ORIGINAL RESEARCH

Plasma Fibroblast Growth Factor 23: Clinical Correlates and Association With Cardiovascular Disease and Mortality in the Framingham Heart Study

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***Background*—**Fibroblast growth factor 23 (FGF23) is emerging as a novel biomarker of bone metabolism, chronic kidney disease, and cardiovascular disease (CVD). However, its clinical correlates and potential predictive role in a community-based setting are incompletely understood.

***Methods and Results*—**We evaluated participants of the Framingham Heart Study (seventh examination cycle of the Offspring cohort plus second examination cycle of the multiethnic Omni cohort) to identify clinical correlates of plasma FGF23 (N=3236) and examine its cross-sectional association with vascular function (N=2209), and longitudinal association with 10-year incidence of CVD (N=2823), and all-cause mortality (N=3223).Circulating FGF23 concentrations were positively related to African-American and Asian ethnicity, waist circumference, current smoking, serum glucose, history of CVD, and antihypertensive medication use; and negatively related to male sex, hormone replacement therapy, and estimated glomerular filtration rate. Multivariable-adjusted cross-sectional analyses showed no consistent association of FGF23 with vascular function measures. During a median follow-up time of 10.8 years, 347 incident CVD events and 412 deaths occurred. Multivariable-adjusted Cox regression models revealed a positive association of FGF23 with all-cause mortality (hazard ratio [HR] per SD increase, 1.31; 95% CI, 1.20–1.42), but not with incident CVD (HR per SD increase, 1.05; 95% CI, 0.94–1.17).

***Conclusions*—**In our large, community-based sample, FGF23 was associated with mortality risk, but not with vascular function or incident CVD. **(*J Am Heart Assoc*. 2016;5:e003486 doi: 10.1161/JAHA.116.003486)**

Key Words: cardiovascular disease risk factors • epidemiology • metabolism • mineral

Fibroblast growth factor 23 (FGF23) is a circulating peptide hormone secreted by bone cells, regulating phosphate and vitamin D metabolism.[1] In chronic kidney disease (CKD) patients, the decline in renal function is paralleled by a rise in FGF23 concentrations, with often dramatically elevated levels in end-stage renal disease. Additionally, several recent observational studies reported an independent association of circulating FGF23 with several cardiovascular disease (CVD) risk factors,[2] including left ventricular hypertrophy and vascular dysfunction, CVD progression,[3–5] and incident clinical CVD events[6] and mortality.[7–9] These adverse prognostic implications of high FGF23 concentrations have stimulated interest in exploring the potential role of FGF23 as mainly a marker of disordered mineral metabolism.[10]

Accordingly, we used data from the Framingham Heart Study (FHS) to (1) evaluate clinical correlates, heritability, and

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Accompanying Tables S1 and S2 and Figures S1 and S2 are available at http://jaha.ahajournals.org/content/5/7/e003486/DC1/embed/inline-supplementary-material-1.pdf

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genome-wide linkage of FGF23 concentrations and (2) examine the potential association of this novel biomarker with vascular function measures, incident 10-year CVD, and all-cause mortality in a community-based sample.

Methods

Study Population

The FHS is a community-based, longitudinal, epidemiological cohort initiated in 1948 in Framingham, Massachusetts, to investigate CVD and its risk factors. The FHS Offspring Study started in 1971, to examine 5124 adult children (and offspring spouses) of the original FHS cohort approximately every 4 to 8 years.[11] In 1994, the FHS Omni cohort began with the recruitment of 506 men and women aged between 40 and 74 years who identified themselves as African American, Hispanic, Asian, Indian, Pacific Islander, or native American residents of Framingham, Massachusetts, and 24 surrounding towns.[12] From the 3539 participants of in-person clinical evaluations at the seventh Offspring examination cycle (1998–2001) and 405 participants at the second Omni examination cycle (1999–2001), we excluded participants because of missing FGF23 data (N=627), incomplete covariate data (N=50), prevalent CKD (N=7), and serum creatinine concentrations ≥2 mg/dL (N=24), yielding a final study population of 3236 individuals (2846 Offspring, 390 Omni) for the cross-sectional analyses of clinical correlates.

Cross-sectional associations between FGF23 and vascular function were examined in 2209 individuals (1937 Offspring, 272 Omni) after the additional exclusion of 396 and 631 individuals with missing vascular function measures and/or covariate data, respectively. Longitudinal analyses of 10-year all-cause mortality and incident CVD events were performed in 3223 individuals (2844 Offspring, 379 Omni) and, after the additional exclusion of individuals with prevalent CVD, in 2823 individuals (2485 Offspring, 338 Omni), respectively. The study protocol was reviewed and approved by the Institutional Review Board of the Boston University Medical Center (Boston, MA). All participants gave written informed consent.

