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## The Associations of Fibroblast Growth Factor 23 and Uncarboxylated Matrix Gla Protein With Mortality in Coronary Artery Disease: The Heart and Soul Study

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### Abstract

**Background**—Fibroblast growth factor 23 (FGF23), uncarboxylated matrix Gla protein (ucMGP), and fetuin-A are regulators of mineral metabolism and inhibitors of vascular

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**Reproducible Research Statement:** *Study protocol:* Available at <http://dgim.ucsf.edu/heartandsoulstudy/index.html>. *Statistical code:* Available from Dr. Ix (joeix@ucsd.edu). *Data set:* Restricted access; see <http://dgim.ucsf.edu/heartandsoulstudy/index.html>.

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calcification. Whether circulating levels of each are associated with cardiovascular disease (CVD) events or mortality in populations without end-stage renal disease is unknown.

**Objective**—To evaluate the associations of FGF23, ucMGP, and fetuin-A with mortality and CVD events.

**Design**—Observational study.

**Setting**—12 outpatient clinics in the San Francisco Bay area.

**Patients**—833 outpatients with stable coronary artery disease (CAD), recruited from 11 September 2000 to 20 December 2002.

**Measurements**—Fibroblast growth factor 23, ucMGP, and fetuin-A concentrations were measured at baseline. Participants were followed until 1 December 2008 for mortality and CVD events.

**Results**—During a median follow-up of 6.0 years, 220 participants died and 182 had CVD events. Compared with participants with FGF-23 levels in the lowest tertile, those in the highest tertile had 2-fold greater risk for mortality (hazard ratio [HR], 2.15 [95% CI, 1.43 to 3.24]) and CVD events (HR, 1.83 [CI, 1.15 to 2.91]) after adjustment for traditional CVD risk factors, C-reactive protein levels, and kidney function. The highest ucMGP tertile was associated with lower mortality risk (HR, 0.48 [CI, 0.31 to 0.75]) and showed a nonsignificant trend toward lower CVD event risk by tertile analysis (HR, 0.65 [CI, 0.40 to 1.05])—an association that was significant when modeled continuously ( $P = 0.029$ ). No significant association of fetuin-A with mortality (HR, 0.84 [CI, 0.55 to 1.27]) or CVD events (HR, 0.99 [CI, 0.64 to 1.55]) was observed.

**Limitation**—Participants had prevalent CAD.

**Conclusion**—In outpatients with stable CAD, higher FGF23 and lower ucMGP levels are independently associated with mortality and CVD events.

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People with cardiovascular disease (CVD) are at high risk for recurrent CVD events and mortality (1), and many traditional CVD risk factors are not strongly associated with CVD events in this setting (2,3). New insights into mechanisms for recurrent events might ultimately provide novel therapeutic targets or prevention strategies.

Abnormalities in mineral metabolism are associated with CVD risk in the general population. For example, higher phosphorus levels promote arterial calcification in vitro (4,5) and are associated with coronary artery calcification and CVD events in the general population, even among persons with normal kidney function (6–9). Similarly, lower serum vitamin D levels and higher serum intact parathyroid hormone levels are associated with CVD events in the general population (10,11). Recently, several novel regulators of arterial calcification, including fibroblast growth factor 23 (FGF23), uncarboxylated matrix Gla protein (ucMGP), and fetuin-A, have been identified. Yet, the relationship of circulating levels of these proteins with CVD events and mortality is unknown.

Fibroblast growth factor 23 is a bone-derived protein that promotes renal phosphorus wasting and inhibits conversion of 25-hydroxyvitamin D to the active 1,25-dihydroxyvitamin D form (12–16). Studies of mice have suggested that elevated FGF23 levels may reflect a response to dietary phosphorus burden and serve as a marker of arterial calcification (17). Matrix Gla protein (MGP) is produced by chondrocytes and vascular smooth-muscle cells and prevents arterial calcification through local effects in the arterial wall (18–21). Its precursor form is ucMGP, which has affinity for hydroxyapatite and may be depleted from blood in the setting of arterial calcification. Therefore, low serum levels may be a marker of vascular disease (22). Fetuin-A is a liver-secreted protein that complexes

with calcium and phosphorus in circulation and prevents the precipitation of these minerals from serum (23,24). In mice, knockouts of genes encoding FGF23, ucMGP, or fetuin-A result in arterial or soft-tissue calcification or both (24–26). In humans, FGF23 and fetuin-A have been most extensively studied in cohorts with end-stage renal disease (ESRD), where higher FGF23 and lower fetuin-A levels are associated with mortality and CVD events (27–30). Although the temporal association of ucMGP with mortality is uncertain, patients with ESRD or atherosclerosis have lower ucMGP levels than healthy controls (31–34). Because these 3 proteins have complementary biological functions, we measured blood levels of all 3 and evaluated associations with mortality and CVD events in an ambulatory cohort with prevalent coronary artery disease (CAD) and a spectrum of kidney function from normal to moderate chronic kidney disease. On the basis of directions of association observed in cohorts with ESRD, we hypothesized that higher FGF23, lower ucMGP, and lower fetuin-A levels would each be associated with higher CVD events and mortality, independent of traditional CVD risk factors, kidney function, and one another.

