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Disorders of phosphorus homeostasis

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Abstract

Purpose of Review—The study of phosphorus physiology and investigations into clinical disorders of phosphorus metabolism has blossomed over the past decade. Recent work has confirmed and further extended our knowledge of basic mechanisms of phosphorus metabolism.

Recent findings—This review will focus on FGF-23 and Klotho, and on the recent further dissection of their roles in phosphorus and skeletal metabolism. Additionally, this review will detail recent studies that implicate a role for these phosphaturic and vitamin D regulating factors in extraskeletal calcification, including that occurring in soft tissue and vascular beds.

Summary—These findings in total provide fertile ground for investigations into the cause and treatment of abnormal skeletal and extraskeletal calcification in patients with inherited hypophosphatemic disorders. More importantly, and certainly with wider potential clinical application, these studies likewise imply a role for these factors in the pathogenesis of accelerated cardiovascular disease that occurs in patients with the most common hyperphosphatemic disorder, chronic kidney disease. Future studies are needed to confirm a harmful or possibly even beneficial role for FGF-23 and other factors in these disease states, and to determine whether therapeutic manipulation of these factors does truly affect clinical outcomes in patients with hypo- and hyperphosphatemia.

Keywords

Hypophosphatemia; hyperphosphatemia; FGF-23; Klotho

Introduction

There has been a tremendous interest in disorders of phosphorus homeostasis over the past decade. This review will attempt to define the recent important observations that have further extended and defined the role of FGF-23 (fibroblast growth factor 23), Klotho (the obligatory co-receptor for FGF-23) and other phosphorus regulating hormones in the most clinically relevant hypophosphatemic and hyperphosphatemic disorders, with specific attention to pathophysiology, diagnosis/clinical outcomes and treatment/prognosis of both inherited and acquired disorders.

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Of note, a recent genome wide association study (GWAS) investigated which genes were most strongly associated with serum phosphorus concentration in a large prospective cohort of over sixteen thousand subjects (1). Interestingly, the strongest association was with tissue nonspecific alkaline phosphatase, which is responsible for hydrolyzing pyrophosphate into phosphorus in the skeleton and other tissues. Strong association were also noted for FGF-23, SLC34A1 (encodes the kidney specific type IIa sodium phosphate co-transporter, Npt2a) and CASR (calcium sensing receptor), and well as several possible novel regulators of phosphorus metabolism.

1. Pathogenesis

a. Inherited hypophosphatemic disorders

The acquired hypophosphatemic disorders, X-linked hypophosphatemic rickets (XLH), autosomal dominant hypophosphatemic rickets (ADHR) and autosomal recessive hypophosphatemic rickets (ARHR) have been previously determined to be due to mutations in the genes encoding PHEX (phosphate regulating gene with homology to endopeptidases), FGF-23, and DMP-1 (dentin matrix protein-1). Recently, mutations in the gene encoding ecto-nucleotide pyrophosphatase/phosphodiesterase 1, which was previously shown to cause generalized arterial calcification of infancy (GACI), were confirmed by 2 different investigators concomitantly to cause ARHR as well (2, 3). The observation in these patients of elevated or inappropriately normal FGF-23 levels established the presence of another factor that regulates FGF-23 in an upstream fashion, and also suggests an as yet unidentified mechanism that balances arterial calcification with bone mineralization.

This recent review of acquired hypophosphatemic disorders would not be complete without detailing the recent critical studies on the FGF-23 and Klotho signaling and regulation. Segawa confirmed the importance of both Npt2a and Npt2c in phosphorus regulation in mice homozygous for the deletion of both transporters (4). Furthermore, Tomoe established that both Npt2a and Npt2c, as well as to a lesser extent the type III Na-dependent Pi transporter PiT2, are necessary for the phosphaturic activity of FGF-23 (5). With regard to FGF-23 mechanism and site of action, Gattineni demonstrated that FGF receptor 1 (FGFR1) was the predominant receptor for the hypophosphatemic action of FGF-23 in vivo (6). Furthermore, Farrow confirmed that initial FGF-23 signaling occurs in the distal convoluted tubule (DCT) in concert with membrane bound co-receptor α -Klotho via a MAP-kinase dependent pathway (7), though the exact mechanism by which this co-receptor complex in the DCT modulates Npt2a and Npt2c in the proximal convoluted tubule remains unknown.

