The Changing Face of Hypophosphatemic Disorders in the FGF-23 Era

Janet Y. Lee, MD, M.P.H and **Erik A. Imel, MD.**

Departments of Medicine and Pediatrics, Indiana University School of Medicine, Indianapolis, IN

Abstract

In the past decade, research in genetic disorders of hypophosphatemia has significantly expanded our understanding of phosphate metabolism. X-linked hypophosphatemia (XLH) is the most common inherited form of rickets due to renal phosphate wasting. Recent understanding of the mechanisms of disease and role of fibroblast growth factor 23 (FGF-23) in XLH and other hypophosphatemic disorders have opened new potential therapeutic avenues. We will discuss the current standard of treatment for XLH as well as promising future directions under study.

Keywords

X-linked hypophosphatemia; phosphate; FGF-23; 1,25(OH)2D

Case

A 12-month-old girl presents with bowed legs and a family history of hypophosphatemia in her mother and sister. Although she is not yet walking, she has had progressive worsening of leg bowing since she started standing. She is short for age at −2.5SD. She has mild frontal bossing and widened wrists. She has moderate bowing and torsion at the tibia bilaterally. X-rays indicate rachitic changes at the wrists and knees. Laboratory testing indicates low serum phosphate for age, 2.1 mg/dL (normal 3.6–6.5 mg/dL), calcium 9.3 mg/ dL (normal 8.5 to 10.5 mg/dL), alkaline phosphatase 645 Unit/L (normal for age 102–400 Unit/L), creatinine 0.2 mg/ dL (normal 0.2–0.7 mg/dL), 25-hydroxyvitamin D (25OHD) 28 ng/dL and 1,25-dihydroxyvitamin D (1,25(OH)$_2$D) of 17 pg/dL (normal 15–75 pg/ml). Parathyroid hormone was 37 pg/ml (normal 10–65 pg/ml). TmP/GFR is low for age at 1.9 mg/dL, indicating renal phosphate wasting as the cause of hypophosphatemia. A clinical diagnosis of XLH is made and confirmed by mutation analysis. Her intact FGF23 concentration is elevated (173 pg/ml).

Inherited hypophosphatemic disorders are most often due to renal phosphate wasting, with XLH being the most common such disorder. Medical management of X-linked hypophosphatemia (XLH) is more complicated than that of the more common nutritional

Corresponding author: Erik A. Imel, MD, Departments of Medicine and Pediatrics, Indiana University School of Medicine, Indianapolis, IN, Tel: 317-274-1339, eimel@iu.edu.

rickets. Many patients demonstrate some improvement with current therapy, although the response is quite variable and there are several significant side effects. Current treatment is often unsatisfactory to patients and providers alike due to limitations in the ability to fully correct skeletal features of the disease. However, investigations into emerging therapies to address the pathophysiology of FGF23 excess have potential to improve treatment effectiveness and safety.

Background: Phosphate Metabolism

Phosphorus is an important component of many biological functions. Phosphorus exists as organic and inorganic phosphate in the human body. Most of the body's phosphate is associated with calcium as skeletal hydroxyapatite where it provides structural strength and is accessible, via resorption, when required for other homeostatic functions. Most extra-skeletal phosphate is intracellular, serving as components of lipids, proteins, nucleic acids, signaling molecules, and energy exchange. Only one percent or less of total-body phosphate is extracellular. (1) Normal serum phosphate concentrations vary greatly between infancy and adulthood.(2) In infancy, higher concentrations of phosphate are necessary for adequate skeletal mineralization, and levels within the adult normal range are insufficient for infants and young children, resulting in rickets. Furthermore, chronic hypophosphatemia at any age impairs the mineralization of osteoid, causing osteomalacia. In fact, hypophosphatemia in the growth plate has recently been considered a common pathway mediating the development of rickets in a variety of mouse models of nutritional and genetic forms of rickets.(3)

Two main families of proteins, SCL20 (PiT-1 and PiT-2) and SCL34 (NPT2a, NPT2b, and NPT2c), mediate transport of phosphate into cells. These proteins use the sodium electrochemical gradient to transport phosphate against the phosphate electrochemical gradient.(4) The sodium-potassium-ATPase pump transports sodium out of the cell, maintaining the sodium gradient.(1) The SCL34 family of proteins provide for regulated phosphate transport in the intestine and the kidneys. The SCL20 proteins are ubiquitously expressed and have been considered as housekeeping genes to providing phosphate transport for necessary intracellular processes. However, recent work suggests that they also have importance in mineral metabolism.(4) PiT-1 in vascular smooth muscle cells has been implicated as a mediator of vascular calcifications. (5) In hypertrophic chondrocytes, PiT-1 mediated phosphate uptake is an important factor impacting intracellular ATP production, activation of caspases and induction of appropriate apoptosis and mineralization.(6) Factors decreasing Pit-1-mediated phosphate transport thus result in a disorganized and widened hypertrophic chondrocyte zone consistent with rickets.(6) Recently, mutations impairing PiT-2 phosphate transport were found to cause familial idiopathic basal ganglia calcification.(7)

