Regulation of renal phosphate transport by FGF23 is mediated by FGFR1 and FGFR4

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Submitted 25 April 2013; accepted in final form 19 November 2013

Fibroblast growth factor family members are known to bind to four known fibroblast growth factor receptors (FGFRs) and initiate intracellular signaling (8, 33). The four FGFRs are numbered 1–4 and are coded by four genes. Alternative splicing of FGFR1, 2, and 3 results in a total of seven isoforms, and these seven FGFRs have ligand binding specificity (32, 34). FGFR2 does not bind to FGF23 and is not present in the proximal tubule (12, 28). Previous data from our laboratory suggested that deletion of a single FGFR was not responsible for the phosphaturic actions of FGF23 (12). However, kidney conditional Fgfr1−/−/Fgfr4−/− mice and global Fgfr4−/− mice had an ~1.4-fold increase in FGF23 levels at baseline compared with wild-type mice and had comparable serum phosphorus levels to wild-type mice. In response to exogenously administered pharmacological doses of FGF23, kidney conditional Fgfr1−/−/Fgfr4−/− mice did not become hypophosphatemic and had an attenuated hypophosphatemic response compared with wild-type mice (12). To determine whether FGFR1 and FGFR4 together were the joint renal receptors for FGF23, we examined phosphate homeostasis in Fgfr1−/−/Fgfr4−/− mice.

METHODS

Generation of Fgfr1−/−/Fgfr4−/− mice (Pax3creTyr; Fgfr1−/−lox/lox; Fgfr4−/− mice). We have previously studied individual kidney conditional Fgfr1−/− and Fgfr4−/− mice (12). As previously described, Fgfr4−/− mice are from a mixed 129/Black Swiss background. Conditional Fgfr1−/− mice were generated using the lox-p/cre recombinase technique as global Fgfr1−/− mice are embryonically lethal and are from mixed 129/Sv and C57BL/6J backgrounds as previously described (7, 12, 17, 26, 38). Pax3 cre recombinase is expressed in the metanephrine mesenchyme and not in the ureteric bud (7). Mice expressing Pax3 cre when mated with mice that have the Fgfr1 gene flanked by lox-p sites will generate mice that have Fgfr1 deleted from the metanephrine mesenchyme. Metanephrine mesenchyme gives rise to nephron segments from the proximal tubule, the primary site of phosphate reabsorption (2), to the distal convoluted tubule (30). Fgfr4−/− mice were mated with mice that had the critical regions of Fgfr1 gene flanked by lox-p sites (Fgfr1Lox/Lox) (17) to generate mice...
that are both null for Fgfr4 and also have lox-p sites for Fgfr1 (Fgfr1Lox/Lox/Fgfr4−/−). Next, Fgfr1Lox/Lox/Fgfr4−/− mice were mated with mice transgenic for Pax3 cre (26) to finally generate Pax3cre/Fgfr1Lox/Lox/Fgfr4−/− mice (Fgfr1−/−/Fgfr4−/− mice). Wild-type littersmates that did not express the Pax3 cre (Fgfr1Lox/Lox/Fgfr4−/− or Fgfr1Lox/−/Fgfr4−/−) and Fgfr4−/− mice that did not express the Pax3 cre (Fgfr1Lox/−/Fgfr4−/− or Fgfr1Lox/Lox/Fgfr4−/−) were studied for comparison. We also studied Fgfr1+/−/Fgfr4+/+ mice, and the data were similar to the data obtained from the wild-type littersmates described above (not shown). The mice were genotyped before the study and were studied at 2.5–4 mo of age. The mice were cared for at the animal facility at the University of Texas Southwestern Medical Center where the mice were on a 12:12-h day-night cycle. The mice were fed on a Teklad Global 16% protein standard rodent diet (TD 2016), and they were given free access to food and water. To study the effects of dietary phosphate depletion on the regulation of FGF23, mice were placed on a phosphate-depleted diet for 5 days (TD 110794). The phosphate-depleted diet had comparable amounts of calcium, vitamin D, and other minerals as the standard 16% protein diet (TD 2016). The Institutional Animal Care and Use Committee at the University of Texas Southwestern Medical Center approved these studies.