Recent studies have also confirmed and extended the nature of the coordinated regulation of phosphorus by FGF-23 and Klotho. Nakatani demonstrated through two separate double-knockout mice studies (Fgf23^{-/-}/klotho23^{-/-} and Hyp^{-/-}/klotho23^{-/-}) that FGF-23 is unable to regulate neither systemic phosphate nor vitamin D homeostasis in the absence of Klotho (8, 9). Furthermore, Bai and colleagues also confirmed complete reversal of systemic ion derangements in Klotho deficient transgenic mice producing proteolytic-resistant FGF-23 (R176Q) (10). While it is well known that complete deficiency of either FGF-23 or Klotho in mice results in disordered systemic ion homeostasis and premature death (11), and in the case of Klotho deficiency, one that resembles premature aging (12), recent studies have further defined the potentially modifiable factors that may mediate these outcomes. Ohnishi confirmed the key role of normophosphatemia in mediating the soft tissue and vascular calcifications in Klotho deficient mice through induction of hypophosphatemia via targeted deletion of NaPi2a (13) or dietary restriction of phosphorus (14), an effect which was reversible in the latter with institution of a high phosphorus diet. These observations are even more striking given the dramatic elevations of both calcium and 1,25 dihydroxyvitamin D (1,25 OH₂ D) that occur in these animals. Finally, Ohnishi also showed that deletion of

1,25 OH₂ D can likewise reverse the phenotypic abnormalities and elevation of FGF-23 that occurs in Klotho-deficient mice (15).

In addition to the aforementioned studies, recent work has suggested that additional factors may actively regulate phosphorus homeostasis. Specifically, overexpression of a nuclear high molecular weight FGF2 isoform in mice caused phosphaturia, hypophosphatemia and osteomalacia in mice via increased expression of FGF-23 and Klotho that was partially rescued by high phosphate diet (16). Additionally, Tsuji described the positive regulation of FGF-23 expression by leptin in leptin-deficient *ob/ob* mice, perhaps shedding light on a possible integrated brain-bone-kidney axis that regulates nutrient homeostasis (17). Although much work needs to be done, there does appear to be strong evidence based on the aforementioned studies and work to date for a bone-kidney-intestine-parathyroid axis through which FGF-23 and Klotho regulate phosphorus and calcium homeostasis (18) (Figure 1).

b. Inherited hyperphosphatemic disorders

Familial tumoral calcinosis (FTC) is an autosomal recessive disorder characterized by ectopic, calcified masses in soft tissues, typically found around major joints including the hip, shoulder, and knee (19, 20). Molecular genetic analyses have demonstrated FTC can result from mutations in genes encoding FGF23, GALNT3, or Klotho. Mutations in exons 2 and 3 of the GALNT3 gene, encompassing the initiation codon and a portion of the glycosyl-transferase domain, respectively, have been previously reported (21). Ichikawa reported 4 patients with novel GALNT3 mutations (22), including a novel missense mutation in exon 2 adjacent to the location of a previously reported mutation, suggesting this region of GALNT3 may be susceptible to increased mutation rate.

As previously well-described, Fgf23-null and Klotho-deficient mice have shortened life spans. Recently, Ichikawa generated Galnt3 null mice through replacement of exons 2 and 3 (23). Homozygous mice had 50-60% lower levels of serum FGF23 compared to wild type and heterozygous mice. C-terminal Fgf23 expression was increased in bone, serum phosphorus levels were elevated and 1,25 OH₂ D levels were inappropriately normal. Interestingly, the mice did not develop ectopic calcifications.

