X-linked hypophosphatemia and growth

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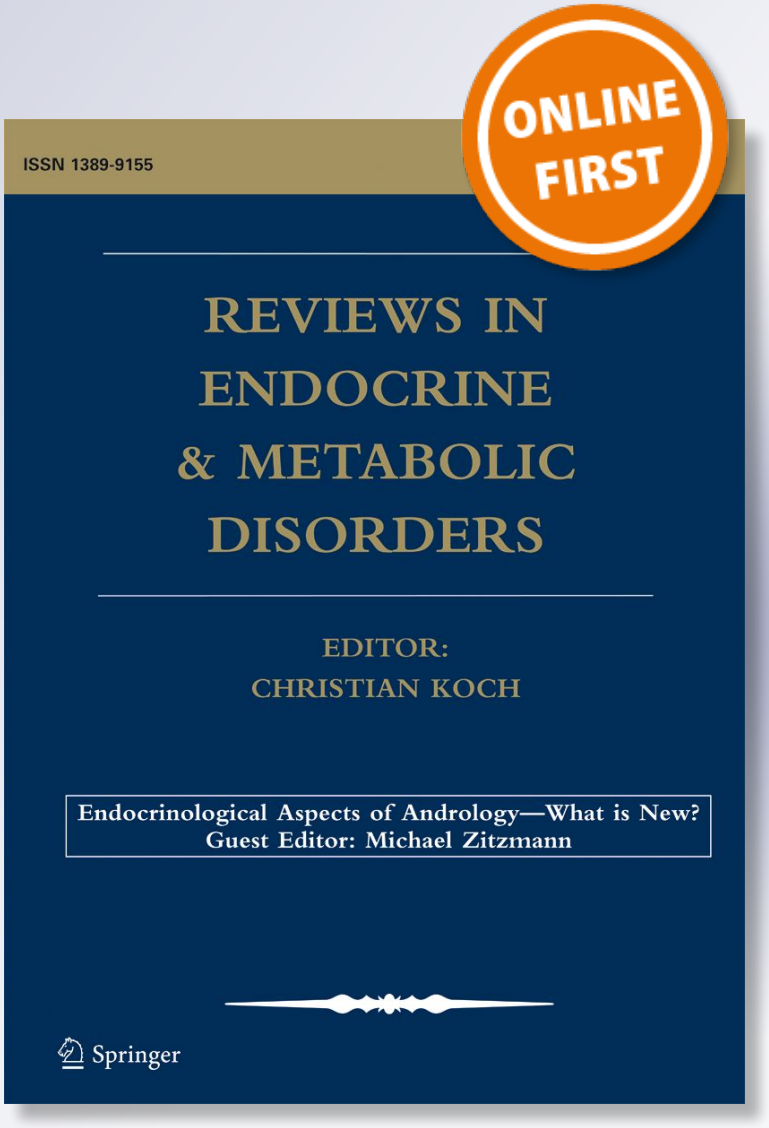

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X-linked hypophosphatemia and growth

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Abstract X-Linked hypophosphatemia (XLH) is the most common form of hereditary rickets caused by loss-of function mutations in the *PHEX* gene. XLH is characterized by hypophosphatemia secondary to renal phosphate wasting, inappropriately low concentrations of 1,25 dihydroxyvitamin D and high circulating levels of fibroblast growth factor 23 (FGF23). Short stature and rachitic osseous lesions are characteristic phenotypic findings of XLH although the severity of these manifestations is highly variable among patients. The degree of growth impairment is not dependent on the magnitude of hypophosphatemia or the extent of legs´ bowing and height is not normalized by chronic administration of phosphate supplements and 1α hydroxyvitamin D derivatives. Treatment with growth hormone accelerates longitudinal growth rate but there is still controversy regarding the potential risk of increasing bone deformities and body disproportion. Treatments aimed at blocking FGF23 action are promising, but information is lacking on the consequences of counteracting FGF23 during the growing period. This review summarizes current knowledge on phosphorus metabolism in XLH, presents updated information on XLH and growth, including the effects of FGF23 on epiphyseal growth plate of the Hyp mouse, an animal model of the disease, and discusses growth hormone and novel FGF23 related therapies.