Although most phosphate absorption takes place in the proximal intestine through active and passive transport mechanisms, phosphate absorption can occur throughout the small intestine.(8) The sodium-phosphate transporter 2b (NPT2b) mediates active intestinal phosphate absorption and is regulated by $1,25(OH)_2D$, which upregulates expression of NPT2b, and by dietary intake of phosphate. (8) Approximately 80 percent of dietary

phosphorus is absorbed, and isolated dietary phosphorus deficiency is a rare occurrence outside of specific populations such as premature infants or individuals with excessive intake of phosphate binding agents.(9)

Plasma phosphate concentration is largely determined by renal tubular phosphate handling. Phosphate is filtered at the glomerulus. Regulation of phosphate reabsorption, primarily at the level of the proximal tubule, allows reclamation or excretion of phosphate during periods of phosphate deprivation or excess. Normal tubular reabsorption of phosphate (TRP) is in the range of 90 percent. Proximal tubular reabsorption is mediated by brush border sodium-dependent phosphate cotransporters (NPT2a and NPT2c). The expression of these cotransporters may be modified by parathyroid hormone (PTH) and fibroblast growth factor 23 (FGF23).(1)

Parathyroid hormone (PTH) is an 84 amino acid protein produced by parathyroid gland chief cells.(10) PTH primarily regulates serum calcium in response to signaling of the calcium-sensing receptor (CASR), but PTH also regulates phosphate, directly or indirectly, in a variety of organs including kidney, bone and intestine. PTH strongly inhibits phosphate reabsorption in both proximal and distal renal tubules, by rapidly sequestering NPT2a and NPT2c. (1) Additionally, PTH also induces transcription of 1α-hydroxylase, which stimulates synthesis of $1,25(OH)_2D$ in the proximal tubule. $1,25(OH)_2D$ in turn, increases NPT2b-dependent phosphate absorption from the intestine(8), and suppresses PTH gene transcription(10), thus raising serum phosphate concentration. At very high concentrations, $1,25(OH)_2D$ stimulates bone resorption, although not at lower concentrations.(11) In a feedback loop, low serum phosphate concentrations due to dietary restriction also increase $1,25(OH)_2D$ production.(12)

FGF23 is a 32-kDa (251 amino acid) protein hormone (13), produced primarily by osteocytes.(14, 15) FGF23 has a protease recognition site between Arginine 179 and Serine 180, making it prone to cleavage into inactive N-terminal and C-terminal fragments(16). FGF23 glycosylation, mediated by GalNAc-transferase T3, limits this cleavage and allows secretion of intact FGF23, though some fragments are still secreted.(17)

Receptor specificity for intact FGF23 is accomplished by interaction of FGF23 with FGF receptors (FGFR1, 3, or 4) and the co-receptor α-Klotho resulting in activation of the mitogen-activated protein kinase (MAPK) pathway and phospho-ERK.(18, 19) Both the N-terminal and C-terminal portions of FGF23 are necessary for this complete interaction.(20) As a transmembrane protein without an intracellular signaling motif, α-Klotho, also exists as a soluble circulating protein. The precise location of initial FGF23/ α-Klotho activity in the kidney remains controversial. The effects on phosphate reabsorption and $1,25(OH)_2D$ occur in the proximal tubule. However, in some studies, α-klotho was not detected in the proximal tubule (21, 22), while in others, lower levels of α-klotho protein expression were reported. (23, 24) These differences may relate to different antibodies used. Some authors have demonstrated co-localization of phospho-ERK signaling with α-klotho in the distal convoluted tubule in response to FGF23 injection, prior to proximal tubular alterations in NPT2a in both wild-type and hyp mice. (21, 22) In these studies, α-klotho expression and phospho-ERK signaling were not detected in the proximal tubule with NPT2a. An

unidentified signal from the distal tubule to the proximal tubule was thus postulated. In contrast, other studies do identify proximal tubular α-klotho and direct signaling of FGF23 and α-klotho within proximal tubule cells.(23, 24)

FGF23 signaling leads to inhibition of phosphate reabsorption via reduced NPT2a and NPT2c.(25, 26). Furthermore, FGF23 inhibits 1α-hydroxylase expression, opposing the effect of PTH.(25) Thus, FGF23 produces phosphaturia and suppresses circulating 1,25(OH)2D levels. In addition, some data suggest that FGF23 interaction with different FGF receptors may have proportionately different effects on FGF23 target actions.(27) More specifically, FGFR1 appears to be more involved in regulating phosphate reabsorption (28), while FGFR3 and FGFR4 may have greater impact on $1,25(OH)_2D$ metabolism.(27, 29)

Conversely, dietary phosphate intake, high phosphate levels, and calcitriol stimulate expression of FGF23 providing feedback regulation.(12, 30, 31) Furthermore, $1,25(OH)_2D$ upregulates Klotho expression in renal tubules facilitating FGF23 signaling for feedback regulation of vitamin D and phosphate metabolism.(32)

