FGF23 gene variation and its association with phosphate homeostasis and bone mineral density in Finnish children and adolescents

Minna Pekkinen a,b*, Christine M. Laine a,b, Riikka Mäkitie a, Eira Leinonen a, Christel Lamberg-Allardt c, Heli Viljakainen d, Outi Mäkitie a,b,c,f

a Folkhälsan Institute of Genetics, Biomedicum Helsinki, Helsinki, Finland
b Department of Endocrinology, Sahlgrenska University Hospital and Institute of Medicine, Sahlgrenska Academy, Sweden
c Department of Pediatrics, Children's Hospital, Helsinki University Central Hospital and University of Helsinki, Helsinki, Finland
d Department of Molecular Medicine and Surgery, Karolinska Institutet, Stockholm, Sweden
e Department of Endocrinology, Sahlgrenska University Hospital and Institute of Medicine, Sahlgrenska Academy, Sweden
f Folkhälsan Institute of Genetics, Biomedicum Helsinki, Helsinki, Finland

Abstract

Fibroblast growth factor 23 (FGF23), a bone-derived hormone, participates in the hormonal bone–parathyroid–kidney axis, which is modulated by PTH, 1,25-dihydroxyvitamin D, plasma phosphate (Pi), and diet. Inappropriately high serum FGF23, seen in certain genetic and acquired disorders, results in urinary phosphate wasting and impaired bone mineralization. This study investigated the impact of FGF23 gene variation on phosphate homeostasis and bone health. The study included 183 children and adolescents (110 girls) aged 7–19 years (median 13.2 years). Urine and blood parameters of calcium and phosphate homeostasis were analyzed. Bone characteristics were quantified by DXA and peripheral quantitative computed tomography (pQCT). Genetic FGF23 variation was assessed by direct sequencing of coding exons and flanking intronic regions. Nine FGF23 polymorphisms were detected; three of them were common: rs3832879 (c.212-37insC), rs7955866 (c.716C>T, p.T239M) and rs11063112 (c.2185A>G, p.P729S). Four different haplotypes and six different diplotypes were observed among these three polymorphisms. The variations in FGF23 significantly associated with plasma PTH and urinary Pi excretion, even after adjusting for relevant covariates. FGF23 variations independently associated with total hip BMD Z-score, but not with other bone outcomes. In instrument analysis, genetic variance in FGF23 was considered a weak instrument as it only induced small variations in circulating FGF23, PTH and Pi concentrations (F statistic less than 10). The observed associations between FGF23 variations and circulating PTH, and Pi excretion and total hip BMD Z-scores suggest that FGF23 polymorphisms may play a role in mineral homeostasis and bone metabolism.

Keywords:
Phosphate
FGF23
PTH
Child
Adolescent
Bone morphometry

Introduction

Fibroblast growth factor (FGF) 23 is a member of the FGF family of polypeptides, which regulates diverse functions in metabolism and development. FGF23 is a hormone mainly produced by osteoblasts and osteocytes and regulates phosphate homeostasis and vitamin D metabolism via a specific FGF receptor-α-klotho-complex in tubular kidney cells, thereby participating in the hormonal bone–kidney axis [1–3]. FGF23 acts to decrease urinary phosphate reuptake by downregulating a sodium-dependent phosphate co-transporter, by inhibiting the CYP27B1 enzyme responsible for 1-hydroxylation of 25-hydroxyvitamin D (25(OH)D), and by inducing 1,25-dihydroxyvitamin D (1,25(OH)2D) inactivation [4,5]. Phosphate is an essential mineral for skeletal mineralization, cellular energy maintenance and for buffering blood pH levels, but high plasma phosphate levels may be a risk for soft tissue calcification [6]. Phosphate is mainly bound to hydroxyapatite in bone and to intracellular components, and only approximately 1% circulates in the blood. The circulating phosphate concentration is regulated by FGF23, 1,25(OH)2D and PTH levels [1].