Measures

Clinical covariates, sociodemographic and behavioral characteristics, as well as medical history and medication use, were assessed by standardized questionnaires, physical examination, and laboratory tests. Current smokers were defined as those who reported having smoked ≥1 cigarette per day regularly during the year preceding the examination. Waist circumference, height, and weight were measured and body mass index (BMI) was calculated (kg/m^2). Blood pressure (BP) was measured twice in the left arm of the seated subject with a mercury column sphygmomanometer. The average of the 2 readings was used as the examination BP, and hypertension was defined as systolic BP ≥140 mm Hg, or a diastolic BP ≥90 mm Hg, or self-reported antihypertensive medication use. Type 2 diabetes mellitus (T2DM) was defined by a fasting glucose level ≥126 mg/dL, or self-reported use of insulin, or oral hypoglycemic medications. Kidney function was assessed using the estimated glomerular filtration rate (eGFR) calculated by the CKD-Epi study equation.[13] Vascular function measures were assessed and defined as previously described in detail[14–16] using a commercially available tonometer (SPT-301; Millar Instruments, Houston, TX). Simultaneous electrocardiogram was used to obtain arterial waveforms from the carotid, brachial, radial, and femoral arteries (all on the right side), as well as supine brachial systolic and diastolic BP. CVD was assessed according to previously reported standardized protocols (including *coronary heart disease* [recognized or unrecognized myocardial infarction, angina pectoris, coronary insufficiency, or coronary heart disease death]; *cerebrovascular disease* [stroke or transient ischemic attack]; or *congestive heart failure*) and confirmed with the aid of medical histories, physical examinations at the Heart Study clinic, review of hospitalization records, and final adjudication by the endpoint committee.[11] Similarly, mortality data were obtained by review of all medical records and from death certificate evaluation according to previously described criteria.[17]

Laboratory Measurements

Serum samples were drawn from the antecubital vein in the supine position between 8:00 and 9:00 AM, after an overnight fast of around 10 hours. Samples were aliquoted and immediately stored at −80°C and remained frozen until the time of assay. Plasma total cholesterol and high-density lipoprotein (HDL) cholesterol concentrations were measured using standard enzymatic methods, as previously described.[18] Fasting plasma glucose was measured in fresh specimens with a hexokinase reagent kit (A-Gene glucose test; Abbot, South Pasadena, CA) and triglyceride was measured enzymatically with intra-assay coefficients of variation (CV) <3%. Plasma FGF23 concentrations were measured using the Human FGF23 (C-TERM) ELISA kit (Immutopics, San Clemente, CA). The lower and upper limit of detection was 18 and 435 RU/mL, respectively. The functional sensitivity of the assay was 1.5 RU/mL with an intraassay CV of 7.5% at the low level and 4.3% at the high level.

Statistical Analyses

Study sample characteristics were presented based on pooled data from Offspring and Omni cohort participants. We natural logarithmically transformed FGF23 concentrations to normalize

its distribution. To identify clinical correlates of FGF23, age-, sex-, and ethnicity-adjusted step-wise multivariable linear regression, models implemented the following covariates by $P<0.05$ inclusion criteria: BMI, waist circumference, systolic BP, diastolic BP, smoking, total/HDL cholesterol ratio, triglycerides, glucose, T2DM, history of CVD, medications (lipid-lowering, antihypertensive, or hormone replacement therapy), cohort (Offspring and Omni), and kidney function (eGFR). To compare the relative effects across different correlates, estimates were expressed as standardized linear regression coefficients (β) and their corresponding 95% CI. We also examined nonlinear age effects by including a quadratic term for age into the multivariable regression models and assessing the *P* value for the term. Because the "squared age" term was not statistically significant at the $P<0.05$ level and did not alter the final set of covariates, we did not include it in further regression modeling.

Heritability analysis

Heritability analyses were performed among FHS offspring cohort members with available phenotypic and genetic data (N=2611). Heritability was estimated based on the covariates that remained in the step-wise regression models using the Sequential Oligogenic Linkage Analysis Routines (SOLAR) package to fit a variance-components model.[19] Thus, heritability denotes the proportion of phenotypic variance explained by genetic effects, after accounting for covariates. We determined a multivariable-adjusted heritability estimate including age, sex, ethnicity, waist circumference, current smoking, glucose, history of CVD, antihypertensive medication, hormone replacement therapy, and eGFR as covariates.

Vascular function measures

In a first step, we examined cross-sectional age-, sex- and height-adjusted linear regression models including FGF23 as a continuous dependent variable. Multivariable modeling included adjustment for age, sex, height, weight, waist circumference, ethnicity, heart rate, mean arterial pressure, total/HDL-cholesterol ratio, triglycerides, glucose, T2DM, history of CVD, antihypertensive medication, antilipidemic medication, smoking, hormone replacement therapy, and eGFR. Estimates were expressed as beta coefficients (β) together with their 95% CI.

10-year risk of incident CVD and mortality

First, we assessed penalized splines to explore potential nonlinear associations of continuous FGF23 concentrations with 10-year CVD and mortality risk. Next, age- and sex-adjusted, as well as multivariable-adjusted, Cox proportional hazard regression analyses modeled FGF23 on a continuous scale, as well as categorized into sex-specific quartiles (with first quartile as a reference category). Multivariable modeling included adjustment for age, sex, BMI, systolic BP, antihypertensive treatment, total/HDL cholesterol ratio, smoking, T2DM, and cohort. Multivariable analyses of 10-year all-cause mortality were additionally adjusted for prevalent CVD. For cause-specific mortality analyses, causes of death were categorized into CDV death, cancer death, and other death. Estimates were expressed as hazard ratio (HR) together with their 95% CI. We estimated C statistics according to methods previously described by Pencina and D'Agostino.[20] The validity of the proportional hazards assumption was assessed by the inclusion of a multiplicative interaction term of ln (FGF23) and log(follow-up time) into the multivariable models. For CVD, the proportionality of hazards assumption was valid, whereas a significant interaction term indicating nonproportionality for the mortality outcome ($P=0.01$) was handled using different baseline hazards for the 3 follow-up time intervals 0 to 5, 5 to 10, >10 years. Dropout analysis revealed that, individuals excluded for missing FGF23 were significantly older, predominantly white, and showed higher BMI and lower HDL-cholesterol. Given these differences in terms of age and cardiovascular risk factor burden, the exclusion of these higher-risk individuals with missing FGF23 may have potentially contributed to an underestimation of the true association of FGF23 with CVD and mortality (Table S1). Two-sided probability values <0.05 were considered statistically significant. All statistical analyses except for heritability and linkage analyses were performed using SAS statistical software (version 9.3; SAS Institute Inc., Cary, NC).

