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Biomarkers of mineral metabolism and progression of aortic valve and mitral annular calcification: The Multi-Ethnic Study of Atherosclerosis (MESA)

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Conception or design of the work: AEB and JRK. Data collection: BK, JHI, NSJ, IdB, MJB, and JSG. Data analysis and interpretation: AEB, SX, RK, BK, JI, NSJ, IdB, EDM, GT, DSS, MJB and JRK. Drafting the article: AEB, RK and JRK. Critical revision of the article: AEB, SX, RK, BK, JI, NSJ, IdB, EDM, GT, DSS, MJB and JRK. Final approval of the version to be published: AEB, SX, RK, BK, JI, NSJ, IdB, EDM, GT, DSS, MJB and JRK

Conflict of Interest

JRK reports stock ownership in Amgen, Gilead Sciences, Johnson & Johnson, and Pfizer. During 2017-18, AEB was a site principal investigator for multi-center trials funded by Abbott, AstraZeneca, CSL-Behring, Sanofi-Aventis, and NIH in 2017-18 for which her institution received compensation. The other authors have nothing to disclose.

Conflict of Interest Form

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Abstract

Background and aims: Previous research has implicated dysregulation of phosphate, calcium-phosphate solubilization and bone turnover in cardiovascular calcification, but epidemiologic studies evaluating their longitudinal association with valvular or annular calcification by computed tomography (CT), a highly sensitive imaging modality, are lacking. Our primary aim was to investigate the association of mineral biomarkers with incidence and progression of aortic valve calcification (AVC) and mitral annular calcification (MAC).

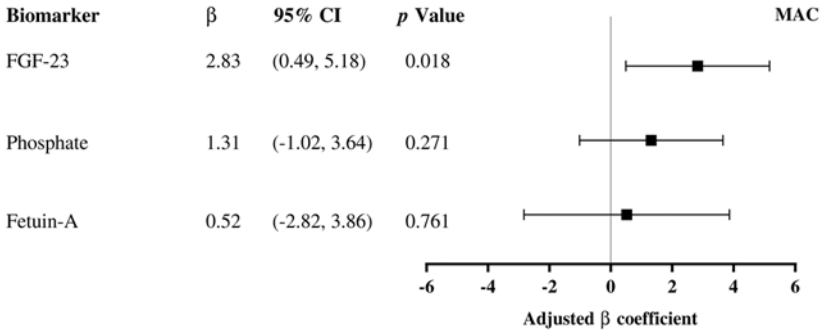
Methods: We evaluated the association of serum FGF-23 (n=6547 participants), phosphate (n=6547), fetuin-A (n=2550) measured at baseline in the community-based Multi-Ethnic Study of Atherosclerosis with AVC and MAC on CT performed at baseline and at a median 2.4 (1.6, 3.1) years later. We used linear mixed effect models to account simultaneously for prevalence, incidence and progression of AVC and MAC.

Results: After adjustment for demographic and clinical characteristics, a significant association was documented for FGF-23 with accelerated annual progression of MAC (2.83 Agatston units (AU), 95% CI=0.49, 5.17 AU, per standard deviation (18.46 pg/mL) of FGF-23), but this was not seen for phosphate or fetuin-A. None of these biomarkers was associated with accelerated annual AVC progression.

Conclusions: This study provides evidence relating serum FGF-23 to accelerated annual MAC progression. Whether this mineral regulator is a risk marker or involved in pathogenesis merits further investigation.

Graphical Abstract

The association of mineral biomarkers with annual acceleration in the progression of mitral annular calcification (MAC). β is annual acceleration in Agatston units per standard deviation (SD) increment of the biomarker, after full adjustment for demographic, clinical and laboratory covariates. For fibroblast growth factor (FGF)-23, SD=18.46 pg/mL; for phosphate, SD=0.519 mg/dL; for fetuin-A, SD=0.105 g/L. Greater annual progression in MAC was associated with increased FGF-23, a regulator of urinary phosphate excretion, but not phosphate itself or fetuin-A, a solubilizer of calcium-phosphate.