The significance of FGF23 in the pathogenesis of hypophosphatemic disorders was unveiled when FGF23 was discovered as the causative gene behind autosomal dominant hypophosphatemic rickets (ADHR), and tumor-induced phosphate wasting was associated with increased FGF23 synthesis. High FGF23 in these diseases leads to excessive urinary...
phosphate excretion, inappropriately low 1,25(OH)2D and osteomalacia [5,7,8]. FGF23 is normally inactivated by enzymatic cleavage, but FGF23 mutations in ADHR render the protein’s cleavage site resistant to degradation, thereby elevating circulating FGF23 [9,10]. In tumor-induced osteomalacia the tumor itself produces excess FGF23 and hypophosphatemia can be reversed by tumor removal [5].

A functional allelic variant rs7955886 (c.716C>T, p.T239M) in FGF23 has recently been linked to renal phosphate leak in calcium nephrolithiasis [11]. FGF23239T subjects had lower plasma phosphate (P-Pi) and reduced renal tubular phosphate reabsorption compared with FGF23716T subjects. In addition, the p.T239M change increased FGF23 secretion and induced a higher activation of the FGF receptor/ERK pathway compared to FGF23239T [11].

The impact of FGF23 gene variation on healthy populations has received little attention in research. The aim of this study was to explore genetic variations in the FGF23 gene and to study whether the gene variants associate with biochemical parameters of phosphate and calcium homeostasis and with bone outcomes (measured with DXA and pQCT) in healthy children and adolescents.

Methods

Study population

A total of 183 children and adolescents, 110 girls (median age 13.3, range 7.4–18.8 years) and 73 boys (median age 12.6, range 7.7–18.1 years), were included in this school-based cross-sectional study in the capital region of Helsinki, in southern Finland (latitude 61°). The primary aim of the original study was to evaluate skeletal health in relation to vitamin D status during childhood and puberty; the secondary aim was to explore FGF23 gene variation and its role in bone health and mineral metabolism. The original cohort included 195 subjects [12] who were recruited from one primary and one secondary school; DNA was obtained for 183 of these subjects (94% of the original cohort), who were included in the present study. More than 95% of the subjects were Caucasian. All participants and their parents gave informed written consent before entering the study. The study was approved by the Research Ethics Committee of Helsinki University Hospital and performed according to the Declaration of Helsinki.

Background and clinical data

The subjects completed a questionnaire on overall health, medical and fracture history, medications, age at menarche, use of supplements and details about their physical activity. If necessary, additional information was obtained by interview. Dietary vitamin D and calcium intakes during the previous month were estimated using a food frequency questionnaire (covering over 70 foods), which has been validated against S-25(OH)D and 3-day food records [13–15]. The calculations of the food nutrient contents were performed using the Finnish National Food Composition Database (Fineli®, version 2001, National Institute for Health and Welfare). The recorded physical activity data included regular every-day activities (e.g. walking to school), activity at school, and both guided and unguided leisure-time activities during two preceding years. The duration, frequency and intensity of activity sessions were evaluated. A total physical activity score was obtained by adding the indices and intensity, as described in detail previously [12]. Heights and weights were measured and compared with Finnish normative data [16,17]. In the absence of Finnish normative data, body mass index Z-scores were calculated according to WHO (http://www.who.int). Pubertal development was scored either pre-, mid- or postpubertal based on serum hormone concentrations by a pediatric endocrinologist (OM).

Biochemistry

Blood samples and second void urine were collected at 8–10 am after an overnight fast. All samples were collected between November and March (wintertime). Plasma calcium (Ca), phosphate (Pi), alkaline phosphatase (ALP) and urinary concentrations of Ca, Pi and creatinine were measured using standard methods. Reference ranges for plasma ALP were age- and sex-dependent and the measured values were transformed into Z-scores using normal values to allow for cross-sectional comparison. S-25(OH)D was assayed with high-performance liquid chromatography (HPLC, evaluated Vitamin D External Quality Assessment Scheme, DEQAS), and plasma fasting parathyroid hormone (PTH) by an immunoluminometric method. Total serum intact FGF23 was analyzed by ELISA assay (FGF23 Kit, Kainos laboratories INC., Tokyo, Japan). Bone turnover markers N-terminal propeptide of type I procollagen (PINP) and C-terminal telopeptide of type I collagen (ICTP), reflecting bone formation and resorption, were measured from serum by radioimmunoassay (UniQ, Orion Diagnostica, Espoo, Finland) and results were interpreted in comparison to in-house age-specific reference values and transformed into Z-scores. All blood and urine measurements were analyzed at the Central Laboratory of Helsinki University Central Hospital.

