FGF23 AND SYNDROMES OF ABNORMAL RENAL PHOSPHATE HANDLING

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Abstract

Fibroblast growth factor 23 (FGF23) is part of a previously unrecognized hormonal bone-parathyroid-kidney axis, which is modulated by $1,25(OH)_2$-vitamin D ($1,25(OH)_2D$), dietary and circulating phosphate and possibly PTH. FGF23 was discovered as the humoral factor in tumors that causes hypophosphatemia and osteomalacia and through the identification of a mutant form of FGF23 that leads to autosomal dominant hypophosphatemic rickets (ADHR), a rare genetic disorder. FGF23 appears to be mainly secreted by osteocytes where its expression is up-regulated by $1,25(OH)_2D$ and probably by increased serum phosphate levels. Its synthesis and secretion is reduced through yet unknown mechanisms that involve the phosphate-regulating gene with homologies to endopeptidases on the X chromosome (PHEX), dentin matrix protein 1 (DMP1) and ecto-nucleotide pyrophosphatase/phosphodiesterase 1 (ENPP1). Consequently, loss-of-function mutations in these genes underlie hypophosphatemic disorders that are either X-linked or autosomal recessive. Impaired O-glycosylation of FGF23 due to the lack of UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylgalactosaminyl-transferase 3 (GALNT3) or due to certain homozygous FGF23 mutations results in reduced secretion of intact FGF23 and leads to familial hypophosphatemic tumoral calcinosis. FGF23 acts through FGF-receptors and the coreceptor Klotho to reduce $1,25(OH)_2D$ synthesis in the kidney and probably the synthesis of parathyroid hormone (PTH) by the parathyroid glands. It furthermore synergizes with PTH to increase renal phosphate excretion by reducing expression of the sodium-phosphate cotransporters NaPi-IIa and NaPi-IIc in the proximal tubules. Loss-of-function mutations in these two transporters lead to autosomal recessive Fanconi syndrome or to hereditary hypophosphatemic rickets with hypercalciuria, respectively.

INTRODUCTION

Identification of the genetic causes of rare familial disorders of renal phosphate handling has provided, over the past decade, important novel insights into the regulation of mammalian phosphate homeostasis. This chapter will provide an update on the current knowledge of the

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pathophysiology, the clinical presentation, diagnostic evaluation and therapy of FGF23-dependent and -independent disorders of phosphate homeostasis and tissue mineralization.

AUTOSOMAL DOMINANT HYPOPHOSPHATEMIC RICKETS (ADHR, OMIM 193100)

Genetics of ADHR

In 1971 Bianchine et al described a small family, in which male-to-male transmission suggested an autosomal dominant form of hypophosphatemic rickets (ADHR, for a full list of abbreviations see Table 2).[1] Subsequently, Econs and McEnery identified a large family, in which numerous members are affected by ADHR.[2] The authors were able to map the disorder to a locus on chromosome 12p13,[3] which ultimately allowed identification of the genetic mutation leading to this rare inherited form of hypophosphatemia.[4] The incidence of ADHR is unknown; thus far, only a few families have been described in which hypophosphatemia follows an autosomal dominant trait.[4–6] ADHR thus appears to be much less frequent than X-linked hypophosphatemia (XLH), which affects approximately 1:20,000 births.[7] The ADHR patients described to date carry a heterozygous mutation in fibroblast growth factor 23 (FGF23, chromosome 12p13.3) at amino acid positions 176 or 179, a site for cleavage by subtilisin-like proptein convertases (SPC)[4–6] (Fig. 1). Although the exact mechanism is unknown, it appears that ADHR mutations enhance resistance towards SPC leading to deminished intracellular cleavage of intact FGF23. The resulting secretion of inappropriate amounts of biologically active FGF23 suggests that the identified amino acid changes are "gain-of-function" mutations, which lead to renal phosphate wasting, possibly combined with an abnormal feed-back regulation of FGF23 synthesis, as discussed below.

The phosphaturic action of FGF23 requires the coreceptor alpha Klotho (KL, also see below). A genetic defect leading to a disorder resembling ADHR has recently been reported in a single sporadic case of hypophosphatemia.[8] The patient, a 13 month old girl, presented with rickets due to excessive renal phosphate excretion and hyperparathyroidism and cytogenetic studies revealed a de novo chromosomal translocation with the breakpoint being located adjacent to the gene encoding KL. As a result, plasma levels of soluble KL and KL-associated beta-glucuronidase activity were increased, along with increased levels of immunoreactive FGF23. It remains uncertain, however, whether the elevated levels of FGF23 and/or PTH are solely responsible for the increased urinary excretion of phosphate, or whether the elevated levels of soluble Klotho contribute to the abnormal renal handling of this mineral.

FGF23 Synthesis and Secretion

The main sources of FGF23 are osteocytes and osteoblasts in the skeleton, but low levels of uncertain biological significance can be detected in the ventrolateral thalamic nucleus, the thymus, the small intestine and the heart.[9] FGF23 synthesis is stimulated by dietary phosphate[10–12] and the application of 1,25(OH)2D.[13,14] Conversely, the phosphate-regulating gene with homologies to endopeptidases on the X chromosome (PHEX), dentin matrix protein 1 (DMP1) and ecto-nucleotide pyrophosphatase/phosphodiesterase 1(ENPP)

appear to have an important role in suppressing FGF23 synthesis. PHEX and DMP1 were shown to be genetically up-stream of FGF23, as the lack of either of these two proteins leads to an up-regulation of FGF23 expression in bone, most likely through indirect mechanisms.[15] After cleavage of the signal sequence comprising 24 amino acids and O-glycosylation by UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylgalactosaminyl-transferase 3 (GALNT3), mature FGF23(25–251) is secreted into the circulation. O-glycosylation of FGF23 occurs in the 162–228 region[16] and this posttranslational modification, which may involve residue 178, appears to protect FGF23 from cleavage by subtilisin-like proprotein convertases as shown with recombinant peptides in vitro.[17] O-glycosylation is also essential for secretion and processing of intact FGF23 in CHO cells.[17,18] Furthermore, in HEK293 cells, expression of GALNT3 is stimulated by extracellular phosphate and suppressed by extracellular calcium and 1,25(OH)2D.[19] This suggests that GALNT3 may be an important component of an as-of-yet incompletely understood circuit regulating FGF23 secretion in vivo, which may be disrupted by ADHR mutations. The two FGF23 amino acid residues that are mutated in patients affected by ADHR, R176 or R179, constitute, as mentioned above, the site for cleavage by subtilisin/furin-like endopeptidases. Just like O-linked glycosylation of residue T178 in wild-type FGF23,[17] mutation of either residue 176 or 179 appears to protect FGF23 against intracellular proteolytic cleavage and degradation,[20] leading to the secretion of intact FGF23, although it remains unclear, how production of the ADHR mutant escapes feed-back regulation by phosphate and $1,25(OH)_2D$.