Keywords

valve; mineral; metabolism; fibroblast growth factor; fetuin-A

Introduction

Calcification of the aortic valve and mitral annulus are disorders leading to major sequelae with advancing age.[1, 2] Among older adults, the echocardiographic prevalence of aortic valve calcification (AVC) and mitral annular calcification (MAC) are 25% and 40%, respectively.[1, 2] Severe AVC and MAC restrict leaflet opening, resulting in aortic and mitral stenosis or impaired leaflet coaptation, leading to regurgitation.[1, 2] AVC is associated with cardiovascular events and MAC with atrial fibrillation and stroke.[3, 4] Surgical and transcatheter aortic valve replacement are widely used for treatment of severe AVC, but MAC makes surgical replacement challenging, and may adversely impact transcatheter approaches.[2, 5]

Mendelian randomization studies identified low density lipoprotein (LDL) cholesterol and lipoprotein (Lp) (a) as causal risk factors for AVC and triglycerides (TG) for MAC.[6-8] Yet randomized trials of statins failed to show benefit for AVC progression, and no pharmacotherapies of proven efficacy currently exist.[9] Thus, treatment may have to target different pathways in order to be effective, like those involved in calcium or phosphorus handling.

Dysregulation of mineral metabolism is a potentially important determinant of calcific valvular disease. Knockout models demonstrate that calcification occurs when genes for fibroblast growth factor (FGF) 23 and fetuin-A are disrupted.[10, 11] FGF-23 inhibits sodium-phosphate cotransporters in the kidney to increase phosphate excretion, while blocking activation of vitamin D to reduce phosphate absorption in the gut.[12] FGF-23 also binds to cardiac FGF receptors, activating the calcineurin – nuclear factor of activated T cells pathway implicated in vascular and valvular calcification.[13-17] Meanwhile, fetuin-A solubilizes calcium and phosphate, but also binds the insulin receptor, causing insulin resistance.[18] Elevated phosphate itself stimulates vascular smooth muscle cells to differentiate into an osteogenic phenotype.[19]

Previous work demonstrated cross-sectional associations of phosphate with echocardiographic AVC and MAC, as well as FGF-23 and fetuin-A with MAC, but not AVC.[20] Phosphate, vitamin D and FGF-23 were studied in relation to AVC in the Multi-Ethnic Study of Atherosclerosis (MESA), in which participants underwent computed tomography (CT), a more sensitive technique for calcium quantification.[21, 22] There was an association of phosphate with prevalent, though not incident AVC. For FGF-23, only an association between its highest quartile and incident AVC was detected. The inverse association of vitamin D with MAC attenuated and became non-significant after adjustment.[22] The relationship of phosphate and FGF-23 with MAC was not assessed, nor was fetuin-A evaluated in relation to either AVC or MAC. To address existing gaps, we investigated the longitudinal associations of FGF-23, phosphate, and fetuin-A with AVC and MAC in MESA. We hypothesized that these markers would be preferentially associated with MAC.

Patients and methods

Multi-Ethnic Study of Atherosclerosis (MESA)

MESA is a prospective cohort study of risk factors for cardiovascular disease (CVD) in a sample of white, African American, Hispanic/Latino and Chinese adults. Individuals with known CVD were excluded.[23] Men and women ages 45-84 years (n=6814) were enrolled in 2000-02 (Exam 1) at 6 urban field centers.[23, 24] MESA protocols were approved by the Institutional Review Boards at each site. Study protocols conformed to the ethical guidelines of the Declaration of Helsinki and participants provided written informed consent.

All participants (n=6814) underwent cardiac CT and collection of serum for storage at −80°C at Exam 1 (baseline) (Figure 1). A random half underwent repeat cardiac CT in 2002-04 (Exam 2) and the remaining half had cardiac CT performed in 2004-05 (Exam 3, n=6058 in combined Exams 2/3). Accordingly, there were no significant differences in baseline characteristics in participants who underwent cardiac CT in Exam 2 versus those who did so in Exam 3 (data not shown). Cardiac CTs at Exam 1 (baseline) and Exam 2/3 were read for AVC (n=6812 at baseline, n=6058 at visits 2/3) and MAC (n=6814 at baseline, n=5895 at visits 2/3).[21, 25] FGF-23 and phosphate measurements were completed in baseline specimens from n=6547 participants,[21] while fetuin-A was also measured in baseline specimens from a random subset of n=2550 participants.[26]

