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Inherited hypophosphatemic disorders in children and the evolving mechanisms of phosphate regulation

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Abstract Phosphorous is essential for multiple cellular functions and constitutes an important mineral in bone. Hypophosphatemia in children leads to rickets resulting in abnormal growth and often skeletal deformities. Among various causes of low serum phosphorous are inherited disorders associated with increased urinary excretion of phosphate, including autosomal dominant hypophosphatemic rickets (ADHR), X-linked hypophosphatemia (XLH), autosomal recessive hypophosphatemia (ARHP), and hereditary hypophosphatemic rickets with hypercalciuria (HHRH). Recent genetic analyses and subsequent biochemical and animal studies have revealed several novel molecules that appear to play key roles in the regulation of renal phosphate handling. These include a protein with abundant expression in bone, fibroblast growth factor 23 (FGF23), which has proven to be a circulating hormone that inhibits tubular reabsorption of phosphate in the kidney. Two other bone-specific proteins, PHEX and dentin matrix protein 1 (DMP1), appear to be necessary for limiting the expression of fibroblast growth factor 23, thereby allowing sufficient renal conservation of phosphate. This review focuses on the clinical, biochemical, and genetic features of inherited hypophosphatemic disorders, and presents the current understanding of hormonal and molecular mechanisms that govern phosphorous homeostasis.

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1 Introduction

Phosphorous is a key molecule required for many cellular processes. It is an essential part of nucleic acids and the cell membrane, serves as an important mediator of intracellular signaling, modifies protein activity, and contributes to cellular energy in the form of ATP. In addition, phosphorous ions are indispensable for the formation and maintenance of bone mineral. Thus, serum phosphorous level is well regulated, with levels being maintained higher in young children than adults. Lower than age-appropriate levels of serum phosphorous are associated with severe skeletal defects and growth failure, unless appropriately treated [1–3]. Hypophosphatemia, independent of the levels of serum calcium and PTH, has been shown to have an adverse effect on endochondral bone formation by impairing apoptosis of hypertrophic growth plate chondrocytes [4].

Most of the dietary phosphorous is absorbed in the intestinal tract regardless of the amount. Once in the circulation, phosphorous is either taken up by cells, accumulates in bone matrix, or is excreted in the urine. The primary site of regulation is in the proximal tubule where the filtered phosphorous is reabsorbed through either high-capacity low-affinity or low-capacity high-affinity sodium phosphate co-transporters [5–7]. Dietary phosphorous loading or depletion significantly affects the expression and subcellular distribution of the renal low capacity high affinity transporters, which include NPT2a and NPT2c.

PTH and vitamin D have long been known as important regulators of serum calcium and phosphorous [8, 9]. PTH, the levels of which are primarily controlled by the level of

serum calcium, exerts its actions by binding to a G protein-coupled receptor, the PTH/PTHrP receptor. In bone, PTH increases turnover with a net increase in bone resorption, thereby mobilizing both calcium and phosphorous into the circulation. In the distal renal tubules, PTH furthermore increases the expression of the calcium channel TRPV5 and, thus, the reabsorption of calcium, thereby limiting renal calcium losses [10]. In the proximal tubules, PTH inhibits reabsorption of phosphorous via effects on NPT2a and NPT2c protein levels [5–7]. The action of PTH on the proximal tubule also includes induction of mRNA encoding 25 hydroxy vitamin D 1-α hydroxylase, the enzyme catalyzing the formation of 1,25-dihydroxy vitamin D ($1,25(OH)_2D$). Proximal tubular biosynthesis of 1,25 $(OH)_2D$ is also induced by low serum phosphorous. Circulating $1,25(OH)_2D$, which exerts its actions via a nuclear receptor, enhances the intestinal absorption of calcium and, to a lesser extent, phosphorous, and suppresses the biosynthesis and secretion of PTH [8, 11]. In bone, elevated $1,25(OH)_2D$ levels increase osteoclastic bone resorption, mobilizing calcium and phosphorous into the circulation. Thus, $1,25(OH)_2D$ acts in intestine and bone to ensure that levels of calcium and phosphorous are sufficient to allow mineralization of the collagen matrix. The physiologic and molecular actions of PTH and 1,25 $(OH)_2D$ are reviewed in detail elsewhere [8, 9].