FGF23 Mode of Action

The phosphaturic actions of FGF23 require KL as a coreceptor. Consequently, a homozygous inactivating KL mutation was shown to lead to FGF23 resistance in a patient with familial hyperphosphatemic tumoral calcinosis type 3 (HFTC3, see below)[21] and mice that are null for Kl, the murine ortholog of KL, show a corresponding phenotype.[22,23] Immunoprecipitation experiments, surface plasmon resonance (SPR) spectroscopy and functional assays measuring the mitogenic response of BaF3 cells or activation of the MAPK-pathway in HEK293 cells have shown that KL, a protein with a single membrane-spanning domain, forms a ternary complex with FGF23 in conjunction with FGFR1c and other FGF receptors.[24,25] Recent work using neutralizing anti-FGF23 antibodies indicates that the N-terminal portion of FGF23 interacts with FGFR1c, while the C-terminus binds to KL and both interactions appear to be important for bioactivity in vitro and in vivo.[26] Low affinity binding of FGF23 to FGFR1c and other FGF receptors in the absence of KL has been shown to occur in vitro.[27–29] However, this coreceptor appears to be essential for high-affinity binding of FGF23 to the receptor and its phosphate-regulating effects in vivo. The ablation of either FGFR3 or FGFR4, individually or in combination, was unable to rescue the hyp phenotype in mice suggesting that these two FGF receptors are not involved in mediating the renal effects of FGF23.[30]

The site of FGF23 action in the kidney is still controversial. While FGF23 decreases NPT2a and NPT2c expression[31–33] and CYP27B1 activity in the proximal tubules,[34–36] the coreceptor KL is expressed mainly in the distal tubules. In addition, mice injected with recombinant FGF23 show phosphorylation of MAPK and an up-regulation of the early

growth-response gene 1 (Egr-1) in the distal tubules.[37] These findings suggest either that FGF23 uses a noncanonical signal-transduction pathway in the proximal tubules or that it induces the secretion of an "intermediary phosphatonin" from the distal tubules, which acts in a paracrine fashion on the proximal tubules. KL may furthermore have ligand-independent actions by regulating expression of the calcium channels TRPV5[38] and the potassium channel ROMK in the distal tubules.[39]

Although the transgenic overexpression of soluble KL can prolong the life span of mice,[24,40] no data on FGF23 levels or mineral ion homeostasis have been reported to date in these animals. Increased FGF23 levels in the patient described by Brownstein et al (see above) suggests that KL may interfere with the normal feed-back inhibition of FGF23 synthesis and/or secretion; however, the exact mechanism of hypophosphatemia in this patient remains unclear.

Clinical Findings in ADHR

Chronic hypophosphatemia in ADHR can lead to abnormal bone growth and mineralization, i.e., rickets in growing children and osteomalacia in adults. As in nutritional rickets, osteoid is undermineralized, leading to a blurring of the microtrabecular architecture and pseudo-fractures (Looser zones) on radiographs. The clinical consequences of rickets or osteomalacia in children or adults, respectively, are bone pain and impaired mechanical properties of the affected bones leading to deformities of the lower extremities, often leading to characteristic wind-swept deformities. Lack of chondrocyte apoptosis in the growing skeleton furthermore leads to an expansion of the epiphyses in children, giving rise to swollen wrists and rachitic rosary.[41] Serum biochemical findings indicative of rickets and osteomalacia include elevated bone-specific alkaline phosphatase, osteocalcin, procollagen, pyridinoline cross-links as well as N- and C-telopeptides.[42,43] Chronic hypophosphatemia also leads to muscle weakness, which when compared to the effects on the skeleton, is less well understood and may be related to the role of phosphate in intracellular signal transduction and synthesis of ATP or creatine phosphate.[42,43]

While rickets or osteomalacia can be observed to variable degrees in all hypophosphatemic disorders, including ADHR, subtle but important differences can guide the differential diagnostic and therapeutic decisions. When compared to other types of hypophosphatemic rickets, ADHR appears to have a quite variable clinical phenotype. The kindred described by Econs et al[2] can be divided into two subgroups. Group 1 consisted of nine female patients who presented with renal phosphate-wasting during adolescence and adulthood. These individuals presented with bone pain, weakness and insufficiency fractures, but without deformities of the lower extremities. Group 2 consisted of nine male and female patients, with onset of symptoms, including bone deformities, during childhood. Two of these nine individuals lost the renal phosphate-wasting defect later in life, which is consistent with observations in the affected members of two other unrelated kindreds, in which five of eight carriers of a heterozygous, "activating" FGF23 mutation had lost the renal phosphate-wasting defect later in life.[5,6] Enthesopathies, which refer to painful or indolent mineral deposits near the insertion sites of tendons usually at the spine and lower extremities, can occur in patients with XLH, ADHR, or autosomal recessive hypophosphatemia (ARHP) (see

below) and are readily identified on radiographs.[44] The development of these lesions may involve FGFR3 and Klotho, which are expressed in fibrocartilage cells of the entheses.[45] Dental cysts or dental dysplasia, respectively, were described in patients with XLH and ADHR, but these abnormalities do not appear to be as common in ADHR as in XLH.[2,5] Likewise, midfacial hypoplasia and frontal bossing may also be observed in ADHR.[5] Although these findings may be related to the severity of hypophosphatemia, therapy with $1,25(OH)_2D$ and phosphate supplements often cannot reverse or prevent these changes. Thus, local effects of FGF23 excess and activation of canonical FGF receptor signaling (Klotho-independent) or other factors up-stream of FGF23 may play a role.[44,46]

Diagnostic Evaluation of ADHR and Other Disorders of Phosphate Homeostasis

The clinical presentation of ADHR and other hypophosphatemic disorders includes renal phosphate wasting, leading to rickets and/or osteomalacia and abnormal vitamin D metabolism. The diagnosis therefore depends on careful evaluation of phosphate homeostasis, which can be challenging since serum phosphate concentrations are influenced by the time of day, relationship to meals and age of the subject and none of the methods for determination of tubular phosphate reabsorption are entirely satisfying. To determine the cause of abnormal serum phosphate levels in a patient, who has normal parathyroid and renal function, we generally first assess his or her tubular reabsorption for phosphate (%TRP). For this purpose the patient is asked to collect a 3-hour timed urine for measurement of phosphate and creatinine along with the corresponding serum parameters after 8 hours of fasting. %TRP is calculated according to Formula 1 and the tubular maximum of reabsorption for phosphate (TmP/GFR) is derived from a nomogram, which was devised by Walton and Bijvoet[47] to correct for the nonlinear relationships of %TRP and TmP/GFR when %TRP is higher 80%.

$$\%TRP = 100 \times (1 - (U\text{-}P \times S\text{-}creat)/(S\text{-}P \times U\text{-}creat)) \text{ using timed 3-hour urine and blood creatinine and phosphate concentrations}$$