## Methods

### Study Participants

The Heart and Soul Study is an observational study designed to investigate the influence of psychosocial factors on the progression of CVD. Methods are described elsewhere (35). In brief, participants were recruited from out-patient clinics in the San Francisco Bay area if they met at least one of the following inclusion criteria: history of myocardial infarction (MI); angiographic evidence of greater than 50% stenosis in 1 or more coronary vessels; evidence of exercise-induced ischemia by treadmill or nuclear testing; and history of coronary revascularization or documented diagnosis of CAD by an internist or cardiologist. Participants were excluded if they could not walk 1 block, had MI within the past 6 months, or were likely to move out of the area within 3 years. The study protocol was approved by the institutional review boards of participating institutions. All participants provided written informed consent. From September 2000 to December 2002, a total of 1024 participants enrolled and had a baseline study appointment.

### Measurements

**Predictor Variables**—Fasting (12-hour) serum and plasma samples were obtained at the baseline visit, frozen at  $-70^{\circ}\text{C}$ , and later used for measurement of FGF23 (in 2007), ucMGP (in 2007), and fetuin-A (in 2004). Blood levels of each were measured twice in each participant, and results were averaged (the Appendix, available at [www.annals.org](http://www.annals.org), includes measurement details).

**Outcome Variables**—From the baseline examination to 1 December 2008, we conducted annual telephone interviews with participants (or their proxies), inquiring about hospitalizations, cardiac procedures, or death. Two independent, blinded adjudicators reviewed all events, medical records, electrocardiography results, death certificates, and coroner's reports. In the event of disagreement, the adjudicators conferred, reconsidered their classification, and requested consultation from a third blinded adjudicator, as necessary.

We considered the composite of MI, stroke, transient ischemic attack, or heart failure as a CVD event. Myocardial infarction was defined by cardiac biomarkers, electrocardiography results, and cardiac symptoms or signs according to the American Heart Association criteria (36). We defined "stroke" as a new neurologic deficit not secondary to brain trauma, tumor, infection, or other cause. Transient ischemic attack was defined as a focal neurologic deficit (in the absence of head trauma) lasting more than 30 seconds and no longer than 24 hours,

with rapid symptom evolution to the maximal level of deficit in less than 5 minutes and with subsequent complete resolution. We defined “heart failure” as hospitalization for a clinical syndrome involving at least 2 of the following changes from usual status: paroxysmal nocturnal dyspnea, orthopnea, elevated jugular venous pressure, pulmonary rales, third heart sound, and cardiomegaly or pulmonary edema on chest radiography. Mortality was determined by death certificates and coroner’s reports.

**Other Measurements**—We determined age, sex, race or ethnicity, and smoking status by questionnaire. We instructed participants to bring their medication bottles to the baseline appointment, and study personnel recorded medications. Waist-to-hip ratio was calculated after measuring waist circumference midway between the lower rib margin and iliac crest and hip circumference at the level of the greater trochanters. We determined prevalent hypertension and diabetes by questionnaire. Systolic and diastolic blood pressures were measured after 5 minutes of rest in the supine position. We calculated estimated glomerular filtration rate (GFR) by incorporating creatinine concentrations, age, sex, and race in the abbreviated (4-variable) Modification of Diet in Renal Disease Study equation (37). High-sensitivity C-reactive protein levels were measured with the Roche assay (Indianapolis, Indiana) and the Beckman Extended Range assay (Galway, Ireland) (38). We measured total cholesterol, high-density lipoprotein cholesterol, calcium, and phosphorus levels by using standard clinical chemistry analyzers. Participants had transthoracic echocardiography at baseline, which we used to measure left ventricular ejection fraction. We did a symptom-limited, graded exercise treadmill test according to the standard Bruce protocol and defined peak exercise capacity as total number of metabolic equivalent tasks achieved.

### Statistical Analysis

We excluded 5 participants (<1%) who were missing outcome data and 186 participants (18%) who did not have baseline blood specimens for measurement of fetuin-A, ucMGP, or FGF23, resulting in a final sample size of 833 participants. Compared with those of excluded participants, demographic characteristics and event rates were similar among participants who were retained in this analysis (Appendix Table 1, available at [www.annals.org](http://www.annals.org)). For descriptive statistics, we compared demographic and clinical variables among participants with and without CVD events or mortality during follow-up by using the *t* test or the Kruskal–Wallis test for continuous variables and the chi-square test or Fisher exact test for categorical variables, as appropriate. We used graphical methods to determine the distribution of FGF23, ucMGP, and fetuin-A in the study sample. Linear regression was used to compare associations of FGF23, ucMGP, and fetuin-A with calcium, phosphorus, and other baseline cardiovascular risks.

We elected to evaluate tertiles of each protein as our primary predictor variables. The lowest tertile served as the reference category. Because the functional form was fairly linear for each, we also evaluated each protein on a continuous scale, to minimize the chance of a type II error. We used Kaplan–Meier curves to evaluate unadjusted time to events and Cox proportional hazards models to evaluate adjusted associations of each novel mineral regulator with CVD events and mortality. Three sequential models were developed: one adjusted for age, sex, and race; one adjusted for age, sex, race and other CVD risk factors (smoking, hypertension, diabetes, waist-to-hip ratio, systolic and diastolic blood pressures, total and high-density lipoprotein cholesterol levels, estimated GFR, C-reactive protein level, ejection fraction, peak exercise capacity, and medications used [Table 1]); and one adjusted for the CVD risk factors plus serum calcium, phosphorus, and the other 2 novel mineral regulators, because we considered it possible that the association of each novel mineral regulator might be mediated through calcium, phosphorus, or the other 2 novel mineral regulators. We assessed proportional hazards assumptions by visually inspecting

log-minus-log plots and plots of Schoenfeld residuals versus survival time. No evidence of violation was observed. We used multiplicative interaction terms to evaluate effect modification by diabetes status and for the presence or absence of chronic kidney disease (estimated GFR <60 mL/min per 1.73 m<sup>2</sup> vs. ≥60 mL/min per 1.73 m<sup>2</sup>). Analyses were done using Stata Statistical Software, version 9.2 (StataCorp, College Station, Texas).