Cardiac computed tomography (CT)

Cardiac imaging was performed with electron beam CT (EBCT) or multi-detector (MD) CT at Exam 1, with subsequent exams using only MDCT. Cardiac CTs were performed in duplicate and scores were averaged.[27] There was excellent agreement for the presence and amount of calcification with kappa >0.90 between and within readers.[28] Prevalent calcification was defined as a score >0 at baseline. Incident calcification was defined as a score >0 on follow-up when baseline calcification was absent. Calcification progression was defined as a positive difference between scans.

Mineral biomarkers

Fetuin-A was measured (Epitope Diagnostics), as previously described.[26] The mean intra-assay coefficient of variation (CV) was 3.0%. The mean inter-assay CVs were 5.3% and 4.8% at low and high fetuin-A concentrations, respectively. FGF-23 was measured as previously described (Kainos Laboratories, Tokyo, Japan).[29] The CVs for high and low controls were 6.7% and 12.4%, respectively.[30] Serum phosphate was measured using timed rate colorimetry (Beckman-Coulter UniCel DxC, Indianapolis IN) with an inter-assay CV of 4.2%.

Covariates

Body mass index (BMI) was calculated as weight (kg)/height (m^2). Seated blood pressure was measured three times with a Dinamap model Pro 100 automated oscillometric sphygmomanometer and the last two measurements averaged. Diabetes was defined as use of antihyperglycemic medication or fasting glucose ≥126 mg/dL. Calcium, 25 hydroxy (OH) vitamin D, intact parathyroid hormone, urine albumin to creatinine ratio (UACR), total

cholesterol and high density lipoprotein (HDL) cholesterol were measured and LDL cholesterol calculated, as previously described.[21, 22] Estimated glomerular filtration rate (eGFR) was calculated from the Chronic Kidney Disease Epidemiology Collaboration (CKD-Epi) equation.[31]

Statistical analysis

Continuous variables were expressed as mean and standard deviation (SD) or median and interquartile range for skewed distributions, and analyzed by t test or Wilcoxon rank sum test as appropriate. Categorical variables were expressed as frequency and proportion and analyzed by Pearson's chi-square test. The analytical approach for multivariable models focused on incidence or progression of AVC and MAC in participants with or without any calcification at baseline using linear mixed-effects models (LMMs), as detailed previously.[32] This was based on LMMs' advantages over the approach of dividing the analysis by baseline presence or absence of calcification and examining incidence and progression separately. LMMs include all available observations, both at baseline and follow-up, without relying on the assumption that observations are missing at random, and accounts for both cross-sectional and longitudinal associations of the exposure of interest. The functional form of the association between each biomarker and outcome measure was examined using generalized additive model plots (Supplementary Figure 1). As these revealed no obvious departure from linearity, continuous levels of predictors were used with effect estimates reported per SD increment.

For the biomarkers, we used LMMs to test if there was acceleration in AVC and MAC score over time (i.e., change in slope) per SD increment in biomarker concentration.[33] The LMM was $Y_{ij} = \beta_0 + \beta_1 t_{ij} + \beta_2 x_i + \beta_3 t_{ij} x_i + b_i + \varepsilon_{ij}$, where b_i is the random intercept for subject i and ε_{ij} is the error term for the j^{th} measurement of the i^{th} subject. The dependent variable Y_{ij} was the repeated measure of AVC or MAC Agatston score. The main predictors were biomarker level (x_i), years after baseline (t_{ij}), and the interaction term between the two, the parameter of interest. The within-person correlation was adjusted with a random intercept.