Serum phosphorous levels can decrease as a result of many different conditions. A common cause of hypophosphatemia in children is decreased intestinal absorption, which can be the result of malabsorption, malnutrition, or defective vitamin D metabolism [11]. Various metabolic conditions can also lead to hypophosphatemia by inducing a transcellular shift of phosphorous from extracellular to intracellular space, such as sepsis, nutritional repletion, or insulin therapy. Hypophosphatemia can also result from excess loss of phosphate into the urine, i.e. insufficient renal phosphate conservation. Multiple conditions, such as hyperparathyroidism, renal tubular defects, or diabetic ketoacidosis, can lead to renal phosphate wasting. Certain inherited or acquired diseases also lead to hypophosphatemia due to diminished phosphate reabsorption in the renal tubules [3, 12–14]. In this review, we focus on the genetic, clinical, and biochemical aspects of inherited hypophosphatemic diseases that affect pediatric patients and discuss how the study of these disorders has shaped our current understanding of phosphate homeostasis at the molecular level.

2 Genetic disorders of hypophosphatemia

Until recently, our understanding of normal phosphate homeostasis has been limited to the actions of PTH and $1,25(OH)_2D$ [8, 9]. Yet, clinical and biochemical findings from patients with various inherited or acquired disorders of phosphate metabolism and the results from many elaborate investigations of pertinent mouse models have suggested that, in addition to these well characterized hormones, at least one additional circulating molecule, termed "phosphatonin", is involved in the regulation of serum phosphorous levels [15]. Recent molecular and genetic studies of hypophosphatemic and hyperphosphatemic disorders have indeed revealed several novel proteins as being essential for normal phosphate handling (Table 1). Among these, fibroblast growth factor-23 (FGF23), which is mutated in patients with autosomal dominant hypophosphatemic rickets (see below), appears to be the most likely phosphatonin candidate, although several other molecules with phosphaturic actions have also been described [14, 16]. FGF23 appears to be the key mediator in several genetically distinct hypophosphatemic disorders, which are associated with inappropriately normal or low serum $1,25(OH)_2D$. Conversely, some other inherited hypophosphatemic disorders are caused by genetic defects directly affecting renal phosphate transporters, and are thus characterized by low serum FGF23 and elevated serum $1,25(OH)_2D$ levels.

2.1 Hypophosphatemic rickets with inappropriately normal or low $1,25(OH)_2D$

2.1.1 Autosomal dominant hypophosphatemic rickets

First described in 1971 by Bianchine et al. [17], ADHR is characterized by hypophosphatemia due to isolated renal phosphate wasting that results from a genetic defect that can be transmittable from male to another male, i.e. this disorder is genetically different from the X-linked form of hypophosphatemia (see below). Children with ADHR present with skeletal defects including severe bowing of

Table 1 Biochemical and genetic features of inherited hypophosphatemic disorders

	FGF23	TRP	$1,25(OH)_2D$	PTH	Serum calcium	Urinary calcium	Mutation
XLH	High/inappropriately normal	Low	Low–normal	High–normal	Normal	Normal	PHEX
ADHR	High/inappropriately normal	Low	Low–normal	High–normal	Normal	Normal	FGF23
ARHP	High/inappropriately normal	Low	Low–normal	High–normal	Normal	Normal	DMP1
HHRH	Low/normal	Low	High	Low	Normal/elevated	High	NPT2c

long bones and widening of the metaphyseal regions of the bones that is most prominent at costochondral joints. Investigation of a large ADHR family has revealed some important features of this disorder, including a variable onset (pre-pubertal vs. post-pubertal), incomplete penetrance, and spontaneous recovery of renal phosphate reabsorption [18–20].