Formula 1

TmP/GFR reflects the threshold of the serum phosphate concentration above which phosphate is no longer fully reclaimed from the glomerular filtrate in the proximal tubules. While the TmP/GFR derived from the Walton and Bijvoet nomogram is generally sufficient in adults, the nomogram does not accommodate the higher normal range of serum phosphate values in newborns and toddlers.[48] Thus, calculating TP/GFR provides a more accurate assessment of renal phosphate handling in the pediatric population[49] (Formula 2).

$$TP/GFR = S\text{-}P - (U\text{-}P \times S\text{-}creat/U\text{-}creat), \text{ using simultaneous urine and blood creatinine and phosphate concentrations}$$

Formula 2

Inappropriately low %TRP in the setting of hypophosphatemia is suggestive of a proximal renal tubular defect as the underlying cause, which can be further classified by determining the patient's vitamin D status: In patients with ADHR, %TRP and $1,25(OH)_2D$ levels are concordantly (inappropriately) reduced, suggesting excess FGF23 activity. In contrast, appropriately elevated $1,25(OH)_2D$ levels suggest an FGF23-independent, renal tubular defect leading to abnormal serum phosphate levels, which can for example be seen in hereditary hypophosphatemic rickets with hypercalciuria (HHRH).[50] If hypophosphatemia occurs without an obvious increase in urinary phosphate excretion, nutritional deficiencies, malabsorption, or liver disease should be considered; in rare cases, a primary intestinal defect involving reduced NPT2b expression has been described in pulmonary alveolar microlithiasis (PAM)[51] (see Table 1).

Excess production of $1,25(OH)_2D$ due to FGF23-independent hypophosphatemia may lead to increased absorption of calcium in the gut, resulting in hypercalciuria and some suppression of PTH production. Measuring the levels of $1,25(OH)_2D$ and PTH, as well as serum and urinary calcium, may therefore help to distinguish FGF23-dependent from FGF23-independent forms of hypophosphatemia. It is important to keep in mind that vitamin D deficiency and secondary hyperparathyroidism may mask the findings as described for HHRH and serum and urinary studies need to be repeated after repletion of vitamin D stores.[52]

Measurement of FGF23 Levels

Circulating FGF23 levels can be determined from EDTA plasma or serum using several commercially available enzyme-linked immunometric assays.[53,54] The intact FGF23 assay (referred to as iFGF23 assay) uses antibodies directed against N-terminal and C-terminal portions of the peptide for capture and detection, respectively (Kainos, Tokyo, Japan). In contrast, the C-terminal FGF23 assay (referred to as cFGF23 assay) (Immutopics, Inc., San Clemente, CA) uses two antibodies directed against distinct epitopes within the C-terminal region of FGF23 and thus detects intact FGF23 as well as C-terminal fragments. Both assays can help establish the diagnosis of FGF23-dependent disorders of phosphate homeostasis, when FGF23 levels are elevated above the normal range or are inappropriately normal.[55,56] They can also help establishing the diagnosis of FGF23-dependent hypophosphatemic disorders such as HHRH or Fanconi syndrome, albeit with significant differences in sensitivity, which is currently best for the iFGF23 assay.[54,57] The measurement of FGF23 with both assays can help establish the diagnosis of tumoral calcinosis (HFTC), since homozygous inactivating mutations in GALNT3 (HFTC1) or FGF23 (HFTC2) result in low levels of intact FGF23, yet often significant elevations of C-terminal FGF23 fragments while both intact and C-terminal FGF23 are elevated in HFTC3 (see below for details).[21,58–60]

Treatment of ADHR

The clinical course of ADHR is usually comparable to mild forms of XLH, which will be described in more detail below. As a result phosphate and 1,25(OH)$_2$D supplementation are often required only during skeletal growth in childhood and adolescence. These therapeutic interventions provide symptomatic relieve and improves the bone abnormalities, but are usually unable to normalize serum phosphate levels. Treatment can be complicated by the development of secondary hyperparathyroidism, hypercalciuria and nephrocalcinosis.[44,46] Thus, treatment needs to be monitored carefully for these complications and the use of calcium-sensing receptor agonists such as cinacalcet, which has been successfully used to normalize parathyroid hormone secretion and to reduce the magnitude of phosphaturia in XLH patients,[61,62] may have a role in ADHR as well. We prefer potassium-containing phosphate supplements over sodium-containing phosphate supplements since the former seem to induce less sodium-related phosphaturia, although formal studies to support this practice are still missing. Thiazide diuretics may be helpful in slowing the progression of nephrocalcinosis.[63] Anti-FGF23 antibodies hold promise to become a therapeutic option for humans with ADHR since treatment has been successful in hyp mice, which like patients with ADHR have high circulating FGF23 levels.[26,64]

OTHER DISORDERS OF RENAL PHOSPHATE EXCRETION

Acquired Hypophosphatemic Disorders

Tumor Induced Osteomalacia (TIO)—Tumor-induced osteomalacia (TIO), also referred to as oncogenic osteomalacia (OOM),[65] is an acquired disorder of FGF23 excess,[66] or possibly FGF7 excess.[67] Tumors secreting the phosphaturic factor are usually benign mixed connective tissue tumors. Other factors such as matrix extracellular protein (MEPE)[68] or secreted frizzled related protein 4 (sFRP4)[69] were also isolated from TIO tumors and may contribute to the abnormal regulation of renal phosphate handling.[70] Tumor-induced osteomalacia is a relatively rare condition, with only slightly more than one hundred cases described in the literature to date.[65] Drezner reviewed 120 cases of tumor-induced osteomalacia and identified four distinct morphologic patterns:[1] primitive-appearing, mixed connective tissue tumors;[2] osteoblastoma-like tumors;[3] non-ossifying fibroma-like tumors; and[4] ossifying fibroma-like tumors. Hypophosphatemia was also described in patients with widespread fibrous dysplasia of bone, neurofibromatosis and linear nevus sebaceous syndrome (see further below) and concurrent with breast carcinoma, prostate carcinoma, oat cell carcinoma, small cell carcinoma, multiple myeloma and chronic lymphocytic leukemia. Proof of a causal relationship has been that removal of the tumor resulted in appropriate biochemical and radiographic improvements; however, since most cases were reported before the discovery of FGF23 (and of MEPE and sFRP-4), the phosphaturic factor secreted by these previously reported tumors has not been determined. The tumors are frequently located in the visceral scull or in the tendons of hands and feet and may only be a few millimeters large and indolent. They commonly escape detection by physical exam and computed tomography scans and may require more sensitive techniques for localization including whole-body octreotide-scans,[71] PET-CT scans using [^{18}F]-FDG,[72] or [^{68}Ga]-DOTANOC[73] as tracers. Selective vein sampling[74] can permit localization, even in individuals with only mildly elevated circulating FGF23 levels.[75–77] Therapy consists of

surgical tumor excision, once its location has been revealed, which usually results in normalization of serum phosphate levels within 24 hrs. In those patients, where localization of the tumor is impossible or if tumor resection is incomplete, symptomatic therapy, as described in more detail above, should be continued.