Differential Diagnosis of Hypophosphatemic Disorders

The differential diagnosis for hypophosphatemia can divided into increased urinary losses, rapid movement of phosphate from the extracellular to the intracellular space, and phosphate malabsorption or severe dietary deficiency.(9) This review focuses on chronic renal phosphate wasting. In order to identify an underlying cause of hypophosphatemia, testing must determine whether urinary phosphate excretion is appropriate. The tubular maximal re-absorption of phosphate (TmP) adjusted for glomerular filtration rate (TmP/GFR) indicates roughly the serum phosphate level at which significant urinary phosphate losses occur. Typically TmP/GFR is estimated using a nomogram or an algorithm. (33, 34) However, the published nomograms were obtained from measurements in adults and do not include the full normal range in children. Consequently some prefer to use an alternate equation, especially in children (with the term abbreviated TP/GFR to distinguish from the other methods): TP/GFR = Serum phosphate – (Urine phosphate × Serum creatinine / Urine creatinine).(35) Like serum phosphate, TmP/GFR is higher in young children than adults.(2) As a rule of thumb, the normal range of TmP/GFR (or TP/GFR) in mg/dL for age, is approximately similar to the normal serum phosphate range in the same units of measurement. A low TmP/GFR during hypophosphatemia indicates failure to conserve phosphate appropriately.

Specific diseases with features of hypophosphatemia due to impaired proximal tubular re-absorption of phosphate include X-linked hypophosphatemia (XLH), autosomal dominant hypophosphatemic rickets (ADHR), autosomal recessive hypophosphatemic rickets (ARHR), tumor-induced osteomalacia (TIO), and hypophosphatemia associated with fibrous dysplasia of bone (McCune-Albright syndrome), all of which are mediated by FGF23 excess. In contrast, hereditary hypophosphatemic rickets with hypercalciuria (HHRH), and Fanconi syndrome are FGF23-independent forms of renal phosphate wasting. Some inherited disorders will present in infancy, while others may present in later childhood or

adulthood. Acquired disorders such as tumor induced osteomalacia (TIO) or some forms of Fanconi syndrome may present at any age.

X-Linked Hypophosphatemia

The most common genetic cause of hypophosphatemic rickets is XLH, inherited in an X-linked dominant fashion, with an estimated incidence reported to be 1/20,000. XLH is caused by a variety of inactivating mutations in the phosphate-regulating gene with homologies to endopeptidases on the X chromosome (*PHEX*; MIM no. 300550)(36), and is expressed in osteocytes and odontoblasts. Inactivating mutations in *PHEX* result in enhanced synthesis and secretion of FGF23.(37) The resulting increased circulating concentrations of FGF23 cause the biochemical phenotype of XLH: phosphaturia, hypophosphatemia and inappropriately low or normal $1,25(OH)_2D$ concentrations.

XLH disease manifestations are modeled in multiple mice with *phex* gene mutations including deletions (38) or a point mutation causing a premature stop codon.(39) In these mice, serum FGF23 concentrations are several-fold higher than in wild-type mice.(39, 40) This excess FGF23 results in decreased renal expression of NPT2a and NPT2c, and also decreased expression of 1α-hydroxylase, resulting in the biochemical phenotype of XLH. Hypophosphatemia results in decreased apoptosis of hypertrophic chondrocytes at the growth plate, and the disorganized histologic appearance of rickets.(3) The effect of hypophosphatemia on chondrocyte differentiation in XLH is further compounded by alterations in chondrocyte Pit-1. *In vitro* studies of hyp mouse chondrocytes indicate reduced expression of PiT-1, resulting in reduced phosphate uptake by these cells and reduced chondrocyte apoptosis and mineralization. (6) Overexpression of PiT-1 in hyp chondrocytes increased phosphate uptake and ATP production, as well as the downstream steps of apoptosis and mineralization in culture (6).

Abolition of FGF23 from the phex-deficient (hyp) mouse results in reversal of the hypophosphatemia, and mice with a phenotype more similar to the FGF23 knockout mouse. (41) Conversely, breeding a phex-deficient mouse to the Galnt3-null mouse, which cannot glycosylate FGF23 properly, results in impairment of intact FGF23 secretion and some improvement in intact FGF23 and phosphate concentrations. (39) However, the double mutant mouse upregulates *FGF23* gene expression above that of the phex-deficient mouse, maintaining elevated *FGF23* concentrations and some hypophosphatemia, suggesting possible altered phosphate sensing in the absence of phex.

Clinical features of XLH are summarized in Table 1. Even when the family history is known, children often are not identified until bowing leg deformities develop, usually after weight-bearing begins. These deformities often result in an abnormal waddling gait. Additional physical examination features of rickets may be evident. Biochemical features include hypophosphatemia, reduced TmP/GFR, and suppressed $1,25(OH)_2D$ concentrations. Generally patients are eucalcemic, but secondary hyperparathyroidism is common, both before treatment, and as a potential consequence of treatment with phosphate.(42, 43)

In adulthood, patients may develop bone pain from osteomalacia, along with joint pain, stiffness and decreased joint mobility from enthesopathy. Enthesopathy and osteoarthritis

are among the most significant complaints of adults with this disorder. Enthesopathy does not appear to be influenced in either a positive or negative way by current standard medical treatment of XLH. However, emerging evidence of expression of FGFR and Klotho in sites that develop enthesopathy suggest that FGF23 might have a direct role (44).

