGF23 is a glycoprotein with 251 amino acids. There is a signal peptide of 24 amino acids in the N-terminal portion of the FGF23 protein. Next to the signal peptide is the FGF homology region, which binds to FGF receptors (FGFR) in the tissue. Its C-terminal peptide binds to its co-receptor Klotho which is also a transmembrane protein. Both the N and C terminals are participants in the hormone’s activity. The intact FGF23 is cleaved prior to secretion between Arg179 and Ser180 by furin recognizing Arg176-X-X-Arg179 motif. Both C-terminal FGF23 and N-terminal FGF23 are inactive. Figure 1 is the structure and function of FGF23. Mutations near this site in the RXXR furin-like cleavage domain of FGF23 (R176Q and

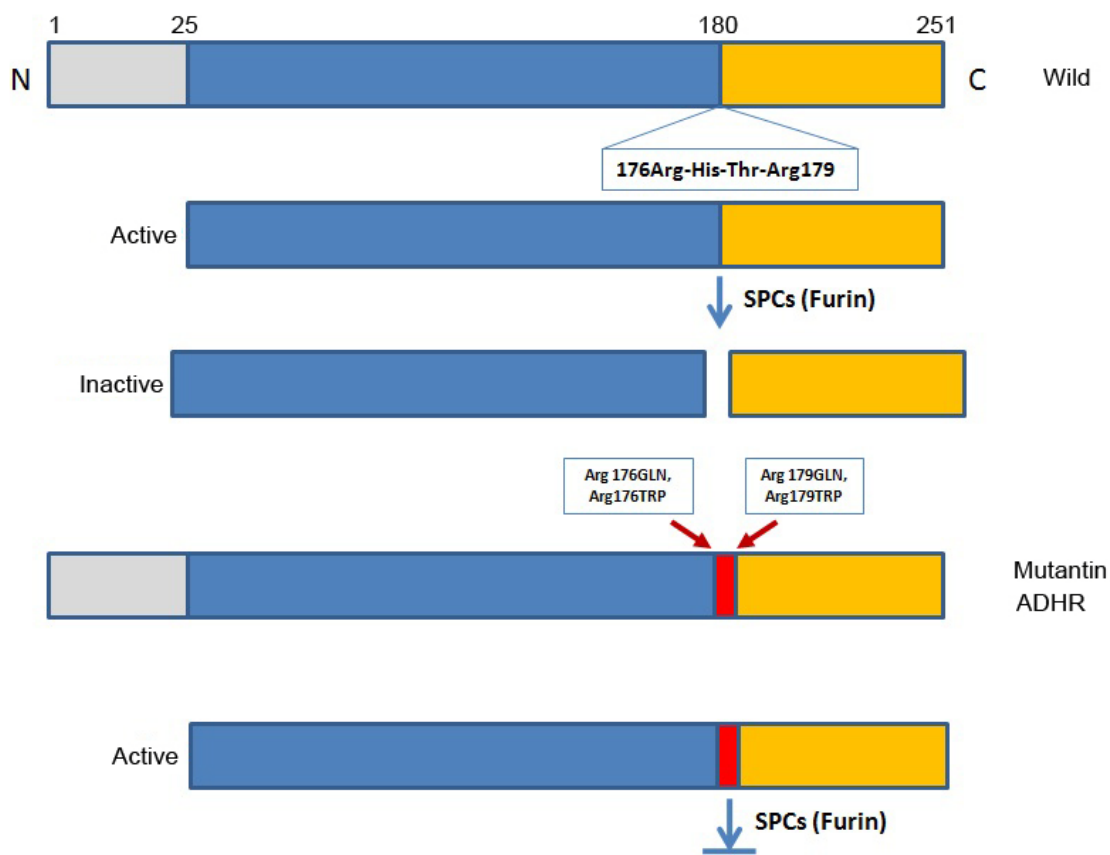


Figure 1 Schematic structure of fibroblast growth factor (FGF) 23. The FGF23 structure is schematically illustrated. FGF23 has a disulfide bond in the FGF-like sequence and internal cleavage site immediately after the R176X177X178R179 consensus sequence for convertase and cleaved into two peptides.