Bone density and body composition measurements

BMD, bone mineral content (BMC) and bone area (BA) were measured with dual X-ray absorptiometry (DXA, Hologic Discovery A, pediatric software version 12.4, Bedford, MA, USA) for lumbar spine (LS) (L1–L4), total hip and whole body (WB). All measured values were transformed into Z-scores using equipment-specific age- and sex-adjusted reference data for US Caucasian children; all subjects were of normal height. Body composition was analyzed with DXA to distinguish between lean and fat mass. Calibration of the measurements was performed with a spine phantom; inter-CV% for the phantom BMC, BA, and BMD was 0.35%, 0.21%, and 0.41%, respectively. The reproducibility of the DXA measurement for bone, fat, and lean mass is 1.2%, 1.9% and 0.7%, respectively, in children between 10 and 18 years of age [18]. Age- and gender-specific reference values were utilized to derive Z-scores for fat percentage [19,20].

Volumetric BMD and bone geometry were measured from nonom- inant radius with pQCT ( XCT-2000; Stratec, Pforzheim, Germany, software version 5.50) as described previously [21,22]. The scans were analyzed using contour mode 2 (45%) and peel mode 1 to assess total bone (TB) and trabecular bone (Trab) parameters at the 4% site. At the 66% site, cortical bone (Cort) was detected with separation mode 1 and a threshold of 710 mg/cm3. In addition, we calculated age- and sex-specific Z-scores for total cross-sectional area (CSA), bone mineral content (BMC), Cort mineral density, TB mineral density and stress and strain index (SSI) using the published Cole’s formula, which is based on mostly Caucasian reference data [23,24].

Genetic analysis

Patient DNA was extracted from peripheral blood by standard methods. Primers for FGF23 (hg18/uc001qmq1) were designed with Primer3 v.0.4.0 (http://rodo.wi.mit.edu/primer3/) for all three exons, UTRs and a minimum of 30 bases of flanking introns. Due to the length of exon 3 and the 3′UTR, this segment was sequenced with four overlapping primer pairs. PCR amplification was performed with AmpliTaq Gold (Applied Biosystems, Foster City, CA, USA). The DNA fragments were then visualized with ethidium bromide on a 1.2% agarose gel, purified with ExoSAP (USB, Cleveland, OH, USA) and labeled with BigDye Terminator v3.1 Cycle Sequencing kit (Applied Biosystems). After sequencing with an ABI3730 sequencer (Applied Biosystems), chromatograms were analyzed with Sequencer v4.7 (Gene Codes Corporation, Ann Arbor, MI, USA). Primer sequences and detailed PCR protocols are
available upon request from the authors. The haplotype analysis was performed with Haploview 4.2. The statistical analysis was performed on diplotypes due to the relatively small study population.

Statistical analysis

Descriptive data are reported as medians and ranges or as means ± SD. Association of variables was tested with Pearson or Spearman correlation, as appropriate. Partial correlation was used to describe the association after controlling for confounding factor(s). ANOVA was applied for comparisons between three or more groups, followed by Bonferroni post hoc test (normally distributed data). Associations between FGF23 single nucleotide polymorphisms (SNP) and diplotypes, and biochemical findings and bone variables were analyzed with T-test or ANOVA. Part of the associations was further tested with analysis of covariance (ANCOVA) with relevant covariates. Before multiple linear regression analyses several variables were log-transformed to obtain (approximate) normal distribution (for instance for PTH concentration and calcium intake). Simple regression analysis was first performed to screen potential predictors for site-specific BMD with backward method. All calculations were performed using PASW version 18.0 for Windows. A p-value of less than 0.05 was considered statistically significant and p-values between 0.05 and 0.10 were considered to indicate trends.