Other Acquired Syndromes of Renal Phosphate Wasting

Another increasingly recognized acquired syndrome of renal phosphate wasting is postrenal transplant hypophosphatemia, which often cannot be attributed to tertiary hyperparathyroidism alone.[78,79] Bhan et al[80] and Pande et al[81] recently showed that posttransplant hypophosphatemia correlates inversely with serum FGF23 levels and coined the term "tertiary hyperphosphatoninism" due to persistent production of FGF23, which persists longer than would be expected from the half-life of the hormone.[80,82] Hypophosphatemia in the setting of inappropriate renal phosphate excretion has also been recognized with severe burn-injuries[83,84] and after partial hepatectomy,[85] although these forms of hypophosphatemia appear to be independent of FGF23.[86,87]

INHERITED HYPOPHOSPHATEMIC DISORDERS OTHER THAN ADHR(SEE FIG. 2)

X-Linked Autosomal Hypophosphatemia (XLH, OMIM 307800)

XLH, the most common form of hypophosphatemia, was first recognized by Albright and coworkers in 1937.[88] Lack of male-to-male transmission was observed by Winters in 1958,[89] which suggested an X-linked mode of inheritance. Using a positional cloning approach, the genetic defect was ultimately identified in 1995[90] and a large number of different loss-of-function mutations in PHEX, phosphate-regulating gene with homologies to endopeptidases on the X chromosome, have since been reported.[91] Deletion of the Phex gene in hyp-mice leads to increased FGF23 gene transcription in osteocytes resulting in elevated circulating levels of FGF23 and thus renal phosphate wasting,[92,93] which is similar to findings in human XLH patients.[53,54] It was therefore concluded that PHEX may be involved in the feed-back regulation of FGF23 secretion,[94] which may explain why males and females are equally affected, although the exact mechanism remains unclear.

Although some patients have normal growth, most XLH patients show stunted growth despite treatment with phosphate and active vitamin D analogs.[95] Additional clinical features include craniosynostosis, frontal bossing and mid-facial hypoplasia as described above.[44,46] Owen et al recently examined female monozygotic twins with a documented PHEX mutation; one twin had overt XLH with hypophosphatemia leading to abnormal growth and rickets, while the other displayed normal renal phosphate handling, normal growth and no evidence for rickets. The authors suggested that nonrandom expression of the normal PHEX allele in critical tissues may be responsible for the discordant XLH phenotype.[96]

As outlined above for ADHR, treatment of XLH consists of oral phosphate supplementation and active vitamin D analogs, which provides symptomatic relieve and improves the bone abnormalities, but is usually unable to normalize serum phosphate levels. Treatment may be

complicated by the development of secondary hyperparathyroidism, hypercalciuria and nephrocalcinosis[44,46] and needs to be monitored carefully for these complications. The calcium-sensing receptor agonist cinacalcet has been successfully used to normalize parathyroid hormone secretion and to reduce the magnitude of phosphaturia in XLH patients, who had developed severe secondary or tertiary hyperparathyroidism.[61,62] However, parathyroidectomy may ultimately be required, which results generally in improved phosphatemic control, although the postoperative course may be complicated by severe hungry bone syndrome.[97] Thiazide diuretics may be helpful in slowing the progression of nephrocalcinosis.[63] Replacement of the membrane-anchored PHEX with a soluble form of PHEX did not prove effective to reverse hypophosphatemia in hyp mice, while treatment with anti-FGF23 antibodies has been successful in these animals and holds promise to become a therapeutic option for humans with XLH.[26,64] Growth hormone therapy was reported to improve linear growth in some patients, although it remains unclear, whether the observed improvements were in part attributable to an increase in tubular reabsorption of phosphate during growth hormone treatment.[98]

Autosomal Recessive Hypophosphatemia (ARHP, OMIM 241520)

ARHP is caused by homozygous, presumably loss-of-function mutations in DMP1[99,100] or in ecto-nucleotide pyrophosphatase/phosphodiesterase 1 (ENPP1).[101,102] Intact DMP1 is cleaved into a 35 and a 57 kDa fragment, possibly by bone morphogenic protein 1 (BMP1),[103] which, in turn, is activated by a complex consisting of the subtilisin propeptide convertase SPC2 and the co-activator 7B2.[104] Transgenic over-expression of the C-terminal 57 kDa DMP1 fragment is both necessary and sufficient to rescue the bone phenotype (and probably the hypophosphatemia resulting from increased FGF23 secretion) of DMP1-null mice.[105] This DMP1 fragment appears to have nuclear effects, which are required to prevent excess FGF23 secretion, renal phosphate wasting and hypophosphatemia.[105] Thus, the C-terminal fragment may be required for suppression and/or feed back regulation of FGF23 gene transcription and/or FGF23 secretion.[99] ENPP1 is a membrane-bound ecto-enzyme responsible for the generation of the mineralization inhibitor pyrophosphate (PPi).[106] Loss-of function mutations in this enzyme are the cause of generalized arterial calcifications of infancy (GACI),[107,108] which was associated in some patients with mild hypophosphatemia.[109] Recently, two groups identified homozygous loss-of-function mutations in ENPP1 in kindreds with hypophosphatemic rickets due to FGF23-dependent renal phosphate-wasting.[101,102] Further evaluation of the phenotypic expression of ENPP1 mutations indicated that members of the same kindred, carrying the same homozygous mutation can present either with arterial calcifications or with rickets. Furthermore, subtle changes, i.e., mild hypophosphatemia was observed in individuals with GACI and thickening of the cardiac valves in individuals with rickets were observed, suggesting the presence of modifiers (genetic or environmental) that underlie the variable expressivity of the disorders. The cause of FGF23 excess may be directly related to lack of PPi production, or due to accumulation of precursors, such as ATP in the extracellular space. However, presence of mild hyperphosphatemia in individuals suffering from hypophosphatasia, which is caused by loss-of-function mutations in the PPi-degrading enzyme, namely tissue nonspecific alkaline phosphatase (TNALP),[110] suggests that PPi may directly or indirectly suppress FGF23 production.

Treatment of ARHP is currently symptomatic and relies, like the treatment of XLH and ADHR, on oral phosphate supplementation and repletion of $1,25(OH)_2D$ to prevent the development of hyperparathyroidism. In the future, it may be possible to treat these groups of patients with monoclonal, inactivating antibodies against FGF23.