R179W) impair proteolytic inactivation of FGF23, resulting in high FGF23 levels and leading to autosomal dominant hypophosphatemic rickets (ADHR) (23).

Regulation of FGF23

FGF23 is almost exclusively produced by osteocytes and osteoblasts in response to high serum phosphate levels and 1,25(OH)₂D (17, 24), although aberrant production may occur in mesenchymal tumors associated with hypophosphatemic osteomalacia (25) and in the tissue of fibrous dysplasia, as in McCune-Albright syndrome with rickets (26). However, it is unclear how FGF23 secretion by bone cells is regulated. Serum phosphate and active vitamin D are positive regulators of FGF23. When serum phosphate or vitamin D levels are high, FGF23 level is elevated to increase renal phosphate wasting and to decrease active vitamin D levels. In addition to being regulated by phosphate and vitamin D, some clinical evidence suggests that FGF23 production is regulated by PHEX, DMP-1, and ENPP1 genes, which encode distinct protein products, but the molecular mechanisms whereby FGF23 is regulated by these

factors are unknown (27-31). PHEX, DMP-1, matrix extra cellular phosphoglycoprotein (MEPE), and acidic serine aspartate-rich MEPE associated motif (ASARM) peptides have been proposed to dynamically regulate FGF23 expression in bone (31-32). Normally, the PHEX-DMP-1 binding initiates a signaling pathway that reduces FGF23 expression, but in XLHR and ARHR, mutations in PHEX or DMP-1, respectively, result in hypophosphatemia through increased FGF23 expression and stability which causes phosphate wasting (25, 33-35).

FGF23 mode of action as a phosphatonin

The physiologic effect of FGF23 is on phosphate metabolism. Although receptors to FGF23 are present in many tissues, only the kidney and parathyroid gland respond to the hormone. The reason is that the phosphaturic actions of FGF23 require FGF receptors and essential cofactor Klotho to form a heterotrimer complex (36-38). Previous studies have found that the N-terminal portion of FGF23 interacts with FGFR 1c, while the C-terminal binds to Klotho and both interactions appear to be important for bioactivity of FGF23 (29).

Klotho, a single-pass transmembrane protein, is predominantly expressed in distal convoluted tubules in the kidney and the epithelium of the choroid plexus in the brain (39) and to a lesser extent, in the parathyroid glands (40). It serves as an obligate co-receptor, enabling FGF23 to interact with its receptor. Thus, Klotho is the modifier dictating which tissues will respond to FGF23.

As mentioned above, FGF23 acts on the kidney to promote phosphate excretion (13, 41-43). The site of FGF23 action in the kidney is controversial. A "cross-talk" between the distal and proximal tubules is postulated for FGF23-induced phosphaturia based on the original notion that Klotho is exclusively expressed in the distal convoluted tubule, and phosphate reabsorption and regulation solely resides in the proximal tubule (44). There may be some proximal tubule expression, but it is clear that the majority of renal Klotho expression is in the DCT (45). While a distal-proximal cross-talk is still possible, FGF23 likely also has direct action on the proximal tubule (46). Another important function of FGF23 is to regulate serum vitamin D levels. The active form of vitamin D (1,25-dihydroxyvitamin D₃) is synthesized in the kidney from its inactive precursor (25-hydroxyvitamin D₃) with 1- α -hydroxylase encoded by the *Cyp27B1* gene and is inactivated with 24-hydroxylase encoded by the *Cyp24* gene. FGF23 suppresses *Cyp27B1* gene expression and increases *Cyp24* gene expression, so that 1- α -hydroxylase (*CYP27B1*) activity is decreased and 24-hydroxylase (*CYP24*) activity is increased, leading to reduced 1,25-(OH)₂D concentrations (47).