We added potential confounders in sequential models, chosen on the basis of known biology or prior associations. We first adjusted for age, sex, race/ethnicity, and scanner type (EBCT or MDCT) (Model 1). Next, we additionally adjusted for education (≥high school, some college or college graduate vs. <high school), BMI, systolic blood pressure, antihypertensive use, diabetes, smoking (ever vs. never), LDL-C, HDL-C (and for MAC: TG), use of statins, eGFR and UACR (Model 2). Last, we examined whether the association was independent of other measures of mineral metabolism by adjusting for calcium, phosphate, 25-OH vitamin D, and/or FGF-23 (Model 3). In the case of fetuin-A, adjustment was for calcium and phosphate as possible mediators. For FGF-23, adjustment was made for 25-OH vitamin D. For FGF-23, phosphate and 25-OH vitamin D may be partial mediators. For phosphate, Model 3 adjustment was for calcium, 25-OH vitamin D, and FGF-23 as potential confounders. In sensitivity analyses, we excluded participants taking warfarin, which might increase calcification.[34] A two-tailed $p<0.05$ was used to define statistical significance.

For each marker, we performed exploratory analyses for three-way interactions by pre-specified covariates. Interactions with continuous levels of potential effect modifiers were

first examined; when significant, interactions were assessed categorically. Accounting for multiple comparisons, we used a two-tailed $p<0.01$ to define statistical significance for interactions.

For all biomarkers, sensitivity analyses examined AVC and MAC progression only among those with baseline calcification, using LMM (Supplementary Table 2). Linear regression was used to examine the relationship with average annual change in calcium score, and compared to LMM in the subset of participants with a follow-up calcium score measure (Supplementary Table 3). Analyses were performed in R 3.3.1 (Vienna, Austria), Stata 15 (College Station, TX), and GraphPad Prism 7 (La Jolla, CA).

Results

Baseline characteristics

The mean age of the MESA cohort was 62.1 ± 10.2 (range 44-84) years. Participants with baseline AVC or MAC were older, more frequently white and less commonly Chinese, were less often college graduates, more commonly had hypertension, diabetes, and statin use, and had lower eGFR and higher UACR as compared to those without calcification (Table 1). Participants with AVC were more likely to be male and those with MAC were more likely to be female, than those without. Concentrations of FGF-23 were higher, and fetuin-A lower, in participants with AVC or MAC as compared to those without.

Progression of calcification on serial CT

The median follow-up interval between scans was 2.3 (1.6, 3.1) years for participants with FGF-23 and phosphate measures, and 3.1 (2.9, 3.4) years for participants with fetuin-A measures. Among the 5,884 participants with a follow-up CT, annual change in AVC was 2.1±39.7 Agatston units (AU), while annual change in MAC was 8.4±96.1 AU. Overall, the incidence of AVC was 1.71 per 100 person-years, while that of MAC was 1.84 per 100 person-years.

There were statistically significant, sex-specific differences in the incidence and progression of AVC, as previously noted.[35] Men had a higher incidence rate of AVC than women (2.09 vs. 1.40 per 100 person-years, p=0.006) and greater progression of AVC (3.3±51.8 vs. 1.0±24.1 AU/year, p=0.040). There were no significant sex differences in incidence of MAC (1.94 vs. 1.73 per 100 person-years in women vs. men, p=0.262 or its progression (10.2±104.0 vs. 6.4±86.5 AU/year, p=0.425).

Mineral biomarkers and progression of AVC and MAC

There were statistically significant associations of FGF-23 with accelerated AVC and MAC progression upon minimal adjustment (Table 2). After additional adjustment, the association between FGF-23 and AVC progression was modestly attenuated and non-significant. However, FGF-23 remained significantly associated with accelerated annual MAC progression. The annual increase of MAC was accelerated by 2.83 (95% CI=0.49, 5.17) AU for every SD increment (18.46 pg/mL) in FGF-23 (Model 2). This relationship was not changed by adjustment for calcium, phosphate, and 25-OH vitamin D, which could be

partial mediators (Model 3). By contrast, no significant associations were observed for phosphate or fetuin-A with rate of annual AVC or MAC progression at any level of adjustment (Table 2). None of the findings was altered after excluding n=24 participants receiving warfarin.

Assessment of effect modification

Exploratory analyses of three-way interactions for mineral biomarkers with time and pre-specified covariates, or with pre-specified covariates only, are detailed in Supplementary Table 1. There was statistically significant effect modification at the $p<0.01$ level for the association of accelerated MAC progression with FGF-23 by sex, race/ethnicity, age, and diabetes, and for fetuin-A by race/ethnicity and eGFR (Fig. 2).