The genetic lesion responsible for ADHR has been identified through positional cloning, which revealed heterozygous mutations within the gene encoding FGF23 [16, 21]. FGF23 is expressed in various tissues, but its levels are most abundant in bone, particularly in osteocytes and lining cells [22–24]. The FGF23 protein is a 251-amino acid polypeptide comprising a 25-amino acid N-terminal signal sequence. After removal of the signal peptide, FGF23 appears as a 32-kDa protein on Western blots. Mutations identified in ADHR are missense mutations, and in each case, the mutation alters an arginine residue located at either position 176 or 179. These residues are within a subtilisin-like pro-protein convertase cleavage site ($R^{176}XXR^{179}$) [14, 25–27]. Based on Western blot analyses, wild-type FGF23 is subject to cleavage and secreted as the full-length 32 kDa and a 12 kDa C-terminal fragment. Mutagenesis studies have shown that mutant FGF23 proteins resulting from mutations found in ADHR patients are unable to undergo this proteolytic process and patients affected by this disorder therefore secrete primarily the full-length hormone, suggesting that the accumulation of full-length FGF23 results in the phosphate wasting observed in ADHR. Consistent with this notion, full-length FGF23 is able to induce hypophosphatemia when injected into rodents (26). Furthermore, mice transplanted with CHO cells stably overexpressing wild-type FGF23, but not those transplanted with non-transfected CHO cells, develop renal phosphate wasting and hypophosphatemia, combined with elevated serum alkaline phosphatase activity [14, 25]. The N-terminal FGF23 fragment appears to lack phosphaturic action upon injection into rodents [26], but it is currently less clear whether the C-terminal FGF23 fragment shows a phosphaturic effect. Earlier studies demonstrated a lack of action by the C-terminal fragment [26], whereas a recent study showed that the full length and C-terminal FGF23 can similarly induce hypophosphatemia when injected into rats at equimolar concentrations [28]. Expression analysis, cloning, and subsequent functional studies have identified FGF23 in tumors that cause hypophosphatemia and osteomalacia (oncogenic osteomalacia) as a relatively abundant protein and one that is able to induce hypophosphatemia [25, 29, 30], thus supporting the notion that FGF23 levels are directly correlated with renal phosphate wasting.

Physiological role of FGF23 in the regulation of phosphate homeostasis has been revealed by the study of mouse models in which Fgf23 is ablated and the findings in patients who carry inactivating mutations in the FGF23 gene. FGF23 null mice display severe hyperphosphatemia, as well as hypercalcemia secondary to elevated serum 1,25 $(OH)_2D$ levels [22–24]. Furthermore, homozygous inactivating mutations of FGF23 or GALNT3, an enzyme required for normal O-glycosylation of FGF23, cause tumoral calcinosis, a disorder in which ectopic calcifications are associated with hyperphosphatemia and often mild hypercalcemia [31, 32]. These findings demonstrate that FGF23 is required for normal phosphate homeostasis and acts by suppressing both the reabsorption of phosphate in the renal tubule and the biosynthesis of $1,25(OH)_2D$. The latter is consistent with the inappropriately low or normal $1,25(OH)_2D$ levels observed in patients with ADHR. In human studies, and particularly in rodents, changes in serum phosphorous levels have been found to regulate serum FGF23, underscoring the importance of this molecule in the physiological regulation of phosphate [33–36].

2.1.2 X-linked hypophosphatemia (XLH)

The most common inherited phosphate-wasting disorder, XLH, frequently becomes manifest during late infancy when the patient begins walking [13, 14]. The patient demonstrates skeletal deformities that primarily include bowing of the long bones and widening of the metaphyseal region. The latter is most prominent at costo-chondral junctions (rachitic rosary). These deformities are accompanied by diminished growth velocity, often resulting in short stature. Later in life, the patients can show osteomalacia, enthesopathy, degenerative joint disease, continued dental disease, particularly tooth decay and dental abscesses. Hypophosphatemia in XLH patients is associated with inability of the renal proximal tubule to reabsorb phosphate, as evidenced from reduced measurements of tubular phosphate reabsorption and TmP/GFR. Despite the low serum phosphorous, serum $1,25(OH)_2D$ is not elevated and it is in fact frequently below the normal range. In addition, serum alkaline phosphorous is elevated. Serum calcium and PTH are typically normal, although some elevation of serum PTH is observed.

Genetic linkage analysis of XLH kindreds and subsequent genomic studies have revealed inactivating mutations in *PHEX*, a gene located on Xp22.1 [37, 38]. PHEX protein is expressed in various tissues, including the kidney, but it is most abundant in mature osteoblasts and odontoblasts. Significant peptide sequence homology exists between PHEX and the members of the M13 family of zinc metallopeptidases, which are integral membrane glycoproteins that show proteolytic activity immediately outside the cell. Since it does not seem to circulate in blood, and since inactivating mutations lead to phosphate wasting through a circulating molecule, i.e. phosphatonin, it has been postu-

lated that PHEX mediates inactivation, by proteolytic cleavage, of phosphatonin. The best candidate is FGF23, whose gene is mutated in patients with ADHR. Consistent with this conclusion, serum FGF-23 concentrations are elevated in about two thirds of patients with XLH [39–41] and in all *Hyp* mice, i.e. the murine model of XLH [42, 43]. Furthermore, the phenotype of Fgf23-null mice is indistinguishable from the phenotype of animals that are null for both, Phex and Fgf23, indicating that Fgf23 is required for the development of hypophosphatemia and that it is downstream of Phex [23, 24]. Interestingly, preliminary studies revealed that *Hyp* mice, which were injected with inactivating antibodies to FGF23, normalized their blood phosphorous concentration and furthermore healed their rachitic changes, thus supporting the conclusion that Phex is directly or indirectly involved in the metabolism of FGF23 [42]. However, PHEX-dependent cleavage of FGF23 could not yet be demonstrated *in vivo.* Also, FGF23 cleavage *in vitro* was shown only in a single study and this could not be confirmed in others [27, 44]. Currently, the physiological substrate of PHEX remains unknown.