### Role of the Funding Source

The Heart and Soul Study was supported by the Department of Veterans Epidemiology Merit Review Program; Department of Veterans Affairs Health Services Research and Development service; National Heart, Lung, and Blood Institute; American Federation for Aging Research; Robert Wood Johnson Foundation; and Ischemia Research and Education Foundation. This ancillary study was supported by the American Heart Association and the National Heart, Lung, and Blood Institute. The funding sources had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, approval, or decision to submit the manuscript for review.

### Results

Among the 833 participants, the mean age was 67 years (SD, 11), 81% were men, 40% were nonwhite, 22% had moderate chronic kidney disease (estimated GFR <60 mL/min per 1.73 m<sup>2</sup>), and 27% had type 2 diabetes mellitus. Cardiovascular disease events occurred among 182 individuals during 3913 person-years of follow-up (median, 5.2 years), and 220 deaths occurred during 4360 person-years of follow-up (median, 6.0 years). Participants who had events were older; were more frequently men; were more likely to be receiving an angiotensin-converting enzyme inhibitor or angiotensin-receptor blocker and anticoagulants; and had lower peak exercise capacity, lower ejection fraction, greater waist-to-hip ratio, greater prevalence of diabetes, lower estimated GFR and high-density lipoprotein cholesterol levels, and higher C-reactive protein levels (Table 1).

The distribution of FGF23 was right-skewed, with a median of 43.1 RU/mL (interquartile range, 28.9 to 72.3 RU/mL), so the variable was natural log-transformed to approximate a normal distribution. The distributions of ucMGP and fetuin-A were approximately normal with means of 3290 nM (SD, 1178) and 0.65 g/L (SD, 0.14), respectively (Appendix Figure, available at [www.annals.org](http://www.annals.org)). After adjustment for age, sex, race, and estimated GFR, associations of mineral regulators with calcium, phosphorus, and one another demonstrated that a higher FGF23 level was strongly associated with phosphorus and weakly associated with calcium and that a higher ucMGP level was associated with a higher fetuin-A level (Appendix Table 2, available at [www.annals.org](http://www.annals.org)). The association of each novel mineral regulator with major demographic variables and traditional CVD risk factors varied by risk factor (Appendix Table 3, available at [www.annals.org](http://www.annals.org)), but persons with diabetes had higher levels of each of the 3 mineral regulators.

Both higher FGF23 and lower ucMGP levels were associated with greater risk for mortality and CVD events in Kaplan–Meier analysis. In contrast, fetuin-A levels were not associated with either outcome (Figure). Table 2 shows the adjusted associations of each novel mineral regulator with CVD events and mortality. Compared with participants in the lowest FGF23 tertile, those in the highest tertile had approximately 3-fold greater risk for CVD events and mortality in models adjusted for age, sex, and race. Additional adjustment for traditional CVD risk factors, kidney function, C-reactive protein level, ejection fraction, peak exercise capacity, and medication use (multivariate model 1) attenuated this association; however, the highest tertile remained associated with an approximately 2-fold greater risk for either outcome. When FGF23 was evaluated as a continuous predictor, each doubling in FGF23 level was associated with a 41% higher mortality risk and 24% higher CVD event risk.

Further adjustment for ucMGP, fetuin-A, calcium, and phosphorus produced essentially unaltered associations. Table 3 shows the associations of FGF23 with the individual components of the composite CVD event outcome, in which associations were qualitatively strongest for heart failure and stroke or transient ischemic attack.

Serum ucMGP levels were also associated with mortality risk, but in the opposite direction. Compared with participants in the lowest ucMGP tertile, participants in the highest tertile had approximately 50% lower risk for mortality in models adjusted for age, sex, and race (Table 2). This association remained after adjustment for traditional CVD risk factors, kidney function, C-reactive protein level, ejection fraction, peak exercise capacity, and medication use. The association of ucMGP with CVD events was in the same direction but of lesser magnitude. When ucMGP was evaluated as a continuous predictor variable, each 1000-nM higher ucMGP level was associated with a 22% lower risk for mortality and a 16% lower risk for CVD events. Further adjustment for FGF23, fetuin-A, calcium, and phosphorus had little effect on these associations. Among the components of the composite CVD outcome, MI risk was qualitatively more strongly associated with lower ucMGP levels (Table 3).

Compared with participants in the lowest fetuin-A tertile, participants in the highest tertile were not at statistically significantly higher risk for either CVD events or mortality. When fetuin-A was evaluated as a continuous predictor variable, its associations with mortality and CVD events were near unity (Tables 2 and 3).

We evaluated whether these associations were modified by diabetes or chronic kidney disease status. Association of all 3 mineral regulators with mortality or CVD events was similar, regardless of the presence or absence of chronic kidney disease ( $P \geq 0.41$  for interaction in multivariate model 1 for all). With the exception of the association of ucMGP with mortality, results were also similar in persons with or without diabetes ( $P \geq 0.41$  for interaction for all). The association of ucMGP with mortality was limited to participants without diabetes ( $P = 0.035$  for interaction). In these persons ( $n = 610$ ), each 1000-nM higher ucMGP level was associated with 33% lower mortality risk (hazard ratio, 0.67 [95% CI, 0.56 to 0.81];  $P < 0.001$ ), whereas there was no significant association of uc-MGP with mortality in 221 participants with diabetes (hazard ratio, 1.10 [CI, 0.85 to 1.41];  $P = 0.47$ ).