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1 Phosphate homeostasis

Phosphate is an essential mineral, indispensable for a normal bone and tooth development, it is critical for a wide array of cellular processes and it is an integral component of the nucleic acids. Normal values of serum phosphate in adults vary from 2.4 to 4 mg/dL whereas children have higher levels, i.e. between 4.8–7.4 mg/dL in the first 3 months of life and between 4.5–5.8 mg/dL at 1–2 years [1]. In mammals including humans, circulating concentration of inorganic phosphate (Pi) is determined by renal and intestinal (predominantly in the jejunum) uptakes and by its distribution between bone and soft tissues [2]. To maintain a neutral balance, the amount of absorbed phosphate has to be equal to the amount excreted in the urine. Approximately, 75–85% of the phosphorus filtrated by the glomeruli is reabsorbed in the proximal tubule. This reabsorption of filtrated Pi is carried out by sodium-coupled phosphate (NaPi) co-transporters localized at the apical membrane of tubular cells [3]. In the brush border membrane three cotransporters have been identified so far: NaPi-IIa (gen *SLC34A1*), NaPi-IIc (gen *SLC34A3*) and, more recently, Pit-2 (gen *SLC20A2*) [3–5]. In the intestine, NaPi-IIb cotransporter mediates the transcellular phosphate transport.

The serum phosphate concentration is regulated by parathyroid hormone (PTH) and 1,25 dihydroxyvitamin D [1,25$(OH)_2$D]. PTH decreases the proximal tubular reabsorption of phosphate by reducing NaPi-IIa and NaPi-IIc activities. This reduction is achieved by internalization of NaPi proteins from the lumen side of the proximal tubular epithelial cells [6]. PTH also mobilizes phosphate from the skeletal into the bloodstream, possibly by enhancing osteoclastic bone resorption. In

addition, PTH increases the production of $1,25(OH)_2D$ by inducing the renal expression of 1-α-hydroxylase (CYP27B1) and stimulates intestinal phosphate absorption [7]. CYP27B1 enzyme, located in the proximal tubule of the kidney and a variety of other tissues, including bone osteoblasts [8], catalyzes the hydroxylation of 25 hydroxyvitamin D to $1,25(OH)_2D$, the bioactive form of vitamin D.

In addition to PTH and vitamin D, other hormones can affect renal phosphate handling. Growth hormone, insulin, and thyroid hormone all increase phosphate tubular reabsorption, whereas calcitonin, glucocorticoids, and atrial natriuretic factor decrease it.

The major recent breakthrough in understanding the active regulation of phosphate homeostasis was accomplished by the identification of fibroblast growth factor 23 (FGF23). FGF23 is an hormone mainly synthesized by osteocytes, but it has also been identified in the endothelial cells of the venous sinusoids of the bone marrow and in the thymus of Hyp mouse [9]. Its synthesis and secretion are positively regulated by vitamin D and serum phosphorus [10, 11]**.** When phosphorus levels are too high, FGF23 inhibits the renal tubular reabsorption. FGF23 suppresses CYP27B1 transcription in the kidney activity and stimulates catabolism of $1,25(OH)_2D$ through 24-hydroxylase enzyme (CYP24) (Fig. 1). Although elevated FGF23 levels are involved in the pathogenesis of some forms of hereditary and acquired hypophosphatemic rickets [12], its physiological role in the regulation of phosphate metabolism is still to be defined.

The protein klotho (KL) has been found to be critical for FGF23 signaling [13]. Klotho is an essential cofactor for FGF23. Membrane-bound-klotho interacts with FGF receptors (FGFR) in proximal tubular cells to form a high affinity receptor for FGF23. FGFR4 or the IIIc isoforms of FGFR1 and FGFR3 [14–16] form an heteromeric complex, FGF23-KL-FGFR, which activates the mitogen activated protein kinase (MAPK) cascade and induces the phosphorylation of ERK1/2 (p-ERK1/2) [17, 18]. In the end, activation of this pathway results in the reduction of NaPi-IIa and NaPi-IIc protein expression in the kidney (Fig. 2) and in the inhibition of $1,25(OH)_2D$ synthesis [19]. Reduction of the $1,25(OH)_2D$ levels may lead to a decrease in intestinal type NaPi-IIb transporter and also to a reduction of intestinal Pi absorption. In fact, KL-knockout mice exhibit overexpression of NaPi-IIa and NaPi-IIc proteins with concomitant hyperphosphatemia [20].