2.1.3 Autosomal recessive hypophosphatemia (ARHP)

Hypophosphatemic rickets in consanguineous kindreds has been reported but it was not until recently that this type of hypophosphatemia was defined at the molecular level [45–47]. Study of the clinical and biochemical findings of the affected individuals in several different ARHP kindreds indicates a great deal of similarity to ADHR and XLH. Clinical features include rickets, skeletal deformities, and dental defects, and affected individuals develop osteosclerotic bone lesions and enthesopathies later in life (Fig. 1). Hypophosphatemia, which results from renal phosphate wasting, is accompanied by normal or low $1,25(OH)_2D$ levels and high alkaline phosphatase activity. FGF23 levels appear to be either elevated or normal in ARHP patients, i.e. inappropriate for the level of serum phosphorous [48, 49].

ARHP is caused by homozygous mutations, which affect the gene encoding dentin matrix protein 1 (DMP1). DMP1 belongs to the SIBLING protein family, which includes osteopontin, matrix extracellular phosphoglycoprotein, bone sialoprotein II, and dentin sialoprotein, and whose genes are clustered on chromosome 4q21. DMP1 is a bone and teeth specific protein [50], which is involved in the regulation of transcription in undifferentiated osteoblasts [51, 52]. DMP1 undergoes phosphorylation during the early phase of osteoblast maturation and is subsequently exported into the extracellular matrix where it regulates the nucleation of hydroxyapatite. Furthermore, preliminary studies have shown that a 57 kDa DMP1 fragment suppresses FGF23 secretion [53].

Of the several different DMP1 mutations identified thus far, one mutation alters the translation initiation codon (M1V), two mutations are located in different intron–exon boundaries, and three are frame-shift mutations within exon 6. Thus these mutations appear to be inactivating, suggesting that the loss of DMP1 results in hypophosphatemia. Accordingly, Dmp1 null knock-out mice show severe defects in dentine, bone, and cartilage, as well as hypophosphatemia and osteomalacia [54, 55]. Furthermore, FGF23 levels in osteocytes and in serum are drastically elevated in these animals [49]. Based on these findings, another role of DMP1 appears to be in the inhibition of FGF23 expression, thereby regulating phosphate homeostasis. Given the established importance of DMP1 in osteoblast function, loss of DMP1 actions in osteoblasts and extracellular matrix may also contribute to the phenotype of patients with ARHP. Consistent with this hypothesis, a high calcium/phosphate diet capable of rescuing osteomalacia in VDR null mice does not seem to

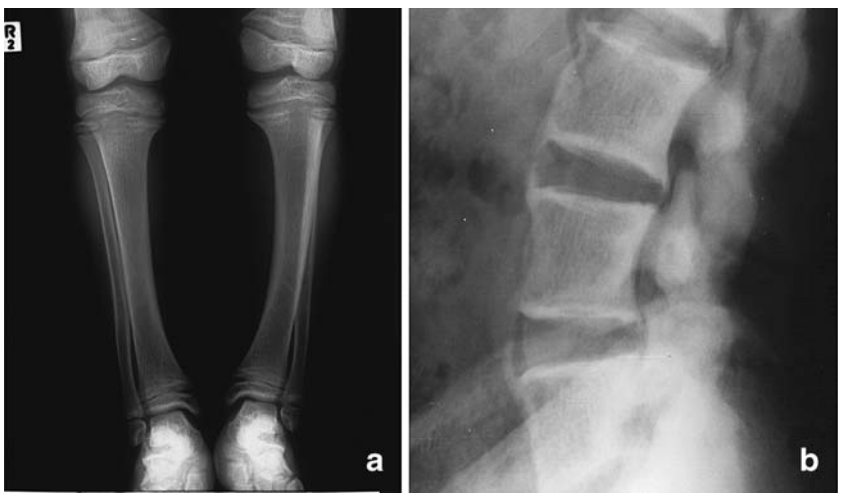

Fig. 1 Radiologic features of genetic hypophosphatemic disorders. Rickets in children is characterized by widened, expanded metaphyses and growth plate irregularities that are typically associated with bowing of long bones (**a**). Later in life, the patients often develop enthesopathy in the form of calcified ligaments and tendo-osseus junctions (**b**)

prevent the bone and dentine mineralization defect in Dmp1 null mice [56]. However, current clinical data in humans, which is limited, do not reveal significant differences between the skeletal findings of patients with ADHR, XLH, and ARHP.