Individual SNPs and FGF23 diplotypes, which combine data on multiple SNPs, were tested as instrumental variables to estimate causal effects of serum FGF23 on BMD. Shared covariates (sex, age, height, lean and fat mass) were chosen for the model. In addition to S-FGF23, P-PTH and P-Pi were also tested as modulators (Fig. 1). These analyses were performed with Stata version 11.0 (with the ivreg2 command).

Results

Clinical findings, biochemistry and skeletal characteristics

Of the 183 subjects who participated in the present study, 60% (N = 110) were girls and 40% (N = 73) were boys. The participants’ age distribution, pubertal stage, height, fat percentage, total intake of calcium and vitamin D (combined intakes from diet and supplements), and physical activity are presented in Table 1. The median total intake of calcium and vitamin D were in accordance with of official recommendations [25]. However, individual intakes showed great variation. The participants’ physical activity scores of 13.5, 17 and 20 correspond to 1, 1.5 and 2 h daily activity. BMD = bone mineral density, LS = lumbar spine and WB = whole body.

![Fig. 1. Directed acyclic graph for Mendelian randomization analysis of FGF23 genotype on phenotype and bone outcomes among children aged between 7 and 19 years. Arrows indicate associations, BMD = bone mineral density, LS = lumbar spine and WB = whole body.](image)

### Table 1
Clinical, biochemical and bone densitometry findings in the study subjects. Data are given as medians and ranges.