Interestingly, KL exists also as a circulating form that likely has a distinct mode of action. Recent studies have shown that secreted KL can act independently from FGF23 and directly inactivate the expression of NaPi-IIa inducing phosphaturia [21, 22]. It has been suggested that secreted KL can influence Na^+-K^+-ATPase activity, which results in an increased Na^+ ion gradient and enhances trans-epithelial calcium transport in the choroid plexus and the kidneys [23]. Indeed, secreted KL has been shown to increase the tubular cell surface expression of TRPV5 [24, 25], a channel involved in calcium reabsorption, probably through FGFR1 and ERK 1/2 WNK4 pathway**.**

2 X-linked hypophosphatemia

X-Linked hypophosphatemia (XLH) (OMIM 307800) is the most prevalent form of hereditary rickets and the most common inherited defect of renal tubular phosphate transport in humans [26, 27]. Its frequency has been estimated to be 1 in 20,000. XLH is characterized by hypophosphatemia secondary to renal phosphate wasting, inappropriately low concentrations of $1,25(OH)_2D$ and high levels of FGF23. Although there is a large phenotypic variability, XLH patients exhibit some degree of disproportional dwarfism with predominant shortening of lower limbs, low mineral density and rickets or osteomalacia [28, 29].

2.1 Genetic basis

XLH is caused by loss of function of the *PHEX* gene and its transmission follows a X-dominant heritance [30–32]. A wide array of mutations has been described [33], and among them a significant number of sporadic cases resulting from *de novo* *PHEX* mutations [34, 35]. The *PHEX* gene has 22 exons and encodes a 749-amino acid protein that putatively consists of an intracellular, transmembrane, and extracellular domain. The Hyp-mouse, discovered in 1976 by Eva M. Eicher, is the best completely characterized animal model of XLH [29, 30]. Hyp gene is homologous with the X-linked human gene, which encodes a protein that belongs to the neutral endopeptidase family of zinc metalloproteinase [34]. Studies in the Hyp mouse have shown that *Phex* is expressed chiefly in osteoblast and osteoclast of bone and teeth but also in lung, brain, ovary, testicle and muscle [31, 36].

2.2 Biochemical and molecular findings

Characteristic biochemical findings of XLH are hypophosphatemia secondary to decreased tubular reabsorption of phosphate, insufficiently high concentrations of serum $1,25(OH)_2D$, elevated circulating levels of FGF23 and normal serum calcium and PTH [37]. Serum alkaline phosphatase, a marker of bone formation, is elevated in children who develop rickets, and usually returns to normal in adulthood.

Studies carried out in parathyroidectomized Hyp mice indicated that the exacerbated phosphaturia is not caused by PTH [38] but more likely results from the decreased expression of the Pi transporters [39]. The identification of FGF23 has disclosed a major role of this hormone in the pathogenesis of the disease. Elevated circulating levels of FGF23 have been noted in Hyp mice [40] as well as in XLH patients [41]. Probably FGF23 increases renal phosphate excretion by