2.1.4 Others

Osteoglophonic dysplasia (OGD) Osteoglophonic dysplasia (OGD) is an autosomal dominant disorder characterized by skeletal abnormalities and frequently changes in mineral ion homeostasis. Patients affected by this disorder present with craniosynostosis, prominent superorbital ridges, and mild-facial hypoplasia, rhizomelic dwarfism, and nonossifying bone lesions, and most of them seem to develop hypophosphatemia due to renal phosphate-wasting associated with inappropriately normal 1,25(OH)2 vitamin D levels [57, 58]. White et al. recently identified several different heterozygous missense mutations in FGFR1 that are all located within or close to the receptor's membrane-spanning domain. These mutations all affect amino acid residues that are highly conserved across species and seem to lead to constitutive receptor activation [59, 60]. Heterozygous activating FGFR1 mutations are found in a patient with Pfeiffer syndrome [61] and in a case with the skeletal findings of Jackson–Weiss syndrome [62]. However, the mutations leading to these disorders are located between the second and third putative Ig domains of FGFR1, just like mutations in FGFR2 and in FGFR3 that cause Crouzon syndrome, and hypo- or achondrodysplasia, respectively; note that patients affected by these other disorders do not develop hypophosphatemia. Surprisingly, some patients with OGD were reported to have elevated FGF23 levels [59, 60]. This could indicate that the skeletal lesions develop because the constitutive activation of the FGFR1 leads to an up-regulation of FGF23 secretion in the metaphyseal growth plate and consequently renal phosphate-wasting. Consistent with this view, patients with more radiographic evidence for lesions appear to develop more profound hypophosphatemia.

Linear nevus sebaceous syndrome (LNSS)/epidermal nevus syndrome (ENS) Linear nevus sebaceous syndrome (LNSS), also known as epidermal nevus syndrome (ENS) or Schimmelpenning–Feuerstein–Mims syndrome, is a rare, highly variable congenital sporadic disorder that can feature, papillomatous epidermal hyperplasia and excess sebaceous glands, brain and eye abnormalities, focal or generalized skeletal disease, and in rare cases, hypophosphatemia leading to the development of rickets [63, 64]. Two recent reports have described elevated FGF23 levels in two patients implying that this phosphaturic hormone contributes to the renal wasting of phosphate. While the skin lesions appeared to be the source of FGF23 in one patient [63], the bone lesions were thought to secrete this hormone in the other patient [64].

Fibrous dysplasia (FD) Fibrous dysplasia (FD) refers to a disorder characterized by fibrous skeletal lesions and associated localized mineralization defects. Some patients affected by FD develop systemic hypophosphatemia due to renal phosphate-wasting, which may lead to the development of generalized rickets/osteomalacia [65]. When these skeletal findings occur in conjunction with abnormal skin pigmentation and premature sexual development, the disease is referred to as McCune–Albright syndrome (MAS). FD and MAS are caused by heterozygous, postzygotic mutations in exon 8 of *GNAS*, the gene encoding the alpha-subunit of the stimulatory G protein (Gsα), which affect the arginine residue at position 201 (R^{201}) (less frequently other *GNAS* mutations have been identified) and lead to constitutive Gsα activity [66, 67]. Recent studies have shown that FD can be associated with increased FGF23 serum levels, which are inversely correlated with serum phosphorous and $1,25(OH)_2D$ levels [68, 69]. Both FGF23 mRNA and protein are expressed in dysplastic lesions, including osteoblasts and fibrous cells [68], thus raising the possibility that FGF23 plays an important role in the pathogenesis of the renal phosphate-wasting observed frequently in patients with FD/MAS. Treatment with bisphosphonates was shown to reduce serum FGF23 levels, which in turn led to a reduction in renal phosphate excretion [70]. Although the mechanisms underlying the elevated FGF23 level in these patients and its reduction in response to bisphosphonates remain to be explored, it appears plausible that the production of FGF23 by osteoblasts and/or osteocytes is regulated at least partially by cAMP-dependent mechanisms.

