Genetic Variants Associated with Circulating Fibroblast Growth Factor 23

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ABSTRACT

Background Fibroblast growth factor 23 (FGF23), a bone-derived hormone that regulates phosphorus and vitamin D metabolism, contributes to the pathogenesis of mineral and bone disorders in CKD and is an emerging cardiovascular risk factor. Central elements of FGF23 regulation remain incompletely understood; genetic variation may help explain interindividual differences.

Methods We performed a meta-analysis of genome-wide association studies of circulating FGF23 concentrations among 16,624 participants of European ancestry from seven cohort studies, excluding participants with eGFR$<$30 ml/min per 1.73 m^2 to focus on FGF23 under normal conditions. We evaluated the association of single-nucleotide polymorphisms (SNPs) with natural log–transformed FGF23 concentration, adjusted for age, sex, study site, and principal components of ancestry. A second model additionally adjusted for BMI and eGFR.

Results We discovered 154 SNPs from five independent regions associated with FGF23 concentration. The SNP with the strongest association, rs17216707 (P=3.0$\times10^{-24}$), lies upstream of *CYP24A1*, which encodes the primary catabolic enzyme for 1,25-dihydroxyvitamin D and 25-hydroxyvitamin D. Each additional copy of the T allele at this locus is associated with 5% higher FGF23 concentration. Another locus strongly associated with variations in FGF23 concentration is rs11741640, within *RGS14* and upstream of *SLC34A1* (a gene involved in renal phosphate transport). Additional adjustment for BMI and eGFR did not materially alter the magnitude of these associations. Another top locus (within *ABO*, the ABO blood group transferase gene) was no longer statistically significant at the genome-wide level.

Conclusions Common genetic variants located near genes involved in vitamin D metabolism and renal phosphate transport are associated with differences in circulating FGF23 concentrations.

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Fibroblast growth factor 23 (FGF23) is secreted from bone and plays a role in both phosphate homeostasis and the vitamin D endocrine system. FGF23 induces urinary phosphate excretion by suppressing the expression of the sodium phosphate cotransporter in kidney proximal tubular cells. FGF23 also suppresses the synthesis of calcitriol [1,25$(OH)_2$D] by inhibiting its production *via*

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1,25α-hydroxylase (CYP27B1) and stimulating its degradation *via* 1,24,25-hydroxylase (CYP24A1).[1,2] In turn, calcitriol directly enhances the transcription of FGF23 to complete a feedback loop. The effects of FGF23 on phosphate and vitamin D homeostasis require klotho as an obligatory coligand, which activates fibroblast growth factor receptors and their downstream signaling molecules.[3] Optimal phosphate balance is important for many physiologic functions, from cell signaling to energy metabolism and skeletal mineralization. In humans, high FGF23 concentrations contribute to the pathogenesis of mineral and bone disorders in CKD and are associated with ventricular hypertrophy, cardiovascular events, and premature death.[4–12]

Despite intense investigation, central elements of FGF23 regulation remain unknown. The primary role of FGF23 in phosphate homeostasis suggests regulation by serum phosphate concentrations; however, experimental studies have not detected direct actions of serum phosphate on FGF23. Rare heritable disorders are characterized by dysregulated FGF23 metabolism, yet only two (autosomal dominant hypophosphatemic rickets and familial hyperphosphatemic tumoral calcinosis) involve direct mutation of the FGF23 gene.[13–15] Mutations in other bone metabolism genes cause familial tumoral calcinosis (*GALNT3*, klotho), X-linked phosphatemic rickets (*PHEX*), and autosomal recessive hypophosphatemic rickets (*DMP1*, *FAM20C*, *ENPP1*), that subsequently alter FGF23 metabolism through mechanisms that are not completely understood.[16–23] We performed a meta-analysis of genome-wide association studies of circulating FGF23 concentrations among 16,624 individuals of European ancestry from seven cohort studies, and replicated in 4443 individuals of African ancestry from three cohorts, to investigate the role of common genetic variants on this hormone.

Significance Statement

Fibroblast growth factor 23 (FGF23), a bone-derived hormone that regulates phosphorus and vitamin D metabolism, contributes to the pathogenesis of mineral and bone disorders in CKD and is an emerging cardiovascular risk factor. The authors performed a meta-analysis of genome-wide association studies of circulating FGF23 concentrations among 16,624 individuals of European ancestry from seven cohort studies. After adjusting for age, sex, study site, and principal components of ancestry, they found that common genetic variants are associated with differences in circulating FGF23 concentration; several are closely linked with enzymes, transporters, and receptors known to be critical to vitamin D metabolism and regulation of phosphate levels. Future study of such variants may help illuminate the mechanism and clinical implications of FGF23's role in vitamin D and phosphate homeostasis.