Elevated concentrations of FGF23 are responsible for impaired bone mineralization. Induced hypophosphatemia is largely responsible for the features of rickets and osteomalacia, since serum phosphorus concentration plays an important role in the process of growth plate mineralization. What is less clear is whether or not hypophosphatemia is solely responsible for the osteomalacia. Recent studies have found that FGF23 (and soluble Klotho) may directly impact bone in diseases with elevated

FGF23 levels (48-49).

High FGF23 and disorders with abnormal phosphate metabolism

Since its discovery in 2000, FGF23 has been found to be related to a number of hereditary and acquired phosphate wasting disorders. Genetic disorders include X-linked dominant hypophosphatemic rickets, autosomal recessive hypophosphatemic rickets (35, 50-51), autosomal dominant hypophosphatemic rickets (1), hypophosphatemic rickets associated with McCune-Albright syndrome (52-54) and Linear sebaceous nevus syndrome (55-57). Acquired disorders include tumor induced osteomalacia. Table 2 lists a number of FGF23-mediated hypophosphatemic disorders. Table 3 lists the biochemical findings of the various forms of hypophosphatemic rickets.

XLH

Clinical features

XLH is the most commonly inherited form of the renal phosphate wasting disorder with a prevalence of 1/20 000 (58). The defective gene is on the X-chromosome, and female carriers are affected (i.e., an X-linked dominant disorder). Clinical manifestations vary in severity. It frequently manifests during late infancy when the child begins walking. The patient develops skeletal deformities that primarily include bowing of the long bones and widening of the metaphyseal region. The latter is most common at costochondral junctions (rachitic rosary) (59-60). These deformities are associated with diminished growth velocity, often resulting in short stature. Later in life, patients develop osteomalacia, enthesopathy (calcified ligaments and teno-osseous junctions), degenerative joint disease, and continued dental disease in particular tooth decay and dental abscesses. With medical therapy these abnormalities can be improved, but cannot be completely resolved.

Table 2 FGF23 mediated hypophosphatemic disorders

| Phosphate wasting disorders | OMIM number | Gene Location | Gene/Locus involved |
|--|------------------|---------------|---------------------------|
| X-linked dominant hypophosphatemic rickets (XLH) | 307800 | Xp22.1 | PHEX |
| Autosomal dominant hypophosphatemic rickets (ADHR) | 193100 | 12p13.32 | FGF23 |
| Autosomal recessive hypophosphatemic rickets 1 (ARHR1) | 241520 | 4q.22.1 | DMP-1 |
| Autosomal recessive hypophosphatemic rickets 2 (ARHR2) | 613312 | 6q23.2 | ENPP1 |
| Autosomal recessive hypophosphatemic rickets 3 (ARHR3) or Nonlethal variant of Raine syndrome | 241520 or 259775 | 7p22.3 | FAM20C |
| Hypophosphatemic rickets and hyperparathyroidism (HRHPT) | 612089 | 13q13.1 | Translocation with Klotho |
| McCune-Albright syndrome (MAS) | 174800 | 20q13.32 | GNAS |
| Osteoglophonic dysplasia (OGD) | 166250 | 8p11.2-p11.1 | FGFR1 |

Table 3 Biochemical findings of the various forms of hypophosphatemic rickets due to genetic mutation/translocation

| Disorder | Ca | P | ALP | UCa | UP | TmP/GFR | FGF23 | PTH | 25OHD | 1,25(OH) ₂ D |
|-----------------|----|---|-----|-----|----|---------|-------|-----|-------|-------------------------|
| XLH | N | ↓ | ↑ | N | ↑ | ↓ | N↑ | N | N | N [^] ,↓ |
| ADHR | N | ↓ | ↑ | N | ↑ | ↓ | N↑ | N | N | N [^] ,↓ |
| ARHR1 | N | ↓ | ↑ | N | ↑ | ↓ | N↑ | N | N | N [^] ,↓ |
| ARHR2 | N | ↓ | ↑ | N | ↑ | ↓ | N↑ | N | N | N [^] ,↓ |
| HRHPT | N | ↓ | ↑ | N | ↑ | ↓ | N↑ | ↑ | N | N [^] ,↓ |
| McCune–Albright | N | ↓ | ↑ | N | ↑ | ↓ | N↑ | N | N | N [^] ,↓ |
| ENS | N | ↓ | ↑ | N | ↑ | ↓ | N↑ | N | N | N [^] ,↓ |
| NF | N | ↓ | ↑ | N | ↑ | ↓ | N↑ | N | N | N [^] ,↓ |
| OGD | N | ↓ | ↑ | N | ↑ | ↓ | N↑ | N | N | N [^] , |