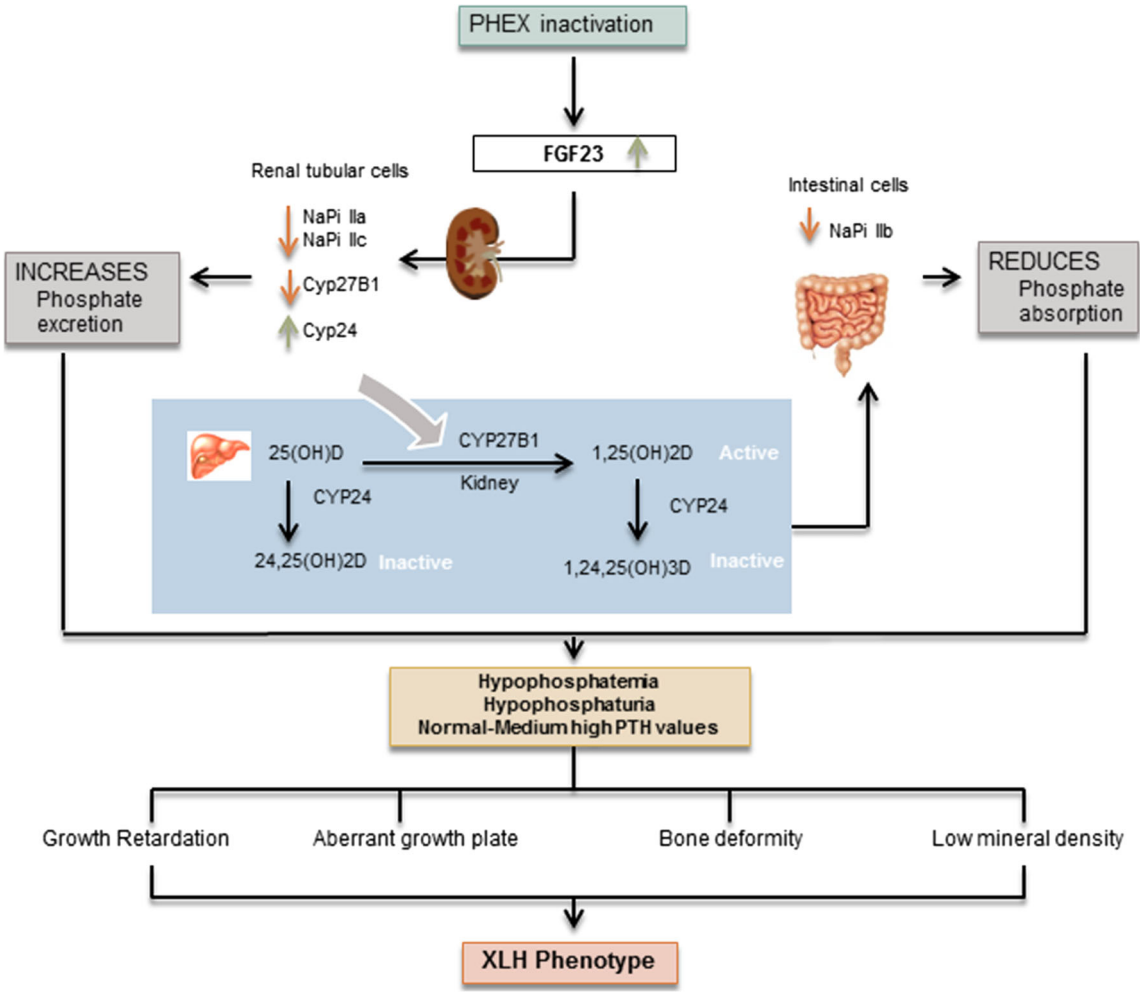

Fig. 1 Phosphorus metabolism in XLH**.** Inactivation of *PHEX* gene results in increased circulating levels of FGF23. FGF23 directly suppresses renal sodium-phosphate cotransporters (NaPi-IIa and NaPi-IIc) and increases the urinary excretion of phosphate. Likewise, FGF23 suppresses the expression of 1-α hydroxylase (CYP27B1) and stimulates the production of 24-hydroxylase (CYP24), so reducing $1,25(OH)_2D$ levels and hence the phosphate intestinal absorption mediated by sodium-phosphate cotransporter NaPi-IIb. Prolonged phosphate restriction it should increase not only $1,25(OH)_2D$ level but also PTH, nevertheless XLH is associated with normal or slightly elevated serum parathyroid hormone. Therefore *PHEX* inactivation induces hyperphospaturia and Hypophosphatemia and in the end XLH patients develop bone complications and growth retardation

reducing expression and activity of NaPi cotransporters as well as by inhibiting the CYP27B1 enzyme [42] (Fig. 1).