METHODS

Study Populations

Seven cohorts contributed to the meta-analysis, by providing study-specific genome-wide analyses of FGF23 concentrations, for a total of 16,624 individuals of European ancestry (Table 1). Contributing studies included the Atherosclerosis Risk in Communities Study (ARIC; number of individuals of European ancestry, n=8594),[24] the Indiana Sisters Study (n=1128),[25] Osteoporotic Fractures in Men Study–Goteborg (MrOS GBG, n=937),[26] the Multi-Ethnic Study of Atherosclerosis (MESA; number of individuals of European ancestry, n=2163),[27] MrOS Malmo[26] (n=894), the Osteoporosis Prospective Risk Assessment Study (OPRA, n=920),[28] and the Cardiovascular Health Study (CHS; number of individuals of European ancestry, n=1988).[29] Detailed information on the study cohorts and methods is provided in Supplemental Table 1 and Table 1.

We calculated the eGFR using the creatinine-based CKD Epidemiology Collaboration equation equation,[30] and excluded participants with eGFR$<$30 ml/min per 1.73 m^2. This exclusion was chosen because our intent was to study FGF23 under normal conditions, because the strong influence of kidney disease may overwhelm potentially more subtle influences of individual SNPs on circulating FGF23. The individual studies were approved by the local research ethics committees, and informed consent was obtained from all participants.

Measurement of FGF23

Circulating FGF23 concentrations were measured by ELISA (Kainos Laboratories, Inc., Tokyo, Japan), which detects the full-length, biologically intact FGF23 molecule *via* midmolecule and distal epitopes, in the ARIC, Indiana Sisters, MrOS GBG and Malmo, and MESA cohorts. In the CHS and OPRA cohorts, FGF23 was measured using a C-terminal ELISA kit (Immutopics, San Clemente, CA).

Genotyping and Quality Control

Genome-wide genotyping and imputation with reference to the 1000 Genomes Phase 3 genotypes were performed independently in each cohort. Each cohort applied sample and SNP-based quality control measures for missingness, minor allele frequency, and Hardy–Weinberg equilibrium (Supplemental Table 1). Poorly imputed SNPs were excluded if $R^2$$<$0.3 or proper-info was $<$0.4. Population stratification and relatedness were assessed using the ancestry principal components as previously described.[31] All cohorts used EIGENSTRAT for principal components of ancestry computation.

Statistical Analyses

Each SNP was tested for association with natural log–transformed FGF23 using linear regression in two additive genetic models.[32] Model 1 was adjusted for age, sex, and the first ten principal components of ancestry.

FGF23 concentrations are strongly associated with eGFR and body mass index (BMI). To detect FGF23 loci independent of these pathways and to diminish associations with effects modulated through these factors, model 2 added

Table 1. Cohort and study participant (European descent) characteristics

Characteristic	ARIC	CHS	Indiana	MESA	MrOS GBG	MrOS Malmo	OPRA
Individuals of European descent	8594	1988	1128	2163	937	894	920
Age, yr	57.1 (5.7)	78.0 (4.4)	36.4 (8.5)	62.6 (10.3)	72.3 (5.7)	73.2 (5.8)	75.2 (0.1)
Men, *n* (%)	3962 (46.1)	754 (38.3)	0 (0)	1025 (47.3)	937 (100)	894 (100)	0 (0)
BMI, kg/m^2	27.3 (5.0)	26.6 (4.5)	26.3 (6.0)	27.7 (5.1)	27.5 (3.7)	27.5 (3.7)	26.2 (4.1)
eGFR (CKD-EPI), ml/min per 1.73 m^2	87.8 (13.2)	72.1 (17.7)	103.5 (7.4)	76.0 (17.1)	71.6 (19.3)	68.8 (19.3)	68.0 (12.6)
FGF23, pg/ml (intact)	45.5 (17.3)	—	39.4 (17.4)	41.9 (17.6)	46.2 (22.5)	55.9 (27.7)	—
FGF23, pg/ml (C-terminal)	—	12.9 (21.3)	—	—	—	—	8.3 (9.1)

Data are mean (SD) or number (%), as appropriate. Indiana, Indiana Sisters Cohort; GBG, Goteburg; CKD-EPI, CKD Epidemiology Collaboration equation; —, not available.