Results

Clinical Correlates and Heritability Analysis

Baseline characteristics of the N=3236 individuals in the pooled study sample are displayed in Table 1. We identified positive correlations of plasma FGF23 with age, African-American and Asian ethnicity, waist circumference, current smoking, glucose, history of CVD, and antihypertensive medication. Correlates inversely associated with plasma FGF23 concentrations included male sex, hormone replacement therapy, and eGFR (Table 2). Next to male sex, the strongest single clinical correlate of FGF23 was kidney function (β per 1 unit increase in eGFR, −0.16 RU/mL; 95% CI, −0.20 to −0.12 RU/mL). Interaction analysis yielded a highly significant interaction between age and eGFR ($P=0.002$), but not for smoking and eGFR ($P=0.288$). The multivariable-adjusted heritability estimate was 0.188 ($P=0.002$), and 9 statistical models with increasing levels of complexity were used to partition variance in FGF23 determined by heritability and covariates. Without any covariates, heritability explained 17.2% of the interindividual variability in

Table 1. Baseline Characteristics of the Study Population (N=3236)

Characteristic	
Age, y	58.8±11.2
Male sex, %	45.6
Ethnicity, %	
White	88.0
African American	4.8
Hispanic	4.2
Asian	3.0
Native American	0.1
Omni cohort, %	12.1
Current smoker, %	13.0
BMI, kg/m^2	28.1±5.4
Waist circumference, cm	99.6±14.5
Fibroblast growth factor 23, RU/mL	67.0 (54.0, 85.0)
Estimated glomerular filtration rate, mL/min per 1.73 m^2	70.6±16.3
Serum creatinine, mg/dL	1.04±0.2
Fasting serum glucose, mg/dL	104±30
Type 2 diabetes mellitus, %	11.2
Triglycerides, mg/dL	135±83
Total cholesterol, mg/dL	200±37
HDL cholesterol, mg/dL	54±17
LDL cholesterol, mg/dL	120±34
Ratio total: HDL-cholesterol, mg/dL	4.0±1.4
Antilipidemic medication, %	19.8
Systolic blood pressure, mm Hg	127±19
Diastolic blood pressure, mm Hg	75±10
Pulse pressure	52±16
Heart rate	64±10
Vascular measures	
Carotid-femoral pulse wave velocity, m/s	9.9±3.3
Mean arterial pressure, mm Hg	91.7±12.1
Primary pressure wave amplitude, mm Hg	40.3±12.7
Flow-mediated dilatation, %	2.4 (0.8, 4.4)
Baseline flow, cm/s	8.0±4.7
Hyperemic flow, cm/s	51.3±21.2
Augmentation index, %	15.2±12.8
Antihypertensive medication, %	32.8
Hypertension, %	45.7
History of cardiovascular disease, %	12.4
Hormone replacement therapy, %	18.6

Data are absolute number (percentages), mean±SD, or median (Q1, Q3). HDL indicates high-density lipoprotein; LDL, low-density lipoprotein.

Table 2. Clinical Correlates of Fibroblast Growth Factor 23 Based on Multivariable Analyses (N=3236)

	FGF23	
Correlates	Beta Coefficient (95% CI)	*P* Value
Age	0.011 (−0.037, 0.059)	0.650
Male sex	−0.194 (−0.232, −0.157)	<0.001
Ethnicity, African American	0.044 (0.006, 0.082)	0.024
Ethnicity, Hispanic	−0.005 (−0.042, 0.032)	0.794
Ethnicity, Asian	0.057 (0.021, 0.093)	0.002
Ethnicity, Native American	−0.002 (−0.035, 0.031)	0.904
Waist circumference	0.098 (0.062, 0.134)	<0.001
Smoking	0.118 (0.084, 0.151)	<0.001
Fasting serum glucose	0.052 (0.017, 0.087)	0.004
History of CVD	0.099 (0.064, 0.134)	<0.001
Antihypertensive medication	0.059 (0.023, 0.095)	0.001
Hormone replacement therapy	−0.078 (−0.114, −0.041)	<0.001
eGFR	−0.162 (−0.202, −0.122)	<0.001

FGF23 was naturally log-transformed; therefore the coefficient (coef.) indicates an e^{β}-fold change FGF23. For example: β for age (log-FGF23)=0.01→$e^{0.01}$=1.01-fold increase in FGF23 concentration per year. BMI indicates body mass index; CVD, cardiovascular disease; eGFR, estimated glomerular filtration rate; FGF23, fibroblast growth factor 23.

plasma FGF23 concentrations. Further step-wise adjustment increased the explained variability in FGF23 by the covariates constantly up to 9.6% in the complete multivariable model, with a concurrent heritability estimate of 18.8% (Figure S1).

Vascular Function Measures

After relating FGF23 to primary (hyperemic flow, carotid femoral pulse wave velocity [PWV], mean arterial pressure, primary pressure wave, and augmented pressure) and secondary (augmentation index) measures of arterial stiffness in cross-sectional multivariable-adjusted models, we detected only a borderline statistically significant association (β, 0.46; 95% CI, 0.01–0.92) with baseline flow (Table 3).