As *PHEX* is the only gene having pathogenic variants known to cause XLH, Liu et al. (2007) examined the mechanism whereby FGF23 is regulated by *Phex* in transplanting Hyp bones in wild type mice and vice-versa [40]. They found that wild type bone explanted into Hyp mice developed osteomalacia and failed to normalize systemic FGF23 levels, suggesting that the defective bone mineralization was caused by circulating factors. On the other hand, FGF23 was increased in Hyp osteocytes before and after explantation into wild type mice, but it was not modified in wild type mouse osteocytes after explantation into Hyp mice, suggesting that FGF23 overexpression resulted from an intrinsic bone *Phex* defect. Unexpectedly, they also observed a paradoxical suppression of FGF23 in juvenile Hyp bone explanted into adult Hyp mice, indicating the presence of an age-dependent systemic inhibitor of FGF23 that can be the real *PHEX* substrate. This finding could explain why the disease appears to lessen in severity with age [43, 44]. In addition, FGF23 levels are highly variable in XLH patients [45], supporting the presence of others factors that regulate FGF23. It remains unclear how loss of function of *PHEX* results in elevated FGF23 levels.

2.3 XLH and growth

Short stature is a phenotypic hallmark of XLH. Adults with XLH have a significantly reduced final height up to 20 cm, mean standard deviation score (SDS) of −1.9 [44], but there is a great variability among individuals. XLH patients are not abnormally small at birth [46]; XLH girls and boys seem to have normal growth spurt and adult men show a more severe