<table>
<thead>
<tr>
<th></th>
<th>Girls (N = 110)</th>
<th>Boys (N = 73)</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>13.3 (7–19)</td>
<td>12.7 (8–17)</td>
<td>0.035</td>
</tr>
<tr>
<td>Pubertal stage</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prepubertal</td>
<td>22</td>
<td>38</td>
<td></td>
</tr>
<tr>
<td>Pubertal</td>
<td>34</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>Postpubertal</td>
<td>53</td>
<td>23</td>
<td></td>
</tr>
<tr>
<td>Height (cm)</td>
<td>160 (122.5–178.2)</td>
<td>151.4 (118.5–199.9)</td>
<td>0.131</td>
</tr>
<tr>
<td>Fat %</td>
<td>28.6 (13.6–49.8)</td>
<td>22.8 (9.5–43.7)</td>
<td>0.044</td>
</tr>
<tr>
<td>Fat % (Z-score)</td>
<td>–0.5</td>
<td>–0.4</td>
<td>0.185</td>
</tr>
<tr>
<td>Calcium intake (mg/day)</td>
<td>1407 (477–3618)</td>
<td>1506 (743–2976)</td>
<td>0.063</td>
</tr>
<tr>
<td>Vitamin D intake (μg/d)</td>
<td>9.2 (2–7)</td>
<td>10 (3–4)</td>
<td>0.918</td>
</tr>
<tr>
<td>Physical activity score</td>
<td>18 (6.5–27)</td>
<td>19 (10–26)</td>
<td>0.545</td>
</tr>
<tr>
<td>S-25(OH)D (nmol/L)</td>
<td>41 (18–82)</td>
<td>45 (17.7–77)</td>
<td>0.097</td>
</tr>
<tr>
<td>P-PTH (ng/L)</td>
<td>39.5 (14–135)</td>
<td>38 (3–136)</td>
<td>0.236</td>
</tr>
<tr>
<td>S-FGF23 (ng/L)</td>
<td>36 (7–101)</td>
<td>37 (10–105)</td>
<td>0.658</td>
</tr>
<tr>
<td>P-Ca (mmol/L)</td>
<td>2.32 (2.1–2.6)</td>
<td>2.32 (2.2–2.6)</td>
<td>0.605</td>
</tr>
<tr>
<td>P-Pi (mmol/L)</td>
<td>1.35 (1–1.82)</td>
<td>1.46 (0.83–2.11)</td>
<td>0.203</td>
</tr>
<tr>
<td>U-Ca/U-Crea (mmol/L/mmol/L)</td>
<td>0.14 (0.03–0.68)</td>
<td>0.15 (0.02–0.88)</td>
<td>0.223</td>
</tr>
<tr>
<td>U-Pi/U-Crea (mmol/L/mmol/L)</td>
<td>0.15 (0.05–4.98)</td>
<td>1.72 (0.19–17.7)</td>
<td>0.375</td>
</tr>
<tr>
<td>DXA findings</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lumbar spine BMD (Z-score)</td>
<td>–0.1 (–2.2–2.7)</td>
<td>+0.1 (–1.3–2.6)</td>
<td>0.182</td>
</tr>
<tr>
<td>Total hip BMD (Z-score)</td>
<td>+0.2 (–1.9–2.5)</td>
<td>+0.1 (–1.6–1.9)</td>
<td>0.052</td>
</tr>
<tr>
<td>Whole Body BMD (Z-score)</td>
<td>+0.05</td>
<td>+0.0 (–1.5–2.4)</td>
<td>0.138</td>
</tr>
<tr>
<td>pQCT findings</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMC 4% (Z-score)</td>
<td>–0.22</td>
<td>–0.2 (–2.7–2.1)</td>
<td>0.531</td>
</tr>
<tr>
<td>Trabecular vBMD 4% (Z-score)</td>
<td>–0.1 (–2.7–2.1)</td>
<td>–0.2 (–2.7–2.1)</td>
<td>0.735</td>
</tr>
<tr>
<td>Total vBMD 4% (Z-score)</td>
<td>–0.3 (–2.7–2.8)</td>
<td>+0.7 (–2.9–3.6)</td>
<td>0.672</td>
</tr>
<tr>
<td>SSI 66% (Z-score)</td>
<td>–0.35 (–5.4–2.1)</td>
<td>–0.01 (–2.4–3.0)</td>
<td>0.518</td>
</tr>
<tr>
<td>BMC 66% (Z-score)</td>
<td>–0.1 (–2.6–2.3)</td>
<td>+0.4 (–3.0–3.2)</td>
<td>0.465</td>
</tr>
<tr>
<td>Cortical vBMD 66% (Z-score)</td>
<td>–0.34</td>
<td>–0.7 (–4.0–1.6)</td>
<td>0.462</td>
</tr>
<tr>
<td>Muscle CSA 66% (Z-score)</td>
<td>–1.16</td>
<td>–1 (–6.7–1.3)</td>
<td>0.301</td>
</tr>
</tbody>
</table>

BMD = bone mineral density, BMC = bone mineral content, CSA = cross-sectional area; physical activity scores of 13.5, 17 and 20 correspond to 1, 1.5 and 2 h daily activity. -0.05 p-Values are in bold.

S-ICTP, P-Ca and P-Pi, or U-Pi/U-Crea. An inverse association between S-FGF23 concentrations and fat % Z-score was observed (r = –0.196, P = 0.031) and it remained after adjusting for calcium intake, S-25(OH)D and P-PTH levels, and physical activity (r = 0.208 P = 0.020). Physical activity had an effect on S-FGF23 (r = 0.621 P = 0.044) after adjusting for calcium intake, S-25(OH)D, P-PTH and fat % Z-score. No association between S-FGF23 concentrations and bone outcomes was observed.

Genetic findings in FGF23

In the screening of the FGF23 gene we discovered nine variations. Three of these variations were observed in several individuals and are summarized in Table 2; these were selected for further analysis. Four of the nine variations occurred in only one individual: c723G>A (P241P) in exon 3 and rs59390594, rs71583766, and c2681A>G in the 3’UTR. In addition, two subjects of African descent carried variations rs13312795 and c2139-2141delTTC, both in the 3’UTR. The subjects with rare variations did not have hypo- or hyperphosphatemia and did not differ in other biochemical and skeletal parameters from the others. The three selected polymorphisms rs3832879 (c.212-37insC), rs7955866 (c.716C>T, p.T239M) and rs1106112 (c.2185A>T) occurred in four different haplotype and six different diplotype combinations.
The combined haplotypes were Haplotype 1 (−CA 58.1%), 2 (−CT 20.8%), 3 (CCA 10.9%), and 4 (−TT 9.8%), and diplotypes were Diplotype 1 −CA/−CA (32.2%), 2 −CA/−TT (16.9%), 3 −CA/−CT (29%) 4 CCA/CCA (14.8%), 5 CCA/−CT (4.9%), and 6 CCA/−TT (2.2%) (Fig. 2).