## Discussion

We demonstrate that among persons with stable CAD and a range of kidney function from normal to moderate chronic kidney disease, higher FGF23 levels and lower ucMGP levels are associated with mortality and CVD events, independent of one another, traditional CVD risk factors, kidney function, and C-reactive protein levels. In contrast, fetuin-A levels were not statistically significantly associated with mortality or CVD events.

Higher FGF23 levels are associated with higher mortality in cohorts with ESRD (30). However, to our knowledge, the association of FGF23 with mortality and CVD events in other settings has not previously been evaluated. The mechanisms responsible for this association are uncertain, but several possibilities exist. Fibroblast growth factor 23, an endocrine regulator of phosphorus metabolism, may be a marker of higher time-averaged serum phosphorus levels, and by extension, FGF23 may be a marker of vascular calcification. We and others (30) have demonstrated that higher FGF23 levels are associated with higher serum phosphorus levels, and others have shown that higher serum phosphorus levels are associated with vascular calcification and CVD events in community-living samples with and without severe kidney disease (7–9,39). However, in our study and in a previous study among patients with ESRD, the associations of FGF23 with mortality were

independent of serum phosphorus levels measured at one point in time. It may be that a single serum phosphorus measurement does not accurately represent cumulative phosphorus burden, given the high intraindividual biological variability in serum phosphorus levels when measured repeatedly over time (40). Because active vitamin D regulates the renin–angiotensin axis (41) and proliferation of cardiomyocytes (42), FGF23 may also affect the cardiovascular system through its inhibition of vitamin D activation (43). Fibroblast growth factor 23 is associated with left ventricular hypertrophy (44,45), a relation that may be explained by the previously mentioned mechanisms, but it is also possible that FGF23 may influence cardiac remodeling through other mechanisms (46–48). Future studies that elucidate potential direct or indirect effects of FGF23 are required to determine these mechanisms.

To our knowledge, the relationship of ucMGP with mortality has not previously been demonstrated in any setting. Vascular smooth-muscle cells produce MGP, and vitamin K–dependent posttranslational activation produces carboxylated MGP, a locally acting and potent inhibitor of arterial calcification (18,19,25). Its precursor, ucMGP, accumulates in arteries near calcium deposits (22)—a process that may lower its blood concentration. Persons with ESRD, calcific uremic arteriolopathy, or cardiac valvular calcification have lower blood ucMGP levels than healthy control participants (31,32), and ucMGP is inversely correlated with coronary artery calcification (33). The present analysis is novel in demonstrating that a lower ucMGP level is associated with mortality in persons with CAD, and that this association was limited to persons without diabetes. The mechanisms responsible for the effect modification by diabetes are uncertain, but the absence of diabetes may reflect a different underlying biology predisposing to ectopic calcification. We have shown previously that a lower ucMGP level was independently associated with cardiac valve calcification. This association, however, was also limited to persons without diabetes (49).

Synthesized by the liver and secreted into blood, fetuin-A is a circulating inhibitor of arterial calcification that increases the solubility of hydroxyapatite precursors in the bloodstream, reminiscent of mechanisms through which apolipoproteins increase the solubility of lipids (50). In cohorts with ESRD, lower fetuin-A levels have consistently been associated with higher mortality risk (27–29). Whether similar associations extend to nondialysis cohorts is uncertain. In persons with stage 3 or 4 chronic kidney disease, we observed no association of fetuin-A levels with all-cause or CVD mortality (51). Similarly, we observed no association of fetuin-A levels with mortality or CVD events among persons with normal to near-normal kidney function. Studies by other investigators in cohorts without ESRD have provided conflicting results. In 1 study, lower fetuin-A levels were associated with mortality among patients hospitalized with acute MI (52), whereas another study reported that higher fetuin-A levels were associated with MI in a community-living cohort (53). In cohorts with ESRD, fetuin-A has been suggested as a negative acute-phase reactant (27–29), but this association is not consistently observed in other settings (51,54). In our analysis, we found no statistically significant association between fetuin-A and C-reactive protein levels after correction for other cardiovascular risks and estimated GFR (55). The reasons for conflicting results between ESRD cohorts and other settings remain uncertain. Because persons with ESRD have a high prevalence and severity of vascular calcification and frequently have calcium and phosphorus levels well outside the normal laboratory ranges, we hypothesize that this extreme phenotype may be required to observe the association of low fetuin-A levels with CVD events. Finally, although higher fetuin-A levels have also been associated with insulin resistance and risk for type 2 diabetes mellitus (56), the association of fetuin-A with CVD events and mortality in our study was similar among participants with or without diabetes.

Our study has important limitations. Parathyroid hormone and vitamin D levels were not measured, and whether participants used calcium or vitamin D supplements and bisphosphonates is unknown. All participants had prevalent CAD, and most were older men. Results may differ in younger persons, women, and persons without CAD. Although most deaths in older persons with prevalent CAD are related to cardiovascular events (1), the associations between FGF23 and ucMGP levels with mortality may also reflect noncardiovascular events, and the causes of death were not specifically adjudicated in our study. Heart failure was considered an ischemic event in this cohort with prevalent CAD, yet some heart failure events may have been nonischemic in origin. For the association of fetuin-A and events, our sample size was limited to 833 persons, and it is possible that a true association was missed or that results may differ with other assays; the results should be interpreted within the confines of the 95% CIs.