^: decreased relative to the serum phosphate concentration; 1,25(OH)₂D: 1,25-dihydroxyvitamin D; 25-OH-D: 25-hydroxyvitamin D; ALP: serum alkaline phosphatase; Ca: serum calcium; P: serum phosphate; PTH: parathyroid hormone; TmPO₄/GFR: maximum rate of renal tubular reabsorption of phosphate normalized to the glomerular filtration rate; UCa: urinary calcium excretion; UP: urinary phosphate excretion; N: normal values; ↓: decreased values; ↑: increased values

Biochemical findings

Hypophosphatemia and low-normal circulating 1,25-dihydroxyvitamin D [1,25(OH)₂D] levels are typical biochemical findings. Serum alkaline phosphatase (ALP) activity and 24-hour urine phosphate are elevated in children, while serum calcium is normal, as is circulating 25-OHD. Tubular reabsorption of phosphate (TRP) is decreased.

Genetics

Genetic linkage analysis has revealed inactivating mutations in the phosphate regulating gene with homology to endopeptidases on the X chromosome (PHEX), a gene located on Xp22 (61-62). PHEX protein is expressed in various tissues, including the kidney, but is most abundant in mature osteoblasts and odontoblasts. PHEX is a member of the M13 family of neutral endopeptidases. It is an integral membrane glycoprotein, which activates or degrades peptides. PHEX is secreted largely by osteocytes, but also by osteoblasts, and is important for normal matrix mineralization. Its role in the mineralization process is unclear at present. Serum intact FGF23 level is elevated in most XLH patients (63). FGF23 was initially referred to as a substrate of PHEX (64), but subsequent research found that FGF23 is not a direct substrate of PHEX (65). The mechanism by which PHEX regulates FGF23 remains unclear. It is now thought that it might control mineralization by binding proteins such as DMP-1 and matrix extracellular phosphoglycoprotein, which are both members of the SIBLING proteins and contain ASARM peptides, preventing their proteolysis and the release of ASARM peptides which inhibit mineralization (66).

ADHR

Clinical features

ADHR is a rare disorder that was first described by Bianchine *et al* in 1971 (58). Clinical and biochemical findings of ADHR patients are similar to those of XLHR patients. But ADHR, unlike XLHR, shows variable and incomplete penetrance with variable symptomatology and biochemical findings depending on the age at presentation. Patients who manifest the disease in their childhood develop short stature, rickets, bone pain, lower extremity deformities, and dental abscess. Some of the children have spontaneous resolution of symptoms during adulthood. On the other hand, patients with ADHR who manifest the disease in adulthood have symptoms similar to patients with TIO. Adults have bone pain, weakness, osteomalacia, and fractures/ pseudo-fractures, but do not have short stature nor lower-extremity deformities. Interestingly, the majority of patients who develop the disease in adulthood are women, and pregnancy triggers the onset of symptoms (67). Studies in ADHR humans have suggested that iron deficiency may be a trigger for dysregulation of FGF23, thus inducing active disease (68). Therefore, the onset of the disease is the product of gene-environment interactions. Imel *et al* also showed that serum iron was negatively correlated to both C-terminal FGF23 and intact FGF23 in ADHR patients (67). These studies suggested that iron status may regulate FGF23 metabolic pathways, and that low iron status results in increased FGF23 mRNA.