Associations between FGF23 variations and biochemical or skeletal characteristics

Variation in rs3832879 (c.212-37insC) genotype correlated with P-Pi concentration (p = 0.033) (Table 3A). However, no association were present after controlling for age, gender, pubertal stage and S-25(OH)D (p = 0.398). We identified only 716CC and 716CT genotypes in rs7955866 (c.716C\(\rightarrow\)N, p.T239M). 716CT heterozygotes had significantly lower mean P-PTH levels and higher U-Pi/U-Crea levels than 716CC homozygotes (Table 3A). These differences remained significant when analyzed with ANCOVA, which yielded a p-value of 0.042 for P-PTH with covariates gender, pubertal stage, S-25(OH)D and calcium intake, and p = 0.038 for U-Pi/U-Crea with covariates age, gender, pubertal stage, P-Pi, S-25(OH)D, and calcium intake. No significant correlation between the rs11063112 (c.2185A\(\rightarrow\)N) genotype and other variables was observed.

When analyzed according to diplotypes (Table 3B) S-FGF23 levels did not differ between diplotypes in the primary analysis or after adjustment for S-25(OH)D, P-PTH and calcium intake (r = 0.02, p = 0.84). There was an association between FGF23 diplotype and P-PTH concentrations (ANOVA p = 0.032, Table 3B). After controlling for age, pubertal stage, S-25(OH)D, date of sampling and calcium intake the difference between FGF23 diplotypes and P-PTH concentrations remained in girls, but disappeared in boys (ANCOVA; p = 0.037 and p = 0.636). Of the 16 children with elevated PTH, 94% had the rs7955866 716CC genotype and 63% the −CA/−CA diplotype while in the whole study population the corresponding proportions were 78% and 32%. There was a statistically significant difference between the two groups in the distribution of rs7955866 genotypes (p = 0.018) and the distribution of diplotypes (p = 0.006). There was a trend toward association between higher S-25(OH)D and FGF23 genetic variation (P = 0.097) in the whole group which was masked by the gender interaction: in boys, but not in girls, FGF23 gene variation associated with S-25(OH)D concentrations (p = 0.032).

In both genders, FGF23 gene variation was independently associated with total hip BMD Z-score in a multivariate regression model with additional variables weight, height, pubertal stage, S-25(OH)D, P-Pi Z-score and physical activity (p = 0.002; Table 4), but not with other bone outcomes. The regression model accounted for 14% of the variance (adjusted R²) in total hip BMD Z-score (Table 4). Among all the independent variables, weight, height, S-25(OH)D and FGF23 diplotype were the significant determinants of total hip BMD Z-score. No association between FGF23 gene variation and other BMD Z-scores, measured with DXA, or pQCT parameters was noticed in multivariate regression models.

