Cardiac hypertrophy elevates serum levels of fibroblast growth factor 23

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Several experimental studies have shown that fibroblast growth factor 23 (FGF23) induces left ventricular hypertrophy (LVH). However, the opposite directional relationship, namely a potential effect of LVH on FGF23, remains uncertain. Here we evaluated the effects of LVH on FGF23 using cardiomyocyte-specific calcineurin A transgenic mice. At six weeks, these mice showed severe LVH, with elevated levels of serum intact FGF23. FGF23 levels were elevated in cardiomyocytes, but not osteocytes, of the transgenic animals. Moreover, transverse aortic constriction also upregulated myocardial FGF23 expression in wild type mice. The promoter region of the FGF23 gene contains two putative nuclear factors of activated T cells (NFAT)-binding sites, with NFAT1 activating the promoter in a proximal NFAT-binding site dependent manner. Neither serum, urinary, or fractional excretion values of calcium and phosphate nor serum levels of 1,25$(OH)_2$ vitamin D were different between wild type and transgenic mice. Moreover, the renal expression of FGF receptors and α–Klotho was comparable. However, plasma levels of antidiuretic hormone were significantly increased in the transgenic mice, and aquaporin-2 immunohistochemical staining was mainly positive in the apical membrane of the collecting duct, compared to a primarily cytoplasmic staining in wild type mice. Real-time PCR analyses of kidney CYP27B1 and CYP24A1 expression in wild type mice showed that exogenous antidiuretic hormone blocked FGF23's actions on these vitamin D activating or inactivating enzymes. Finally, the renal resistance of transgenic mice to FGF23 was partly overcome by tolvaptan. Thus, LVH in transgenic mice is associated with an increase in myocardial and serum intact FGF23, with the kidneys being protected against FGF23 excess by elevated antidiuretic hormone levels.

Kidney International (2018) **94,** 60–71; https://doi.org/10.1016/j.kint.2018.02.018

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Received 21 July 2017; revised 25 January 2018; accepted 8 February 2018; published online 8 May 2018

KEYWORDS: FGF23; mineral metabolism; vasopressin

Fibroblast growth factor 23 (FGF23) is a potent phosphaturic hormone that is predominantly produced by osteoblasts and osteocytes.[1,2] In contrast to canonical autocrine/paracrine FGFs, FGF23 requires α-klotho as a cofactor for its signaling.[1,2] Therefore, under physiological conditions, the targets of FGF23 are limited to α-klotho–expressing cells. Several studies have identified tubular cells in the kidney, chief cells in the parathyroid gland, and choroid plexus cells in the brain as the major α-klotho–expressing cell types.[1,2] Among these, the tubular cells are the main target cells that mediate the phosphaturic actions of FGF23.[1,2]

Circulating levels of FGF23 are 1 of the candidate biomarkers that powerfully predict renal and cardiovascular outcomes,[3–8] with compensatory elevation of FGF23 being indispensable for maintaining serum phosphate levels in the presence of a decrease in nephrons.[6,8] Cross-sectional studies have revealed that higher FGF23 is consistently associated with left ventricular hypertrophy (LVH), which can lead to congestive heart failure and subsequent death.[3,4,9] Thus, LVH is likely 1 of the plausible pathologic conditions linking higher FGF23 to greater cardiovascular mortality. Currently, 2 theories explaining the correlation of higher FGF23 with an increased prevalence of LVH have been proposed. Andrukhova *et al.*[10] reported that FGF23 upregulates distal tubular sodium uptake in an α-klotho–dependent manner, leading to volume expansion, hypertension, and subsequent cardiac hypertrophy. In comparison, Faul *et al.*[9,11] proposed that FGF23 induces cardiac hypertrophy via an α-klotho–independent activation of calcineurin A (CnA)–nuclear factor of activated T cells (NFAT) in cardiomyocytes. Although both theories are plausible, the following points indicate that these 2 theories cannot fully explain the association of higher FGF23 with an increased prevalence of LVH. First, observational studies have reported that FGF23 is associated with LVH, even in statistical models adjusted for blood pressure.[4,12–15] Thus, there must be blood pressure–independent mechanisms linking higher FGF23 to a greater risk of LVH. Second, the

association of FGF23 with LVH has been reported not only in patients with end-stage renal disease, whose circulating FGF23 is extremely high, but also in patients with chronic kidney disease and in non–chronic kidney disease populations, whose circulating FGF23 levels are not as high.[4,9,14] Moreover, Mirza *et al.*[16] reported that an increase in FGF23 that remains within the normal range is associated with an increased risk of LVH. Because the α-klotho–independent effect of FGF23 on cardiomyocyte requires extremely high FGF23, the activation of the FGF23-CnA pathway in cardiomyocytes cannot fully explain the underlying mechanisms that link FGF23 to LVH.[9]

In the current study, we examined the effects of ventricular hypertrophy on circulating levels of FGF23 to clarify a novel mechanism that could link LVH to FGF23 rather than solely considering the FGF23 to LVH link. We used transgenic (TG) mice that express the constitutively active form of the CnA catalytic subunit under the control of α-myosin heavy chain (α-MHC) promoter as the CnA-NFAT is the key pathway activated in pathologic, but not physiological, LVH.[17,18]