Dental abscesses of deciduous and permanent teeth are a common complication of XLH and many patients lose their permanent teeth in young adulthood as a consequence of repeated infections. Radiographs may show root dysplasia and enlarged pulp chambers. *Phex* is expressed in teeth and there are structural abnormalities that increase the risk of dental abscesses, including poorly mineralized and thinner dentin and cementum layers, and possibly enamel abnormalities.(45–49) In addition, *FGF23* is expressed in odontoblasts(50), and hypophosphatemia may contribute to the dental pathology.(46) However, it is not clear from studies whether current medical treatment with calcitriol and phosphate actually alters the occurrence of dental abscesses.(51, 52). However, appropriate preventative dental hygiene and regular monitoring by a dentist are recommended.

Although there are over 170 different mutations in PHEX reported, including missense, nonsense, deletions, and splice site mutations, a clear genotype-phenotype correlation has not been demonstrated. Although one study has reported a correlation of more deleterious mutations with tubular reabsorption of phosphate and $1,25(OH)_2D$ concentrations, this study failed to include multiple subjects from the same kindred, limiting the ability to identify intra-genotype variability.(53) In contrast, both clinical observation and other published studies have demonstrated significant variability in growth parameters and skeletal deformities within the same kindred or genotype.(54, 55) Although females with XLH have one normal copy of the gene remaining, studies have not confirmed an effect of gender on disease severity.(54) Additional genetic and environmental factors likely influence the clinical manifestations of XLH.

Even with a clear X-linked dominant family history, a *PHEX* gene mutation is frequently not detected, and sporadic cases are common. Importantly, mutation analysis is not specifically necessary to effectively diagnose and treat children with renal phosphate wasting since the diagnosis of isolated renal phosphate wasting (as opposed to a Fanconi syndrome or HHRH) can be made from appropriate biochemical studies. In addition, for the various *FGF*23-mediated disorders, the current medical treatment remains the same as that for XLH.

Other Renal Phosphate Wasting Disorders

Other inherited renal phosphate wasting disorders are less common than XLH. Autosomal recessive forms of hypophosphatemic rickets (ARHR) are caused by inactivating mutations in dentin matrix protein 1 (*DMP1*) or ectonucleotide pyrophosphatase/phosphodiesterase 1 (*ENPP1*) genes, both of which involve increased FGF23 concentrations.(56, 57) Tumor-induced osteomalacia is a rare acquired disorder caused by increased expression of FGF23 by typically small mesenchymal tumors, though other factors have been implicated in the hypophosphatemia such as FGF7.(58, 59) Fibrous dysplasia lesions may also overexpress FGF23 resulting in hypophosphatemia, especially in the setting of extensive bone lesions. (14)

Autosomal dominant hypophosphatemic rickets (ADHR) is caused by a mutation in *FGF23* itself, which causes the protein to resist cleavage.(13) In this condition, patients may have similar presentations to XLH in childhood, or may have normal serum phosphorus and normal growth until adolescence or adulthood, at which point new hypophosphatemic osteomalacia develops. Other individuals never develop hypophosphatemia despite carrying the gene mutation. In patients with active disease, the biochemical phenotype of hypophosphatemia, phosphaturia, and inappropriately normal or low $1,25(OH)_2D$ concentrations were similar to XLH.(60) Furthermore, some patients waxed and waned with periods of hypophosphatemia and normophosphatemia, coinciding with differences in FGF23 concentrations.(61, 62)

Until recently, it has not been clear why these patients only sometimes generated inappropriate FGF23 and hypophosphatemia. However, two recent studies have now demonstrated, both in ADHR patients and in a mouse model of ADHR, that iron is related to FGF23 production. In humans with ADHR, intact FGF23 was inversely related to serum iron concentrations, while in healthy controls, serum iron was inversely related to C-terminal FGF23 measurements (which include fragments), but not to intact FGF23, suggesting increased production of fragments.(62) Likewise, iron deprivation resulted in increased *FGF23* gene expression in both the ADHR mouse and the wild-type mouse.(63) However, only the ADHR mouse developed elevated intact FGF23 concentrations and hypophosphatemic osteomalacia. These two studies have demonstrated that low iron concentration is able to influence FGF23 expression, but, in the absence of a cleavage defect, normal intact FGF23 concentrations were maintained. In contrast, with certain iron infusion formulations given to iron-deficient individuals, elevated FGF23 concentrations and osteomalacia have been demonstrated.(64–66) The reason for these differences is unclear and further study into the mechanism of iron's effect on FGF23 is required to completely reconcile these disparate findings.

Conversely, primary renal phosphate wasting disorders may occur that are not mediated by FGF23 or other hormones. Hereditary hypophosphatemic rickets with hypercalciuria (HHRH) is caused by mutations in the gene encoding NPT2c, and results in a primary renal phosphate loss.(67) FGF23 is appropriately down-regulated in HHRH. (67) However in npt2c-null mice, serum phosphate is normal at all ages from 2 to 50 weeks (68), while absence of npt2a causes hypophosphatemia in mice at ages from 4 to 20 weeks.(69) This suggests that in mice, npt2a is the more important mediator of renal phosphate transport and is able to compensate for loss of npt2c. However, in humans with HHRH, loss of npt2c is not adequately compensated and results in hypophosphatemic rickets. Both the human disorder of HHRH due to NPT2c mutations and the mouse models of npt2c or npt2a deficiency demonstrate that increased $1,25(OH)_2D$ production results in increased calcium absorption (and hypercalcemia in the mice) and consequent hypercalciuria.(67–69) In the npt2c-null mouse, the hypercalcemia resolved by 9–11 weeks, although $1,25(OH)_2D$ and the urinary calcium/creatinine ratio remained elevated, but renal calcifications did not occur. (68) In contrast, patients with HHRH and npt2a-null mice do demonstrate renal calcifications or nephrolithiasis beginning at young ages. (70–72)