Incident CVD and Mortality

During a median follow-up time of 10.8 years (Q1, 10.0; Q3, 11.4), incident CVD occurred in 347 individuals, and 412 individuals died. Kaplan–Meier curves for incident CVD and all-cause mortality by FGF23 quartiles (Figure) depict that individuals with plasma FGF23 concentrations in the highest quartile experienced significantly lower cumulative survival (P<0.001), but there was no statistically significant difference in CVD incidence (P=0.10). In Table 4, age- and sex-adjusted Cox proportional-hazard regression models revealed a significant positive association of continuous FGF23 with incident CVD (HR

Table 3. Cross-Sectional Associations of Fibroblast Growth Factor 23 With Measures of Vascular Function (N=2209)

	Carotid-Femoral PWV (m/s)	Primary Pressure Wave Amplitude (mm Hg)	Flow-Mediated Dilatation (%)	Hyperemic Flow (cm/s)	Baseline Flow (cm/s)	Augmentation Index (%)
Beta coefficient (95% CI)						
Model 1	5.89 (3.48, 8.30)*	1.18 (−0.03, 2.39)	0.01 (−0.27, 0.28)	−1.04 (−3.02, 0.94)	0.61 (0.14, 1.08)*	0.34 (−0.87, 1.56)
Model 2	1.52 (−0.37, 3.42)	0.33 (−0.79, 1.44)	0.20 (−0.05, 0.45)	1.07 (−0.82, 2.96)	0.46 (0.01, 0.92)*	0.38 (−0.74, 1.50)

Model 1 was adjusted for age, sex, and height. Model 2 was multivariable-adjusted for age, sex, height, weight, waist circumference, ethnicity, heart rate, mean arterial pressure, total and high-density lipoprotein cholesterol ratio, triglycerides, glucose, type 2 diabetes mellitus, history of CVD, antihypertensive medication, antilipidemic medication, smoking, hormone replacement therapy, and estimated glomerular filtration rate. CVD indicates cardiovascular disease; PWV, pulse wave velocity.
*$P<0.05$.

per SD increase, 1.16; 95% CI, 1.04–1.29) and all-cause mortality (HR per SD increase, 1.42; 95% CI, 1.30–1.54). Similarly, individuals with FGF23 concentrations in the highest quartile (vs lowest quartile) showed an increased risk of incident CVD (HR, 1.51; 95% CI, 1.13–2.03) and all-cause mortality (HR, 2.27; 95% CI, 1.69–3.05). After multivariable adjustment, the association of FGF23 with incident CVD was rendered statistically nonsignificant (Table 4), whereas the positive association of FGF23 with all-cause mortality was preserved in continuous and categorical analyses (HR per SD increase, 1.31; 95% CI, 1.20–1.42). During cause-specific analyses, continuous FGF23 was associated with mortality across all different causes, in age- and sex-adjusted models, as well as in multivariable-adjusted models (Table S2). Finally, the spline curves presented in Figure S2 support the revealed positive, almost linear risk association of FGF23 with all-cause mortality, but not with incident CVD.

To assess the uniqueness of FGF23 as a biomarker in comparison with existing (and well-known) risk markers, we performed receiver operating characteristic analyses. At this, we compared multivariable models with and without adjustment for FGF23 and calculated corresponding C statistics with 95% CI. With regard to incident CVD, the model without FGF23 (0.74358; 95% CI, 0.71825–0.76891) and with FGF23 (0.744; 95% CI, 0.718–0.769) yielded a nonsignificant difference in C (0.0001; 95% CI, −0.001 to 0.002). Similarly, outcome analyses of all-cause mortality without FGF23 (0.774; 95% CI, 0.751–0.797) and with FGF23 (0.780; 95% CI, 0.757–0.802) yielded a nonsignificant difference in C (0.00566; 95% CI, −0.001 to 0.011), suggesting that FGF23 does not add any predictive value in terms of incident CVD risk or all-cause mortality risk, over and above the standard covariates already included in the multivariable model.

Discussion

Principal Findings

Using data from a large, multiethnic, community-based cohort, the identified correlates of plasma FGF23 included age, sex, ethnicity, waist circumference, current smoking, glucose, history of CVD, antihypertensive medication, and hormone replacement therapy. We observed that kidney function measured by eGFR was the strongest single clinical correlate of FGF23 after male sex, with genetic factors exerting only modest influences on the total interindividual variability in plasma FGF23 concentrations. Finally, FGF23 showed no consistent association with vascular function measures or incident clinical CVD, but was associated with all-cause mortality.