Hyperostosis-hyperphosphatemia syndrome (HHS) is a variant of FTC with elevated serum phosphorus and abnormal 1,25 OH₂ D levels. As with FTC, patients with HHS have low serum levels of intact FGF23 but high levels of C-terminal FGF23. However, the phenotypic presentation of HHS is characterized by recurrent, transient, and painful lesions of the long bones. Radiographic features include cortical hyperostosis, diaphysitis, and periosteal apposition.

Only mutations in GALNT3 have been shown to result in phenotypic HHS (24). Based on reported cases to date, there is a tendency for missense mutations in GALNT3 to present as HHS and nonsense mutations as FTC. Dumitrescu et. al. reported a patient with compound heterozygous mutations in GALNT3, with phenotypic features of both FTC and HHS, suggesting that the spectrum of disease in FTC might be related to the functional ability of Galnt3 to process FGF-23 (25).

c. Acquired hypophosphatemic disorders

Prolonged hypophosphatemia has recently been reported to occur after infusion of intravenous iron polymaltose, an increasingly utilized therapy for patients with chronic anemia. Interestingly, prospective evaluation of patients receiving iron polymaltose in 2 different series of patients has confirmed an increase in FGF-23 levels and decrease in 1,25 OH₂ D levels, though the specific offending substance in iron polymaltose and the

mechanisms underlying this dysregulation have yet to be determined (26, 27). Hypophosphatemia can also occur during refeeding of malnourished individuals and during treatment of diabetic ketoacidosis due to extra- to intracellular shift of inorganic phosphorus.

d. Acquired hyperphosphatemic disorders

Although hyperphosphatemia can occur acutely due to conditions such as tumor lysis and rhabdomyolysis, the most common clinical disorder is chronic kidney disease (CKD). Previous studies have shown increased FGF23 levels in patients with CKD. FGF23 levels rise in part due to decreased renal excretion as the glomerular filtration rate declines, but also in response to progressively increasing serum phosphate levels. This compensatory mechanism initially results in increased renal phosphate excretion and reduced dietary phosphate absorption via inhibition of 1,25 OH₂ D production. Studies have also shown FGF23 directly decreases parathyroid hormone gene expression and hormone secretion. However, in CKD patients with secondary hyperparathyroidism, parathyroid hormone secretion remains elevated despite high FGF23 levels. Recent studies (28-30) have shown decreased FGF23 receptor 1 (FGFR1) and Klotho mRNA expression and protein production in parathyroid tissue in mice with severe renal impairment, suggesting that FGF23 resistance may result from down regulation of the FGFR1-Klotho receptor complex.

2. Diagnosis

The work-up of phosphorus disorders normally includes measurement of fasting serum phosphorus, 25 OH D, 1, 25 OH₂ D and possibly FGF-23 levels (as indicated by clinical presentation), as well as serum creatinine and calcium in hyperphosphatemic patients. The role of soluble alpha-Klotho, a circulating form that is distinct from membrane bound Klotho, is unclear. Given the known obligatory dependent interaction that is necessary for end-organ effect of FGF-23, and the still as yet unreconciled observation that the sites of FGF-23/Klotho signaling and phosphaturic effect are spatially discordant, measurement of circulating Klotho may provide important insight into the pathogenesis of these disorders. To that end, Yamazaki recently established and preliminarily characterized a sandwich ELISA for soluble alpha-Klotho in healthy adult subjects (31). Future studies in patients with XLH, TIO and other hypo- and hyperphosphatemic disorders will certainly be of keen interest.