In conclusion, higher FGF23 and lower ucMGP levels are each associated with mortality and CVD events in outpatients with stable CAD. These findings need to be replicated in independent populations, but they add to the growing body of literature demonstrating that abnormalities in mineral metabolism may identify persons at greater risk for CVD events and death. Because *in vitro* and animal studies demonstrate that these pathways influence arterial calcification, future studies determining the exact mechanisms may ultimately provide novel therapies for primary or secondary prevention of CVD.

#### Context

Other investigators recently identified novel regulators of mineral metabolism, which have been associated with cardiovascular disease and mortality in patients with end-stage renal disease.

#### Contribution

The authors studied 833 persons and found that those with higher levels of fibroblast growth factor 23 and lower levels of uncarboxylated matrix Gla protein had higher cardiovascular disease event rates and mortality after adjustment for traditional risk factors, C-reactive protein levels, and kidney function.

#### Caution

All study participants had coronary artery disease.

#### Implication

Better understanding of these relationships might lead to new therapies for cardiovascular disease.

—The Editors

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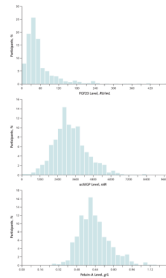
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## Appendix

Plasma FGF23 concentrations were measured by using a C-terminal human enzyme-linked immunosorbent assay (Immunotopics, San Clemente, California) (57). This assay recognizes 2 epitopes on the C-terminal side cleavage site and thus recognizes both full-length FGF23 and C-terminal cleavage fragments of FGF-23. The intra-assay coefficient of variation was 5.0%; the interassay coefficient of variation was 9.9% at a concentration of 36.4 RU/mL and 12.6% at a level 379 RU/mL. Serum ucMGP (nM) was measured by using a competitive enzyme-linked immunosorbent assay developed at VitaK BV, Maastricht University, Maastricht, the Netherlands (58). The assay detects both phosphorylated and nonphosphorylated ucMGP. The lower limit of detection is 98 nM. The intra-assay and interassay coefficients of variation were 6% and 11%, respectively. Fetuin-A was measured by a nephelometric assay (59). This assay uses the same high-specificity antibody as commercially available enzyme-linked immunosorbent assays and has been used in a previous analysis showing an association between fetuin-A and mortality in hemodialysis patients (60). The assay ranges from 0.05 to 3.5 g/L. The intra-assay coefficient of variation was 7.7%, and the interassay coefficient of variation was 8.1%.



**Appendix Figure. Distribution of FGF23\*, ucMGP, and fetuin-A levels among 833 participants with coronary artery disease**

FGF23 = fibroblast growth factor 23; ucMGP = uncarboxylated matrix Gla protein.

\*16 participants with FGF23 levels >480 RU/mL (range, 529 to 3150 RU/mL) were not included because of space limitations.

**Appendix Table 1**

Comparison of Variables in Persons Excluded Because of Missing Measurements

Variable	Included in Analysis ( <i>n</i> = 833)	Excluded From Analysis ( <i>n</i> = 191)	<i>P</i> Value
<b>Major baseline characteristics</b>			
Mean age (SD), y	67 (11)	67 (10)	0.89
Women, <i>n</i> (%)	156 (19)	28 (14)	0.137
Race, <i>n</i> (%)			0.70
White	499 (60)	120 (62)	–
Black	135 (16)	34 (17)	–
Other	198 (24)	41 (21)	–
Diabetes, <i>n</i> (%)	221 (27)	45 (23)	0.37
<b>Outcomes, <i>n</i> (%)</b>			
Heart failure	119 (14)	24 (13)	0.62
Myocardial infarction	88 (11)	21 (11)	0.78
Stroke or transient ischemic attack	36 (4)	12 (6)	0.22
Mortality	221 (27)	43 (23)	0.25
Combined outcomes	301 (36)	65 (35)	0.76

**Appendix Table 2**

Association of Calcium and Phosphorus With ln-FGF23, ucMGP, and Fetuin-A Levels\*

Variable	ln-FGF23		ucMGP		Fetuin-A	
	Unadjusted $\beta$ (95% CI)	<i>P</i> Value	Unadjusted $\beta$ (95% CI)	<i>P</i> Value	Unadjusted $\beta$ (95% CI)	<i>P</i> Value
ln-FGF23	–	–	–0.05 (–0.12 to 0.01)	0.116	0.10 (0.03 to 0.17)	0.004
ucMGP	–0.05 (–0.12 to 0.01)	0.116	–	–	0.27 (0.21 to 0.34)	<0.001
Fetuin-A	0.10 (0.03 to 0.17)	0.004	0.27 (0.21 to 0.34)	<0.001	–	–
Calcium <sup>†</sup>	0.11 (0.04 to 0.18)	0.001	0.06 (0.00 to 0.13)	0.064	0.08 (0.01 to 0.14)	0.027

Variable	ln-FGF23		ucMGP		Fetuin-A	
	Unadjusted $\beta$ (95% CI)	P Value	Unadjusted $\beta$ (95% CI)	P Value	Unadjusted $\beta$ (95% CI)	P Value
Phosphorus	0.27 (0.21 to 0.34)	<0.001	0.10 (0.03 to 0.16)	0.006	0.09 (0.02 to 0.16)	0.008
	Adjusted $\beta$ (95% CI) <sup>‡</sup>		Adjusted $\beta$ (95% CI) <sup>‡</sup>		Adjusted $\beta$ (95% CI) <sup>‡</sup>	
ln-FGF23	–	–	–0.03 (–0.1 to 0.04)	0.39	0.06 (–0.01 to 0.13)	0.111
ucMGP	–0.03 (–0.09 to 0.04)	0.39	–	–	0.25 (0.19 to 0.32)	<0.001
Fetuin-A	0.05 (–0.01 to 0.12)	0.111	0.25 (0.18 to 0.31)	<0.001	–	–
Calcium <sup>†</sup>	0.09 (0.02 to 0.15)	0.008	0.04 (–0.03 to 0.11)	0.22	0.05 (–0.02 to 0.12)	0.147
Phosphorus	0.19 (0.12 to 0.25)	<0.001	0.08 (0.01 to 0.15)	0.025	0.02 (–0.05 to 0.09)	0.62

ln-FGF23 = natural log-transformed fibroblast growth factor 23; ucMGP = uncarboxylated matrix Gla protein.