Recombinant FGF23 administration. Recombinant mouse FGF23 (R&D Systems, Minneapolis, MN), at 200 ng/g body wt, was injected into mice transgenic for Pax3 cre (1:1,000, Sigma, St. Louis, MO), and 100 μl of the solution (Millipore, Billerica, MA) was used to quantitate the protein abundance.

Sodium-dependent BBMV phosphate transport activity. The rapid Millipore filtration technique was employed to measure sodium-dependent phosphate (3P) transport in BBMVs. The intravesicular buffer (300 mM mannitol and 16 mM HEPES titrated to a pH of 7.5 using Tris) was used to suspend BBMVs, and 100 μg (10 μl) of BBMVs were used to assay phosphate transport. BBMVs were then mixed with extravesicular buffer (90 μl) (150 mM NaCl and 16 mM HEPES, 0.1 mM KH2PO4 titrated to a pH 7.5 with Tris) as described previously (1, 39). Phosphate uptake was terminated at 20 s by using ice-cold stop solution (135 mM NaCl, 16 mM HEPES, 10 mM sodium arsenate, pH 7.5), and 0.45-μm filters were used to filter the resulting solution (Millipore, Billerica, MA). The uptake measurements were performed at least in triplicate.

Metabolic studies. Mice were first acclimatized to metabolic cages (Hatteras Instruments, Hatteras, NC) with free access to food and water for 3 days. After acclimatization, 24-h food intake was measured and 24-h stool was collected to estimate gastrointestinal phosphate absorption. The TD 2016 standard diet has 0.7 g of phosphate/100 g of diet (Harlan Laboratories, Madison, WI). Twenty-four-hour stool samples were first dissolved by incubation at 95°C for 12–14 h in the presence of 2 ml of 1.2 M nitric acid. Each sample was then diluted and solubilized with 5 ml of water (3). The supernatant was collected, and stool phosphorus was measured using the Fiske and Subbarow method (11).

RNA isolation and quantitative PCR. Kidneys were homogenized in lysis solution, and RNA was extracted using a GenElute mammalian total RNA kit (Sigma-Aldrich, St. Louis, MO). Calvarial bones were harvested and frozen in liquid nitrogen until further assay. TRizol was used to extract RNA from the bones. RNA quality and purity were determined before cDNA synthesis. cDNA was synthesized from 4 μg of RNA as per kit instructions from the Thermoscript RT-PCR System (Invitrogen Life Technologies, Grand Island, NY). GAPDH primers were used to verify cDNA synthesis. An iCycler PCR Thermal cycler (Bio-Rad, Hercules, CA) was used to carry out quantitative PCR. Taqman gene expression assay and universal master mix were used to quantify Klotho mRNA expression. Fgfr3, NaPi-2a, NaPi-2c, and 28S mRNA expression were quantified using SYBR Green master mix (Bio-Rad). The following primer sequences were used: FGF23, forward 5′-TTT CCC AGG TTC GTC TAG G-3′ and reverse 5′-CTC GCA GGT GAC TCT CAG-3′ (45); NaPi-2a, forward 5′-CAAC TGC CAT CCT ATC CAA CGA-3′ and reverse 5′-GAC GCT GAC AAT GAT GG-3′; and NaPi-2c, forward 5′-ATC TCC GGT CTT ATT CCA GGA-3′ and reverse 5′-GTA GAG CTT TCC CAG GAC-3′.

Statistical analysis. All data are expressed as means ± SE. Student’s t-test was used to assess the difference between two groups. ANOVA was employed to study the differences between multiple groups with a post hoc Student-Newman-Keuls test. A P value <0.05 was considered significant.

RESULTS

Baseline characteristics. It was our hypothesis that FGFR1 and FGFR4 are the critical receptors for the phosphaturic
actions of FGF23. Thus we examined whether deletion of both 
Fgfr1 and Fgfr4 in the kidney would result in hyperphos-
phatemia, demonstrating resistance to FGF23. In Table 1, we 
compare the serum phosphorus, FGF23, 1,25(OH)2Vitamin 
D3, and PTH levels in wild-type, Fgfr1—/-/Fgfr4—/-, and 
Fgfr4—/- mice. Compared with Fgfr4—/- and wild-type mice, 
Fgfr1—/-/Fgfr4—/- mice had 20- to 50-fold higher FGF23 
levels and yet had higher serum phosphorus levels, consistent 
with FGR1 and FGR4 being the proximal tubule receptors 
regulating phosphorus transport. Serum 1,25(OH)2Vitamin D3 
levels were also elevated in Fgfr1—/-/Fgfr4—/- mice compared 
with wild-type and Fgfr4—/- mice. Values are means ± SE.