The rate of annual MAC progression per SD increment in FGF-23 was accelerated particularly in female and diabetic participants, but was decelerated in Chinese participants. By contrast, the rate of annual MAC progression per SD increment (SD=0.105 g/L) in fetuin-A was accelerated for Chinese but not for other race/ethnic groups. There was slower annual MAC progression per SD increment in fetuin-A in participants with a lower eGFR. However, dichotomizing eGFR at 60 and 30 ml/min/1.73 m^2 did not show statistically significant interactions, likely due to a small number of participants in these subgroups (eGFR ≥60 and <60 ml/min/1.73 m^2 are shown in Fig 2.; eGFR <30 ml/min/1.73 m^2, n=9, β=−88.09 AU per SD*year, 95% CI −315.30, 139.13, and eGFR ≥30 ml/min/1.73 m^2, n=2762, β=0.78 AU per SD*year, 95% CI, −2.55, 4.11). Additional interactions at the $p<0.05$ level (Supplementary Table 1) showed the rate of annual AVC progression per SD increment in FGF-23 and fetuin-A was accelerated in women. These also signalled accelerated rates of annual MAC progression per SD increment in FGF-23 in older adults ≥65 years; per SD increment in fetuin-A levels in diabetic participants; and per SD increment (SD=0.519 mg/dL) in phosphate levels in non-Chinese participants.

Sensitivity analyses

There were no statistically significant associations of biomarkers with the acceleration of annual AVC or MAC progression among participants with prevalent calcification (AVC or MAC score > 0, Supplementary Table 2). There were statistically significant associations of FGF-23 with MAC progression by linear regression and LMM when the analysis was restricted to those having a follow-up calcification measure on CT (Supplementary Table 3).

Discussion

In this multi-ethnic cohort, we found that higher levels of FGF-23 were associated with modestly accelerated rates of annual MAC progression, but not annual AVC progression. Neither circulating fetuin-A nor phosphate levels were associated with changes in the rates of MAC or AVC progression. In exploratory analyses, higher FGF-23 levels were particularly associated with greater annual MAC progression in women and participants with diabetes, and decreased annual MAC progression in Chinese participants. By comparison, higher fetuin-A levels were associated with acceleration of annual MAC progression specifically among participants of Chinese race-ethnicity and with lower eGFR.

The strengths of the present study include serial CT determinations of AVC and MAC, and analytic power of LMMs to assess three key biomarkers of mineral metabolism with incidence and progression of calcification. Our study builds on previous work in MESA that examined FGF-23 and phosphate in relation to AVC using these same data, but divided the analysis into separate evaluations of prevalent AVC, incident AVC among participants without baseline AVC, and progression of AVC among those with baseline AVC score >0.[21] LMMs integrate data from all scans and do not depend on the assumption that missingness of data is random, offering clear analytic advantages.[32] The previous analyses showed association of phosphate with prevalent AVC, but not with incidence or progression of AVC. Continuous FGF-23 levels were not associated with AVC prevalence, incidence or progression. The highest FGF-23 quartile was associated with incident AVC alone. Our current analyses did not reveal apparent departure from linearity in FGF-23's associations in LMMs, showing no obvious threshold effects. The lack of significant associations for FGF-23 or phosphate provides further evidence excluding an all but modest relationship of either biomarker with progression of AVC over middle-term follow-up in this cohort. The present study provides new longitudinal assessment of progression of MAC, demonstrating a statistically significant association for serum FGF-23.

Our findings strengthen the premise that systemic perturbations in mineral metabolism are particularly relevant for MAC. The relationship of biomarkers of mineral metabolism with MAC, but not AVC, is consistent with our previous findings in the Cardiovascular Health Study (CHS), where mineral regulators were cross-sectionally associated only with echocardiographic MAC.[20] Consistent with these results, cross-sectional data from the Framingham Heart Study have linked echocardiographic MAC, but not AVC, to chronic kidney disease, a disorder marked by dysregulation of mineral metabolism and, typically, elevated FGF-23 levels.[36]