\* Data are coefficients of regression ( $\beta$ ) with 95% CIs representing SD change in ln-FGF23, ucMGP, and fetuin-A levels per SD change in predictor variables (FGF23 was log-transformed for regression analysis because of nonparametric distribution).

<sup>†</sup> Adjusted calcium = total calcium + (4.0 – serum albumin)  $\times$  0.8.

<sup>‡</sup> Adjusted for age, sex, race, and estimated glomerular filtration rate.

### Appendix Table 3

Association Between Baseline Factors and ln-FGF23, ucMGP, and Fetuin-A Levels\*

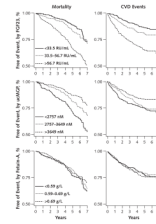
Variable	ln-FGF23		ucMGP		Fetuin-A	
	$\beta$ (95% CI)	P Value	$\beta$ (95% CI)	P Value	$\beta$ (95% CI)	P Value
Age (per 10 y)	–0.05 (–0.11 to 0.01)	0.107	–0.21 (–0.27 to –0.15)	<0.001	–0.18 (–0.24 to –0.12)	<0.001
Women	0.40 (0.23 to 0.57)	<0.001	0.15 (–0.02 to 0.32)	0.093	0.26 (0.09 to 0.43)	0.004
Nonwhite race	–0.04 (–0.18 to 0.10)	0.56	0.06 (–0.08 to 0.20)	0.37	0.07 (–0.07 to 0.21)	0.35
Smoking status	0.36 (0.19 to 0.53)	<0.001	0.13 (–0.04 to 0.30)	0.128	0.08 (–0.09 to 0.25)	0.36
Hypertension	0.14 (–0.01 to 0.29)	0.077	–0.05 (–0.20 to 0.11)	0.54	–0.02 (–0.17 to 0.14)	0.84
Diabetes	0.27 (0.12 to 0.42)	0.001	0.26 (0.11 to 0.41)	0.001	0.27 (0.12 to 0.43)	<0.001
Estimated GFR <sup>†</sup>	–0.32 (–0.39 to –0.26)	<0.001	0.20 (0.13 to 0.27)	<0.001	–0.01 (–0.08 to 0.06)	0.83
Waist-to-hip ratio <sup>†</sup>	–0.03 (–0.10 to 0.04)	0.36	0.03 (–0.04 to 0.10)	0.40	0.04 (–0.03 to 0.11)	0.21

Variable	ln-FGF23		ucMGP		Fetuin-A	
	$\beta$ (95% CI)	P Value	$\beta$ (95% CI)	P Value	$\beta$ (95% CI)	P Value
Total cholesterol level <sup>†</sup>	0.01 (−0.06 to 0.08)	0.82	0.16 (0.10 to 0.23)	<0.001	0.35 (0.29 to 0.42)	<0.001
HDL cholesterol level <sup>†</sup>	−0.05 (−0.11 to 0.02)	0.184	−0.05 (−0.11 to 0.02)	0.185	−0.17 (−0.24 to −0.10)	<0.001
ln-hsCRP level <sup>†</sup>	0.20 (0.13 to 0.27)	<0.001	0.01 (−0.06 to 0.07)	0.88	0.08 (0.01 to 0.15)	0.020
Ejection fraction <sup>†</sup>	−0.11 (−0.18 to −0.05)	0.001	0.05 (−0.02 to 0.12)	0.149	−0.07 (−0.14 to 0.00)	0.050
Max METS <sup>†</sup>	−0.23 (−0.30 to −0.16)	<0.001	0.10 (0.03 to 0.17)	0.006	−0.04 (−0.11 to 0.02)	0.21
Medication use						
$\beta$ -Blocker	0.04 (−0.10 to 0.18)	0.55	0.02 (−0.11 to 0.16)	0.73	0.06 (−0.08 to 0.20)	0.40
ACE inhibitor or ARB	0.12 (−0.02 to 0.25)	0.092	0.04 (−0.10 to 0.18)	0.56	0.02 (−0.12 to 0.16)	0.79
Aspirin	−0.23 (−0.39 to −0.06)	0.006	−0.06 (−0.23 to 0.10)	0.45	−0.09 (−0.25 to 0.07)	0.26
Statin	−0.13 (−0.27 to 0.01)	0.079	0.08 (−0.06 to 0.22)	0.26	−0.09 (−0.24 to 0.05)	0.198
Anticoagulant	0.63 (0.38 to 0.89)	<0.001	−0.04 (−0.30 to 0.22)	0.78	−0.09 (−0.35 to 0.17)	0.51

ACE = angiotensin-converting enzyme; ARB = angiotensin-receptor blocker; GFR = glomerular filtration rate (based on the 4-variable Modification of Diet in Renal Disease Study equation); HDL = high-density lipoprotein; ln-FGF23 = natural log-transformed fibroblast growth factor 23; ln-hsCRP = natural log-transformed high-sensitivity C-reactive protein; max METS = maximum attainable metabolic equivalent tasks (obtained by using the modified Bruce protocol); ucMGP = uncarboxylated matrix Gla protein.