2.2 Hypophosphatemic rickets due to defective renal phosphate transporters

The homozygous ablation of *Npt2a* in mice ($Npt2a^{-/-}$) results, as expected, in increased urinary phosphate excretion leading to hypophosphatemia [71]. Due to the hypophosphatemia *Npt2a*-ablated mice show an appropriate elevation in the serum levels of $1,25(OH)_2D$ leading to hypercalcemia, hypercalciuria and decreased serum PTH levels, and increased serum alkaline phosphatase activity. These biochemical features are typically observed in patients with hereditary hypophosphatemic rickets with hypercalciuria (HHRH) [72, 73]. Most HHRH patients present with rickets, have short stature, and increased renal phosphate clearance (TmP/GFR is usually 2 to 4 standard deviations below the age-related normal range). Hyper-

calciuria occurs despite normal serum calcium levels, reflecting elevated serum 1,25$(OH)_2$D that leads to increased intestinal absorption of calcium and phosphorus. Serum FGF23 is low to low–normal in HHRH [74, 75]. Patients with HHRH frequently develop renal stones as a result of increased urinary excretion of both calcium and phosphate. Long-term phosphate supplementation as the sole therapy leads, with the exception of persistently decreased TmP/GFR, to reversal of the clinical and biochemical abnormalities [72, 73].

Unlike HHRH patients, *Npt2a*$^{-/-}$ mice do not have rickets or osteomalacia. Instead, they have poorly developed trabecular bone and retarded secondary ossification, and in older animals there is a dramatic reversal and eventual overcompensation of the skeletal phenotype. Consistent with these phenotypic differences, mutations in *SLC34A1*, the gene encoding the sodium-phosphate co-transporter NPT2a were excluded in the affected members of several unrelated kindreds, including the one in whom this syndrome was first described [76]. On the other hand, recent studies have led to the identification of homozygous or compound heterozygous mutations in *SLC34A3*, the gene encoding the sodium-phosphate co-transporter NPT-2c, in patients affected by HHRH [74, 77, 78]. These findings indicate that NPT-2c has a more important role in phosphate homeostasis than previously thought.

Two different heterozygous mutations (A48P and V147M) in *SLC34A1*, the gene encoding NPT2a, have been reported in patients with urolithiasis or osteoporosis and persistent idiopathic hypophosphatemia due to decreased tubular phosphate reabsorption [79]. When expressed in *Xenopus laevis* oocytes, the mutant NPT2a showed impaired function and dominant negative properties, when co-expressed with the wild-type co-transporter. However, these *in vitro* findings were not confirmed in another study using oocytes and OK cells, raising the concern that the identified NPT2a mutations alone cannot explain the findings in the described patients [80]. On the other hand, additional heterozygous NPT2a variations, either in-frame deletions or missense changes, have recently been identified upon analyzing a large cohort of hypercalciuric stone-forming kindreds; however, these genetic variations do not seem to cause functional abnormalities [81].

2.3 Laboratory investigations and treatment

Laboratory investigations in serum and urine should be performed prior to treatment. These should include the following measurements (a) in serum/plasma: calcium, phosphorous, alkaline phosphatase, creatinine, PTH, 1,25$(OH)_2$D, 25-hydroxyvitamin D, and, if available, FGF23; and (b) in fasting urine: calcium, phosphate, and creatinine for measurement of calcium:creatinine ratio and of tubular reabsorption of phosphate (TRP). TRP is the fraction of excreted phosphate that is reabsorbed by the kidney, and is calculated using the following formula: 1 − (urine phosphorus × serum creatinine/serum phosphorus × urine creatinine) (normal range: 0.85–0.98). From the TRP, the tubular threshold maximum for phosphorus per glomerular filtration rate (TMP/GFR) can be calculated using a nomogram developed by Walton and Bijvoet [82]. The normal adult range is 2.5–4.2 mg/dl, and the range is higher for children. For urine collection, patients should be asked to empty their bladder in the morning after an overnight fast, allowed to drink only water, and asked to collect the next urine sample for laboratory measurements.

Treatment of the different hypophosphatemic conditions depends on the underlying genetic defect. Disorders caused by genetic mutations associated with low or inappropriately normal 1,25$(OH)_2$D levels (presumably because of elevated FGF23 levels that suppress the renal 1-alpha hydroxylase) are generally treated with oral phosphate and oral 1,25$(OH)_2$D3 (Rocaltrol or Calcitriol), while hypophosphatemic conditions caused by mutations in the sodium-phosphate co-transporters, NPT2a or NPT2c, should be treated with oral phosphate alone.