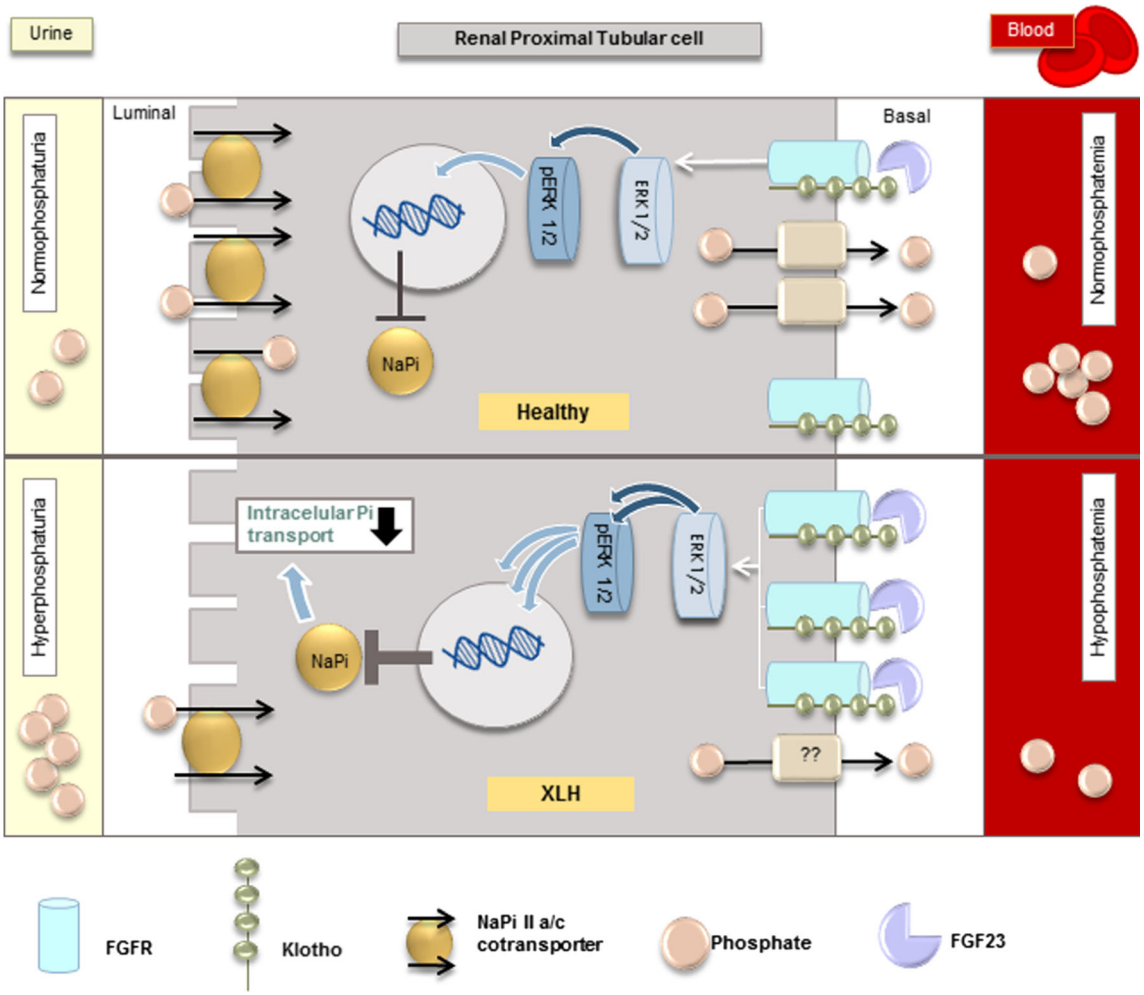

Fig. 2 Model of FGF23-αKlotho signaling in renal distal tubular cells. This figure shows the possible mode of action of FGF23/klotho in producing hypophosphatemia and hyperphospaturia in XLH renal proximal tubular cell. FGF23 binds to the basolateral FGFR-Klotho complex in proximal tubular cells and activates ERK1/2 by phosphorylation. pERK1/2 acts on the nucleus and induced a downregulation of NaPi-IIa/c cotransporters expression. In a XLH cell, the pathogenic high levels of FGF23 lead to ERK1/2 over-activation and to a dramatic reduction of NaPi II a/c expression. Low presence of NaPi cotransporters in the apical border membrane reduces the intracellular transport of phosphate in proximal tubular cells, increment the phosphate excretion through the urine and leads to the hyperphospaturia and hypophosphatemia

XLH phenotype than women [46–49]. Otherwise, serum phosphate concentrations and stature SDS have been shown to keep a poor positive correlation in children with XLH. Adults who began treatment with phosphate and $1,25(OH)_2D$ earlier, manage to grow taller despite having a similar degree of hypophosphatemia [50, 51]. In this regard, Jehan et al. (2008) described changes in growth that are associated with different vitamin D receptor promoter haplotypes, providing a possible explanation for some of the clinical variability observed in XLH [52]. On the other hand, defects in growth hormone (GH) secretion have been reported in only a few patients [47], and associated metabolic, inflammatory or nutritional disorders have not been described in XLH individuals. Thus, the adverse effect of XLH on growth seems to be not dependent on GH disordered metabolism or nutritional deficiencies.

A relevant point is that dwarfism of XLH patients is disproportional, longitudinal shortening and the degree of the deformity being more severe in lower extremities than in the rest of the body [44, 50, 53] Bowing and/or twisting tend to appear once a child begins to bear weight on his or her legs. In fact, the diagnosis is frequently made in the first two years of life, when lower-extremity bowing becomes evident. Corrective surgical osteotomy is often required when correction of the torsion is not reversed by medical therapy. Nevertheless, it is not clear that growth retardation can be only explained by the bone bowing itself.

Bone mineral density is also decreased in children and adolescents [54], often persists low into adulthood, and not always improves with standards therapies [43, 54, 55]. This bone weakness may affect the child's daily life and increases the possibility of developing arthritis over the years. Qiu et al.

(2004) showed in Hyp mice that there were not gender differences in bone mineral accrued between males and females Hyp mice, but there were differences in vertebra length, indicating that long bones' rickets and osteomalacia follow different mechanism and are not equally affected by gen dose [56].

Epiphyseal growth plate is responsible for the longitudinal endochondral ossification and determines the final length of bones. Defects in the development of this organ or fractures can lead to growth complications. Physiologically, the rate and extent of growth are determined by the combination of chondrocyte proliferation, matrix production, and enlargement of hypertrophic chondrocytes [57]. The finding that *PHEX* is strongly expressed not only by osteoblasts, but also by chondrocytes, and its expression is influenced by transcription factor of chondrogenesis [58], suggests that the loss of *PHEX* function might directly contribute to the pathogenesis of growth retardation and cartilage abnormalities. The anatomic characteristics of the growth plate make its study extremely difficult in the clinical setting and justify the use of animal models for a better understanding of the mechanisms interfering with normal growth in XLH patients. Hyp mice have profound abnormalities in the growth plate although a detailed analysis of the structure and dynamics of growth plate cartilage in this animal model of XLH remains to be performed. It is known that epiphyseal growth plates are proximally–distally thicker than normal growth plates [59]. These findings suggest that the primary skeletal defect in Hyp mice is not caused by abnormal osteoblast differentiation but rather by an impairment in mineralization by osteoblasts [60]. Recent findings indicate that FGF21, a member of the FGF family that like FGF23 functions as an endocrine factor, directly inhibits chondrocyte proliferation and differentiation at the growth plate; so that FGF23 might be playing a causal role in the abnormal process of growth plate [61].