3. Clinical outcomes

Although the skeletal outcomes of inherited and acquired hypophosphatemic, osteomalacic syndromes have been well-characterized to date, the extra-skeletal outcomes have not. Liang found an extremely high prevalence of enthesopathy in young adults with XLH (32). In addition, they determined for the first time in the Hyp mouse model that mineralizing insertion sites of tendons and ligaments were due to a significant expansion of mineralizing fibrocartilage, not due to bone-forming osteoblasts. The finding of FGFR3 and Klotho expression in the fibrocartilaginous cells suggests a plausible mechanism by which elevated FGF-23 levels may facilitate abnormal extraskelatal calcification in patients with XLH.

Perhaps the greatest interest in clinical outcomes and FGF-23 was generated by studies in the acquired hyperphosphatemic disorder CKD, wherein it was demonstrated that increased FGF23 levels are associated with increased mortality in patients on hemodialysis (33). In a recent prospective study by Jean et. al. (34), the 2-year survival rate was significantly lower among HD patients within the highest quartile of FGF23 level compared to those in the lowest quartile (HR 2.5, 95% CI 1.3 - 5.0). Patients in this study were treated with long HD (5 to 8 hours thrice weekly) for better control of hyperphosphatemia; therefore, serum phosphate levels were lower in this study population. Similar to previous reports, a lower

odds of mortality was observed in patients with lower FGF23 levels at all tertiles of phosphatemia but was only statistically significant in the lowest tertile.

Levels of FGF23 are also elevated in animal models of Klotho deficiency. Moreover, mice null for Klotho have a premature aging phenotype and shortened lifespan. Based on this, relative Klotho deficiency may be causally associated with increased mortality among CKD patients. Friedman et. al. studied 12 single nucleotide polymorphisms (SNPs) within the Klotho gene in 1307 patients during their incident year on hemodialysis (35). A significant association was found between a C/C genotype of SNP rs577912 and increased 1-year mortality (RR 1.76, 95% CI 1.19 – 2.59). rs577912 is located within an intron and therefore has no obvious functional significance. However, gene expression profiling did suggest an association with lower Klotho mRNA expression.

Previous studies have demonstrated that calcium-phosphate abnormalities are associated with increased cardiovascular morbidity and mortality among patients with CKD. Whether the increased mortality associated with FGF23 levels is due to cardiovascular events is an area of active investigation (36-39). Parker et. al. studied 833 patients with stable CAD and renal function ranging from normal to moderate CKD (40). Compared to patients with FGF23 levels in the lowest tertile, those in the highest tertile had a significantly greater risk for cardiovascular events including MI, stroke, or heart failure (HR 1.83, 95% CI 1.15 – 2.91) (Figure 2). Furthermore, there was a 2-fold increase in all-cause mortality (HR 2.15, 95% CI 1.43 – 3.24). In another report, Seiler et. al. (41) measured plasma C-terminal FGF23 levels in 149 CKD patients not on dialysis. Patients with FGF23 levels above 104 rU/mL had an increased risk of a composite of cardiovascular events (HR 2.49, 95% CI 1.40-4.39) compared to those below this level. The specific mechanism of FGF-23 effect and the role of Klotho remain to be determined.

4. Treatment/Future Directions

An important unresolved issue in the treatment of hereditary FGF-23 dependent hypophosphatemic disorders is whether standard treatment with phosphorus and activated vitamin D analogues is truly helpful or potentially harmful. Certainly, the skeletal and growth benefits of this therapy provide more benefit than harm in pediatric patients with XLH. In adults, the risks and benefits are less clear. Based on mouse data suggesting that 1,25 OH₂ D treatment promotes expression of FGF-23 and was possibly a counter-regulatory hormone to FGF-23 (42), Imel and colleagues demonstrated that treatment with calcitriol and phosphate increased FGF-23 levels 123 % compared with untreated controls (43). The true pathophysiological consequences of these changes in FGF-23, however, are unknown at present.

