Current and Future Therapeutical Aspects of X-Linked Hypophosphatemia in Children With Special Attention to Klotho/Fibroblast Growth Factor 23 System

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To The Editor

X-chromosomal hypophosphatemia is a disease in which too much phosphate is excreted in the urine, which should actually be recovered in the kidney from the pre-urine. This leads to a severe bone growth disorder, because phosphate affects the storage of calcium in the bones. The disease manifests itself as early as infancy and occurs in an average of one in 325,000 people [1-27]. Girls are affected twice as often as boys but show milder courses of the disease [3-5]. The probability of a so-called first mutation is about 1:25,000,000 [3]. The incidence is 1:20,000 newborns. The disease is not contagious or transmissible. The disease is caused by a mutation for a gene on the short arm of the X chromosome (Xp22.1). This mutation is dominant; one affected allele is sufficient to cause the disease [1-27]. The exact mechanism of development is still unclear; it is assumed that the mutated gene contains the building instructions for a membrane protein that regulates the recovery of phosphate in the kidney via fibroblast growth factor (FGF)23 [9]. The mutation is based on the *PHEX* gene, where disturbed *PHEX* protein will be expressed [7].

The *PHEX* protein encoded by this gene is a transmembraneous endopeptidase that is classified to the type II integral membrane zinc-dependent endopeptidase group. The protein is thought to be involved in bone and dentin mineralization and renal phosphate reabsorption. The bone and dentin protein osteopontin which inhibits mineralization in the skeleton and in teeth is a substrate for *PHEX*. The absence of functional *PHEX* in the mouse model of X-linked hypophosphatemia, and in human X-linked hypophosphatemia, where *PHEX* activity is decreased or absent, increased circulating FGF23 hormone, resulting in low serum phosphate and insufficient level of this mineral ion in the blood in transit to mineralized tissues, compared to the normal amount that is required for proper bone and tooth mineralization. The enthesopathy of the disease is a mechanical adaptation to osteomalacia. A major comorbidity of X-linked hypophosphatemia is fibrocartilaginous tendinous insertion site mineralization resulting in painful enthesophytes that contribute to the adult clinical picture and significantly impact physical function. Enthesophytes in Hyp mice, a murine model of the disease, is the result of a hyperplastic expansion of resident alkaline phosphatase, Sox9-positive mineralizing fibrochondrocytes. Mineralizing fibrochondrocyte expansion of the disease occurs as a compensatory adaptation to the soft bone matrix.

As a result of the mutation on the *PHEX* gene, FGF23 overactivity impairs this recovery [1, 4, 8, 9, 21]. FGF23 derives from the FGF19 subfamily [4, 7]. In the kidney, a klotho-FGFR1-FGF23 trimeric complex will be built with activation of the mitogen-activated protein kinase (MAPK) pathways [9]. Moreover, a downregulation of type IIa and c sodium transporters in the proximal tubulus of the kidney is present [1-20]. As a result, phosphate excretion in the kidney is elevated [1-27]. In the body, the amounts of phosphate and calcium dissolved in the blood are coupled together, the calcium-phosphate product. If the amount of phosphate in the blood decreases, at the same time less calcium is incorporated into the bones. However, this calcium is needed in large quantities for the strength of the bones, and the bones are no longer able to cope with the higher loads imposed by the growing child [3, 6]. This leads to the bone deformations that are typical of the disease. In addition, too low a phosphate level in the healthy body would trigger the release of vitamin D, which in turn leads to an increase in phosphate absorption from the intestine [15]. In this disease, however, this control loop is also disturbed: despite the lowered phosphate level in the serum, calcitriol secretion does not increase. The disease usually manifests itself from the second year of life in the form of skeletal deformities with strong genua and coxa vara (bow legs), wide-legged waddling gait, short stature, impaired tooth development, and middle ear deafness if no treatment is given, due to the deficient development of the auditory ossicles. As a secondary disease, calcium deposits may develop in the kidneys. The clinical picture, the age at which the first symptoms appear, and the presence of the disease in the family are typical. The clinical picture is particularly determined by the analysis of blood values and the evaluation of radiographs [1, 2, 6, 8, 9, 20]. In the blood, there is a decreased phosphate level, increased alkaline phosphatase with normal levels for calcium, parathyroid hormone (PTH),