Hereditary Hypophosphatemic Rickets with Hypercalciuria (HHRH, OMIM 241530)

HHRH is a rare disorder with autosomal recessive inheritance that was first described in 1985 in a large consanguineous Bedouin kindred.[50] Unlike patients with XLH, individuals affected by HHRH do not develop dental abscesses or craniofacial abnormalities (i.e., craniosynostosis, frontal bossing, scaphocephaly, Chiari I malformation)[111,112] and different from patients affected by XLH, ADHR and ARHR, HHRH patients show suppressed or low-normal FGF23 levels.[113] This reduction in FGF23, combined with the hypophosphatemia, contributes to the compensatory increase in the plasma level of $1,25(OH)_2D$ typically observed in HHRH. This appropriate rise in the concentration of the biologically active form of vitamin D results in absorptive hypercalciuria, the cardinal feature that distinguishes HHRH from most other Mendelian hypophosphatemic disorders. Measurement of $1,25(OH)_2D$ and urinary calcium excretion are thus essential for establishing the diagnosis of HHRH, although both may be normal, if vitamin D deficiency and/or secondary hyperparathyroidism are present.[52]

HHRH is caused by homozygous or compound heterozygous loss-of-function mutations in NaPi-IIc/SLC34A3.[113–115] Expressivity can be quite variable and may be affected like in other hypophosphatemic forms of rickets by vitamin D status. Some heterozygous individuals may have an increased urinary calcium excretion and occasionally some of the above biochemical features of HHRH, while bone changes are generally missing. Likewise, individuals with two mutated SLC34A3 alleles can initially present with renal stones alone even in the absence of clinical symptoms associated with rickets or osteomalacia.[52,116]

In contrast to patients with XLH, ADHR or ARHP, who are usually treated with multiple daily doses of oral phosphate and high doses of calcitriol (i.e.,$1,25(OH)_2D$), the effective therapy of individuals affected by HHRH consists of oral phosphate supplementation alone. The prescription of biologically active vitamin D analogs is contraindicated and may lead to hypercalcemia, hypercalciuria, nephrocalcinosis and possibly renal insufficiency.[52,116]

Hypophosphatemia with Osteoporosis and Nephrolithiasis Due to NaPi-IIa (OMIM 612286) or NHERF-1 Mutations (OMIM 604990)

Prie et al investigated a heterogeneous group of patients with idiopathic hypercalciuria, osteoporosis and renal stones. Using a candidate gene approach they found in 2 of 20 individuals heterozygous for nonsynonymous SNPs in NaPi-IIa/SLC34A1.[117] Consistent with their findings, Lapointe et al identified additional heterozygous mutations in other patients affected by calcium nephrolithiasis and a renal phosphate leak.[118] These latter NaPi-IIa mutations did not lead to functional abnormalities of sodium-dependent phosphate cotransport, when tested in vitro. In contrast, Prie et al presented experimental in vitro evidence for dominant negative effects of the NaPi-IIa/SLC34A1 alterations on proximal renal tubular phosphate reabsorption; these findings, however, were challenged by others.[119]

The same group more recently identified heterozygous, nonsynonymous amino acid changes in NHERF-1; three different changes were identified in 7 of 94 individuals with idiopathic hypercalciuria, osteoporosis and renal stones.[120] However, two of those amino acid changes are listed in the NCBI dbSNP database as low frequency polymorphisms,[121] raising the possibility that these are either not contributing to the clinical and laboratory findings in the investigated cohort or that these changes may lead more frequently to hypophosphatemia, albeit without readily appreciated clinical abnormalities. Further studies are thus required to prove that mutations in NaPi-IIa or NHERF-1 are indeed responsible for idiopathic hypercalciuria, osteoporosis and renal stones.

Autosomal Recessive Fanconi Syndrome (ARFS)

In 1988, Tieder et al described a consanguineous Arab kindred with childhood rickets and defective proximal tubular handing of phosphate, amino acids and glucose consistent with renal Fanconi syndrome.[122] Distinct from other forms of Fanconi, their patients also had elevated $1,25(OH)_2D$ levels and absorptive hypercalciuria. Recently, homozygosity mapping of this kindred showed linkage of the disease to chromosome 5q35.1–3 and subsequent sequence analysis of the SLC34A1/NaPi-IIa gene in the linked interval revealed a homozygous duplication g.2061_2081dup (p.I154_V160dup).[123] Expression of the mutant NaPi-IIa protein in *Xenopus* oocytes and *Opossum* kidney cells showed complete loss-of-function and lack of membrane insertion, respectively. The two patients described in the 1988 report, who are now 39 and 43 years old, continue to have low TmP/GFR and their FGF23 and PTH levels were recently shown to be low-normal (despite impaired renal function), suggesting that their hypophosphatemia is FGF23- and PTH-independent. However, their previously documented absorptive hypercalciuria due to increased $1,25(OH)_2D$ levels, had normalized in the setting of vitamin D deficiency. Although symptoms of rickets were present in childhood, both patients have been relatively asymptomatic during adulthood and discontinued phosphate supplementation. Both homozygous carriers developed CKD stage 2–3 in their 30s, which is in contrast to the other familial hypophosphatemia disorders described above. Heterozygous carriers of p.I154_V160dup had normal renal function and no evidence for renal phosphate leak or proximal tubulopathy, arguing strongly against dominant negative effects of the mutant NaPi-IIa. However, it is still possible that intracellular accumulation of the mutant transporters in homozygous carriers may be involved in the pathogenesis of their proximal tubular defect, since no comparable abnormalities were observed in mice that are null for the murine ortholog of NaPi-IIa.[124, 125]

Other Inherited Forms of Renal Phosphate Wasting

Osteoglophonic dysplasia (OGD, OMIM166250) is an autosomal dominant disorder caused by activating missense mutations in the gene encoding fibroblast growth factor receptor-1 (FGFR1).[126,127] Affected individuals may develop hypophosphatemia and renal phosphate wasting due to an increased production of FGF23.[128,129] Other clinical features include those seen in other syndromes that are caused by fibroblast growth factor receptor-1 mutations, such as craniosynostosis, midfacial hypoplasia, prognatism and rizomelic chondrodysplasia. In addition, individuals affected by OGD have symmetrical radiolucent

metaphyseal defects, which may be the source of their excess FGF23 production, although direct histological evidence to support this hypothesis is to date lacking.

Opsismodysplasia (OSD, OMIM 258480)[130] is an autosomal recessive skeletal dysplasia that is characterized by a delay in epiphyseal ossification, platyspondyly, metaphyseal cupping, resulting in brachydactyly with short metacarpals and phalanges. The genetic defect is unknown. Like OGD, opsismodysplasia can go along with FGF23 excess leading to renal phosphate wasting.[131]

Linear nevus sebaceous syndrome (LNSS)/epidermal nevus syndrome (ENS) (OMIM163200) is characterized by sebaceous nevi, often in the face, abnormalities of the central nervous system, ocular anomalies, including coloboma and skeletal defects.[132–135] Most patients with LNSS or ENS carry mosaic FGFR3 mutations.[136] Some patients develop hypophosphatemic rickets[137,138] and recently some nevi were shown to secrete FGF23 thus providing an explanation for the underlying renal phosphate wasting.[139–141] However, it is unknown whether the FGFR3 mutations alone or additional unknown somatic mutations lead to the increase in FGF23 production and thus renal phosphate-wasting.

