The elevation of circulating fibroblast growth factor 23 without kidney disease does not increase cardiovascular disease risk

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High circulating fibroblast growth factor 23 (FGF23) levels are probably a major risk factor for cardiovascular disease in chronic kidney disease. FGF23 interacts with the receptor FGFR4 in cardiomyocytes inducing left ventricular hypertrophy. Moreover, in the liver FGF23 via FGFR4 increases the risk of inflammation which is also found in chronic kidney disease. In contrast, X-linked hypophosphatemia is characterized by high FGF23 circulating levels due to loss of function mutations of the phosphate-regulating gene with homologies to an endopeptidase on the X chromosome (*PHEX*), but is not characterized by high cardiovascular morbidity. Here we used a novel murine X-linked hypophosphatemia model, the *Phex*^{C733RMhda} mouse line, bearing an amino acid substitution (p.Cys733Arg) to test whether high circulating FGF23 in the absence of renal injury would trigger cardiovascular disease. As X-linked hypophosphatemia patient mimics, these mice show high FGF23 levels, hypophosphatemia, normocalcemia, and low/normal vitamin D levels. Moreover, these mice show hyperparathyroidism and low circulating soluble αKlotho levels. At the age of 27 weeks we found no left ventricular hypertrophy and no alteration of cardiac function as assessed by echocardiography. These mice also showed no activation of the calcineurin/NFAT pathway in heart and liver and no tissue and systemic signs of inflammation. Importantly, blood pressure, glomerular filtration rate and urea clearance were similar between genotypes. Thus, the presence of high circulating FGF23 levels alone in the absence of renal impairment and normal/high phosphate levels is not sufficient to cause cardiovascular disease.

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End-stage renal disease (ESRD), chronic kidney disease (CKD), and also reduced renal function increase the risk of mortality and cardiovascular disease.[1] Left ventricular hypertrophy (LVH) is likely the primary manifestation of uremic cardiomyopathy.[2] LVH affects approximately 70% of patients during intermediate stages of CKD[3] compared with 16% to 21% affected individuals in the general population.[4] CKD patients show high circulating fibroblast growth factor 23 (FGF23), which likely participates in the pathogenesis of left ventricular hypertrophy in these patients.[5] FGF23 together with parathyroid hormone (PTH) and 1,25$(OH)_2$ vitamin D (1,25$(OH)_2$D) is responsible for the regulation of phosphate homeostasis in the organism.[6] FGF23 is synthetized in bone, and after posttranslational modification the active form is secreted into circulation. The action of FGF23 is mediated by coupling to a fibroblast growth factor receptor (FGFR) in the target cell or organ. At least in kidney the presence of the coreceptor αKlotho is required for an effective

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FGF23-FGFR binding.[7] αKlotho is also present in the circulation as soluble Klotho. Its function is still not fully understood, but together with the other major endocrine regulators of phosphate homeostasis, its levels are altered in CKD: FGF23 and PTH are elevated already in the first phases of CKD, whereas soluble Klotho and $1,25(OH)_2D$ are decreased. Phosphate increases in plasma only in later stages of CKD.[8] FGF23 has been suggested as the inductor of left ventricular hypertrophy in chronic kidney disease, by binding to FGFR4 in myocytes in the absence of αKlotho.[5,9] Other authors suggest that αKlotho and phosphate rather than FGF23, PTH, and $1,25(OH)_2D$ levels correlate with the appearance of cardiac hypertrophy and fibrosis.[10] Therefore, we wanted to test in an X-linked hypophosphatemia (XLH) mouse model that has chronic high circulating FGF23 levels whether these animals have a higher risk of cardiovascular disease. Of note, FGF23 levels in XLH are comparable to levels in CKD.[11,12]

XLH (OMIM 307800) is the most common form of hereditary rickets affecting 1 in 20,000 individuals.[13,14] The main feature of this disease is high circulating levels of the hormone FGF23, which lead to hypophosphatemia due to decreased renal phosphate reabsorption. Affected individuals frequently present with bone pain, lower limb deformity, dental anomalies, and muscular symptoms, although the severity grade varies greatly between individuals and between relatives. XLH is caused by mutations in the phosphate-regulating gene with homologies to endopeptidases on the X chromosome (PHEX).[15] The exact physiological function of PHEX is still unknown, but mutations in this gene lead to high synthesis of FGF23 in the bone and secretion to the circulation. A large variety of mutations have been identified in the *PHEX* gene.[16] These mutations include large and small deletions, insertions, nonsense, missense, and splice site mutations. Several XLH mouse models are available.[17,18] The most used mouse XLH model is the Hyp mouse.[19] We have recently reported 2 new mouse XLH models, C3Heb/FeJ-PhexBAP012, and C3Heb/FeJ-PhexBAP024 ($Phex^{C733RMhda}$).[20] $Phex^{C733R}$ mice have a missense mutation in the cysteine at position 733 causing a change to an arginine residue. This mutation has also been described in 2 patients causing a change to a serine residue or a stop codon,[16,21] respectively. Here, we show that these mice are a good XLH model. Second, as the CVD risk increases with age we studied the effect of the primary elevation of FGF23 levels in plasma on indicators of CVD and systemic inflammation in 27-week-old $Phex^{C733R}$ mice.