Table 1. Baseline serum characteristics of wild-type, Fgfr1—/-/Fgfr4—/-, and Fgfr4—/- mice

<table>
<thead>
<tr>
<th></th>
<th>Wild-Type</th>
<th>Fgfr1—/-/Fgfr4—/-</th>
<th>Fgfr4—/-</th>
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<tbody>
<tr>
<td>Serum phosphorus, mg/dl</td>
<td>7.1 ± 0.2 (n = 25)</td>
<td>9.2 ± 0.3* (n = 16)</td>
<td>7.5 ± 0.3 (n = 8)</td>
</tr>
<tr>
<td>Serum FGF23, pg/ml</td>
<td>108.1 ± 7.3 (n = 10)</td>
<td>4,953.6 ± 675.0* (n = 10)</td>
<td>265 ± 30.9 (n = 10)</td>
</tr>
<tr>
<td>Serum 1,25(OH)2D3, pmol/l</td>
<td>115.7 ± 8.2 (n = 27)</td>
<td>182.5 ± 22.4* (n = 17)</td>
<td>80.5 ± 12.1 (n = 10)</td>
</tr>
<tr>
<td>Serum PTH, pg/ml</td>
<td>143.0 ± 26.7 (n = 16)</td>
<td>66.4 ± 20.7* (n = 17)</td>
<td>181.6 ± 23.1 (n = 12)</td>
</tr>
<tr>
<td>24-h Gastrointestinal phosphorus absorption, mg/g body wt</td>
<td>0.6 ± 0.1 (n = 6)</td>
<td>0.8 ± 0.2 (n = 6)</td>
<td>0.7 ± 0.1 (n = 6)</td>
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Values are means ± SE. Using an unpaired t-test, FGF23 levels are significantly higher in Fgfr4—/- mice compared with wild-type mice but not using ANOVA comparing all 3 groups, *P < 0.001, Fgfr1—/-/Fgfr4—/- vs. wild-type and Fgfr4—/- mice.

We next examined the cause for the increase in serum phosphorus despite an increase in FGF23. In Fig. 1, we show that BBMV phosphate transport was increased in Fgfr1—/-/ Fgfr4—/- mice compared with wild-type and Fgfr4—/- mice. We next compared BBMV NaPi-2a and NaPi-2c protein abundance to determine whether either of the proximal tubule brush border transporters had increased protein expression. NaPi-2c protein expression relative to β-actin was increased in Fgfr1—/-/ Fgfr4—/- mice compared with both wild-type and Fgfr4—/- mice (Fig. 2), while NaPi-2a protein expression was comparable in wild-type, Fgfr1—/-/Fgfr4—/-, and Fgfr4—/- mice (Fig. 3). The relative mRNA expression of NaPi-2a/28s and NaPi-2c/28s was comparable in wild-type and Fgfr1—/-/Fgfr4—/- mice (Fig. 4, A and B). Additionally, we examined whether gastro-intestinal absorption of phosphorus played a role in the elevated serum phosphorus seen in Fgfr1—/-/Fgfr4—/- mice. Twenty-four-hour stool phosphorus and phosphorus intake were measured. Gastrointestinal phosphorus absorption was comparable in wild-type, Fgfr1—/-/Fgfr4—/-, and Fgfr4—/- mice (Table 1).

We examined the relative expression of Fgfr23 mRNA in calvarial bone to determine whether the elevated level of FGF23 in Fgfr1—/-/Fgfr4—/- mice was due to increased expression. In Fig. 5, we show that Fgfr23 mRNA expression is significantly higher in Fgfr1—/-/Fgfr4—/- mice compared with wild-type mice. We did not find Fgfr23 mRNA expression in the kidneys of Fgfr1—/-/Fgfr4—/- mice (data not shown). These data indicate that bone is the source of increased FGF23 levels in Fgfr1—/-/Fgfr4—/- mice.
different in wild-type and Klotho protein expression from whole kidney lysate were not FGF23 levels in wild-type and wild-type mice, demonstrating resistance to phosphaturic ac-

recombinant FGF23, while demonstrated increased pMAPK protein abundance 1 h after receiving signaling of FGF23. As shown in Fig. 7, wild-type mice demon-

strated increased pMAPK protein abundance (Fig. 7). Wild-type mice did not dem-

onstrate increased pMAPK protein abundance (Fig. 7).