RESULTS

CnA-TG mice develop cardiac hypertrophy

We confirmed the phenotypes of CnA-TG mice. CnA-TG mice show severe ventricular hypertrophy by 6 weeks of age (Figure 1a). Real-time polymerase chain reaction (PCR) analysis of the left ventricle confirmed the upregulation of hypertrophy-related genes (atrial natriuretic peptide [ANP], brain natriuretic peptide [BNP], and β-myosin heavy chain) in the CnA-TG mice (Figure 1b). Masson's trichrome–stained heart tissue sections revealed that perivascular fibrosis was evident in the CnA-TG mice (Figure 1c). Fibrosis-related genes (collagen I, collagen III, and fibronectin), but not osteogenic differentiation marker genes (osteoprotegerin, osteocalcin, osterix, and runt-related transcription factor 2) in the CnA-TG mice were upregulated (Figure 1d and e). The CnA-TG mice used in this study express a constitutively active form of CnA under the control of an α-myosin heavy chain (α-MHC) promoter.[17] mRNA for α-MHC was not detected in the femur, bone marrow, kidney, or brain of either the wild-type (WT) or CnA-TG mice, indicating that the transgene was not expressed in these organs (Figure 1f). The level of α-MHC in the left ventricle was suppressed in the CnA-TG mice (Figure 1f).

We identified a 2-fold increase in the weight of the heart of TG mice compared with WT mice (Table 1). Measurements of all other parameters were comparable between TG and WT mice: the body weight, systolic blood pressure, food intake, water intake, urinary volume, lung weight, spleen weight, kidney weight, serum creatinine, creatinine clearance, and urinary *N*-acetyl-β-D-glucosaminidase/creatinine ratio (Table 1). There were no differences in Masson's trichrome– and periodic acid–Schiff-stained kidney sections between TG and WT mice (Figure 2a and b). Levels of mRNA for injury-related molecules, kidney injury molecule 1 and neutrophil gelatinase–associated lipocalin, and fibrosis-related molecules in the kidney were comparable in TG and WT mice (Figure 2c).

Hypertrophic cardiomyocyte expresses FGF23 in an NFAT-dependent manner

We analyzed the effects of cardiac hypertrophy on FGF23 in the CnA-TG mice. Serum levels of intact FGF23 (iFGF23) and c-terminal FGF23 were higher in the CnA-TG mice than in WT mice (Figure 3a). As bone is the major FGF23-producing organ under physiological conditions, we analyzed the expression level of FGF23 in the femurs of the animals. Both real-time PCR and immunohistochemistry indicated comparable levels of FGF23 in the femur of TG and WT mice (Figure 3b and c). mRNA levels of FGF23 in bone marrow were also comparable in TG and WT mice (Figure 3b). In contrast, higher levels of FGF23, at both mRNA and protein levels, were expressed in hypertrophic hearts (Figure 3b–d). Immunohistochemistry of the left ventricle showed that expression of FGF23 in TG mice was increased in hypertrophic cardiomyocytes, but not in interstitial or vascular cells (Figure 3c). Because Western blot analysis indicated that hypertrophic cardiomyocytes expressed an intact form of FGF23, we investigated *N*-acetylgalactosaminyltransferase 3 (GALNT3) levels in the left ventricle and found that GALNT3 was upregulated in CnA-TG mice (Figure 3e). We additionally examined the effects of 3 hypertrophy-related hormones, ANP, BNP, and antidiuretic hormone (ADH), on the mRNA levels of FGF23 in the femur. All 3 hormones suppressed mRNA for FGF23 in the femur (Supplementary Figure S1).

We further analyzed whether ventricular hypertrophy induced by transverse aortic constriction (TAC) increases FGF23 in the cardiomyocytes of C57BL/6 mice. Similar to the results obtained with CnA-TG mice, mRNA levels of hypertrophy- and fibrosis-related genes, but not osteogenic differentiation marker genes, were upregulated in the left ventricle of TAC-operated mice (Figure 4a). Masson trichrome–stained heart tissue sections demonstrated fibrosis of the left ventricle in TAC-operated mice (Figure 4b). The mRNA level of cardiac FGF23 was upregulated in TAC-operated mice, whereas the increase in GALNT3 did not reach statistical significance (Figure 4a). Both immunohistochemistry and Western blot analyses confirmed the increase in FGF23 in the left ventricle of TAC-operated mice (Figure 4c and d).

We subsequently analyzed the promoter region of FGF23. *Homo sapiens*, *Rattus norvegicus*, and *Mus musculus* had similar promoter sequences for FGF23 (Figure 5a). Due to the presence of 2 putative NFAT-binding sites in the region, we examined whether these 2 sites were actually responsible for the transcriptional regulation of FGF23. Among 4 classic members in the *NFAT* gene family (i.e., *NFAT1*, *2*, *3*, and *4*), Bourajjaj *et al.*[19] reported that NFAT1 is an indispensable mediator of calcineurin-dependent cardiac hypertrophy. Thus, we analyzed the specific effects of *NFAT1* on the regulation of FGF23 using a dual luciferase assay system. We found that *NFAT1* activates the promoter of FGF23 (Figure 5b). The activation by *NFAT1* was abrogated in a