Undoubtedly, manipulation of FGF-23 levels will have a much broader application in patients with CKD, wherein at present it is unclear whether attempts to reduce FGF-23 levels will be beneficial or potentially harmful. Modulation of FGF-23 activity could be achieved via indirect and direct mechanisms. Regarding the former, Wetmore (44) recently reported significantly lower FGF23 levels in patients treated with cinacalcet and low-dose calcitriol analogues compared to those treated with standard dose calcitriol analogues alone ($p < 0.002$).

Intuitively, a more targeted approach to reduce the production and/or function of the presumably pathogenic factor FGF-23 in inherited hypophosphatemic disorders and in CKD would be a logical therapeutic approach to management of these patients. Indeed, Aono did perform this proof of principle by injecting Hyp mice with neutralizing FGF23 IgG₁ antibody, which produced a dose dependent reversal of hypophosphatemia, phosphaturia and low calcitriol levels via expected physiologic mechanism (45) (Figure 3). Indeed, an

ongoing Phase I, double-blind, randomized, placebo-controlled, single-dose, dose-escalation study (NCT00830674) is currently examining the biological effect and safety of such a compound in subjects with XLH. Further on the horizon, although certainly tenable, are peptides derived from the C-terminal tail of FGF-23. Administration of these peptidomimetic and organomimetic compounds to rats antagonized the phosphaturic activity of FGF-23 in vivo (46). Such approaches could likewise be employed in patients with CKD, although demonstration of clinical benefit and safety will ultimately require adequately powered, prospective studies to determine whether reduction of FGF-23 levels diminishes the premature morbidity and mortality associated with CKD.

Conclusion

Our understanding of the mechanisms governing phosphorus homeostasis has been further extended by recent studies in both animals and humans. These studies have also more definitively established the obligatory role of FGF-23 and Klotho in hypo- and hyperphosphatemic disorders, though it remains to be established whether they are harmful or potentially helpful players in patients with hyperphosphatemia, especially CKD. Future studies are definitely needed to confirm this unanswered question, as well the risks and benefits of the therapeutic manipulation of these phosphaturic factors.

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Figure 1. The FGF-23/Klotho axis

FGF-23 produced by bone principally targets the kidney, leading to reductions in serum phosphate and 1,25-dihydroxyvitamin D ($1,25\text{-OH}_2\text{D}$) levels by stimulating the fractional excretion of phosphate and reducing 1α -hydroxylase activity. The receptor for FGF-23 in the kidney is a Klotho: FGFR1 complex located in the distal tubule. There may be a distal-to-proximal feedback mechanism that mediates the effects of FGF-23 on the proximal tubule. FGF-23 also decreases the kidney expression of Klotho, which diminishes renal tubular calcium reabsorption via its interactions with transient receptor potential cation channel, subfamily V, member 5 (TRPV 5), FGF-23 may also directly target the parathyroid gland (PTG) to reduce parathyroid hormone (PTH) secretion. FGF-23 is the principal phosphaturic hormone and may function to counter the hypercalcemic and hyperphosphatemic effects of excess $1,25\text{-OH}_2\text{D}$ through reductions in PTH and elevations in FGF-23 levels. Effects of mutation in PHEX , DMP-1 , and ENPP-1 lead to elevated levels of FGF-23 and attendant downstream effects. Reproduced with permission and modifications from J Clin Invest 2008; 118:3820–3828, Fig. 1, p. 3821.

Figure 2. Unadjusted event-free (MI, stroke, transient ischemic attack, or heart failure) survival, by tertiles of FGF-23

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Figure 3. The Improvement of hypophosphatemia after a single injection of FGF23Ab (4 or 16mg/kg body weight)

Time course of changes in serum phosphate and calcium levels. All results represent mean $\pm$ SE ($n=6$). Statistical analysis was conducted for the results at each time point by Dunnett's method. (a) $P < 0.05$ and (b) $P < 0.001$ vs. WT control. (c) $P < 0.05$ and (d) $P < 0.01$ vs. Hyp control. Hyp, hypophosphatemic; WT, wild type. Reproduced with permission and modifications [45**], Fig. 1, p. 18881.