Since Klotho is a cofactor for the interaction of FGF23 and its receptor, and Klotho−/− mice have elevated FGF23 levels, we measured Klotho protein and mRNA expression in the kidney. Both relative Klotho mRNA expression (Fig. 8A) and Klotho protein expression from whole kidney lysate were not different in wild-type and Fgfr1−/−/Fgfr4−/− mice (Fig. 8B).

We studied the role of a phosphate-depleted diet on serum FGF23 levels in wild-type and Fgfr1−/−/Fgfr4−/− mice. After 5 days on a phosphate-depleted diet, serum phosphorus in the animals was comparable in wild-type and Fgfr1−/−/Fgfr4−/− mice (Fig. 9A), but serum FGF23 levels remained elevated in Fgfr1−/−/Fgfr4−/− mice (Fig. 9B).

In summary, Fgfr1−/−/Fgfr4−/− mice have elevated serum FGF23 levels compared with wild-type mice, and the elevated levels are likely due to increased synthesis of FGF23 from bone. Despite the elevated FGF23 levels, serum phosphorus levels are increased in Fgfr1−/−/Fgfr4−/− mice compared with wild-type mice, demonstrating resistance to phosphaturic ac-

tions of FGF23. In addition, there is abrogation of intracellular signaling of FGF23 in Fgfr1−/−/Fgfr4−/− mice. These results are consistent with our hypothesis that FGRF1 and FGRF4 are the primary receptors for the phosphaturic actions of FGF23.

![Graph](image1)

**Fig. 3.** Baseline BBMV NaPi-2a protein expression in wild-type, Fgfr1−/−/Fgfr4−/−, and Fgfr4−/− mice. Baseline NaPi-2a protein expression relative to β-actin on BBMVs from the renal cortex was comparable in wild-type, Fgfr1−/−/Fgfr4−/−, and Fgfr4−/− mice. Twenty-five micrograms of BBMVs were fractionated on 8% SDS gels.

**Fig. 4.** A: baseline relative expression of NaPi-2a mRNA in wild-type and Fgfr1−/−/Fgfr4−/− mice. Relative NaPi-2a mRNA expression was comparable between wild-type and Fgfr1−/−/Fgfr4−/− mice. B: baseline relative expression of NaPi-2c mRNA in wild-type and Fgfr1−/−/Fgfr4−/− mice. Relative NaPi-2c mRNA expression was similar between wild-type and Fgfr1−/−/Fgfr4−/− mice.

![Graph](image2)

**Fig. 5.** Baseline relative Fgfr23 mRNA expression in calvarial bones of wild-type and Fgfr1−/−/Fgfr4−/− mice. Fgfr23 mRNA expression from cal-

varial bones was significantly higher in Fgfr1−/−/Fgfr4−/− compared with wild-type mice, consistent with the elevated serum FGF23 levels in Fgfr1−/−/Fgfr4−/− mice.
wild-type and Fgfr1

To assess the baseline MAPK signaling, 50 and Fgfr1 Baseline phosphorylation of MAPK (pMAPK) was comparable in wild-type phosphate transport. FGF23 levels were H11011 are the receptors that regulate the effects of FGF23 on renal DISCUSSION

Fig. 6. Baseline MAPK signaling in wild-type and Fgfr1−/−/Fgfr4−/− mice. To assess the baseline MAPK signaling, 50 µg of whole kidney lysate from wild-type and Fgfr1−/−/Fgfr4−/− mice were fractionated on 8% SDS gels. Baseline phosphorylation of MAPK (pMAPK) was comparable in wild-type and Fgfr1−/−/Fgfr4−/− mice.