adjustment for BMI, and both linear and quadratic terms for the eGFR.[30]

Genomic control parameters were estimated for each cohort and appropriate genomic control correction was applied to input statistics before performing meta-analysis to correct for residual cryptic relatedness or population stratification.[33] There was little evidence for population stratification at study level (median genomic inflation factor, λ=1.03) or meta-analysis level (λ=1.007). A fixed-effects inverse-variance weighted meta-analysis using METAL was performed across cohorts on the β coefficient/SEM from each cohort.[34] Secondarily, random-effects DerSimonian and Laird models were performed using Stata 15.1 (Statacorp, College Station, TX).[35]

Genetic differentiation was estimated using the Weir unbiased estimator of the fixation index, calculated using the variance in allele frequencies among European and African ancestry samples from the 1000 Genomes and standardized according to the mean allele frequency in the combined sample.[36]

We examined the consistency of the magnitude and direction of associations across individual studies using forest plots. The Locuszoom tool was used to make regional association plots,[37] and Manhattan and QQ-plots were plotted with the qqman R package.[38]

Proportion of Phenotypic Variance Explained

The proportion of variance (PVE) in circulating FGF23 levels explained by each top novel locus, jointly across all cohorts, was estimated as:

$$PVE_{SNP} = \frac{2\hat{\beta}^2 \cdot MAF \cdot (1 - MAF)}{2\hat{\beta}^2 \cdot MAF \cdot (1 - MAF) + ((se(\hat{\beta}))^2 \cdot 2N \cdot MAF \cdot (1 - MAF))}$$

where $\hat{\beta}$, $se(\hat{\beta})$, N, and MAF are the effect size estimate of each minor allele on the relative concentration of FGF23, SEM of the effect size, sample size, and MAF for the SNP, respectively.[39]

Follow-Up in African Ancestry Cohorts

SNPs found to be associated with FGF23 concentration in individuals of European ancestry were evaluated for replication among individuals of African ancestry from three cohorts: ARIC (n=2464), MESA (n=1510), and CHS (n=469). These analyses were performed using the same quality control and imputation exclusions, and meta-analysis was performed using METAL.[34]

Sensitivity Analyses

A sensitivity analysis was conducted in which we excluded the CHS and OPRA GWAS results, which were on the basis of C-terminal FGF23 measurements, in order to evaluate whether their inclusion had altered the findings.

eQTL and Functionality Prediction

Functional annotations were assigned to all SNPs from the meta-analysis with $P<5\times10^{-6}$ using ANNOVAR software.[40] We used the MetaXcan software to identify genes whose expression levels were significantly associated with circulating FGF23.[41,42] MetaXcan uses the FGF23 GWAS summary results and gene expression levels predicted by genetic variants in a library of tissues from the Genotype-Tissue Expression (GTEx) project to impute the genetic component of gene expression in different tissues (thereby eliminating the need to directly measure gene expression levels) and to correlate the imputed gene expressions with the phenotype of interest.[43,44] For the purposes of this study, we used covariance matrices built for ten relevant tissues from GTEx (*i.e.*, whole blood cells, transformed fibroblast cells, subcutaneous adipose, skeletal muscle, liver, tibial nerve, left ventricle of the heart, coronary artery, aortic artery, and atrial appendage). Of note, reliable data from kidney and bone tissue are not currently available in GTEx.

MetaXcan is an extension of PredixCan,[39] a gene-based approach that uses GWAS summary results to impute the genetic component of gene expression in different tissues (thus eliminating the need to directly measure gene expression levels), and correlates the imputed gene expressions with phenotypes of interest.

Associations with Other Traits

We performed evaluations of SNP associations with publicly available results generated from consortia investigating other traits. Specifically, we evaluated SNPs associated with eGFR from CKD-GEN,[45] coronary artery disease in CARDIOGRAM,[46] parathyroid hormone,[47] and bone mineral density from the GEFOS.[48] In total, we performed 20 tests, corresponding to