and calcitriol [1-27]. Radiographs show rachitic changes in the metaphysis of the forearms, and later of the knee and ankle joints, as well as signs of osteomalacia. In the differential diagnosis, it should be noted that the clinical picture of phosphate diabetes can easily be confused with that of hypophosphatasia. Hypophosphatasia can be found in different diseases like achondrogenesis, osteomalacia due to vitamin D deficiency, or thanatophoric dysplasia [28, 29]. In the latter, however, there is a decreased alkaline phosphatase, so that a different treatment is necessary.

The Klotho-FGF23 system plays an important role in activation kidneys phosphate excretion in these patients [21-27]. Klotho is a proteohormone that can prolong life by approximately 20% to 30% in mice [21]. It is a membrane-bound protein with a size of 130 kDa. In addition, there is a soluble form of the protein called soluble Klotho (sKL). Proteolytic cleavage upstream (α-cut) or downstream (β-cut) of the KL2 domain of the protein, results in either a 130-kDa sKL, or 60 - 70-kDa klotho fragments [21, 23, 27]. In 1997, Makoto Kuro'o at the National Institute of Neuroscience in Tokyo identified a new gene that extends lifespan in mice when expressed in increased amounts. A defect in this gene leads to a syndrome similar to human aging. Affected animals have a reduced life expectancy, are infertile and develop typical diseases of old age such as arteriosclerosis, skin atrophy, osteoporosis, and emphysema. The *Klotho* gene encodes a transmembrane protein with a transmembrane domain. The Klotho protein binds as a co-receptor to several FGFRs. To date, 22 FGFs are known, but only four different receptors. Therefore, it is thought that the specificity of the receptor proteins is mediated by co-receptors. Only the binding of Klotho to FGFR1 subtype IIIc leads to the specific binding of FGF23. FGF23 is a bone-derived hormone that inhibits phosphate reabsorption and vitamin D synthesis in the kidney. Mice treated with a monoclonal antibody against Klotho no longer respond to FGF23 [30]. The Klotho protein is expressed primarily in the kidney, but also in the placenta, prostate, lung, spleen, and parathyroid gland. Current knowledge suggests that the kidney is the major source of sKL, as no sKL circulates in kidney-specific Klotho knockout mice. In the kidney, Klotho inhibits phosphate reabsorption in proximal tubule cells by direct binding to the FGF23 receptor, regulates calcium reabsorption by stabilizing the TRPV5 calcium channel in the cell membrane, Klotho inhibits 1α-hydroxylase and thus the activation of 25(OH) vitamin D3 to calcitriol [21-27]. The effect on calcium and on phosphate transport in the kidney is synergistic with the effects of PTH, whereas the effect on calcitriol synthesis is antagonistic. The extracellular domain of Klotho is cleaved off, becoming a humoral factor [24, 27]. The secreted Klotho protein regulates several signaling pathways, including the insulin/insulin-like growth factor 1 (IGF-1) pathway and the Wnt signaling pathway, as well as the activity of many ion channels. In addition, Klotho protein protects cells and tissues from oxidative stress, but the exact mechanism has not yet been elucidated [21, 27]. In 2007, a homozygous mutation of the *Klotho* gene was described for the first time in humans [31]. It affected a 13-year-old girl who developed severe calcinosis, Teutschlander's disease, with calcifications of the common carotid artery and outer meninges. Disturbances in mineral metabolism were found with elevated serum phosphate, elevated serum calcium, elevated PTH, and elevated FGF23. Expression and secretion of Klotho were markedly reduced, resulting in impaired signal transduction of FGF23 via the FGF23 receptor. FGF23 is a hormone produced in bone osteocytes that regulates phosphate balance. The early increase of FGF23 before and at the onset of renal insufficiency prevents hyperphosphatemia by increasing renal phosphate excretion and, by inhibiting 1-hydroxylase in the kidney, decreases the concentration of $1,25(OH)_2$-vitamin D3 and thus the absorption of calcium and phosphate from the intestine. Until now, it was hypothesized that primarily PTH caused increased excretion of phosphate in the urine. Currently, however, it is believed that FGF23 increases very early and even before PTH and keeps serum phosphate within the normal range via increased phosphate excretion. Phosphate (serum) therefore does not increase until stage 4 (glomerular filtration rate (GFR) 15 - 29 mL/min) of chronic renal failure. Elevated or high-normal phosphate levels in the presence of still normal renal function or incipient renal failure are associated with increased cardiovascular risk. Dialysis patients have markedly elevated FGF23 levels, and their elevation indicates the development of refractory secondary hyperparathyroidism (sHPT). Restriction of phosphate leads to a decrease in FGF23. Approximately 90% of patients with chronic renal failure stage 3 and 4 have elevated FGF23 levels without hyperphosphatemia. Higher FGF23 levels are considered to indicate a more severe progression of chronic renal failure. They are associated with higher cardiovascular risk and mortality. Recent studies further show that the association between high serum phosphate levels and survival is not limited to renal patients but is also found in patients with cardiovascular disease and even in the general population. Already high-normal serum phosphate levels are associated with the occurrence of coronary calcification in young healthy men, and high-normal serum phosphate levels were a predictor of cardiovascular events in the Framingham study [32]. Increased mortality was seen primarily in renally healthy cardiac patients with serum phosphate levels in the upper normal range.