**DISCUSSION**

This study examined whether FGFR1 and FGFR4 together are the receptors that regulate the effects of FGF23 on renal phosphate transport. FGF23 levels were ~50-fold higher in Fgfr1−/−/Fgfr4−/− mice compared with wild-type mice. Despite the elevated FGF23 levels in Fgfr1−/−/Fgfr4−/− mice, these mice have elevated serum phosphorus levels and increased brush border membrane phosphate transport compared with their wild-type and Fgfr4−/− counterparts, indicating that the phosphaturic action of FGF23 is abrogated in Fgfr1−/−/Fgfr4−/− mice. In keeping with increased brush border membrane phosphate transport, brush border membrane NaPi-2c protein expression was increased in Fgfr1−/−/Fgfr4−/− mice in the absence of a change in mRNA expression. This indicates that there is posttranscriptional regulation of NaPi-2c in Fgfr1−/−/Fgfr4−/− mice. In response to exogenous recombinant FGF23 administration, phosphorylation of MAPK, a marker of intracellular signaling of FGF23, is also abrogated, demonstrating that FGFR1 and FGFR4 are critical receptors for FGF23 in the kidney. Gastrointestinal phosphate absorption was comparable in wild-type and Fgfr1−/−/Fgfr4−/− mice, indicating that the elevated phosphorus is primarily due to resistance to the phosphaturic actions of FGF23. Decreasing dietary phosphate content decreased serum phosphate in both wild-type and Fgfr1−/−/Fgfr4−/− mice to comparable levels, but serum FGF23 levels remained elevated in Fgfr1−/−/Fgfr4−/− mice compared with wild-type mice. While there are other possibilities of elevated FGF23 levels in Fgfr1−/−/Fgfr4−/− mice including decreased clearance of FGF23, we demonstrate that in Fgfr1−/−/Fgfr4−/− mice the likely source of elevated FGF23 levels is increased production from bone. The expression of Klotho, an important cofactor for the interaction of FGF23 with its receptors, was comparable in wild-type and Fgfr1−/−/Fgfr4−/− mice. These data together demonstrate that FGFR1 and FGFR4 are critical receptors for the renal phosphaturic effects of FGF23.

Identification of the FGFRs responsible for the various actions of FGF23 has been a topic of significant interest (12, 15, 25, 28, 48). Results from in vitro studies employing cell

**Fig. 6.** Baseline MAPK signaling in wild-type and Fgfr1−/−/Fgfr4−/− mice. Mice were injected intraperitoneally with FGF23 (200 ng/g body wt), and kidneys were harvested 1 h later. Phosphorylation of MAPK (pMAPK) was assessed in total kidney homogenate; 50 µg of whole kidney lysate was fractionated on 8% SDS gels. There was an increase in MAPK phosphorylation in wild-type mice but not in Fgfr1−/−/Fgfr4−/− mice.

*Fig. 7.** Effects of FGF23 on MAPK signaling in wild-type and Fgfr1−/−/Fgfr4−/− mice. Mice were injected intraperitoneally with FGF23 (200 ng/g body wt), and kidneys were harvested 1 h later. Phosphorylation of MAPK (pMAPK) was assessed in total kidney homogenate; 50 µg of whole kidney lysate was fractionated on 8% SDS gels. There was an increase in MAPK phosphorylation in wild-type mice but not in Fgfr1−/−/Fgfr4−/− mice.
lated on 8% SDS gels. Values are means ± SE. B: baseline relative kidney Klotho protein expression in wild-type and Fgfr1−/−/Fgfr4−/− mice. Baseline whole kidney lysate Klotho protein expression relative to β-actin was comparable in wild-type and Fgfr1−/−/Fgfr4−/− mice. Twenty-five micrograms of whole kidney lysate was fractionated on 8% SDS gels. Values are means ± SE.