\* Data are coefficients of regression ( $\beta$ ) with 95% CIs representing SD change in ln-FGF23, ucMGP, and fetuin-A levels per SD change in predictor variables (FGF23 was log-transformed for regression analysis because of nonparametric distribution).

<sup>†</sup> Predictor variable is normalized by its SD for regression analysis.



**Figure. Unadjusted event-free survival, by tertiles of FGF23, ucMGP, and fetuin-A**  
CVD = cardiovascular disease; FGF23 = fibroblast growth factor 23; ucMGP = uncarboxylated matrix Gla protein.



**Table 1**

## Baseline Characteristics of Participants With and Without Events\*

Characteristic	Participants With Events (n = 301)	Participants Without Events (n = 532)	P Value
Mean age (SD), y	71 (12)	65 (10)	<0.001
Men, n (%)	261 (87)	416 (78)	0.002
Race, n (%)			0.080
White	195 (65)	304 (57)	
Black	44 (15)	91 (17)	
Other	61 (20)	137 (26)	
Medication use, n (%)			
Aspirin	231 (77)	413 (78)	0.77
$\beta$ -Blocker	177 (59)	301 (57)	0.53
ACE inhibitor or ARB	176 (58)	254 (48)	0.003
Statin	187 (62)	350 (66)	0.29
Anticoagulant	30 (10)	32 (6)	0.037
Current smoker, n (%)	66 (22)	102 (19)	0.36
Diabetes, n (%)	100 (33)	121 (23)	0.001
Hypertension, n (%)	225 (75)	375 (71)	0.176
Mean systolic BP (SD), mm Hg	135 (22)	133 (20)	0.24
Mean diastolic BP (SD), mm Hg	74 (12)	75 (11)	0.027
Mean waist-to-hip ratio (SD)	0.97 (0.08)	0.95 (0.08)	0.008
Mean peak exercise capacity (SD), METS	6 (3)	8 (3)	<0.001
Mean ejection fraction (SD)	0.59 (0.11)	0.63 (0.09)	<0.001
Mean estimated GFR (SD), mL/min per 1.73 m <sup>2</sup>	70 (25)	80 (21)	<0.001
Median total cholesterol level (IQR)			
mmol/L	4.33 (3.70–5.20)	4.48 (3.89–5.05)	
mg/dL	167 (143–201)	173 (150–195)	0.172
Mean HDL cholesterol level (SD)			
mmol/L	1.17 (0.36)	1.22 (0.36)	
mg/dL	45 (14)	47 (14)	0.052
Median CRP level (IQR), nmol/L	25 (12–63)	19 (8–40)	<0.001

Characteristic	Participants With Events ( <i>n</i> = 301)	Participants Without Events ( <i>n</i> = 532)	<i>P</i> Value
Mean calcium level (SD) <sup>†</sup>			
<i>mmol/L</i>	2.4 (0.13)	2.4 (0.13)	
<i>mg/dL</i>	9.6 (0.5)	9.6 (0.5)	0.96
Median phosphorus level (IQR)			
<i>mmol/L</i>	1.16 (1.07–1.29)	1.20 (1.07–1.29)	
<i>mg/dL</i>	3.6 (3.3–4.0)	3.7 (3.3–4.0)	0.53

ACE = angiotensin-converting enzyme; ARB = angiotensin-receptor blocker; BP = blood pressure; CRP = C-reactive protein; GFR = glomerular filtration rate (based on the 4-variable Modification of Diet in Renal Disease Study equation); HDL = high-density lipoprotein; IQR = interquartile range; METS = metabolic equivalent tasks.

\* Cardiovascular event or death.

<sup>†</sup> Adjusted calcium = total calcium + (4.0 – serum albumin) × 0.8.

**Table 2**  
Association of FGF23, ucMGP, and Fetuin-A Levels With All-Cause Mortality or CVD Events

Variable	HR (95% CI), by Tertile			HR (95% CI), by Continuous Variable in Cox Model	P Value
	Tertile 1	Tertile 2	Tertile 3		
<b>FGF23*</b>	Per Doubling of FGF23 <sup>†</sup>				
All-cause mortality					
Age-, sex-, and race-adjusted	1.00	1.30 (0.89–1.89)	2.86 (2.04–4.01)	1.54 (1.39–1.70)	<0.001
Multivariate model 1 <sup>‡</sup>	1.00	1.38 (0.92–2.08)	2.15 (1.43–3.24)	1.41 (1.23–1.60)	<0.001
Multivariate model 2 <sup>§</sup>	1.00	1.39 (0.93–2.09)	2.07 (1.36–3.13)	1.40 (1.22–1.60)	<0.001
<b>CVD event</b>					
Age-, sex-, and race-adjusted	1.00	1.50 (0.99–2.27)	3.09 (2.12–4.52)	1.49 (1.35–1.66)	<0.001
Multivariate model 1 <sup>‡</sup>	1.00	1.46 (0.94–2.29)	1.83 (1.15–2.91)	1.24 (1.07–1.43)	0.004
Multivariate model 2 <sup>§</sup>	1.00	1.47 (0.94–2.30)	1.83 (1.14–2.94)	1.24 (1.06–1.44)	0.006
<b>ucMGP//</b>	Per 1000-nM Greater ucMGP Level				
All-cause mortality					
Age-, sex-, and race-adjusted	1.00	0.71 (0.52–0.96)	0.51 (0.36–0.74)	0.75 (0.66–0.85)	<0.001
Multivariate model 1 <sup>‡</sup>	1.00	0.69 (0.49–0.96)	0.48 (0.31–0.75)	0.78 (0.68–0.90)	0.001
Multivariate model 2 <sup>§</sup>	1.00	0.70 (0.49–0.99)	0.49 (0.31–0.78)	0.79 (0.68–0.92)	0.002
<b>CVD event</b>					
Age-, sex-, and race-adjusted	1.00	0.92 (0.66–1.29)	0.70 (0.48–1.04)	0.82 (0.71–0.94)	0.005
Multivariate model 1 <sup>‡</sup>	1.00	0.96 (0.66–1.41)	0.65 (0.40–1.05)	0.84 (0.72–0.98)	0.029
Multivariate model 2 <sup>§</sup>	1.00	0.98 (0.66–1.45)	0.64 (0.39–1.05)	0.84 (0.72–0.99)	0.042
<b>Fetuin-A¶</b>	Per 0.1-g/L Greater Fetuin-A Level				