ADHR is caused by heterozygous mutations in the gene encoding FGF23 which is on 12p13 (1, 69). Mutations identified in ADHR are missense mutations, and in each case, the mutation alters an arginine residue at

either position 176 or 179. The mutations, which involve the proprotein convertase (furin) cleavage site, prevent the proteolytic processing of FGF23 to its inactive N- and C-terminal peptides. Mutant FGF23 proteins exhibit increased stability, are more active than wild-type FGF23, *in vivo* (70-71), and are likely present at elevated concentrations in ADHR patients (72). Thus, in ADHR patients, high circulating levels of FGF23 are due to decreased FGF23 degradation.

ARHR

Autosomal-recessive hypophosphatemic rickets (ARHR), is a rare disorder that is recently recognized (3, 35). Clinical and biochemical findings of the affected individuals are similar to ADHR and XLH. Clinical features include rickets, skeletal deformities, dental defects, and affected individuals develop sclerotic bone lesions and enthesopathies. The clinical presentation of ARHR is not found at birth. Affected individuals present signs of rickets/osteomalacia later during childhood and even in adulthood (73-74). ARHR type 1 is caused by inactivating mutations in dentin matrix protein 1 (DMP-1), a member of the small integrin-binding ligand N-linked glycoprotein family of extracellular matrix proteins that augment mineralization (75-76). DMP-1 is widely expressed, but particularly abundant in bone, where it is synthesized by osteoblasts. It is involved in the regulation of transcription in undifferentiated osteoblasts. DMP-1 belongs to the SIB-LING protein family, which includes osteopontin, MEPE, bone sialoprotein II, and dentin sialoprotein, and whose genes are clustered on chromosome 4q21. DMP-1 undergoes phosphorylation during the early phase of an osteoblast's maturation and is subsequently exported into the extracellular matrix where it regulates the nucleation of hydroxyapatite. FGF23 levels are elevated or inappropriately normal for the low serum phosphate levels. Loss of function of DMP-1 results in increased transcription of FGF23 by osteocytes, but the mechanism is not clear (35).

Recently, Levi-Litan *et al*, Lorenz-Depiereux *et al* and Saito *et al* identified an inactivating mutation in the ectonucleotide pyrophosphatase/phosphodiesterase 1 (ENPP1) gene that cause ARHR type 2 (50-51, 77). The gene product ectonucleotide pyrophosphatase/phosphodiesterase 1 is a cell surface enzyme responsible for generating inorganic pyrophosphate, which inhibits mineralization (51). Typically, loss-of-function mutations in ENPP1 cause generalized arterial calcification of infancy (GACI). However, hypophosphatemic rickets has also been found in patients with ENPP1 mutation. It remains unclear how the loss-of-function mutation in ENPP1 causes hypophosphatemic rickets (and not GACI), but it

has been speculated that it might be through increased secretion of FGF23 (24). A recent study demonstrated that the FGF23 level is elevated in patients with mutations in ENPP1 (50). However, the mechanism has yet to be defined.

Another type ARHR with hypophosphatemia, hyperphosphaturia, dental anomalies, intracerebral calcifications and osteosclerosis of the long bones in the absence of rickets, was called nonlethal variant of Raine syndrome or ARHR type 3. Using whole exome sequencing, Rafaelsen *et al* identified compound heterozygous mutations in family with sequence similarity 20, member C (FAM20C) in patients with ARHR type 3. DMP1 is phosphorylated by FAM20C, with partial loss of Fam20c function, DMP1 is not properly phosphorylated, seems to be a mechanism that is involved in the pathways that generate FGF23-dependent hypophosphatemia (78).

Current and future treatments in hypophosphatemic rickets

Basic therapy

Renal phosphate wasting is the principal pathophysiological abnormality that leads to all these disorders. Current treatment for patients with FGF23-dependent hypophosphatemic rickets is based on the association of activated vitamin D metabolites (calcitriol or alfacalcidol) and oral inorganic phosphate salts. Treatment during growth partially corrects leg deformities, decreases the number of surgeries, and may improve adult height. Early initiation of treatment appears to optimize height outcomes (79-80). However, the improvement is often partial (81).