Fibrous dysplasia (FD)/McCune Albright Syndrome (MAS) (OMIM 174800) is caused by somatic activating missense mutations in the alpha subunit of the stimulatory G-protein (encoded by GNAS).[142–144] The classical triad of MAS includes polyostotic FD, café-au-lait spots and precocious puberty. However, a number of other endocrine disorders such as thyrotoxicosis, pituitary gigantism and Cushing syndrome are often present as well.[144] The nonmineralizing bone lesions of FD/MAS may secrete FGF23, which can lead to hypophosphatemic rickets or osteomalacia.[145–148] FGF23-mediated hypophosphatemia can also be observed in Jansen's metaphyseal chondrodysplasia (OMIM 156400), which is caused by heterozygous activating PTH/PTHrP receptor mutations and may be, like in FD/MAS, a consequence of agonist-independent activation of the cAMP/PKA signaling pathway.[149] Finally, hypophosphatemia leading to osteomalacia has been described in some individuals with neurofibromatosis 1 and 2,[150,151] although the mechanism remains to be clarified.

HYPERPHOSPHATEMIC DISORDERS

Hyperphosphatemic Familial Tumoral calcinosis, HFTC (OMIM 211900)

Tumoral calcinosis is a clinically and genetically heterogeneous group of disorders first described by Giard (1898)[152] and then by Duret (1899).[153] Tumoral calcinosis is characterized by calcium-phosphate deposits in different tissues, but osteogenic cells and matrix formation are absent, which distinguishes this disorders from heterotopic ossification. For the purpose of this review, the hyperphosphatemic forms of familial tumoral calcinosis shall be classified as type 1–3 (HFTC1-3), which all follow an autosomal recessive mode of inheritance and all furthermore show inappropriately enhanced renal tubular absorption of phosphate leading to hyperphosphatemia as a common laboratory feature. The activity of renal 1-alpha-hydroxylase is increased resulting in elevated serum $1,25(OH)_2D$ levels and thus increased intestinal absorption of calcium (and phosphate), suppression of parathyroid

hormone production and hypercalciuria. The increased serum calcium-phosphate product results in the characteristic abnormal tissue mineralization observed in tumoral calcinosis.

Ectopic tissue mineralization in HFTC is often seen in juxtaarticular muscular and subcutaneous tissues. Patients with tumoral calcinosis often also show dental pulp stones, which may lead to a complete obliteration of the dental pulp cavities. Other clinical features, which may constitute the only clinical evidence for tumoral calcinosis, can include eye-lid calcifications, vascular calcifications and/or nephrocalcinosis. There can also be mineralization of the juxtaarticular bone marrow cavities, however, the remaining skeleton often shows low bone mineral density due to a mineralization defect, which at the moment is only poorly understood.[154,155]

Familial tumoral calcinosis type 1 (HFTC1) is caused by homozygous loss-of-function mutations in the gene encoding UDP-N-acetyl-alpha-D-galactosamine: polypeptide N-acetylgalactosaminyl-transferase 3 (GALNT3).[58,156] GALNT3 is responsible for O-glycosylation and proper secretion of intact FGF23.[17] Patients with HFTC1 characteristically have low or undetectable intact, but increased C-terminal FGF23 levels. HFTC1 is allelic with the hyperostosis-hyperphosphatemia syndrome (HSS; OMIM 610233). Patients affected by HSS show, besides the characteristic biochemical abnormalities in serum and urine, recurrent albeit transient painful swellings of the long bones and associated radiographic findings that are consistent with periosteal reaction and cortical hyperostosis.[157,158] Similar or identical GALNT3 mutations thus can lead to HSS (bone abnormalities without skin involvement) or to HFTC1, but the genetic modifiers responsible for these differences in disease presentation are currently unknown.

HFTC2 is caused by mutations affecting both alleles of FGF23, which reduce the circulating levels of bioactive intact FGF23, just like in HFTC1 (Fig. 1).[156,159] C-terminal FGF23 fragments are secreted, however and high levels of this fragment are often detected in the circulation.[60] The mechanism by which HPTC2 mutations may lead to loss-of-function of FGF23 is unclear. Consistent with the patients' low circulating intact FGF23 levels, we recently showed that HEK293 or COS-7 cells expressing FGF23 with proline at position 129 ([P129]FGF23) (or with other mutations identified in HFTC2 patients), secrete the intact hormone only poorly into the medium. Interestingly, total lysates of HEK293 or COS-7 cells expressing [P129]FGF23 (and other FGF23 mutants) revealed a 25 kDa form of FGF23 that was also detected in cells expressing wild-type FGF23. However, in contrast to cells expressing wild-type FGF23, a 32 kDa protein species was missing in cells expressing FGF23 mutants.

The 32 kDa protein species of FGF23 is detected with antibodies raised against N-terminal and C-terminal portions of FGF23. Thus full-length FGF23 is larger than predicted from the primary amino acid sequence of mature FGF23(25–251). This suggested that wild-type FGF23 is modified and that this modification is absent in patients carrying one of the FGF23 mutations leading to tumoral calcinosis. Enzymatic deglycosylation of wild-type FGF23 suggested that the modification consists of O-linked glycans (Fig. 3A) and that these modifications are absent in [P129]FGF23 (Fig. 3B) as well as in [G71]FGF23 and [F129]FGF23, other mutant forms of FGF23 that cause tumoral calcinosis. These findings

suggest that a similar mechanism, namely lack of O-glycosylation, underlies the poor secretion observed of these FGF23 mutants. Both, HFTC1 (GALNT3 mutations) and HFTC2 (FGF23 mutations), thus appear to be disorders of abnormal O-glycosylation of FGF23.

The mechanism by which the FGF23 mutations identified in HFTC2 lead to impaired O-glycosylation of FGF23 remains unclear. However, previous reports have indicated that O-glycosylation occurs within the C-terminal portion of FGF23, i.e., the 168–228 region,[16] while all HFTC2 mutations identified to date reside in the N-terminal portion of FGF23; some of these mutations furthermore do not affect potential O-glycosylation sites (H41Q,[160] Q54K).[161] Thus, HFTC2 mutations likely cause misfolding, which may delay or impair O-glycosylation, as previously suggested.[60,159]

The lack of O-glycosylation can be the consequence or the cause of poor secretion of the mutant FGF23 by HEK293 cells; the former scenario would likely result in the secretion of large quantities of an unmodified 25 kDa FGF23 into the medium of cells transfected with plasmids encoding the mutant forms of FGF23. However, even after concentrating 50-fold the conditioned medium from HEK293 expressing the mutant FGF23, the 25 kDa protein species could not be detected. Instead, small amounts of the 32 kDa protein species were observed in concentrated medium, indicating that O-glycosylation can occur, albeit inefficiently for FGF23 mutants and that these mutants can be secreted once glycosylated (Fig. 3C).