Fig. 8. A: baseline relative kidney Klotho mRNA expression in wild-type and Fgfr1−/−/Fgfr4−/− mice. Relative Klotho mRNA expression in whole kidney samples was not different between wild-type and Fgfr1−/−/Fgfr4−/− mice. Values are means ± SE. B: baseline kidney Klotho protein expression in wild-type and Fgfr1−/−/Fgfr4−/− mice. Baseline whole kidney lysate Klotho protein expression relative to β-actin was comparable in wild-type and Fgfr1−/−/Fgfr4−/− mice. Twenty-five micrograms of whole kidney lysate was fractionated on 8% SDS gels. Values are means ± SE.

lines to identify the receptors for FGF23 have been controversial, with one study demonstrating the binding of FGF23 to only FGFR1 in the presence of Klotho (46) while another study demonstrated that FGF23 binds to FGFR1c, 3c, and 4 in the presence of Klotho (23). By studying individual Fgfr1−/− (kidney conditional), Fgfr3−/−, and Fgfr4−/− mice, we have previously shown that FGF23 levels were modestly elevated in Fgfr1−/− (1.5-fold) and Fgfr4−/− (1.3-fold) mice while the serum phosphorus levels remained comparable to their wild-type counterparts, suggesting the presence of compensatory mechanisms for the absence of one receptor at baseline (12). In response to pharmacological doses of FGF23, Fgfr1−/− mice had no decrease and Fgfr4−/− mice had an attenuated decrease in serum phosphorus levels compared with wild-type mice. We concluded that FGFR1 was the predominant receptor responsible for the hypophosphatemic actions of FGF23 with FGFR4 playing a minor role (12). Liu et al. (28) also demonstrated that deletion of Fgfr3 or Fgfr4 in Hyp mice (mouse model of X-linked hypophosphatemic rickets) did not reverse the hypophosphatemic phenotype of the Hyp mice, consistent with the importance of FGFR1 in mediating the actions of FGF23 on phosphate homeostasis.

Li et al. (25) and our laboratory (15) had previously concluded that FGF3 and FGF4 were the predominant receptors in the regulation of 1,25(OH)2Vitamin D3. We demonstrated that Fgfr3−/−/Fgfr4−/− mice had ~2.6-fold higher 1,25(OH)2Vitamin D3 levels at baseline compared with wild-type mice and 1,25(OH)2Vitamin D3 levels did not decrease in response to pharmacological doses of exogenously administered FGF23 (15). In Fgfr1−/−/Fgfr4−/− mice, despite the higher serum phosphorus, lower PTH, and markedly elevated FGF23 levels, 1,25(OH)2Vitamin D3 levels were higher (1.4-fold) than in wild-type mice. The present study suggests that...
FGFR1 in conjunction with FGFR4 also appears to play a role in 1,25(OH)_{2}Vitamin D_{3} homeostasis.

There are several known regulators of FGF23, including 1,25(OH)_{2}Vitamin D_{3}, PTH, and serum phosphorus (22, 27, 36, 40). While 1,25(OH)_{2}Vitamin D_{3} increases FGF23 levels, it is unlikely that a 1.4-fold increase in 1,25(OH)_{2}Vitamin D_{3} levels in F{\text{gfr}}^{1/4}/F{\text{gfr}}^{4/4} mice can explain the ~50-fold increase in FGF23 levels seen in F{\text{gfr}}^{1/4}/F{\text{gfr}}^{4/4} mice compared with wild-type mice. Administration of pharmacological doses of 1,25(OH)_{2}Vitamin D_{3} (calcitriol) increased FGF23 in mice but by an order of magnitude less than what we find in F{\text{gfr}}^{1/4}/F{\text{gfr}}^{4/4} mice (27). PTH has been shown to increase FGF23 levels, as seen in a mouse model of hyperparathyroidism (22) and in patients with Jansen’s metaphyseal chondrodysplasia due to activating mutations of the PTH/PTH-related peptide receptor (41). In addition, parathyroidectomy in mice with early chronic kidney disease decreased FGF23 levels (24). In our study, serum PTH levels were lower in F{\text{gfr}}^{1/4}/F{\text{gfr}}^{4/4} mice than wild-type mice and thus are not contributing to the elevated FGF23 levels in F{\text{gfr}}^{1/4}/F{\text{gfr}}^{4/4} mice.