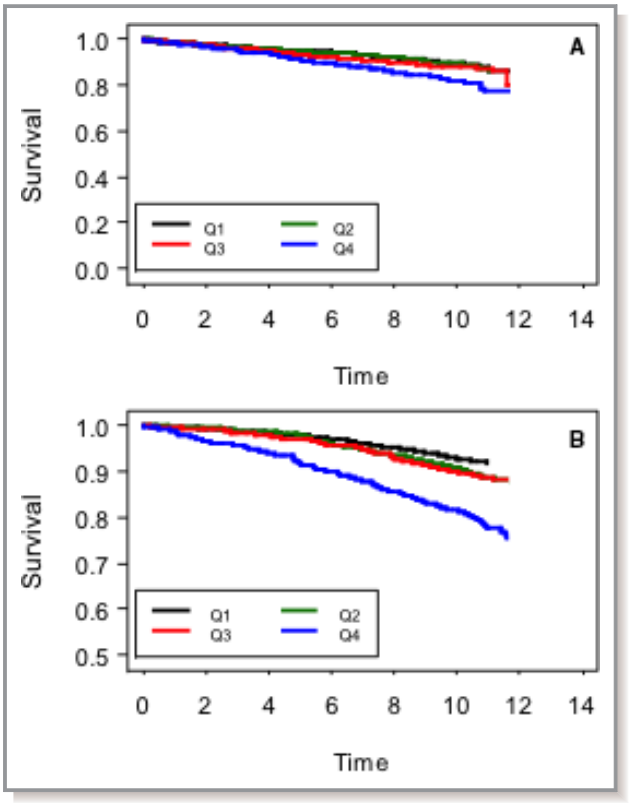

Figure. Kaplan–Meier curves for incident CVD (A) and all-cause mortality (B) by quartiles of plasma FGF23 concentrations. CVD indicates cardiovascular disease; FGF23, fibroblast growth factor 23.

Table 4. Longitudinal Associations of Fibroblast Growth Factor 23 With Incident 10-Year Cardiovascular Disease and All-Cause Mortality

FGF23	Incident CVD HR (95% CI)	All-Cause Mortality HR (95% CI)
Age- and sex-adjusted models		
Continuous (per SD increase)	1.16 (1.04, 1.29)*	1.42 (1.30, 1.54)*
<25th	Ref.	Ref.
25 to 50th	0.92 (0.67, 1.27)	1.23 (0.88, 1.72)
50 to 75th	1.07 (0.78, 1.46)	1.27 (0.91, 1.76)
>75th	1.51 (1.13, 2.03)*	2.27 (1.69, 3.05)*
Multivariable-adjusted models†		
Continuous (per SD increase)	1.05 (0.94, 1.17)	1.31 (1.20, 1.42)*
<25th	Ref.	Ref.
25 to 50th	0.90 (0.65, 1.24)	1.28 (0.92, 1.79)
50 to 75th	0.96 (0.70, 1.31)	1.22 (0.88, 1.70)
>75th	1.17 (0.87, 1.59)	1.87 (1.38, 2.53)*

Incident 10-year CVD and all-cause mortality risk was examined in N=2823 and N=3223 individuals, respectively. CVD indicates cardiovascular disease; FGF23, fibroblast growth factor 23; HR, harzard ratio.

*$P<0.05$.

†Multivariable HRs were adjusted for age, sex, body mass index, systolic blood pressure, antihypertensive medication, total and high-density lipoprotein cholesterol ratio, smoking, type 2 diabetes mellitus, and cohort. Multivariable HRs for all-cause mortality risk were additionally adjusted for prevalent CVD.

The identified clinical correlates of FGF23 are consistent with previous community-based studies linking FGF23 to traditional CVD risk factors, including smoking, adiposity, dyslipidemia, and metabolic syndrome components.[2,21–23] A cross-sectional investigation among 1261 participants of the Health Professionals Follow-up Study reported positive correlations of FGF23 with age, phosphorus intake, creatinine, BMI, smoking, and hypertension.[21] The additional assessment of biochemical parameters showed further correlations of FGF23 with parathyroid hormone, phosphate, uric acid, triglycerides, and inflammatory markers.[21] Parathyroid hormone and eGFR were also significantly correlated with FGF23 in multivariable analyses among 1000 elderly men from the cross-sectional population-based MrOS Sweden study.[24]

Similarly, cross-sectional analyses among 987 community-living individuals from the Heart and Soul study provide confirmatory findings, reporting that participants with higher FGF23 concentrations were more frequently white and hypertensive and showed lower eGFR and higher serum phosphorus concentrations, respectively.[23] Also the negative correlation of FGF23 with hormone replacement therapy, observed in the present study, is consistent with findings from the Heart and Soul study, showing that women on supplementation therapy expose significantly lower FGF23 concentrations.[23] Another cross-sectional investigation among 659 elderly women from the population-based Women's Health and Aging Studies showed FGF23 concentrations positively related to age, BMI, hypertension, and physical activity, respectively; and negatively related to educational level and eGFR.[25] Taken together, these community-based findings extend previous observations among highly selected study populations linking FGF23 concentrations to BMI and dyslipidemia in dialysis patients,[26] as well as to smoking, T2DM, and history of CVD,[22] or eGFR, cystatin C, serum phosphate, parathyroid hormone, hemoglobin,[8] and inflammation[27] in CKD patients.[28]

Given this apparent overlap between altered concentrations of plasma FGF23 concentrations and common cardiovascular risk factors, we were not able to detect any adjusted associations of FGF23 with subclinical or clinical CVD in cross-sectional and longitudinal analyses. Unlike previous reports noting associations of FGF23 with left ventricular hypertrophy,[29] vascular dysfunction,[30,31] incident CVD, and mortality in patient-based study populations with CKD,[8,28,32] coronary artery disease,[33] end-stage liver disease,[34] hemodialysis,[7] or kidney transplants,[35] in the present community-based sample of men and women from the general population FGF23 was not associated with vascular function or with incident CVD. Taken together, studies among healthy, non-CKD populations are much less consistent and suggest rather weak or absent associations of FGF23 with incident CVD and mortality. Whereas some studies reported positive associations of FGF23 with CVD and mortality,[25,36,37] others observed no such associations after multivariable adjustment.[38–40] For example, a 5-year follow-up of 3014 men from the MrOs Sweden study showed no association of FGF23 with all-cause and CVD mortality.[40] Similarly, in a nested case-control study of 1259 healthy men from the Health Professionals Follow-up Study, FGF23 was not related to incident coronary heart disease.[39] The present divergent associations of FGF23 with all-cause mortality, but not with incident CVD, are consistent with findings of a 10-year follow-up in the community-based Cardiovascular Health Study.[37] In this non-CKD subsample of 1979 healthy elderly individuals, higher FGF23 concentrations (highest vs lowest quartiles) were associated with all-cause mortality, but not with incident CVD.