RESULTS

The $Phex^{C773R}$ mouse line is an XLH model showing primary high FGF23 blood levels

First we analyzed whether hemizygous $Phex^{C733R/Y}$ males and heterozygous $Phex^{C733R/+}$ females showed the typical features of XLH patients. Indeed, $Phex^{C733R/Y}$ males and $Phex^{C733R/+}$ females had high circulating intact FGF23 levels at 12 and 27 weeks of age (Figure 1a; Supplementary Figures S1A and S2A and B). C-terminal FGF23 levels were higher than intact FGF23 levels and also elevated in $Phex^{C733R/Y}$ males when compared with wild-type animals (Supplementary Figure S2C). Moreover, $Phex^{C733R/Y}$ males and $Phex^{C733R/+}$ females were hypophosphatemic and normocalcemic (Figure 1b and c; Supplementary Figures S1B and C and 2D–G). Only female $Phex^{C733R/+}$ showed the hyperphosphaturia detected in XLH patients, whereas $Phex^{C733R/Y}$ males were normophosphaturic (Figure 1d and e). This is probably because male $Phex^{C733R/Y}$ excreted less urine and had higher urine creatinine levels (Supplementary Table S2). The hyperphosphaturia observed in XLH patients is due to downregulation of the sodium-phosphate transporter NaPi-IIa (Slc34a1) protein expression in the proximal tubule provoked by high FGF23 levels in blood. $Phex^{C733R/Y}$ males and $Phex^{C733R/+}$ females showed also clearly reduced NaPi-IIa protein expression in renal brush border membranes (Figure 1g and i; Supplementary Figure S1D and E). $Phex^{C733R/Y}$ males and $Phex^{C733R/+}$ females showed a tendency to hypocalciuria ($P = 0.09$ and 0.07, respectively; Figure 1f and Supplementary Figure S1F). Next, we observed that the protein renal expression of the coreceptor αKlotho was lower in $Phex^{C733R/Y}$ males (Figure 1h and j). Plasma soluble αKlotho was also decreased in $Phex^{C733R/Y}$ males (Figure 1k). XLH patients show abnormally low to normal $1,25(OH)_2D$ levels when considering their hypophosphatemic state.[14] $Phex^{C733R/Y}$ males also showed low $1,25(OH)_2D$ levels (Figure 1l), due to decreased synthesis rate and despite increased inactivation (Supplementary Figure S3A–C). Inactivation and synthesis of $1,25(OH)_2D$ levels is mediated by the renal enzyme 24-hydroxylase (Cyp24a1) and 1α-hydroxylase (Cyp27b1), respectively. Female $Phex^{C733R/+}$ had similar $1,25(OH)_2D$ levels as their wild-type littermates (Figure 1m). Despite the high FGF23 levels in blood, *Cyp27b1* mRNA expression and Cyp24a1 protein expression were similar between wild-type and $Phex^{C733R/+}$ females (Supplementary Figure S3D–F). Hyperparathyroidism is mostly seen in XLH patients treated with phosphate supplements, but has also been reported in untreated XLH patients.[22] $Phex^{C733R/Y}$ males and $Phex^{C733R/+}$ females also presented with higher PTH levels in plasma than their wild-type littermates (Figure 1n; Supplementary Figure S1G). Finally, the $Phex^{C733R}$ mouse line showed as XLH patients alterations in their bone structure and bone mineral density. $Phex^{C733R}$ mice had lower bone mineral density and lower cortical bone mineral density[14,23] (Figure 1o and p; Supplementary Table S3). In conclusion, the $Phex^{C733R}$ mouse line shows a phenotype resembling XLH patients with very high FGF23 levels.

$Phex^{C773R}$ mice have normal kidney function

As high FGF23 levels lead to a higher risk of developing cardiovascular disease in chronic kidney disease, we next checked the renal function of $Phex^{C733R}$ mice. $Phex^{C733R/Y}$ males and $Phex^{C733R/+}$ females had a similar glomerular filtration rate (GFR) (Figure 2a; Supplementary Figure S4A). Urea and creatinine levels in plasma were also similar between genotypes (Figure 2b and c; Supplementary Figure S4B and C). Moreover, urea clearance showed no differences