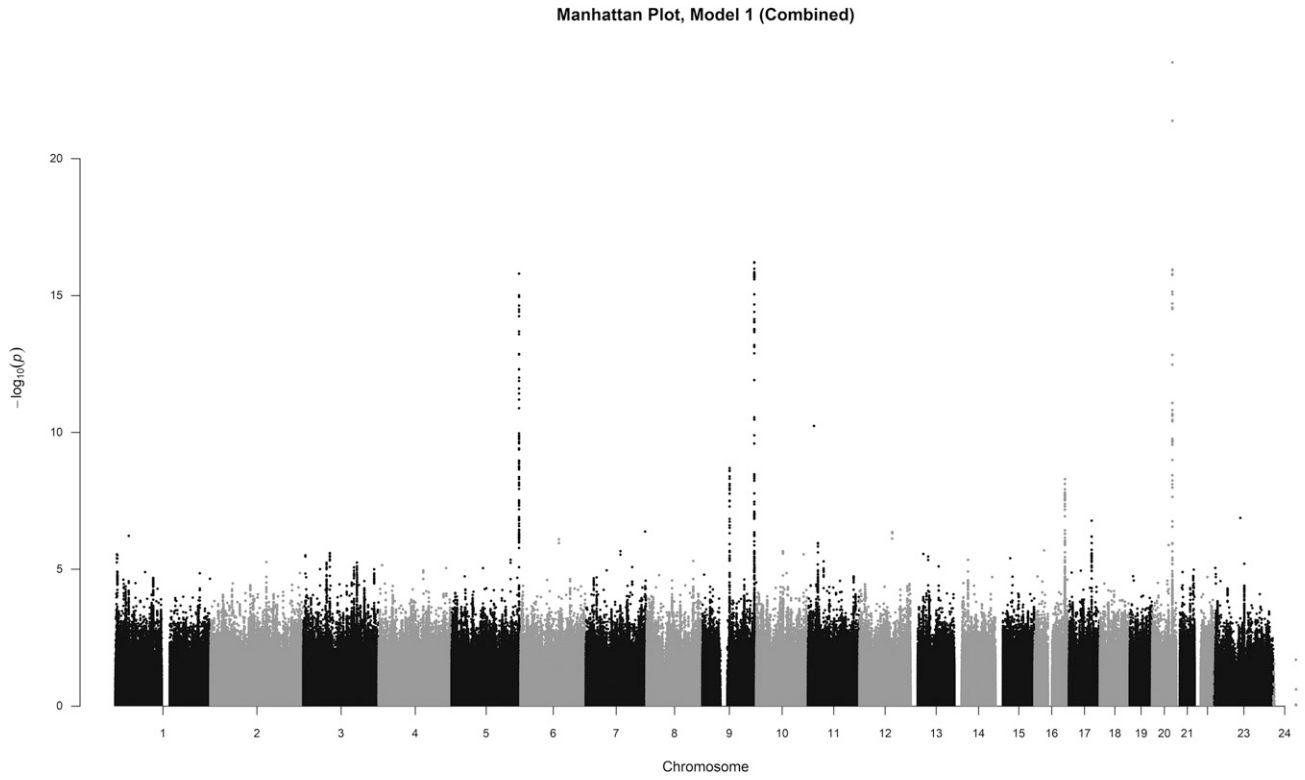

Figure 1. Manhattan plot of SNPs for FGF23. SNPs are plotted on the *x* axis according to their position on each chromosome against association with ln(FGF23) on the *y* axis (shown as –log10 *P* value).

four traits tested for association against the five SNPs. Significance was evaluated at the Bonferroni-corrected level of 0.0025.

RESULTS

The SNP-based meta-analysis identified 192 SNPs associated with circulating FGF23 at genome-wide significance level ($P<5\times10^{-8}$). These SNPs were located in five genomic regions, 5q35.3, 9q21.11, 9q34.2, 16q23.2, and 20q13.2 (Figure 1). The top SNP in each region and genes contained in the region were 20q13.2, rs17216707 ($P=3.0\times10^{-24}$; *CYP24A1*); 9q34.2, rs2769071 ($P=6.1\times10^{-17}$; *ABO*); 5q35.3, rs11741640 ($P=1.6\times10^{-16}$; *RGS14*); 9q21.11, rs17479566 ($P=2.0\times10^{-9}$; *LINC01506*); and 16q23.2, rs9925837 ($P=5.1\times10^{-9}$; *LINC01229*). In aggregate, the top five loci explained 3% of the variability in circulating FGF23. Inspection of the quantile-quantile plot suggested an excess of association signals beyond those expected (Supplemental Figure 1). All 341 SNPs from the meta-analysis with a *P* value less than a suggestive significance threshold of $P<5\times10^{-6}$ are detailed in Supplemental Table 2.