Studies in humans and mice raised the question as to whether normalization of serum calcium, phosphate, or both was required to normalize the growth plate phenotype and therefore the final length. The calcium sensing receptor KO mouse CasR (a murine model of familial hypercalcemia) has high serum calcium, low circulating phosphate levels and develops a rachitic growth plate in the presence of hyperparathyroidism. However, when this mouse is mated with a Gcm2null-mouse (mice lacking parathyroid glands) [62], resulting mice develop hypocalcemia combined with normophosphatemia and mild hypoparathyroidism. Therefore, it can be concluded that the correction of PTH and Pi levels are crucial for a normal cartilage development [63]. Sabbagh et al. (2005) found decreased apoptosis rate, assessed by TUNEL technique and cleaved caspase 3 activity in hypertrophic chondrocytes of Hyp mouse versus wild type mouse growth plate [64], demonstrating that normal phosphorus levels are also required for hypertrophic chondrocyte cell death. Likewise Shiguang Liu et al. (2007) explanted Hyp bones in wild type mice showing that Hyp bones near normalized the growth plate width [40] when they are in a normal phosphorus environment.

Thus, all these studies point to phosphate as well as FGF23 as important regulators of growth plate maturation and bone formation [65].

2.4 XLH treatment

In humans, growth deceleration and rickets in XLH begin to occur during the first 2 years of age when the growth velocity is physiologically maximum. Therefore, the early initiation of treatment is essential, before overt manifestation of rickets and growth retardation develop [46]. Pharmacologic treatment in XLH patients consists of oral phosphate supplementation and 1-alpha hydroxyvitamin D derivatives' administration. Although this therapy usually leads to an improvement of biochemical parameters and amelioration of rickets [66], the effects on longitudinal growth are often disappointing and some patients remain unresponsive [55]. On the other hand, the risk of hypercalcemia, hypercalciuria, hyperparathyroidism and nephrocalcinosis as well as the gastrointestinal tolerance to phosphate limit the progressive increase in the doses of 1-α hydroxyvitamin D derivatives and phosphate [67, 68]. Recent data from a multi-center Austrian - German study on 76 children with XLH showed progressive deterioration of height, expressed as SDS, despite continued treatment with phosphate and calcitriol [50]. Another study in 23 adult patients showed a decreased joint mobility despite the treatment with vitamin D and phosphorus [43]. It is also of note that, despite inappropriate baseline elevations in FGF23 concentrations in XLH patients, standard treatment with calcitriol and phosphate further increases levels of this hormone [69, 70].

2.4.1 Growth hormone treatment

Pediatric patients with XLH have been treated with recombinant human GH (rhGH) [71]. Maintained doses of rhGH have shown an improvement in growth velocity and phosphate concentrations [46, 48, 50, 72], however, there are not clear evidences about the effectiveness of the treatment [50]. A 3-year-study carried out by Haffner et al. (2004) in rhGH treated children showed potential hormone benefit over the final height but failure to normalize the body disproportion [73]. In poorly growing XLH patients, rhGH therapy added to the conventional treatment with vitamin D metabolites and phosphate improved final height, phosphate retention and radial bone mineral density but did not modify the disharmonic development [48]. Animal studies have mostly focused on examine the short-term effect of GH treatment on vitamin D metabolism and renal phosphate transport [74] rather than the long-term effect on growth.

2.4.2 New FGF23 related therapies

Tumor induced osteomalacia (TIO) has been proposed to have an overlapping pathophysiology with XLH, since *PHEX* is also present in the tumors, raising the possibility that TIO and XLH share components of a common pathogenic mechanism. In fact, in some TIO patients high levels of FGF23 have been identified to cause osteomalacia as well as hypophosphatemia [75, 76]. It would be of interest to know whether the inhibition of FGF23 activity is useful or not for patients with hypophosphatemic rickets/osteomalacia caused by excessive activity of FGF23 [77]. In this respect there are not many studies in Hyp mouse [78–84] and only two in humans [85, 86].