Table 2

<table>
<thead>
<tr>
<th>db SNP n:o</th>
<th>Change</th>
<th>Position in gene</th>
<th>Genomic position (hg19/human)</th>
<th>Variants n (% of 183 screened samples)</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs3832879</td>
<td>c.212-37insC</td>
<td>Intron 2</td>
<td>12:4481900-4481999</td>
<td>143 (78) 40 (22)</td>
</tr>
<tr>
<td>rs7955866</td>
<td>c.716C(\rightarrow)T, p.T239M</td>
<td>Exon 3</td>
<td>12:4479549</td>
<td>146 (80) 37 (20)</td>
</tr>
<tr>
<td>Unknown</td>
<td>c.723G(\rightarrow)A, p.F241P</td>
<td>3'UTR</td>
<td>12:4479418</td>
<td>182 (99) 1 (1)</td>
</tr>
<tr>
<td>rs59309594</td>
<td>c.1097 delTTT</td>
<td>3'UTR</td>
<td>12:4479121</td>
<td>181 (99) 2 (1)</td>
</tr>
<tr>
<td>rs13312795</td>
<td>c.1144C(\rightarrow)A</td>
<td>3'UTR</td>
<td>12:4478511</td>
<td>182 (99) 1 (1)</td>
</tr>
<tr>
<td>rs71583766</td>
<td>c.1754C(\rightarrow)G</td>
<td>3'UTR</td>
<td>12:4478127-4478129</td>
<td>181 (99) 2 (1)</td>
</tr>
<tr>
<td>Unknown</td>
<td>c.2139-2141delTTTC</td>
<td>3'UTR</td>
<td>12:4478330</td>
<td>85 (46) 83 (45) 15 (8)</td>
</tr>
<tr>
<td>rs11063112</td>
<td>c.2185A(\rightarrow)T</td>
<td>3'UTR</td>
<td>12:4477584</td>
<td>182 (99) 1 (1)</td>
</tr>
</tbody>
</table>

Bolded variants were included in the statistical analyses.
The causality between the genetic variation in FGF23 and bone outcomes was further investigated by instrument analysis based on the concept of Mendelian randomization [26]. For possible modulators of the effect we tested S-25(OH)D, P-PTH and P-Pi (Table 1). The S-25(OH)D concentration was adjusted for genetic variation, but this explained some of the variance as some differences emerged after adjustment (p = 0.032) and adjustment for genetic variance strengthened this finding (median concentrations 49.6, 46.2, 42.9, and 39.5 ng/mL for the S-25(OH)D, P-PTH, S-25(OH)D, and P-Pi, respectively). However, no significant associations were found after adjustment for confounders. No associations were found for diplotypes and bone outcomes. The strongest association was for total hip BMD (r = 0.6, 95% CI: 0.27–1.53, p = 0.0169), but for others (β) varied between −0.1 and 0.5 and p-values between 0.5 and 0.9. The P-PTH concentration differed significantly between diplotypes (in unadjusted model p = 0.032) and adjustment for genetic variance strengthened this finding (median concentrations 49.6, 46.2, 42.9, and 39.5 ng/mL for the difference 0.019), but the unexplained part of PTH did not associate with bone outcomes. Similarly, in a crude model, P-PTH did not differ between diplotypes (p = 0.208), but the genetic variants of FGF23 explained some of the variance as some differences emerged after adjustment (p = 0.084). Again residuals of P-PTH did not associate with bone outcomes. Thus, genetic variance in FGF23 was considered a weak instrument as it induced rather small variation in S-25(OH)D, P-PTH and P-Pi (r² statistic less than 10%; but higher for P-PTH and P-Pi than for S-25(OH)D) and ultimately no causal effects on skeletal parameters could be seen.