Thus, the observed risk associations among CKD patients may have generated much interest in FGF23 as a novel nontraditional cardiovascular risk marker, but it appears that some reported associations are confined to these highly select patient samples. Consequently, it appears challenging to demonstrate adverse cardiovascular associations that (1) are specific to FGF23, (2) persist after adjusting for other factors of disordered mineral metabolism, and (3) add to established risk factors in terms of CVD risk prediction and discrimination. However, the field of FGF23 and its

association with CVD is still developing, and currently available studies have just laid the groundwork for future research.[10] For example, fractional excretion of phosphorus has been suggested as a modifier of the association of FGF23 with CVD events and all-cause mortality.[41] Others suggested circulating FGF23 concentration as a marker of existing CVD severity, rather than CVD onset or progression.[33]

However, in order to examine the novel role of FGF23 beyond that of a circulating biomarker of disordered mineral metabolism and phosphate homeostasis,[42] experimental and interventional randomized studies are required. Clinical studies assessing the cardiovascular effects of lowering FGF23 concentrations and their potential translation into improved outcomes represent another line of future research.

Strengths and Limitations

Although we investigated a large, longitudinal, multiethnic, community-based study sample including a family-based cohort structure allowing genetic analyses, a number of potential limitations require further consideration.

Given the observational study design, we cannot prove causal relations, wherefore a difference between statistical significance and clinical significance remains. Furthermore, our community-based sample largely involved middle-aged to older adults, limiting the generalizability to other settings and younger adults. Finally, residual confounding may arise from unmeasured markers of mineral metabolism, such as serum phosphorus, calcium, phosphate, parathyroid hormone, or 25 (OH) vitamin D concentrations, as well as dietary intake of phosphate or vitamin D. However, previous reports suggest that the relation of FGF23 with CVD and mortality is rather strengthened by adjusting for these factors.[7,8,33,36]

Conclusions

Examining a large community-based sample from the general population, we identified important associations of plasma FGF23 and traditional CVD risk factors, with FGF23 concentrations only modestly influenced by genetic factors. Longitudinal analyses of 10-year follow-up data revealed an association of FGF23 with all-cause mortality, but not with incident CVD. These findings suggest a limited predictive role of FGF23 as a CVD risk marker among individuals with normal renal function.

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Disclosures

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SUPPLEMENTAL MATERIAL

Table S1: Differences between study population and those excluded for missing FGF23.

Characteristic	**Included in sample** (N=3236)	**Excluded for missing FGF23** (N=627)	**p-value**
Age, years	58.8 ± 11.2	63.7 ± 10.6	<0.001
Male sex, %	45.6	44.8	0.73
Ethnicity, %			
White	88	98	
African American	4.8	0.2	
Hispanic	4.2	0.6	<0.001
Asian	3	1	
Native American	0.1	0.2	
Omni cohort, %	12.1	2.1	<0.001
Current smoker, %	13	14	0.41
Body mass index, kg/m^2	28.1 ± 5.4	28.7 ± 5.0	0.02
Waist circumference, cm	99.6 ± 14.5	101.0 ± 13.1	0.04
Estimated glomerular filtration rate, mL/min/1.73m^2	70.6 ± 16.3	65.3 ± 12.8	<0.001
Serum creatinine, mg/dl	1.04 ± 0.2	1.08 ± 0.24	0.01
Fasting serum glucose, mg/dl	104 ± 30	103 ± 24	0.16
Type 2 diabetes mellitus, %	11	11	0.90
Triglycerides, mg/dl	135 ± 83	141 ± 97	0.24
Total cholesterol, mg/dl	200 ± 37	201 ± 37	0.72
HDL cholesterol, mg/dl	54 ± 17	51 ± 15	0.01
LDL cholesterol, mg/dl	120 ± 34	122 ± 30	0.19
Ratio total : HDL, mg/dl	4.0 ± 1.4	4.2 ± 1.3	0.01
Lipid-lowering medication, %	19.8	21.9	0.23
Systolic blood pressure, mmHg	127 ± 19	127 ± 19	0.97
Diastolic blood pressure, mmHg	75 ± 10	74 ± 10	0.01
Pulse pressure	52 ± 16	54 ± 17	0.15
Heart rate	64 ± 10	66 ± 11	0.03
Vascular measures			
Carotid-femoral pulse wave velocity, m/s	9.9 ± 3.3	10.7 ± 4.4	0.06
Mean arterial pressure, mmHg	91.7 ± 12.1	92.6 ± 12.5	0.41
Primary pressure wave amplitude, mmHg	40.3 ± 12.7	42.4 ± 12.6	0.07
Flow-mediated dilatation, %	2.4 (0.8, 4.4)	2.6 (0.9, 4.8)	0.07
Baseline flow, cm/s	8.0 ± 4.7	7.9 ± 4.7	0.80
Hyperemic flow, cm/s	51.3 ± 21.2	50.3 ± 22.3	0.64
Augmentation index, %	15.2 ± 12.8	14.5 ± 12.7	0.53
Antihypertensive medication, %	32.8	37.0	0.04
Hypertension, %	45.7	46.4	0.74
History of cardiovascular disease, %	12.4	14.8	0.10
Hormone replacement therapy, %	18.6	14.5	0.04

Data are percentages, mean ± standard deviation or median (Q1, Q3).