Heterozygous point mutations altering NPT2a have been reported in patients with hypophosphatemia and nephrolithiasis (73); however, the mechanism for these particular mutations that cause the phenotype was not confirmed by others.(74) Hypophosphatemic rickets was later noted to occur due to a 7-amino acid duplication in exon 5 of NPT2a which caused abnormal accumulation of the protein in the cytoplasm rather than expression at the plasma membrane. The result was a generalized proximal tubulopathy causing rickets due to Fanconi syndrome.(75)

Diagnostic Evaluation

Early recognition requires identifying important family histories and screening in infancy, even before rachitic deformities are evident. Frequently, we recommend testing of serum phosphate and alkaline phosphatase around six months of age in patients with a family history. Patients without a family history are more likely to have delayed diagnosis. However, treatment before one or two years of age may possibly improve treatment outcomes(54) Genetic analysis of a cord blood sample could be pursued in newborns with positive family history of XLH, but in general, screening serum phosphate and alkaline phosphatase levels using appropriate age norms for interpretation suffices.

Diagnostic evaluation is focused on assessment of biochemical features. Fasting serum and urine phosphate and creatinine allow confirmation of hypophosphatemia and determination of TmP/GFR. The inappropriate use of adult serum phosphate norms leads to missed diagnoses in children with XLH. In untreated children and some adults with XLH, alkaline phosphatase activity is generally increased for age. Testing of 25-hydroxyvitamin D allows detection of vitamin D deficiency, while $1,25(OH)_2D$ in XLH is inappropriately low or normal. PTH, if tested, is frequently mildly elevated at diagnosis and is likely secondary to effects of FGF23 on $1,25(OH)_2D$.

Likewise, serum and urine calcium should be checked to confirm normal levels; abnormalities should raise suspicion of different diseases. Hypocalcemia with hypophosphatemia would suggest vitamin D deficiency rickets or, rarely, a defect in vitamin D metabolism due to mutations in the 1α-hydroxylase or vitamin D receptor genes. In contrast, primary hyperparathyroidism may cause hypophosphatemia with hypercalcemia. Hypercalciuria during hypophosphatemia would suggest a Fanconi syndrome or HHRH. A urinary screen for glucosuria, proteinuria or aminoaciduria should raise suspicion of one of many types of Fanconi syndrome.

Plasma FGF23 concentrations are elevated in patients with XLH (76–80), but FGF23 concentrations are quite variable, ranging from just above the normal mean to several-fold elevations in XLH. Currently, serum FGF23 levels are not measured in the typical diagnostic evaluation of hypophosphatemic rickets. However, during hypophosphatemia, intact FGF23 concentration greater than the normal population mean, which in most studies is approximately 30 pg/ml, should be considered inappropriately elevated and likely causative of the hypophosphatemia.(76, 77) Studies have suggested that the normal range of intact FGF23 in children is similar to that in adults.(77) An elevated FGF23 concentration is not specific for XLH, even in the setting of hypophosphatemia, since ADHR, ARHR, TIO

and fibrous dysplasia all cause FGF23-mediated hypophosphatemia.(14, 56, 58, 61, 79, 81, 82) Nevertheless, a low FGF23 concentration in the setting of hypophosphatemia suggests a different cause such as Fanconi syndrome, dietary phosphate deficiency, or malabsorption.

Radiographically, growth plate changes of rickets are usually seen, but are absent in some patients, and this can lead to confusion about the diagnosis.(83) Standing radiographs (or serial weight-bearing radiographs) of the knees are useful in documenting rickets and response to treatment, as well as in determining when the severity of deformity warrants surgical intervention in growing children.

Current Treatment Strategies

The current goals of medical management of XLH treatment are not specifically to normalize the serum phosphate, but rather to improve osteomalacia and rachitic deformities, maximizing growth in affected children. Early diagnosis and treatment beginning prior to walking and the development of more severe leg deformities may be beneficial. Two studies have demonstrated better height standard deviation scores (SDS) when calcitriol and phosphate treatment began prior to one year of age. (54, 84) However, even at an early age, a highly variable response to treatment has been noted, with many children fail to normalize growth, demonstrating the limitations of current strategies. (54, 84, 85) Most children are treated until they are finished growing. The decision to treat adults is more complicated and must be individualized, since many adults do well without treatment, while others develop bone pain and pseudofractures.

Standard medical treatment of FGF23-mediated hypophosphatemic disorders in general (eg, ADHR, TIO, ARHR) is based on treatment of the XLH, due to similarities in pathophysiology and a lack of specific studies in the less common disorders. Of note, most of the published studies treating XLH are uncontrolled or use only historical controls. As FGF23 excess results in both hypophosphatemia and inappropriately low $1,25(OH)_2D$ concentrations, it is necessary to treat with both phosphate salts and with an activated analog of vitamin D (often calcitriol, although paricalcitol, alfacalcidiol and others have been used). (86)

Treatment of XLH with calcitriol is a pharmacologic therapy rather than merely a supplementation. Although some studies describe higher doses of calcitriol and phosphate for XLH, we generally recommend doses of in the range of 30–40 mg/kg/day for phosphate and of 20–30 ng/ kg/day for calcitriol.(86) Treatment does require careful attention to the balance of these two agents. Patients are usually started with smaller doses, and titrated toward these targets to minimize gastrointestinal symptoms. Some patients may continue to grow well on smaller doses as they "outgrow" their doses based on weight, without worsening leg deformity or laboratory values. Others may require doses mildly above these ranges during monitoring. Much higher doses likely contribute to an increased risk of serious side effects as described below.