Recently, a 13-year old girl with a disorder resembling HFTC1 and HFTC2, was reported, who had extremely high circulating levels of intact and C-terminal FGF23.[21] Radiographs of the patient showed osteopenia, patchy sclerosis in the hands, feet, long bones and calvaria, intracranial calcifications and calcifications of the dura and carotid arteries. Interestingly and distinct from the first two forms of HFTC, she had elevated PTH levels due to four-gland parathyroid hyperplasia. Very high circulating levels of intact FGF23 had also been observed in mice that are null for klotho (KL), the coreceptor for FGF23 (see above). The authors therefore decided to analyze the gene encoding KL, which led to the discovery of a homozygous missense mutation in the second putative beta-glycosidase domain that is presumably inactivating, leading to end-organ resistance to FGF23.[21]

Like humans with HFTC, mice that are null for FGF23,[162,163] GALNT3,[164] KL[22] suffer from hyperphosphatemia and increased $1,25(OH)_2D$ levels due to the loss of biologically active FGF23 or due to end-organ resistance to FGF23, respectively. Consistent with an increased intestinal absorption of calcium from the gut, mice with these genetic modifications also display mild hypercalcemia, suppressed PTH levels and hypercalciuria. The resulting increase in the calcium-phosphorus product in each of these disorders is thought to cause the characteristic tissue calcifications. The homozygous ablation of Npt2a,[154] the VDR,[163] or CYP27B1[165] rescues the serum-biochemical abnormalities of FGF23-null mice and the homozygous ablation of CYP27B1 was also shown to rescue the klotho-null mice.[166] Likewise, diets low in phosphate or low in vitamin D[167] can normalize the changes in mineral ion homeostasis of FGF23- and klotho-null animals, although mineralization defects in the skeleton may persist.[154,162,168]

Heterozygous carriers of the genetic mutations that cause HFTC1-3 may have mildly abnormal blood chemistries,[156] but do not develop significant calcified lesions and they do not require specific treatment. Treatment of homozygous individuals currently relies on minimizing the intestinal absorption of phosphate through appropriate binders such as aluminum hydroxide or sevelamer[60] and on inhibiting renal phosphate reabsorption with acetazolamide;[169] in one study treatment with PTH was temporarily able to induce phosphaturia.[170] In the future, patients affected by HFTC may be treated with calcilytic agents that reduce the activity of the calcium-sensing receptor and thus stimulate endogenous PTH secretion[171] or with recombinant FGF23, if this becomes available for use in humans.

CONCLUSION

FGF23 was identified initially as the hypophosphatemic factor that is abundantly expressed in TIO tumors and that is mutated in individuals affected by ADHR. This phosphaturic hormone was subsequently found to be readily detectable in the circulation of healthy individuals and research over the past years strongly suggests that it is an important regulator of normal phosphate homeostasis. Dysregulation of FGF23 occurs in more than 13 acquired and inherited disorders of phosphate homeostasis and it is emerging as a predictor of disease progression in chronic kidney disease and of mortality of incident and long-term dialysis patients. Further investigations are required to understand the regulation of FGF23 expression through changes in dietary intake and/or serum phosphate levels and through PTH and $1,25(OH)_2D$. It will also be important to understand which FGF receptors mediate the actions of FGF23 in parathyroids and kidney and how PHEX, DMP1, ENPP1 and most likely several other proteins contribute to the regulation of FGF23 synthesis and secretion.

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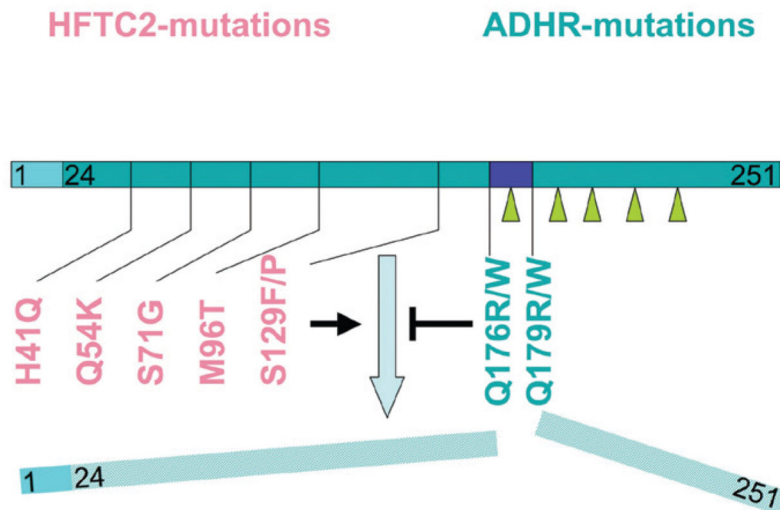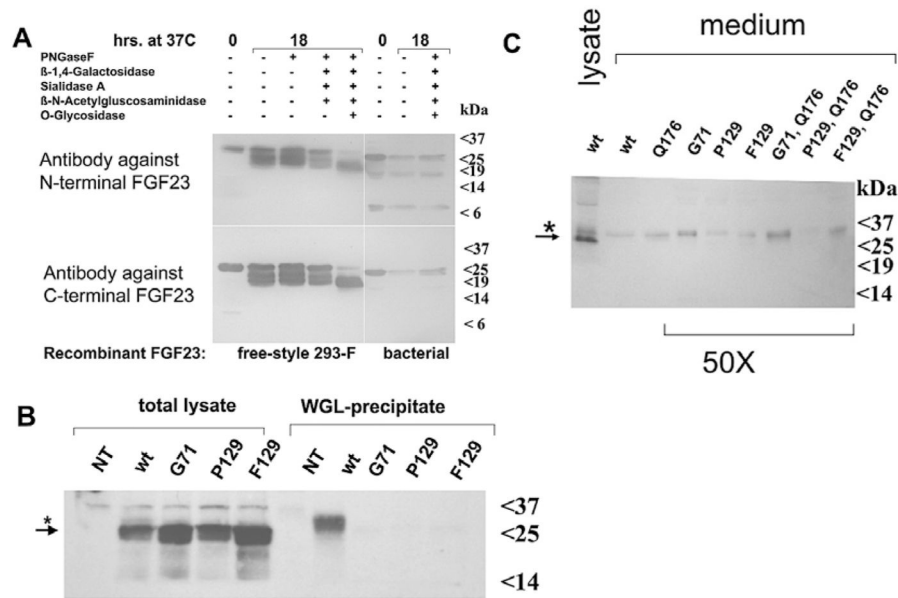
Figure 1.
Known mutations in FGF23. Human mutations leading to hyperphosphatemic tumoral calcinosis type 2 (HFTC2, red) are located in the first half of the FGF23 molecule. These impair, directly or indirectly, O-glycosylation (solid arrows) in the second half of FGF23 and thereby prevent secretion of intact FGF23. Human FGF23 mutations leading to autosomal dominant hypophosphatemic rickets (ADHR, green) change the subtilisin-furin cleavage motif RXXR in amino acid positions 176–179 (blue box) and lead to increased secretion and stability of FGF23 that is independent of O-glycosylation.
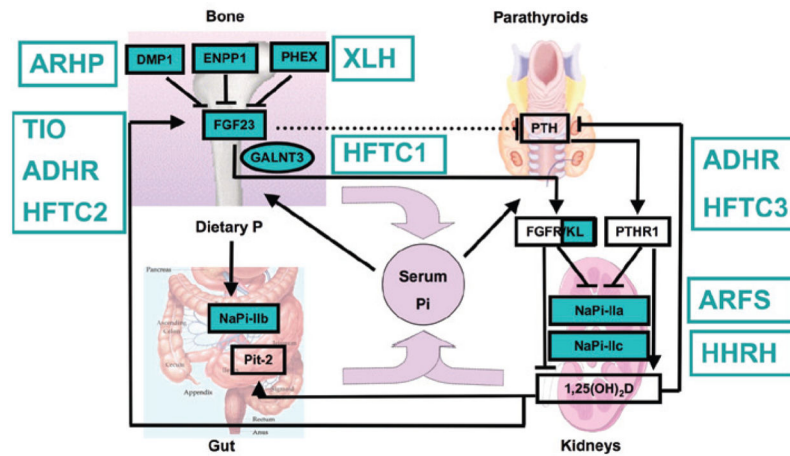
Figure 2.
Disorders of phosphate homeostasis. FGF23 expression in bone is up-regulated by an increase in dietary phosphate intake and by 1,25(OH)$_2$D and down-regulated, through yet unknown mechanisms, by PHEX, DMP1, ENPP1 and probably several additional proteins. FGF23 acts through one or more FGF receptors, with Klotho as coreceptor, to inhibit renal phosphate re-absorption and to decrease circulating 1,25(OH)$_2$D levels and possibly to inhibit PTH secretion by the parathyroid glands (dashed line). The net effect of these PTH-dependent actions is a decrease in serum phosphate levels, yet an increase in serum 1,25(OH)$_2$D levels. 1-alpha hydroxylase activity is also up-regulated by low serum phosphate levels and down-regulated by increased serum calcium and phosphate levels and by increased FGF23 levels. 1,25(OH)$_2$D acts through VDR/RXR heterodimers to enhance the intestinal absorption of phosphate and to stimulate FGF23 synthesis and secretion by osteocytes; it furthermore inhibits PTH synthesis and secretion by the parathyroid glands. The net 1,25(OH)$_2$D effect is an increase in serum phosphate levels. The disorders affecting phosphate homeostasis are indicated in green. Please see the text for a detailed description.