XLH is the most common hypophosphatemic disorder and the medical management is best established for this condition. Similar treatment strategies should therefore be adopted for other hypophosphatemic conditions associated with elevated FGF23 and low/inappropriately low 1,25$(OH)_2$D levels, i.e. ADHR and ARHP. During the first year of life, the treatment should include 250–375 mg of elemental phosphorus daily (provided as either Neutra-Phos or Neutra-Phos K), provided as three to five daily doses. Since intermittent oral phosphate loads lead to the development of secondary hyperparathyroidism, 1,25$(OH)_2$D (available as capsules and an oral solution) should also be given, starting at a dose of 0.25 μg once or twice every day. Later in childhood, 1,25$(OH)_2$D doses are increased, usually to 10–25 ng/kg bodyweight/day, and during puberty even higher doses may be required. Therapy with 1,25$(OH)_2$D needs to be adjusted to avoid the development of hypercalcemia and hypercalciuria, yet to maximize the suppression of PTH synthesis and secretion. The therapeutic goal is thus to maintain serum calcium and PTH levels within the normal range, to improve alkaline phosphatase activity, and to prevent the development of increased urinary calcium excretion. The daily dosage of elemental phosphorus can be increased to 1 to 2 g/day; divided into 3–5 daily doses. Phosphate therapy needs to be adjusted to prevent development of diarrhea and abdominal discomfort, and strict adherence to medication regime is recommended. A renal ultrasound should be performed before treatment and subsequently at 1–2 year intervals. Likewise, radiographs of the knees and over the hand/wrist

should be obtained before treatment and subsequently at yearly intervals.

Treatment of HHRH usually requires the administration of phosphate salts alone. In fact, treatment with biologically active vitamin D analogs may lead to the development of nephrocalcinosis and nephrolithiasis. The treatment goal is to provide sufficient phosphorus to improve mineralization of osteoid, and to decrease the circulating levels of $1,25(OH)_2D$, thereby reducing the intestinal absorption of calcium.

3 Emerging molecular and hormonal mechanisms

Our understanding of FGF23 actions is still evolving. Current data suggest that FGF23 exerts its actions via known FGF receptors. In cultured opossum kidney cells, a cell line with a proximal tubular phenotype, FGF23 binds to the fibroblast growth factor receptor type 3c (FGFR3c) and elicits MAP kinase signaling in a manner sensitive to the inhibitors of the FGF receptor tyrosine kinase activity [83]. However, it seems that Klotho, a membrane bound protein with β-glucuronidase activity, is also required as a co-receptor for FGF23 actions. Klotho can bind FGF23, and its co-expression in cells converts FGFR1(IIIc) into a functional FGF23 receptor [84, 85]. Klotho null mice and Fgf23 null mice have significantly overlapping phenotypes, and furthermore, the Klotho null animals show markedly elevated serum levels of biologically active Fgf23 [84]. Furthermore, a patient with tumoral calcinosis has recently been identified as a carrier of an inactivating Klotho mutation [86]. Thus, FGF23 acts through the known FGFRs but only in those tissues in which Klotho is also expressed, including kidney [87, 88]. It appears that the requirement of Klotho serves to limit FGF23 actions in a spatial manner and thus provides an additional layer of regulation in phosphate homeostasis.

Recent studies have revealed regulatory feedback mechanisms that involve the old and new players of phosphate homeostasis. It has been shown that $1,25(OH)_2D$ acts as a positive regulator of FGF23 expression in bone, as demonstrated by both *in vivo* and *in vitro* assays [89]. Consistent with this finding, $1,25(OH)_2D$ action on chondrocytes is required for maintaining normal FGF23 production in osteoblasts [90]. It also seems that FGF23 acts on the parathyroid gland to inhibit both PTH biosynthesis and secretion [91, 92], a finding that correlates well with the expression of Klotho in the parathyroid [93, 94]. By inhibiting the circulating level of PTH, FGF23 thus appears to counteract its inhibition of tubular phosphate reabsorption and enhance its suppression of $1,25(OH)_2D$ biosynthesis. A murine model of hyperparathyroidism, on the other hand, shows elevated FGF23 levels in bone, along with elevated osteocalcin and alkaline phosphatase levels