Monotherapy with phosphate in XLH is inappropriate and ineffective. Phosphate monotherapy does not adequately heal osteomalacia in XLH, and has significant risk of producing iatrogenic hyperparathyroidism. Oral phosphate alone increases serum phosphate,

decreases ionized calcium, and increases PTH. Unfortunately, phosphate monotherapy has been the cause of two separate reports of severe cardiac valvular and myocardial calcifications, one of which resulted in death.(87, 88) Especially at larger doses, oral phosphate salts cause unpleasant gastrointestinal side effects, notably a laxative effect. Consequently, in order to minimize the severity of symptoms and improve adherence to therapy, gradual titration up to target doses is advised.

The addition of active analogs of vitamin D improves the osteomalacia more significantly than phosphate alone. (89, 90) Calcitriol also increases gastrointestinal calcium and phosphate absorption, and it has a suppressive effect on PTH. Prior to the standard use of calcitriol, XLH was described as a type of vitamin D-resistant rickets and patients were treated with high doses of ergocalciferol or cholecalciferol, which was less effective and carried a higher risk of vitamin D toxicity due to the prolonged half-life, compared to the active vitamin D analogs.

Specific counseling regarding side effects is important because some are potentially severe. Most notably, nephrocalcinosis occurs in up to 71% of individuals in some studies (54, 91) which is likely related to the high throughput of calcium and phosphate induced by medical treatment. Total gastrointestinal absorption of these minerals increases while renal losses of phosphate continue, and hypercalciuria may develop. In fact, nephrocalcinosis does not appear to occur in treatment-naive individuals, and is most likely related to phosphate dose(91), although other studies could not identify specific risk factors.(54) While most nephrocalcinosis is mild and frequently does not impair GFR in the short term(54), it can also become progressive, leading to chronic kidney disease. In addition, treatment with phosphate can cause secondary and tertiary hyperparathyroidism necessitating parathyroidectomy. (42, 43)

Although FGF23 concentrations are elevated in XLH prior to treatment, multiple investigators have now demonstrated that treatment with calcitriol and phosphate increases FGF23 in both the hyp mouse and humans with XLH.(40, 78, 92, 93) Despite the clearly demonstrated benefits of treatment, the clinical consequences of this rise in FGF23 are uncertain. In a cross-sectional analysis of a small number of treated subjects, FGF23 concentrations did not correlate with indices of disease severity.(77)

Many patients experience dramatic improvement of their leg deformities and growth during treatment. However, the response is variable and significant deformities may persist and can ultimately be accompanied by short adult height. Many patients require varying degrees of surgical correction with the goal of improving straightness of the legs. Body disproportion occurs as longitudinal leg growth is more severely affected compared to measures of trunk growth. (94) Patients with delayed onset of treatment experience less catch-up growth, emphasizing the importance of early recognition, diagnosis and therapy.(54, 84)

Growth Hormone (GH)

Although short stature in XLH is not due to a defect in GH levels(95), some studies suggest that recombinant human GH can sometimes increase growth velocity in XLH patients with short stature.(96, 97) In addition to its effects on skeletal growth, GH also increases renal

phosphate reabsorption and 1α-hydroxylation of vitamin D via increased insulin-like growth factor-1 (IGF-I) production.(98) However, most growth hormone treatment studies in XLH patients have been uncontrolled. In one trial, monotherapy with growth hormone improved phosphate, $1,25(OH)_2D$, and longitudinal growth in 12 subjects, but some subjects had apparent worsening of leg deformities.(99) Other studies have also reported worsening body disproportion in XLH. A recent small controlled study indicated improvements in linear growth compared to controls when GH was added to standard therapy, although there were not ultimate height differences between treatment groups at the end of the study.(100) Importantly, this controlled study did not indicate worsening of leg deformities with growth hormone. However, patients with more extreme leg deformities were excluded from the study by design. There remains continued debate and uncertainty about the ultimate risks and benefits of growth hormone treatment in the general population of short children as well as in XLH.

Future Treatment Strategies

The recent understanding of the pathophysiology of XLH can allow for newer targeted and adjunctive therapeutic possibilities. Several novel treatments are in various stages of study, including methods to modulate PTH, to counteract the downstream consequences of FGF23 excess, to alter FGF23 production or degradation, and to block the effects of FGF23. In addition, bypassing FGF23 effect by directly modulating the activity of renal sodium-phosphate cotransporters could be another interesting target. These exciting potential additions require further study.

Calcimimetics

Secondary and tertiary hyperparathyroidism are common complications of XLH and its treatment. Cinacalcet is a calcimimetic, which allosterically modulates the calcium sensing receptor in the parathyroid gland, and was recently approved for treatment of hyperparathyroidism. Cinacalcet decreases PTH level and the serum calcium concentration in patients with hyperparathyroidism. Cinacalcet may have the added potential to indirectly lower FGF23 concentrations.