Discussion

The detrimental effects of abnormal serum phosphate concentrations on bone mineralization and cardiovascular morbidity and mortality, and the association of FGF23 and P-PTH with bone outcomes, suggest that genetic variants of FGF23 may play a role in the regulation of skeletal health.
mortality have been known for long, but only during the last decade have the complex control mechanisms of phosphate metabolism begun to unravel. The discovery of the osteoblast/osteocyte-derived FGF23 as a phosphaturic agent and a regulator of vitamin D metabolism has clarified the hormonal cross-talk between bone tissue, kidneys and parathyroid glands. Still many aspects of phosphate homeostasis and the underlying cellular pathways remain inadequately defined. The approach of this study was to evaluate the impact of genetic variation in the FGF23 gene on phosphate homeostasis and bone outcomes in healthy children and adolescents. Factors such as demographics, dietary intake, fasting status and time of day at sampling, cardiovascular risk factors and kidney function only account for approximately 12% of the variation in serum phosphate levels [27]. Thus other factors, such as genetic variability, are likely to influence phosphate homeostasis. Our hypothesis was that more subtle changes in FGF23 function could cause measurable alterations in phosphate metabolism and bone health. Upon sequencing of the FGF23 gene we discovered nine single nucleotide changes: seven SNPs, one deletion and one insertion. Of these we assessed common: rs3832879, rs7955866 and rs11063112. In two of the SNPs, rs3832879 and rs7955866, the variation was dichotomous; only AA homozygotes and AA heterozygotes were present. Instrument analysis did not show a link between FGF23 genetic variation and S-FGF23 concentration. One reason could be the lack of AA homozygotes in our data and another reason might be that in this study we measured only total intact S-FGF23, not c-terminal FGF23. Rendina et al. [10] have shown association between rs7955866 (FGF23-716T) and calcium nephrolithiasis with renal phosphate leak and lower P-Pi concentrations. In our study, 9% of the subjects had elevated P-Pi concentrations (>74 ng/L) and all had normal P-Ca levels. In addition, results demonstrated association between rs3832873 (c.212-37insC) SNP in the FGF23 gene and P-Pi concentrations. High P-Pi levels, as in chronic kidney failure, cause soft tissue calcification and related cardiovascular diseases [6]. An elevated risk for vascular calcification and morbidity can also be seen in otherwise healthy individuals with elevated circulating phosphate levels [31]. Our study focused on phosphate metabolism and bone parameters, and due to the young age of our subjects no screening for vascular disease was performed. However, as our results indicate that one polymorphism (rs3832879, c.212-37insC) is linked to elevated P-Pi levels even in children, this polymorphism could possibly explain some of the variation in phosphate levels in the general population. Interestingly, the FGF23 variation associated with total hip BMD Z-scores but not with other skeletal parameters. It can be hypothesized that since this skeletal site reflects effects of bone loading, it would be impacted more than other skeletal sites by variation in an osteocyte-specific factor. Unfortunately our data does not allow for more detailed assessment of this association.

Our material is limited, as we assessed only 183 children. The International Society for Clinical Densitometry recommends that in children total body less head BMD rather than total body BMD values are used [32]. However, no normative data were available to calculate total body less head Z-score values and we therefore used total body BMD values. It is unlikely that this impacted our findings. We measured the P-Pi levels once, albeit at the same time of day and after an over-night fasting for all subjects. P-Pi levels normally vary from day to day and during the course of a day, but the most reliable results are achieved in the morning after fasting [27]. The known tendency for variation may affect the validity of our findings. We were unable to evaluate phosphorus intake with a more specific dietary inquiry. In future studies, it would be important to obtain information on phosphorus intake, which is an important variable and provides more information on phosphorus metabolism.

### Conclusion

This is the first study exploring associations between FGF23 polymorphisms and clinical phenotypes, and we succeeded in showing a weak but statistically significant association between rs7955866 (c.716C>T, p.T239M) genotype and P-Pi concentration and U-Pi/U-Crea in healthy school children. In addition, we found an association between FGF23 diplotype and total hip BMD Z-scores, but not with other skeletal parameters. We observed a genetic variant that influences circulatingPTH and phosphate without affecting serum FGF23 concentration. Future studies are needed to confirm our findings in a larger cohort and to elucidate the impact of other genes implicated in phosphate homeostasis [27] on bone density parameters and cardiovascular morbidity as to better clarify the link between gene polymorphisms and diseases secondary to variations in phosphate regulation.
Disclosure

Lamberg-Allardt has received payment for lectures from Roche and Nutricia in Finland. Other authors have no conflicts of interest to report.

Acknowledgments

We are grateful to the children and adolescents who took part in this research. We thank Nea Röman, Heini Karp and Elisa Saarnio for technical assistance. This work was supported by the Foundation for Pediatric Research, the Yrjö Jahnsson Foundation, the Ministry of Education, the Academy of Finland, the Helsinki University Central Hospital research funds, the Sigrid Juselius Foundation and the Folkhälso Research Foundation; all Helsinki, Finland.

References