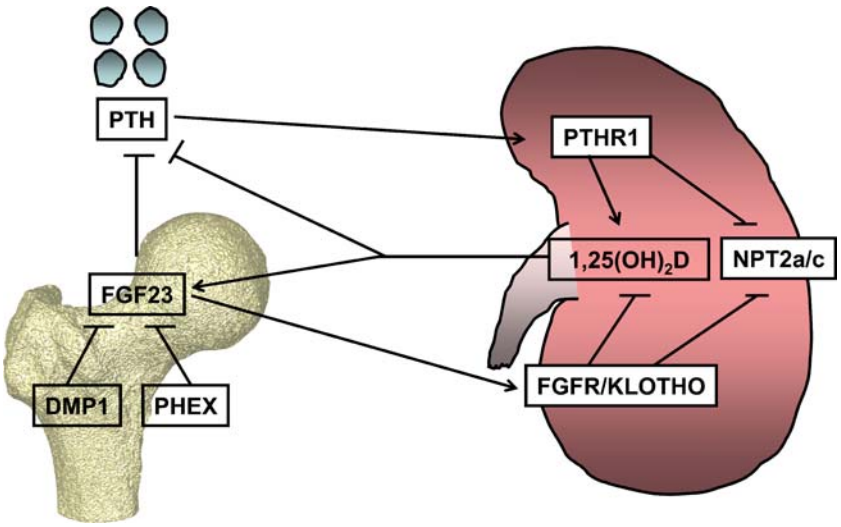

Fig. 2 Involvement of parathyroid, bone, and kidney in the regulation of phosphate homeostasis. Acting on its G protein-coupled receptor (PTH/PTHrP receptor; PTHR1), PTH inhibits phosphate re-uptake in the proximal tubule and stimulates the biosynthesis of $1,25(OH)_2D$. In addition to enhancing phosphate (and calcium) absorption in the gut, the latter suppresses the biosynthesis and secretion of PTH from the parathyroids, thus forming a negative feedback loop. $1,25(OH)_2D$ also stimulates transcription of FGF23 in bone. A key circulating phosphaturic agent, FGF23 expression in bone is normally suppressed by PHEX and DMP1, so that lack of these latter molecules result in increased serum FGF23 and renal phosphate wasting (as seen in patients with XLH and ARHP). Mutant FGF23 molecules that are unable to undergo proteolytic cleavage by subtilisin-like proprotein convertases also result in renal phosphate wasting (as seen in patients with ADHR). In the renal proximal tubule FGF23 inhibits phosphate re-uptake but, unlike PTH, it suppresses the biosynthesis of $1,25(OH)_2D$. FGF23 also inhibits PTH synthesis in the parathyroid

[95], suggesting that PTH also regulates FGF23, although this type of regulation may be indirect.

4 Summary

PTH and 1,25$(OH)_2$D have been investigated as the most important regulators of phosphate homeostasis, although the existence of additional molecules that play equally important roles in phosphate handling have become evident from various genetic diseases associated with hypo or hyperphosphatemia. Recent discoveries of mutations in specific genes in these diseases have revealed most of these additional regulators of phosphate metabolism. These include FGF23, PHEX, and DMP1, which are mutated in ADHR, XLH, and ARHP, respectively. All three proteins are abundantly expressed in bone, and FGF23 is detectable in the circulation. Patients with XLH and ARHP, who lack PHEX or DMP1, respectively, display inappropriately normal or elevated serum FGF23 levels, indicating that PHEX and DMP1 are important negative regulators of FGF23 (Fig. 2). With additional *in vivo* and *in vitro* investigations, it now appears that many significant interactions exist among the key regulators of phosphate homeostasis (Fig. 2). PTH and FGF23 both inhibit proximal tubular phosphate reabsorption. However, whereas PTH stimulates the synthesis of 1,25$(OH)_2$D, FGF23 inhibits this. In turn, 1,25$(OH)_2$D exerts opposite effects on the synthesis of PTH and FGF23, inhibiting PTH and stimulating FGF23 synthesis. The latter also seems to inhibit PTH through actions on the parathyroid. The actions of PTH and 1,25$(OH)_2$D are mediated through a G protein coupled cell surface or a nuclear receptor, respectively. FGF23, on the other hand, appears to act via known FGFRs, but with the requirement of Klotho as a co-receptor. Clearly, our knowledge of the molecular mechanisms underlying phosphate metabolism in physiology and disease have significantly improved, and these recent advances will likely lead to better treatment options for hypophosphatemic rickets.

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