Table S2: Longitudinal associations of fibroblast growth factor 23 with cause-specific 10-year-mortality.

	CVD death	**Cancer death**	**Other deaths**
	HR (95% CI)	**HR (95% CI)**	**HR (95% CI)**
Number of deaths, N	90	168	153
		Age- and sex-adjusted models	
Continuous (per SD increase)	**1.53 (1.29, 1.82) ***	**1.30 (1.14, 1.50) ***	**1.48 (1.29, 1.69) ***
<25th	Ref.	Ref.	Ref.
25-50th	0.44 (0.19, 1.03)	**2.33 (1.35, 4.02) ***	0.98 (0.58, 1.67)
50-75th	1.00 (0.52, 1.95)	**2.11 (1.21, 3.65) ***	0.83 (0.48, 1.43)
>75th	**2.08 (1.17, 3.69) ***	**2.80 (1.65, 4.74) ***	**1.98 (1.26, 3.11) ***
		Multivariable-adjusted models [a]	
Continuous (per SD increase)	**1.32 (1.09, 1.59) ***	**1.23 (1.06, 1.41) ***	**1.40 (1.21, 1.61) ***
<25th	Ref.	Ref.	Ref.
25-50th	0.45 (0.19, 1.05)	**2.41 (1.39, 4.17) ***	1.02 (0.60, 1.73)
50-75th	0.87 (0.44, 1.70)	**2.10 (1.21, 3.66) ***	0.81 (0.47, 1.39)
>75th	1.41 (0.78, 2.55)	**2.42 (1.42, 4.14) ***	**1.71 (1.07, 2.72) ***

FGF23, fibroblast growth factor 23; CVD, cardiovascular disease; SD, standard deviation.
Incident 10-year CVD and all-cause mortality risk was examined in N = 2,823 and N = 3,223 individuals, respectively.
[a] Multivariable hazard ratios (HR) were adjusted for age, sex, body mass index, systolic blood pressure, antihypertensive medication, total and high-density lipoprotein cholesterol ratio, smoking, type 2 diabetes mellitus, and cohort.* $p < 0.05$