The top SNP in the 20q13.2 region, having the strongest association with FGF23 concentration, was rs17216707 ($P=3.0\times10^{-24}$; Supplemental Figure 2A, Table 2). This intergenic SNP lies 38 kb upstream of *CYP24A1* (cytochrome P450, family 24, subfamily A, polypeptide 1). Each additional copy of the rs17216707 T allele was associated with 5.4% higher FGF23 concentration, after adjustment for age, sex, and the first ten principal components of ancestry (model 1). The *CYP24A1* gene encodes the major enzyme responsible for catabolizing calcitriol to water-soluble 1,24,25-trihydroxyvitamin D (1,24,25(OH)$_3$D) and 25(OH)D to 25-hydroxyvitamin D (25(OH)D) to 24,25-dihydroxyvitamin D (24,25(OH)$_2$D) for excretion.[49]

rs2769071, a variant on chromosome 9q34.2, intronic in the *ABO* (ABO blood group transferase gene), was also associated with FGF23 concentrations ($P=6.1\times10^{-17}$; Supplemental Figure 2B, Table 2). Every additional minor allele at the locus was associated with 3.7% higher circulating FGF23 concentrations. The association did not remain statistically significant after adjustment for BMI, eGFR, and eGFR squared ($P=3.0\times10^{-5}$).

Within the 5q35.3 region, the variant most strongly associated with circulating FGF23 concentrations was rs11741640 ($P=1.6\times10^{-16}$; Table 2). This SNP is intronic within *RGS14* (regulator of G-protein signaling 14) and is in linkage disequilibrium (LD) with SNPs in adjacent genes, *SLC34A1* (solute carrier family 34 [type 2 sodium/phosphate cotransporter], member 1, responsible for phosphate reabsorption in the proximal tubule) (*e.g.*, rs12659266, $R^2=0.78$), and *FGFR4*

Table 2. Associations of top single nucleotide polymorphisms with ln-transformed FGF23 concentrations

SNP	Nearest Gene[a]	Chr	Position	FGF23 Increasing Allele	Other Allele	FGF23 Increasing Allele Frequency[b]	Model 1 (*n*=16,624)		Model 2[c] (*n*=15,496)	
							β[d] (SEM)	*P* Value	β (SEM)	*P* Value
rs17216707	*CYP24A1*	20	52732362	T	C	0.80	0.054 (0.005)	3.0×10^{-24}	0.044 (0.005)	1.5×10^{-21}
rs2769071	*ABO*	9	136145974	G	A	0.37	0.037 (0.005)	6.1×10^{-17}	0.031 (0.008)	3.0×10^{-5}
rs11741640	*RGS14*	5	176792743	G	A	0.73	0.039 (0.005)	1.6×10^{-16}	0.038 (0.005)	2.8×10^{-16}
rs17479566	*LINC01506*	9	71198013	T	C	0.22	0.031 (0.005)	2.0×10^{-9}	0.039 (0.009)	6.8×10^{-6}
rs9925837	*LINC01229*	16	79927303	G	A	0.13	0.035 (0.006)	5.1×10^{-9}	0.035 (0.006)	2.1×10^{-9}

Only the top SNP from each region is shown. Model 1 includes age, sex, and first ten principal components of ancestry. SNP, single nucleotide polymorphism; Chr, chromosome.

[a]Nearest gene by physical distance to the lead SNP.

[b]Allele frequency data from 1000 Genomes Phase 1 genotype data.

[c]Results for model 2 were not available for the Indiana Sisters Study.

[d]β-estimates are interpreted as the relative difference in FGF23 concentration per minor allele; *e.g.*, 0.051 is a 5.1% higher FGF23 concentration per additional allele.

(fibroblast growth factor receptor 4) (*e.g.*, rs244707, R^2=0.43) (Supplemental Figure 2C).[11,50,51]

Other top SNPs significantly associated with FGF23 included rs17479566, an SNP in the 9q21.11 region, residing intergenically between *LINC01506* (long intergenic non–protein coding RNA 1506) and *PIP5K1B* (phosphatidylinositol-4-phosphate 5-kinase type 1 β; Supplemental Figure 2D), and rs9925837, an intergenic SNP positioned between *LINC01229* (long intergenic non–protein coding RNA 1229) and *LOC102724084* (uncharacterized noncoding RNA) in the 16q23.2 region (P=5.1×10^{-9}; Supplemental Figure 2E, Table 2).

The primary regression coefficients and interpretation of our results were not affected by further adjustment for BMI, eGFR, and eGFR squared (model 2) for rs17216707, rs11741640, or rs9925837 (Supplemental Figure 3). However, the *P* values for SNPs rs2769071 and rs17479566 were attenuated by factors of 10^{12} and 10^{3}, respectively (Table 2).