Although an impaired growth hormone (GH)-insulin like growth factor-I axis is not the primary cause of short stature in XLHR patients, GH is useful to improve growth in poorly growing XLHR patients (82). However, the results are not conclusive, as some patients do not have a recovery of growth during GH treatment. Orthopedic surgery is indicated when healing of skeletal deformities of the lower limbs by medical treatment is unsatisfactory.

With the recognition of increased FGF23 levels in the pathogenesis of these renal phosphate wasting disorders, new therapeutic strategies are being developed. One study found that subcutaneous injection of salmon calcitonin in XLHR patients causes a significant and sustained drop in circulating levels of FGF23, with an increase in serum phosphate levels (83). Studies in Hyp mice have demonstrated that the inhibition of FGF23 overproduction by anti-FGF23 neutralizing antibodies can improve phosphate levels, renal tubular phosphate reabsorption, and bone mineralization (84-85). These

results indicate that inhibition of FGF23 activity is a promising therapy for FGF23-dependent hypophosphatemia. However, further studies are needed to determine whether these findings in mice can be applied to humans.

Tumor-induced osteomalacia (TIO)

Tumor-induced osteomalacia (TIO), or oncogenic osteomalacia, is a rare paraneoplastic syndrome of abnormal phosphate and vitamin D metabolism caused by typically small endocrine tumors. Clinical symptoms include chronic bone pain, which is usually the first presentation, weakness, and fatigue in association with a high risk of fragility fractures. Due to under-recognition of the disease, the diagnosis is commonly delayed for years. At our hospital, patients frequently present with multiple fractures, height loss, and a generalized debilitated status. Biochemical hallmarks of the disorder are similar to hypophosphatemic rickets. The diagnosis is confirmed by the dramatic improvement of symptoms and correction of metabolic abnormalities following complete excision of the responsible tumor.

The tumors are usually very small in size and their locations are often obscure. They can arise in bone or soft tissue, in any part of the body, and they grow slowly.

Most histologic diagnoses have been classified as phosphaturic mesenchymal tumors (PMT). A characteristic histologic feature of such tumors is a background of spindle cells that tend to have low mitotic activity, prominent vascularity, osteoclast-like giant cells, or the presence of bony tissue. Although most of these tumors are thought to have a benign histologic appearance, malignant presentation and metastases can occur (86-90). While metastases are rare, infiltration of surrounding connective tissue is typically present, which has significant implications for surgical management and emphasizes the importance for wide surgical margins to avoid persistence or recurrence.

Numerous reports show elevation of FGF23 in some, but not all, patients with TIO (91-92). Removal of the tumor is associated with reduction in serum FGF23 concentrations, and there is a temporal association between the reduction in FGF23 concentration and the elevation in serum phosphate, decrease in renal phosphate wasting, and increase in 1,25(OH)₂D₃ concentrations (93-94). The diagnosis of TIO can be challenging because the tumors are often small and difficult to find. Bone scanning, computerized tomography (CT) (95), magnetic resonance imaging (MRI), Indium-111 pentetreotide or octreotide scintigraphy, and positron emission tomography (PET) have all been employed in an effort to localize the tumor (96). A stepwise approach,

first performing functional tests and then anatomical tests, is advocated. In our hospital, we have successfully used ⁹⁹Tcm-OCT scintigraphy to locate tumors in most patients with TIO as we previously reported (94). Therefore, we prefer octreotide scintigraphy as the first approach. As for patients who are octreotide negative, when a tumor is highly suspected, we use FDG-PET/CT. Recently, ⁶⁸Ga-DOTANOC PET/CT has been explored as a means of finding TIO tumors (97). Once suspicious lesions have been identified with functional imaging, one should proceed to anatomical imaging such as X-rays, CT, and/or MRI scans to confirm the location of the tumor.