In a study of non-XLH, dialysis patients with secondary hyperparathyroidism, cinacalcet lowered serum calcium, phosphate, PTH and FGF23 concentrations.(101) Furthermore, in two TIO patients, cinacalcet improved long-term phosphate concentrations while the doses of calcitriol and phosphate were decreased.(102) Single doses of cinacalcet plus an oral phosphate dose increased serum phosphate and TmP/GFR compared to phosphate alone in XLH, while suppressing PTH compared to phosphate plus calcitriol.(103) A case report has indicated some success in managing an XLH patient with complications of nephrocalcinosis, hyperparathyroidism, and renal dysfunction from standard phosphate and calcitriol therapy. (104) Long-term clinical trials are still required to investigate cinacalcet's potential as an adjuvant treatment of XLH.

Calcitonin

Calcitonin stimulates $1,25(OH)_2D$ production in XLH patients and in healthy controls (105), and could alter the biochemical abnormalities of XLH. In addition, calcitonin caused a decrease in serum FGF23 levels in a patient with oncogenic osteomalacia.(106) Recently, calcitonin administration to subjects with XLH was shown to lower FGF23 concentrations for several hours after a single dose. (107) While there was no significant change in the TmP/GFR, there was an increase in serum phosphorus and $1,25(OH)_2D$ following calcitonin administration.(107) Interestingly, for patients with the biochemically opposite disorder of familial hyperphosphatemic tumoral calcinosis (which is due to FGF23 insufficiency), calcitonin was shown to decrease serum phosphate via effects on tubular reabsorption of phosphate.(108, 109) However $1,25(OH)_2D$ did increase in one tumoral calcinosis case report as well.(108) It may be that the net effect of calcitonin on serum phosphate concentration depends on the summation of its effects on phosphate reabsorption and $1,25(OH)_2D$ metabolism, and could bring phosphate abnormalities from either direction closer to normal. Although this seems incongruent, further studies may be justified by these preliminary data.

Hexa-D-arginine

In the hyp mouse model, a decreased expression of 7B2 is thought to be involved in pathogenesis of increased FGF23 concentrations. 7B2 is a helper protein that increases the enzyme activity of subtilisin-like proprotein convertase 2 (SPC2). (110) Phex deficiency results in decreased 7B2 production and decreased 7B2-SPC2 enzyme activity. (110) Pharmacologic inhibition of 7B2-SPC2 both increases FGF23 expression and decreases FGF23 cleavage. (110) Conversely, hexa-D-arginine (D6R) is a small peptide that stimulates 7B2 mRNA expression, enhances 7B2-SPC2 activity and decreases FGF23 mRNA.(110) Likewise, treatment of hyp mice with D6R for 5 weeks resulted in decreased FGF23 expression, increased FGF23 degradation, and improvements in total FGF23 concentrations. (110) Although the FGF23 concentrations of the hyp mice were not completely normalized, there were improvements in the biochemical phenotype of these mice. This peptide, D6R, may provide another way to treat patients with XLH by decreasing excess FGF23 production.

C-Terminal FGF23 Fragments as Antagonists

Conflicting data regarding the relative effects of FGF23 fragments have been reported. Shimada et al reported that only full length FGF23 had biological activity resulting in phosphaturia and diminished $1,25(OH)_2D$ production. (16) Specifically, neither C-terminal nor N-terminal FGF23 fragments had phosphaturic activity. However, Berndt *et al* reported phosphaturic activity of a C-terminal fragment, ranging from 176–251 amino acids.(111) More recently, an antagonistic effect of the C-terminal 180–251 FGF23 fragment on FGF23 biological activity was noted by Goetz et al.(112) Specific binding of the 180–251 fragment to FGFR1 and Klotho was demonstrated along with inhibition of downstream FGFR signaling. Furthermore, this fragment was injected into both normal rats and the hyp mouse, resulting in decreased fractional excretion of phosphate and increased serum phosphate concentrations.(112) The FGF23 180–205 epitope was the minimal necessary binding site

for FGFR1-Klotho in complex, and this fragment was sufficient to increase serum phosphate in hyp mice. Although there is some conflicting data, further animal studies are needed to determine whether antagonistic fragments of FGF23 could block the FGF23 effect significantly enough to allow healing of osteomalacia for potential use in human studies.

FGF23 neutralizing antibodies

Anti-FGF23 antibodies have been studied as possible therapy for XLH. Treating hyp mice with a combination of N-terminal and C-terminal neutralizing antibodies to FGF23 resulted in inhibition of endogenous FGF23 activity. In short and longer term treatment studies, phosphaturia decreased, hypophosphatemia improved and $1,25(OH)_2D$ concentrations increased.(113) Four weeks of treatment resulted in decreased unmineralized osteoid thickness, and decreased growth plate thickness. Furthermore, the length and the shape of the hyp mouse femur improved during treatment with FGF23 neutralizing antibodies.(113) Although more difficult to quantify in humans with XLH, some patients also complain of subjective muscle weakness. Moreover, hyp mice have some evidence of myopathy. The use of the FGF23 neutralizing antibody also improved muscle weakness as measured by improved grip strength and spontaneous motor activity in hyp mice.(114) FGF23 antibodies are currently under investigation in clinical trials in XLH (ClinicalTrials.gov identifiers NCT00830674, NCT01340482, and NCT01571596).