Figure S1

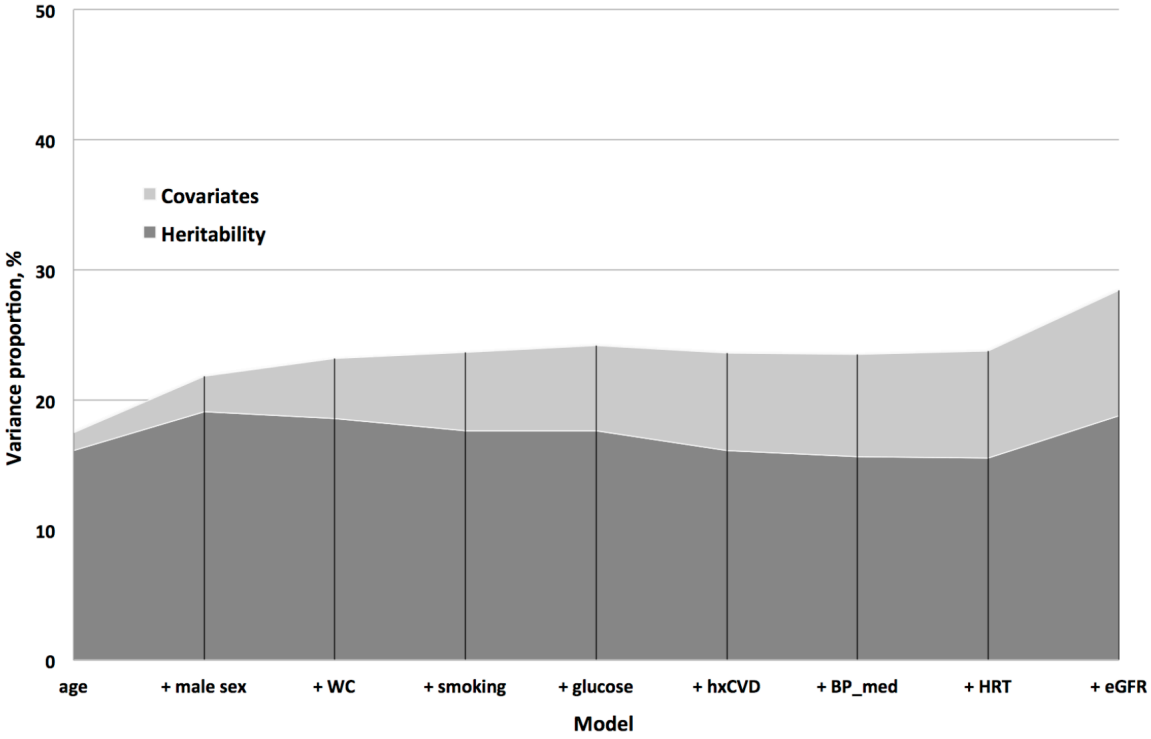
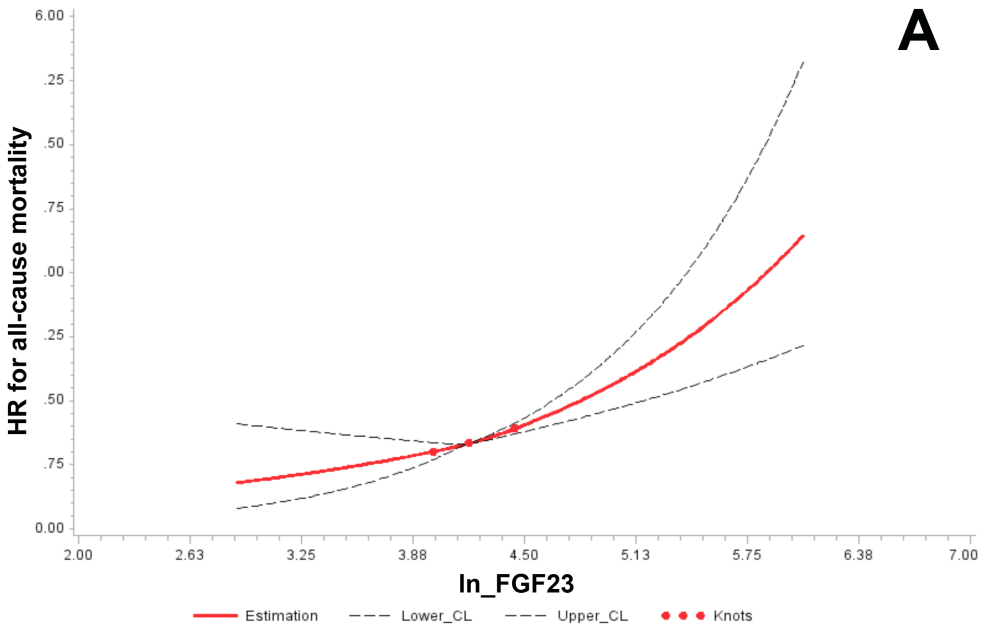
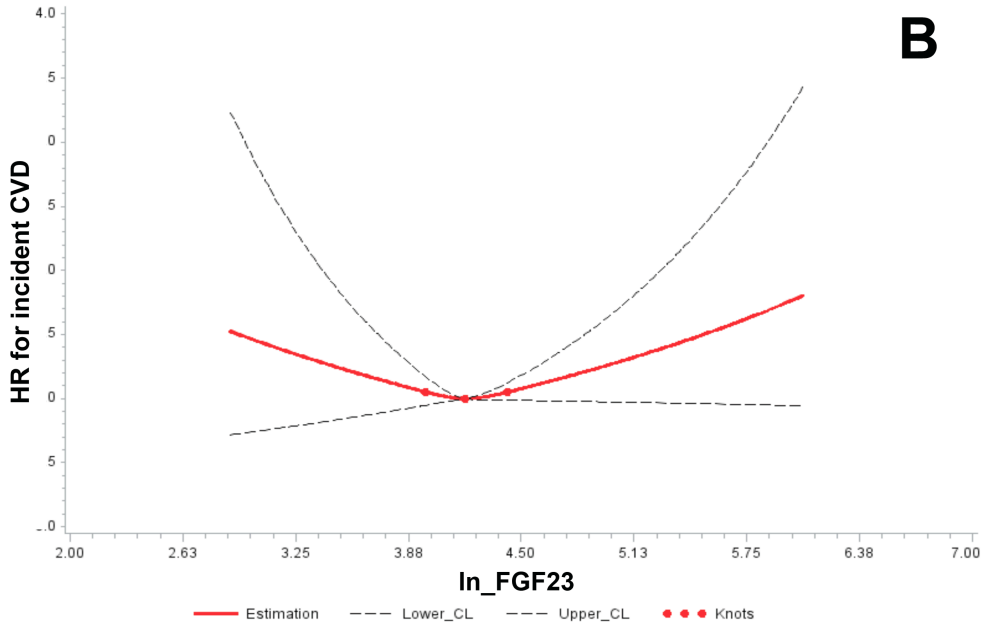

50
40
30
20
10
0
Covariates
Heritability
Variance proportion, %
age
+ male sex
+ WC
+ smoking
+ glucose
+ hxCVD
+ BP_med
+ HRT
+ eGFR
Model

Figure S2


A
HR for all-cause mortality
6.00
.25
.50
.75
.00
.25
.50
.75
0.00
2.00
2.63
3.25
3.88
4.50
5.13
5.75
6.38
7.00
ln_FGF23
Estimation
Lower_CL
Upper_CL
Knots


B
HR for incident CVD
4.0
5
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3.25
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4.50
5.13
5.75
6.38
7.00
ln_FGF23
Estimation
Lower_CL
Upper_CL
Knots

FIGURE LEGENDS

Figure S1 Total variance in fibroblast growth factor 23 concentrations explained by heritability and covariates for increasing model complexity.

Legend: The complete multivariable model included: age, male sex, waist circumferences (WC), smoking, glucose, history of cardiovascular disease (hxCVD), antihypertensive medication (BP_med), hormone replacement therapy (HRT), and estimated glomerular filtration rate (eGFR).

Figure S2 Spline curves for all-cause mortality (A) and incident CVD (B) by continuous plasma FGF23 concentrations.

Legend: Splines were calculated based on multivariable-adjusted models using three knots.