The treatment of choice for TIO is resection of the tumor with a wide margin to ensure complete resection. Resection with a wide surgical margin is very important, as recurrences of these tumors have been reported (89-90, 98). Therefore, intermittent monitoring of patients after tumor resection should be performed. Tumor resection is almost always curative, and following complete resection of the tumor, there is relatively rapid improvement. FGF23 disappears rapidly from the circulation (99) and serum phosphate returns to normal within five days post operation (94). Most patients feel better within days to weeks of tumor removal. Bone healing starts immediately, but depending on the severity of the disease, it may take a year or more for significant clinical improvement.

When the tumor cannot be localized or is not surgically resectable, medical therapy with phosphate supplementation and calcitriol or alfalcidol is used. The treatment regimen that follows is essentially the same as that used in non-TIO hypophosphatemia. When initiating treatment, it is prudent to check weekly labs to guide titration of medications until treatment targets are reached. Future treatment will likely be guided by a better understanding of the biology of FGF23 and the nature of these tumors.

Fibrous dysplasia (FD)/McCune Albright Syndrome

McCune-Albright syndrome (MAS) is characterized by café-au-lait spots, polyostotic fibrous bone dysplasia, and multiple endocrine hyperfunctions, such as precocious puberty, hyperthyroidism, autonomous adrenal hyperplasia, and growth hormone secreting pituitary adenoma (100). Fibrous dysplasia (FD) is a focal and benign fibrous bone lesion that is caused by the activating mutation of the Gsa protein (101-102). Hypophosphatemic rickets is sometimes observed as a complication of MAS (103).

FD and MAS are caused by somatic activating mutations of the guanine nucleotide binding protein, alpha

stimulating gene (GNAS1), the gene encoding the alpha-subunit of the stimulatory G-protein.

Renal phosphate wasting occurs in approximately 50% of patients with MAS and FD of bone. However, the cause of hypophosphatemia is unclear. Recently, Riminucci *et al* (26) reported the important role of FGF23 as a cause of hypophosphatemia in MAS. However, hypophosphatemia is not always associated with MAS patients (104). Hypophosphatemia appears as the age of the MAS patients increases, which is usually accompanied by advanced bone fibrous dysplasia lesions (105). It is plausible that overproduction of FGF23 is dependent on the severity of FD bone lesions, which may be associated with increased serum FGF23 levels, and which could explain the presence or absence of hypophosphatemia in MAS patients. *In situ* hybridization analysis of FGF23 mRNA expression identified "fibrous" cells, osteogenic cells, and cells associated with microvascular walls as specific cellular sources of FGF23 in FD. Production of FGF23 by FD tissue may play an important role in the renal phosphate wasting syndrome associated with FD/MAS (26).

Treatment with bisphosphonates has been shown to reduce serum FGF23 levels, which result in a reduction of renal phosphate wasting. The mechanisms underlying the reduction of FGF23 by bisphosphonates are unclear.

Neurofibromatosis (NF)

Skeletal lesions are not uncommon in neurofibromatosis. Most of them are considered to be dysplastic in nature. Association of osteomalacia or rickets with NF has only rarely been documented (106-107). Osteomalacia occurring in NF is quite distinct from the more common skeletal affection seen in the disease and its pathogenesis is still unknown. Osteomalacia associated with NF1 is characterized by later onset in adulthood, renal phosphate loss with hypophosphataemia, multiple and pseudofractures in typical cases. Treatment with oral phosphate and vitamin D is effective (108-109). It is hypothesized that melatonin deficiency in cases of NF might play a role in the pathogenesis of hyperphosphatemia (110). We have a few patients who are diagnosed with neurofibromatosis and osteomalacia, while FGF23 is positive in the neurofibroma bundle of a few of the patients (data not published yet).