Late complications are renal insufficiency, nephrocalcinosis, osteodystrophy of the bones, and low vitamin D levels with all its clinical features. Diagnosis was made by measuring phosphate level in blood and urine, calcium, parathormon and FGF23 levels in blood. A molecular analysis of *PHEX* gene mutation leads to diagnosis. Therapy is based on supplementation of phosphate, calcium, and vitamin D at therapy of first choice. In more serious cases, burosumab, an immunoglobulin (Ig)1 neutralizing recombinant antibody against FGF23, was introduced in 2018 in children and later also in adults [2, 3, 5, 10, 11, 14, 15, 18]. Long-term results and late effects of burosumab therapy in this disease are missing due to the initiation of this new therapy in 2018. Further studies in children are necessary to get more information concerning late side effects of this new treatment.

Future therapeutical aspects could include inhibiting Klotho-FGFR1-FGF23 trimeric complex to prevent the effect of FGF23 in the kidney. Klotho-FGFR1-FGF23 trimeric complex signaling plays an important role in X-linked hypophos-

phatemia and inhibition of this complex could diminish the level of FGF in the kidney [33].

A validation of a next-generation sequencing (NGS) panel improved the diagnosis of X-linked hypophosphatemia [20]. This NGS panel described 42 pathogenic missense-, nonsense-, splice-sited- and indel *PHEX* mutations and in one the known homozygous *DMP1* mutation [20]. The developed NGS panel seems to be a reliable tool with high sensitivity and specificity for the diagnosis, also to diagnose this disease [20]. MAPK inhibition and growth hormone therapy could also act as promising therapeutic agent [9]. Gene therapy could play a major role in curing the disease, but the clinical management in patients is still in childhood shoes [13, 19]. Cure depends on developing gene therapy options to repair the mutation at *PHEX* gene [13, 19]. The time of gene therapy should then be initiated as early as possible to prevent the child from complications of this very rare disease. A one-time gene therapy approach would be desirable.

Overall, the disease is a serious and very rare phosphate-wasting disease in childhood with different, but to date, not curable therapy option. Research should include possible molecular aspects on inhibiting Klotho-FGFR1-FGF23 trimeric complex as a therapy option in the future and working on developing a curable, desirable one-time approach gene therapy for this disease [21-27].

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Conflict of Interest

There is no conflict of interest.

Informed Consent

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Author Contributions

SB performed research, data collection and references; EL and GV read the manuscript and gave important ideas; EM checked grammar and style of the manuscript.

Data Availability

Any inquiries regarding supporting data availability of this letter should be directed to corresponding author.

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