Replication in Individuals of African Ancestry

In populations of African ancestry, the effect estimates for the five top SNPs were in the same direction as in individuals of European ancestry and one SNP (rs9925837) was nominally associated ($P<0.05$) with FGF23 concentrations (Table 3). Rs17216707, rs11741640, and rs9925837 showed the most allelic differentiation between individuals of African and European ancestry.

Sensitivity Analysis

We repeated our meta-analysis after excluding the CHS and OPRA, in which circulating concentrations of C-terminal FGF23 were measured. This meta-analysis identified 161 SNPs associated with circulating intact FGF23 at genome-wide significance level ($P<5\times10^{-8}$), located in four genomic regions, 5q35.3, 9q21.11, 9q34.2, and 20q13.2. The top SNP in each region and genes contained in the region were 20q13.2, rs17216707 (β [SEM]=0.057 [0.006]; $P=1.0\times10^{-25}$; *CYP24A1*); 9q34.2, rs2769071 (β [SEM]=0.037 [0.005]; $P=7.1\times10^{-16}$; *ABO*); 5q35.3, rs11741640 (β [SEM]=0.038 [0.005]; $P=1.6\times10^{-14}$; *RGS14*); and 9q21.11, rs17479566 (β [SEM]=0.032 [0.005]; $P=3.8\times10^{-9}$; *LINC01506*) (Supplemental Table 3). Although the association at rs9925837 did not meet genome-wide significance, the magnitude of the effect was not meaningfully different after exclusion of CHS and OPRA (β [SEM]=0.032 [0.006], $P=2.2\times10^{-7}$).

Table 3. Associations of top single nucleotide polymorphisms with ln-transformed FGF23 concentrations among individuals of African ancestry (*n*=4443)

SNP	Nearest Gene	Chr	Position	FGF23 Increasing Allele	Other Allele	FGF23 Increasing Allele Frequency[a]	β[b] (SEM)	*P* Value	Fst[c]
rs17216707	*CYP24A1*	20	52732362	T	C	0.97	0.044 (0.037)	0.24	0.162
rs2769071	*ABO*	9	136145974	G	A	0.48	0.010 (0.012)	0.41	0.009
rs11741640	*RGS14*	5	176792743	G	A	0.96	0.008 (0.027)	0.78	0.195
rs17479566	*LINC01506*	9	71198013	T	C	0.93	0.022 (0.020)	0.29	0.087
rs9925837	*LINC01229*	16	79927303	G	A	0.28	0.029 (0.015)	0.05	0.232

Only the top SNP from each region is shown. SNP, single nucleotide polymorphism; Chr, chromosome.

[a]Allele frequency data from 1000 Genome Phase 1 genotype data among African samples.

[b]Effect estimate adjusted for age, sex, and first ten principal components of ancestry. β-estimates are interpreted as the relative difference in FGF23 concentration per minor allele; *e.g.*, 0.038 is a 3.8% higher FGF23 concentration per additional allele.

[c]Fst is an estimate of genetic differentiation calculated using the variance in allele frequencies among European- and African-ancestry samples from the 1000 Genomes and standardized according to the mean allele frequency in the combined sample.[36]

Table 4. Association between replicated SNPs and FGF23-related phenotypes

SNP	Nearest Gene	Allele	Coronary Artery Disease[a]		Bone Mineral Density[b] (standardized mean difference)		Parathyroid Hormone[c] (% difference)		eGFR[d] (ml/min per 1.73 m^2)	
			OR (SEM)	*P* Value	*β* (SEM)	*P* Value	*β* (SEM)	*P* Value	*β* (SEM)	*P* Value
rs17216707	*CYP24A1*	T	0.98 (0.012)	0.13	−0.0036 (0.0044)	0.26	−5.4% (0.053)	4.7×10^{-25}	−0.97% (0.13)	5.4×10^{-15}
rs2769071	*ABO*	G	1.05 (0.01)	3.3×10^{-6}	−0.0197 (0.0036)	2.7×10^{-8}	+1.2% (0.044)	6.3×10^{-4}	+0.01% (0.10)	0.91
rs11741640	*RGS14*	G	0.98 (0.010)	0.04	−0.0049 (0.0038)	0.10	−3.1% (0.031)	3.4×10^{-24}	+0.75% (0.11)	4.5×10^{-12}
rs17479566	*LINC01506*	T	0.98 (0.011)	0.10	+0.0066 (0.0041)	0.18	−1.7% (0.047)	5.1×10^{-5}	+0.56% (0.12)	1.5×10^{-6}
rs9925837	*LINC01229*	G	1.00 (0.013)	0.87	−0.0035 (0.0047)	0.62	+1.0% (0.055)	0.02	+0.51% (0.14)	1.7×10^{-4}

In total, we performed 20 tests, corresponding to four traits tested for association against the five SNPs. Significance was evaluated at the Bonferroni-corrected level of 0.0025. SNP, single nucleotide polymorphism; OR, odds ratio; GWAS, Genome-wide association study.