Linear nevus sebaceous syndrome (LNSS)

Linear nevus sebaceous syndrome (LNSS)/epidermal nevus syndrome (ENS) is a sporadic condition characterized by congenital epidermal nevi associated with anomalies in other organ systems, most commonly the

central nervous system and skeleton (111). Abnormalities in the eyes, heart, or genitourinary system have also been reported (112). Hypophosphataemic rickets is rare (113-115) in ENS; the manifestation usually presents in the first years of life (114, 116). It is generally accepted that it may represent a variant of tumor-induced rickets/osteomalacia (113-114, 117-118) characterized by renal phosphate wasting and inappropriately low serum levels of 1, 25(OH)₂D. The pathogenic mechanism involved in the onset of hypophosphataemic rickets in ENS is not fully clarified. It has been proposed that FGF23 is the putative phosphatonin, based on demonstration of its elevated blood levels in a patient with LNSS (56). Subsequent studies also found the same result (119-120). In typical tumor-induced osteomalacia, symptoms tend to be resolved after removal of the tumor (117). Excision of epidermal lesions with ENS may improve the hypophosphataemic rickets in some patients (47, 56), while it has failed to heal rickets in most patients (115-116, 118). Although it is possible that large amounts of FGF23 are autonomously secreted by LNS lesions, this factor was not found to be directly excreted from the LNS lesions (119). Further study is needed to understand the exact mechanism of how FGF23 is related to hypophosphatemic rickets in LNSS.

Osteoglophonic dysplasia (OGD)

Osteoglophonic dysplasia (OGD), also known as Fairbank-Keats syndrome, is a very rare skeletal dysplasia with craniosynostosis, and multiple lucent metaphyseal defects. It is an autosomal dominant disorder characterized by short stature, although most cases are the result of *de novo* mutations (121). Recently, White *et al* identified several heterozygous missense mutations in fibroblast growth factor receptor 1 (FGFR1) (121). These mutations are in highly conserved residues comprising the extracellular (asparagine 330 to isoleucine, Asn330Ile) and transmembrane domains (tyrosine 374 to cysteine, Tyr374Cys; and cysteine 381 to arginine, Cys381Arg) of FGFR1, which seems to lead to constitutive receptor activation (122-123). Hypophosphatemia, secondary to renal phosphate wasting associated with inappropriately normal 1,25(OH)₂D₃ levels, is present in affected individuals (124-125). FGF23 levels are elevated in some OGD patients. The elevated levels of FGF23 result in renal phosphate wasting seen in this condition (121). It is thought that the skeletal lesions in OGD patients develop because the constitutive activation of the FGFR1 leads to an up-regulation of FGF23 secretion in the metaphyseal growth plate. However, the mechanism is not yet clear.

Hypophosphatemic rickets and hyperparathyroidism (HRHPT)

Hypophosphatemic rickets and hyperparathyroidism (HRHPT) is a syndrome featuring both hypophosphatemic rickets and hyperparathyroidism due to parathyroid hyperplasia as well as other skeletal abnormalities. Brownstein *et al* investigated a patient with hypophosphatemic rickets and hyperparathyroidism due to parathyroid hyperplasia (126). They found no mutation in PHEX, DMP-1, and FGF23. They found a *de novo* translocation with a breakpoint adjacent to alpha-Klotho, which encodes alpha-glucuronidase. Plasma alpha-Klotho levels, beta-glucuronidase activity, and circulating FGF23 levels were markedly elevated. Moreover, emerging evidence indicates that alpha-Klotho is critical for FGF23 signaling. Whether the elevated FGF23 level seen in the patient is the direct result of increased alpha-Klotho (for example, if degradation of FGF23 is prevented by interaction with alpha-Klotho), or is part of a negative-feedback loop responding to hyperparathyroidism is difficult to discern at present.

Conclusion

Rickets, due to inherited or acquired causes, remains a significant problem across the globe. Considerable advances have been made in identifying genes responsible for a number of the inherited causes of hypophosphatemic rickets and to clarify the pathways of regulation of phosphate metabolism. The discovery that FGF23 overproduction is a primary cause of hypophosphatemic rickets may suggest a new approach for the treatment of these disorders.

Dysregulation of FGF23 occurs in a number of acquired and inherited disorders of phosphate homeostasis. Further investigations are required to understand the regulation of FGF23 expression.

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