Two studies in wild type and Hyp mice have shown that the inhibition of the FGFR effectively blocks FGF23 function and it partially corrects the hypophosphatemia of the mutant mouse [77, 78]. Wöle et al. (2013) have assessed the potential use of FGFR inhibitors during long term periods in Hyp mice. They used NVP-BGJ398, a novel FGFR inhibitor, currently being used in phase I clinical trials as cancer therapy. Drug was orally administered during 5 weeks in adult Hyp mice [80] leading to enhanced bone growth, increased mineralization, and reorganization of the disturbed growth plate structure.

As for FGF23 inhibition, a single dose of anti-murine FGF23 antibody given to Hyp mice increased serum calcitriol whereas repeated injections ameliorated rickets and bone mineralization [81, 82]. In the only prolonged study in humans, 4-month administration of KRN23 (a recombinant human IgG1 monoclonal antibody that binds to FGF23 and blocks its biological activity) resulted in significantly increased serum phosphate, renal phosphate reabsorption, and circulating $1,25(OH)_2D$ concentrations in 28 XLH adult patients [85].

It has also been shown that MAPK signaling mediated by ERK1/2 is necessary for the suppressive effects of FGF23 on renal Pi reabsorption and calcitriol production [17, 80, 83] (Fig. 2). In Hyp mice, Zang et al. (2012) showed that the inhibition of this pathway, improved serum phosphate and $1,25(OH)_2D$ concentrations as well as osseous lesions of rickets or osteomalacia regardless the persistently elevated systemic levels of FGF23 [84]. They hypothesized that other molecules apart from FGF23 must be contributing to the pathogenesis of the disease. In this regard, crossing mice homozygous for the null Cyp24 allele, the mitochondrial enzyme responsible for inactivating $1,25(OH)_2D$, with Hyp mice [79] led to offspring having high circulating levels of FGF23 but lacking CYP24 enzymatic activity. In these animals, serum concentrations of phosphate and $1,25(OH)_2D$ did not improve but the rachitic/osteomalacic bone abnormalities and abnormal growth plate were ameliorated independently of the levels of FGF23. Thus, bone and growth plate disturbances in XLH may be dependent on the autocrine/paracrine effects of vitamin D rather than on circulating phosphorus and FGF23 levels.

3 Areas of research and development

XLH, the most prevalent inherited rickets, is caused by loss of function of the *PHEX* gene and biochemically characterized by low serum phosphate concentration, reduced tubular reabsorption of phosphate and high circulating levels of FGF23. The mechanisms whereby inactivation of *PHEX* causes these alterations remain unclear.

XLH patients show a wide phenotypic variability but short stature and body disproportion with some degree of legs' bowing are key clinical manifestations. The reason for growth impairment in XLH is not completely clear because the reduction in height does not entirely depend on the severity of lower extremities' deformity. It is of note that few data are available on the abnormalities of the growth plate in XLH, even in the Hyp mouse which is a good animal model of the disease. The impact of XLH on the normal structure and dynamics of growth plate chondrocytes and the relationship of these alterations on longitudinal growth rate are areas of further research.

Height is not normalized by sustained treatment with phosphate and calcitriol and although GH therapy has been shown to accelerate growth velocity there are some concerns on its potential to exaggerate body disproportion. Studies on the administration of GH in Hyp mice have mostly focused on the ability of GH to improve phosphate and vitamin D metabolism but there is a lack of information on its effects on growth plate. In addition, treatments aimed at blocking FGF23 action are promising but, again, information is lacking on the consequences of counteracting FGF23 on the growth plate.

In conclusion, despite rickets and bone bowing being the most noticeable symptoms of the XLH disorder, most of the treatments are focused on phosphorus and vitamin D metabolism. Consequently, it is essential to understand the mechanism underlying the growing impairment and how conventional and new treatments affect in this regard.

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Compliance with ethical standards The article did not involve human or animal participation. Therefore, informed consent or IRB approval were not needed.

Conflict of interest The authors declare that they have no conflict of interest.

- The manuscript has not been submitted to more than one journal.
- The manuscript has not been published previously.
- No data/figures have been fabricated or manipulated.
- A single study is not split up into several parts.
- No data, text or theories are being plagiarized.
- All authors have been informed about the submission of the paper and all have contributed to the article.

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