[a]Association estimates from GWAS meta-analysis of coronary artery disease among 60,801 cases and 123,504 controls from 48 studies within the Coronary Artery Disease Genome Wide Replication and Meta-Analysis Consortium.[46]

[b]Estimates from meta-analysis of bone mineral density of the femoral neck GWAS among 32,961 individuals from 17 studies within the Genetic Factors for Osteoporosis Consortium.[48]

[c]Estimates from meta-analysis of parathyroid hormone GWAS among 29,155 individuals from 13 cohort studies.[47]

[d]Estimates from meta-analysis of eGFR in 110,517 individuals from 33 studies within the Chronic Kidney Disease Genetics (CKD-Gen) CKDGen Consortium.[45]

Functional Evaluation

Of the 192 SNPs that met genome-wide significance ($P<5\times10^{-8}$) in the primary analysis, 98 were in intergenic regions, 84 overlapped introns, one was exonic (rs5030873, for *SLC34A1*), one overlapped a 3′ untranslated region (rs3812035, for *SLC34A1*), four overlapped the 1-kb region upstream of a transcription start site (all for *RGS14*), and four overlapped the 1-kb region downstream of a transcription end site (three for *RGS14* and one for *LMAN2*) (Supplemental Table 2). Analysis with MetaXcan to evaluate the association with genetically predicted gene expression implicated *RGS14* as the most significant genetic result and revealed a significant association of FGF23 with increased expression at that gene in multiple tissues (Supplemental Table 4).

Assessment for Association of SNPs with Related Traits

Each of the top SNPs was associated with parathyroid hormone concentration; four of the five were significantly associated at the Bonferroni-corrected *P* value threshold of 0.003 (Table 4). We also observed associations of four of the five SNPs with eGFR, and of rs2769071 with coronary artery disease and bone mineral density. At this locus, the FGF23 increasing allele was associated with 4.5% greater odds of coronary artery disease ($P=3.3\times10^{-6}$) and lower BMD ($\beta=-0.0197$, $P=2.7\times10^{-8}$).

DISCUSSION

In this first reported GWAS of FGF23, we identified five loci, located on chromosomes 5, 9, 16, and 20, that were associated with variation in FGF23 concentrations. The strongest association was observed for rs17216707, located 37 kb upstream of *CYP24A1*, encoding the primary catabolic enzyme for calcitriol [1,25$(OH)_2$D]. Every additional T allele at this locus was associated with a 5.1% difference in circulating FGF23. Other significant associations included SNPs located near the gene encoding proteins related to the first discovered blood group system, ABO, and genes encoding the regulator of G-protein signaling 14 and the Na/Pi cotransporter solute carrier family 34 member 1. In replication analyses conducted in blacks, the effect estimates for the top five SNPs were in the same direction as in the individuals of European ancestry; however, confidence intervals were wider, and the results were not statistically significant.

Calcitriol, the activated form of vitamin D, directly promotes FGF23 gene transcription *via* enhancement of the promotor region. The cytochrome p450 enzyme CYP24A1 is critically important for maintaining 1,25$(OH)_2$D concentrations within a tight physiologic range and preventing vitamin D toxicity by catalyzing the conversion of 1,25$(OH)_2$D to 1,24,25$(OH)_3$D for subsequent excretion.[49] We found the T allele of rs17216707 to be associated with greater circulating FGF23 concentrations, suggesting that the associated haplotype may confer suppressed *CYP24A1* activity, with a resultant increase in 1,25$(OH)_2$D and stimulation of FGF23