Case revisited

Treatment of the patient began with calcitriol and potassium phosphate in divided doses. Doses were titrated and adjusted for weight, maintaining a level of about 30 ng/ kg/day of calcitriol and about 28 mg/kg/day of potassium phosphate. On monitoring, serum phosphorus was generally in the range of 2.5 to 3.5 mg/dL (low for age), and she remained normocalcemic. Alkaline phosphatase initially increased after starting treatment, but gradually decreased to 531 Units/L (still moderately elevated). Her urine calcium excretion has remained low, and nephrocalcinosis as not been shown on screening ultrasound. However, increased parathyroid hormone necessitated decreasing her phosphorus doses, and increasing calcitriol doses somewhat. Over the next two years, her wrist-widening resolved, and she had improvements in her bowed tibia, although mild genu varum and tibial torsion remained. Her growth rate did slow, and her height was at the 3rd percentile at age 3. Her gait is more "waddling" than the typical toddler. In comparison, her mother's legs had completely straightened during therapy, while her older sister has mild genu valgum, illustrating the variability within kindred. To date, she has not had issues with dental abscesses. This case illustrates some of the complexities and limitations of current treatment with respect to skeletal outcomes.

Conclusion

The past decade has greatly increased our understanding of the mechanisms of disease involved in hypophosphatemic disorders, including the discovery of FGF23 as a critical factor causing hypophosphatemia and abnormal vitamin D metabolism in several disorders. Insights into the pathophysiologic mechanism of disease may guide future treatment strategies. While current therapies have efficacy in managing and treating XLH,

improvements are still needed. Attempts to modify FGF23 activity make logical sense from a pathophysiologic standpoint, and mark the first major potential therapeutic advances for XLH since the addition of calcitriol to the treatment of this disorder. Furthermore, strategies aimed at decreasing FGF23 expression or activity may increase our understanding of disease mechanisms; they may allow clarification regarding whether some features of the disorder such as enthesopathy and dental abscesses are mediated in part by FGF23 itself or by hypophosphatemia, or by other effects of PHEX inactivation. Exciting investigations are being made in the arena of alternate and adjuvant therapies based on pathophysiologic mechanisms. It is hoped these efforts may lead to greater effectiveness, fewer side effects, and improvements in the patient's long-term quality of life.

Acknowledgments

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Table 1

Clinical features of X-linked hypophosphatemia

Clinical findings	Typical imaging and laboratory findings
Short stature, affecting legs more than trunk leading to increased upper to lower segment ratio	Radiographic rachitic changes at the growth plate (may or may not be present)
Lower extremity bowing (genu varum or valgus)	Hypophosphatemia
Wrist widening, frontal bossing and other exam features of rickets	Low TMP/GFR (indicating inappropriate renal phosphate losses)
Tooth abscesses	Normocalcemia
Enthesopathy/joint stiffness (gradual onset in adolescence or adulthood)	Normal urine calcium excretion
Osteoarthritis (in adulthood)	Secondary hyperparathyroidism
	Low or normal 1,25-dihydroxy-vitamin D
	Normal 25-hydroxy-vitamin D

Table 2

Diseases with hypophosphatemia secondary to FGF23 excess

Disease	Etiology
X-linked hypophosphatemia	PHEX (inactivating mutations)
Autosomal dominant hypophosphatemic rickets	FGF23 (mutations causing resistance to proteolytic cleavage)
Autosomal recessive hypophosphatemic rickets	DMP1 and ENPP1 (inactivating mutations)
Fibrous dysplasia of bone/McCune Albright syndrome	Post-zygotic GNAS activating mutations
Tumor-induced osteomalacia	FGF23 production by mesenchymal tumors
Linear sebaceous nevus syndrome	FGF23 production by characteristic skin lesions
Hypophosphatemia following renal transplantation	"Tertiary" excess FGF23 production persisting after transplantation

Table 3

Current and Investigational Agents for XLH

	Target	Agent	Effect
Current standard of care	Consequence of FGF23 excess (low 1,25(OH)$_2$D)	Calcitriol in divided doses (20–30 ng/kg/day)	Increase gastrointestinal calcium and phosphorus absorption
	Consequence of FGF23 excess (hypophosphatemia)	Phosphate salts in divided doses (30–40 mg/kg/day of elemental phosphorus)	Increase gastrointestinal phosphorus absorption
Potential future agents[a]	Hyperparathyroidism	Cinacalcet [b]	Lower PTH, increase phosphorus
	FGF23 excess and low 1,25(OH)$_2$D	Calcitonin [c]	Decreases FGF23, increases serum phosphate and 1,25(OH)$_2$D
	FGF23 excess	C-terminal FGF23 fragments [d]	Antagonize FGF23 action Block FGF23 action
	FGF23 excess	Neutralizing anti-FGF23 antibodies [e]	

[a] Appropriate clinical trials are necessary for these to become incorporated into clinical care.

[b] Case report and single dose studies.

[c] Single dose study.

[d] Animal model

[e] Animal model, and ongoing early phase clinical trials.