Previous studies have examined the role of changes in dietary phosphorus in the regulation of FGF23. Changing the dietary phosphate content of mice from 0.02 to 1.6% resulted in a change in serum phosphorus from 3.7 to 9.3 mg/dl while the FGF23 levels increased from 9.3 to 65 pg/ml. Thus a 2.5-fold change in serum phosphorus resulted in a 7-fold increase in FGF23 levels (36). Of note, the total body phosphate content and changes in serum phosphorus could have differing roles in their regulation of serum FGF23 levels. In F{\text{gfr}}^{1/4}/F{\text{gfr}}^{4/4} mice, FGF23 levels are ~50-fold higher compared with wild-type mice in the face of a 30% increase in serum phosphorus levels in F{\text{gfr}}^{1/4}/F{\text{gfr}}^{4/4} mice. In response to a phosphate-depleted diet, serum phosphorus levels decrease in wild-type and F{\text{gfr}}^{1/4}/F{\text{gfr}}^{4/4} mice to comparable levels. In animals on a phosphate-depleted diet, serum FGF23 levels also decrease in WT and F{\text{gfr}}^{1/4}/F{\text{gfr}}^{4/4} mice, but serum FGF23 levels still remained elevated in F{\text{gfr}}^{1/4}/F{\text{gfr}}^{4/4} mice compared with wild-type mice. Thus the serum phosphorus level in F{\text{gfr}}^{1/4}/F{\text{gfr}}^{4/4} mice is not the sole factor contributing to the elevated FGF23 levels in F{\text{gfr}}^{1/4}/F{\text{gfr}}^{4/4} mice. As in early chronic kidney disease, the factors regulating the elevated FGF23 levels in F{\text{gfr}}^{1/4}/F{\text{gfr}}^{4/4} mice are unclear at this time. There are several hypotheses that have been put forth for elevated FGF23 levels in early chronic kidney disease, including Klotho deficiency and an increase in total body phosphorus that is not reflected by an increase in serum phosphorus levels. However, these are speculative, and further work is needed to identify the regulatory factors for elevated FGF23.

It is intriguing that F{\text{gfr}}^{1/4}/F{\text{gfr}}^{4/4} mice have significantly elevated serum FGF23 levels, and it appears that the well-known regulators of FGF23 alone do not appear to be the culprits. From our data, it is also apparent that FGF23 is synthesized in bone, and there is likely kidney-to-bone cross talk, as the kidney is the only organ in this mouse model with deletion of both F{\text{gfr}}^{1} and F{\text{gfr}}^{4}. Recently, the use of FGFR1 blockers in bone marrow stem cells in H{\text{yp}} mice has been shown to decrease Fg23 mRNA expression, indicating that FGFR1 plays a role in regulating FGF23 expression (31). It is possible that FGFR1 present in the bones of F{\text{gfr}}^{1/4}/F{\text{gfr}}^{4/4} mice plays an important role in the regulation of FGF23 production, but the signal from kidney to bone needs to be investigated further. Our data are consistent with FGFR1 and FGFR4 being primary renal receptors for the phosphaturic actions of FGF23. However, they are likely not the sole receptors. While F{\text{gfr}}^{1/4}/F{\text{gfr}}^{4/4} mice have significantly elevated FGF23 levels compared with their wild-type counterparts and individual F{\text{gfr}}^{1/4} and F{\text{gfr}}^{4/4} mice, their serum phosphorus levels and serum 1,25(OH)_{2}Vitamin D_{3} levels are not as elevated as the levels seen in F{\text{gfr}}^{2/4}/F{\text{gfr}}^{4/4} mice (43, 44).

Thus FGF23 is still having some action to suppress serum 1,25(OH)_{2}Vitamin D_{3} levels and regulate phosphate transport in F{\text{gfr}}^{1/4}/F{\text{gfr}}^{4/4} mice. This suggests that there is still redundancy of FGFRs and compensatory mechanisms exist even in these F{\text{gfr}}^{1/4}/F{\text{gfr}}^{4/4} mice.

GRANTS
This work was supported by a Children’s Medical Center Research Foundation Grant (J. Gattineni), National Institutes of Health Grants K08DK089295 (J. Gattineni), DK41612 (M. Baum), DK078596 (M. Baum), and the O’Brien Center (P30DK079328; Peter Igarashi, PI).

DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS
Author contributions: J.G. provided conception and design of research; J.G., P.A., Q.Z., and N.M. performed experiments; J.G. and P.A. analyzed data; J.G. and M.B. interpreted results of experiments; J.G. prepared figures; J.G. drafted manuscript; J.G., C.M.B., and M.B. edited and revised manuscript; J.G. and M.B. approved final version of manuscript.

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FIBROBLAST GROWTH FACTOR 23 RECEPTORS