production. The T allele was also strongly associated with lower eGFR in previous GWASs,[52,53] and yet adjustment for eGFR in the association of the SNP with FGF23 did not alter the magnitude of the association. The association of the T allele of rs17216707 with lower serum concentrations of PTH is also consistent with suppressed *CYP24A1* activity, decelerated $1,25(OH)_2D$ catabolism, and increased $1,25(OH)_2D$ levels. In murine homolog models of autosomal dominant phosphatemic rickets (ADHR) and X-linked hypophosphatemic rickets (XLH), high FGF23 states, *CYP24A1* expression in the kidney is elevated.[54,55] Interestingly, when these mouse strains were crossed with *CYP24A1*-null mice, the bone abnormalities improved, without improvements in the serum biochemical profile.[56]

rs2769071 is located within the *ABO* blood group locus on chromosome 9, which encodes alpha 1–3-N-acetylgalactosaminyltransferase, a major determinant of ABO blood type. The C allele at rs2769071 is highly correlated (r^2=0.86) with a single base pair insertion/deletion (indel) variant in exon 6 of *ABO* (rs8176719) which characterizes the human O blood group.[57] This SNP is associated with *ABO* gene expression in most tissues in GTEx. Using available GWAS data of other phenotypes, we observed a significant association of variation at this locus to be associated with higher odds of coronary artery disease, lower bone mineral density, and higher parathyroid hormone levels. In previous published GWASs, this SNP has been found to be associated with plasma vWf and Factor VII,[58] coagulation factors levels,[59] and myocardial infarction in patients with angiographic coronary artery disease.[60] Recent studies have suggested that serum iron may be a regulator of FGF23 homeostasis, and ferritin levels have been shown to differ between blood groups.[61–63] The attenuation of the statistical significance of the association with adjustment for eGFR and BMI suggests that the contribution of genetic risk to FGF23 within the *ABO* loci may be mediated by the SNP's effects on these covariates. However, variation at rs2769071 was not associated with circulating eGFR[45] in the CKD-GEN GWAS or BMI in the GWAS of 700,000 individuals from the GIANT Consortium.[64] The functional relevance of this polymorphism to mineral metabolism and FGF23 regulation awaits further elucidation.

rs11741640 is located within *RGS14*, which encodes a G-protein signal–regulating protein and was the only replicated SNP significantly associated with both intact and C-terminal FGF23. Previous GWASs of serum phosphate and parathyroid hormone concentrations reported strong associations with rs4074995, which is in near-perfect LD with rs11741640 (R^2=0.93).[47,65] In our gene-level expression-based analyses with MetaXcan, *RGS14* was the gene with the strongest association of genetically determined expression with FGF23 levels. We found that increased expression of *RGS14* was associated with higher levels of FGF23 across many tissues, including in heart and muscle tissue, lending biologic support to its implication in mineral metabolism. rs11741640 is also in strong LD with multiple SNPs within the gene coding for type 2a sodium phosphate cotransporter (Npt2a, *SLC34A1*) and fibroblast growth factor receptor 4 (*FGFR4*). FGF23 acts on the kidney to induce phosphaturia by reducing the expression of Npt2a in the brush border of the proximal tubular cells, and FGFR4 has been proposed as a mediator in the associations of FGF23 with left ventricular hypertrophy.[11]

Strengths of our study include the large and diverse sample, the population-based settings, and the comprehensive set of common genetic variants examined. Potential limitations include a restriction to common variants only, discovery efforts in an exclusively European ancestry sample, limited African ancestry and cFGF23 samples, and a lack of kidney or bone tissue in the gene expression–based association methods.

Although the observed variants were associated with small differences in FGF23 concentrations, a difference in circulating FGF23 of 5% is a risk equivalent for heart failure in the MESA study of 15 ml/min per 1.73 m^2 lower eGFR or 5 mm Hg higher systolic BP.[4] Further, it is possible that the effects may be more pronounced among individuals with mineral metabolism disturbances, such as those with CKD mineral and bone disorder. It will be crucial to test these results among individuals with CKD, in whom the biologic implications and potential treatment options are most relevant. The clinical relevance of the GWAS findings may not be specifically the magnitude of the difference in FGF23 concentrations, but the underlying biology that is revealed.

In summary, we demonstrate that common genetic variants are associated with circulating FGF23 in adults from the general population. Several of the genetic variants are closely linked with enzymes, transporters, and receptors known to be critical to vitamin D and phosphate homeostasis. Further studies are needed to confirm the SNPs and target loci and elucidate the biologic role of the implicated genes. Candidate genes may be explored in more comprehensive metabolic studies of FGF23 metabolism and in translational animal models that will shed new light on the mechanisms and clinical implications of FGF23 in vitamin D and phosphate homeostasis and its treatment.

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DISCLOSURES

None.

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