Variable	HR (95% CI), by Tertile			HR (95% CI), by Continuous Variable in Cox Model		P Value
	Tertile 1	Tertile 2	Tertile 3			
All-cause mortality						
Age-, sex-, and race-adjusted	1.00	0.98 (0.71–1.34)	1.08 (0.77–1.50)	0.98 (0.89–1.09)		0.74
Multivariate model 1 <sup>‡</sup>	1.00	0.89 (0.62–1.27)	0.84 (0.55–1.27)	0.92 (0.81–1.05)		0.22
Multivariate model 2 <sup>§</sup>	1.00	0.85 (0.59–1.22)	0.82 (0.53–1.28)	0.91 (0.80–1.04)		0.179
CVD event						
Age-, sex-, and race-adjusted	1.00	0.99 (0.69–1.43)	1.29 (0.90–1.84)	1.10 (0.99–1.23)		0.071
Multivariate model 1 <sup>‡</sup>	1.00	0.86 (0.57–1.30)	0.99 (0.64–1.55)	1.08 (0.94–1.24)		0.28
Multivariate model 2 <sup>§</sup>	1.00	0.92 (0.60–1.39)	1.12 (0.71–1.78)	1.14 (0.98–1.31)		0.081

CVD = cardiovascular disease; FGF23 = fibroblast growth factor 23; HR = hazard ratio; ucMGP = uncarboxylated matrix Gla protein.

<sup>\*</sup> The values for tertiles 1, 2, and 3 are <33.5, 33.5–56.7, and >56.7 RU/mL, respectively.

<sup>‡</sup> HRs for FGF23 as a continuous predictor are expressed as “per doubling of FGF23” because it was natural log-transformed for analysis.

<sup>‡</sup> Adjusted for age; sex; race; waist-to-hip ratio; smoking; hypertension; diabetes; systolic and diastolic blood pressures; estimated glomerular filtration rate; total and high-density lipoprotein cholesterol levels; C-reactive protein level; ejection fraction; peak exercise capacity; and use of aspirin,  $\beta$ -blockers, angiotensin-converting enzyme inhibitors or angiotensin-receptor blockers, statins, or anticoagulants.

<sup>§</sup> Adjusted for covariates in multivariable model 1 plus calcium level, phosphorus level, and the 2 other predictor variables (for example, in the FGF23 model, ucMGP and fetuin-A were included as adjustment covariates).

<sup>||</sup> The values for tertiles 1, 2, and 3 are <2757, 2757–3649, and >3649 nM, respectively.

<sup>¶</sup> The values for tertiles 1, 2, and 3 are <0.59, 0.59–0.69, and >0.69 g/L, respectively.

Table 3

HRs for Individual Events per Change in FGF23, ucMGP, or Fetuin-A Level\*

Variable	Per Doubling of FGF23 <sup>†</sup>		Per 1000-nM Greater ucMGP Level		Per 0.1-g/L Greater Fetuin-A Level	
	HR (95% CI)	P Value	HR (95% CI)	P Value	HR (95% CI)	P Value
Death ( <i>n</i> =220)	1.54 (1.39–1.70)	<0.001	0.78 (0.68–0.90)	0.001	0.92 (0.81–1.05)	0.22
Cardiovascular event ( <i>n</i> =182)	1.49 (1.35–1.66)	0.004	0.84 (0.72–0.98)	0.029	1.08 (0.94–1.24)	0.28
Heart failure ( <i>n</i> =119)	1.31 (1.08–1.59)	0.005	0.88 (0.72–1.08)	0.22	1.04 (0.87–1.25)	0.67
Myocardial infarction ( <i>n</i> =88)	1.05 (0.85–1.30)	0.67	0.75 (0.59–0.95)	0.015	1.04 (0.85–1.26)	0.71
Stroke or transient ischemic attack ( <i>n</i> =36)	1.50 (1.11–2.04)	0.008	1.00 (0.72–1.40)	0.99	1.09 (0.82–1.45)	0.55

FGF23 = fibroblast growth factor 23; HR = hazard ratio; ucMGP = uncarboxylated matrix Gla protein.

\* Adjusted for age; sex; race; waist-to-hip ratio; smoking status; hypertension; diabetes; systolic and diastolic blood pressures; estimated glomerular filtration rate; total and high-density lipoprotein cholesterol levels; C-reactive protein level; ejection fraction; peak exercise capacity; and use of aspirin,  $\beta$ -blockers, angiotensin-converting enzyme inhibitors or angiotensin-receptor blockers, statins, or anticoagulants.<sup>†</sup> HRs for FGF23 as a continuous predictor are expressed as “per doubling of FGF23” because it was natural log-transformed for analysis.