Figure 3.
Tumoral calcinosis mutations impair O-glycosylation and secretion of FGF23. A) Enzymatic deglycosylation of recombinant FGF23: N-linked and O-linked carbohydrates were removed under denaturing conditions using approximately 1 μg of recombinant FGF23 and the E-DEGLY kit (Sigma). The molecular shift observed with O-glycosidase indicates that recombinant FGF23 is O-glycosylated. B) Total lysates from HEK293 cells expressing wild-type FGF23(1–251) (wt) or FGF23 with mutations at residues 71 or 129 (G71, P129, or F129) show equal expression of the 25 kDa protein species; the 32 kDa form of FGF23 is detected only in the glycoprotein fraction (star); NT, nontransfected. C) The 32 kDa, presumably glycosylated form of FGF23, but not the more abundant 25 kDa protein species is detected after concentrating 50-fold conditioned media from HEK293 cells expressing wild-type and mutant FGF23; this suggests that FGF23 carrying one of the mutations that cause tumoral calcinosis can undergo limited O-glycosylation thus allowing secretion (star); see text for details. Reproduced with permission from Bergwitz C et al. J Clin Endocrinol Metab 2009; 94:4267–4274,[172] ©2009 The Endocrine Society.

Table 1

Serum biochemical findings in disorders of phosphate homeostasis

Parameter	Hypophosphatemia		Hyperphosphatemia	
	FGF23-dependent	FGF23-independent	FGF23-deficient	FGF23-resistant
Acquired	TIO, postrenal transplant	Post-hepatectomy	NA	NA
Inherited	XLH, ADHR, ARHP, OGD, OSD, FD/MAS, NF1+2	HHRH, Fanconi with hypercalciuria	HFTC1, 2	HFTC3
S-Ca	NL	NL	NL to HIGH	NL to HIGH
S-PTH	NL to HIGH	NL to LOW	NL to LOW	NL to HIGH
S-1,25(OH)$_2$D	NL to LOW	HIGH	HIGH	HIGH
U-Ca	NL to LOW	HIGH	NL to HIGH	NL to HIGH
S-P	LOW	LOW	HIGH	HIGH
S-FGF23	NL to HIGH	LOW to NL	LOW	HIGH
U-P	HIGH	HIGH	LOW	LOW
Current Rx:	Phosphate and 1,25(OH)$_2$D replacement	Phosphate replacement only	Phosphate binders, acetazolamide	Phosphate binders, acetazolamide

Table 2

Abbreviations

Key-Term/Acronym	Definition
GALNT3	UDP-N-Acetyl-α-D-Galactosamine:Polypeptide N-Acetylgalactosaminyltransferease, isoform 3 appears to be important for O-glycosylation of FGF23
FGF23	Fibroblast growth Factor 23
KL	Alpha Klotho
PHEX	Phosphate-regulating gene with Homologies to Endopeptidases on the X chromosome
FGF23	Fibroblast Growth Factor 23
DMP1	Dentin Matrix Acidic Phosphoprotein 1
MEPE	Matrix extracellular phosphoprotein
FGFR	Fibroblast Growth Factor Receptor
ARHP	Autosomal Recessive Hypophosphatemia
HHRH	Hereditary Hypophosphatemic Rickets with Hypercalciuria
PTHR1	PTH/PTHrP Receptor
VDR	Vitamin D receptor, forms heterodimer with RXR
HFTC	Hyperphosphatemic Familial Tumoral Calcinosis
XLH	X-linked Dominant hypophosphatemia
ADHR	Autosomal Dominant Hypophosphatemic Rickets
SLC34	Solute Carrier Family 34 (sodium-phosphate cotransporter) (also referred to as NaPi-II or NPT2), members 1 (NaPi-IIa) and 3 (NaPi-IIc) are expressed in the proximal renal tubule, member 2 (NaPi-IIb) is expressed in the intestine
CYP27B1	1-alpha-hydroxylase, vitamin D-activating enzyme, which is expressed in the renal proximal tubule
TRPV	Transient receptor potential cation channel, subfamily V, members 5 and 6 are calcium-selective
PMCA	plasma membrane Ca^{2+} ATPase
Pit-2	Solute Carrier Family 20 (sodium-phosphate cotransporter), member 2