Elevated FGF-23 was associated with major adverse cardiac events and mortality following acute coronary syndromes, an association that was independent of kidney function measures, phosphate, and vitamin D levels.[36] Higher levels may be an early marker of kidney disease and impaired capacity for phosphate excretion.[15] Notably, phosphate itself was not significantly associated with MAC progression. This runs counter to experimental findings showing that higher phosphate promotes cellular transdifferentiation to an osteoblastic phenotype, and to previous data linking it to prevalent calcification in MESA (AVC) and CHS (both AVC and MAC).[21, 37, 38] Although the 95% CIs still allow for the possibility of an association for phosphate with MAC progression at least as large as that seen for FGF-23, our findings suggest that FGF-23 may have the more prominent role. This could relate to FGF-23's status as a sensitive marker of kidney dysfunction, potentially rising before phosphate and creatinine levels become abnormal.[39]

FGF-23 binds its receptor Klotho in the kidney, but exhibits direct, Klotho-independent binding to cardiac FGF receptors causing left ventricular hypertrophy, as well as calcification.[13-15, 40] If high FGF-23 triggers calcification, it could be a target for intervention using existing therapies (e.g., cinacalcet, antibodies, soluble Klotho).[12, 41]

Exploratory analyses of effect modification suggested that the rate of annual MAC progression in relation to FGF-23 was particularly accelerated in women and diabetic participants, but decelerated in Chinese participants. As MAC is more prevalent in women, it may have been easier to detect this association, but the finding could also reflect a sex-specific effect of FGF-23 or related pathways.[2, 25] The basis for the differential associations observed for Chinese vs. non-Chinese participants is uncertain, but might have to do with the lower frequency of calcification observed in the former group. As for participants with diabetes, previous work from MESA showed that diabetes-associated kidney disease was especially related to MAC.[42] It is possible that in this cohort, the link between FGF-23 and MAC was more detectable in those with glucose dysregulaton.

No significant associations were detected for serum fetuin-A and progression of calcification. This contrasts with the previously reported cross-sectional associations with echocardiographic MAC.[20, 42] Nonetheless, the 95% CIs, especially for MAC, still allow for the possibility of a meaningful association. Findings of effect modification by race/ethnicity and eGFR will require further investigation, but diminished progression of MAC with higher fetuin-A in the context of lower eGFR does accord with a greater protective impact of this molecule against calciphylaxis as kidney function declines.[42]

There are several limitations to this study. Biomarker measurement was made at baseline and levels could vary over time. MESA is a healthy cohort, reducing the ability to fully explore dose-response relationships and effects in those with severe CKD or known CVD, subgroups in which calcification might progress more rapidly. Moreover, duration of follow-up was short for calcification progression. For FGF-23 and phosphate, valid measures were available in 96% of the baseline cohort, indicating a small proportion of missingness due to random sample collection/processing issues. For fetuin-A, measurement was performed in a random subset of participants, and while limiting power, random sampling reduces the possibility of bias. While we adjusted extensively, we cannot exclude the possibility that other factors upstream or downstream may be mediating the observed association. More generally, serum biomarkers may not be reflective of calcification at the tissue level; however, circulating proteins have been associated with histologic AVC.[43] Furthermore, it must be noted that subclinical calcification detected on CT may become symptomatic disease in some, but not all individuals. Last, the results of secondary analyses of effect-modification must be interpreted cautiously given the size of the subgroups and require additional study with more power.

In conclusion, we detected a significant association for FGF-23, but not phosphate or fetuin-A, with accelerated annual progression of MAC in a multi-ethnic cohort of middle-aged to older adults. None of the three biomarkers was related to the rate of AVC progression. These findings support a role for dysregulation of mineral metabolism in the development of MAC, providing impetus for further investigation of variation in different clinical subgroups. FGF-23 may be a viable target for identification, prevention and/or treatment of MAC progression in individuals at risk.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations:

MAC	mitral annular calcification
AVC	aortic valve calcification
FGF-23	fibroblast growth factor-23

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- Fibroblast growth factor-23 was associated with accelerated annual progression in mitral annular calcification when analyzed longitudinally by mixed models.
- FGF-23 may be a novel pathway to target for intervention on and future study of mitral annular calcification.

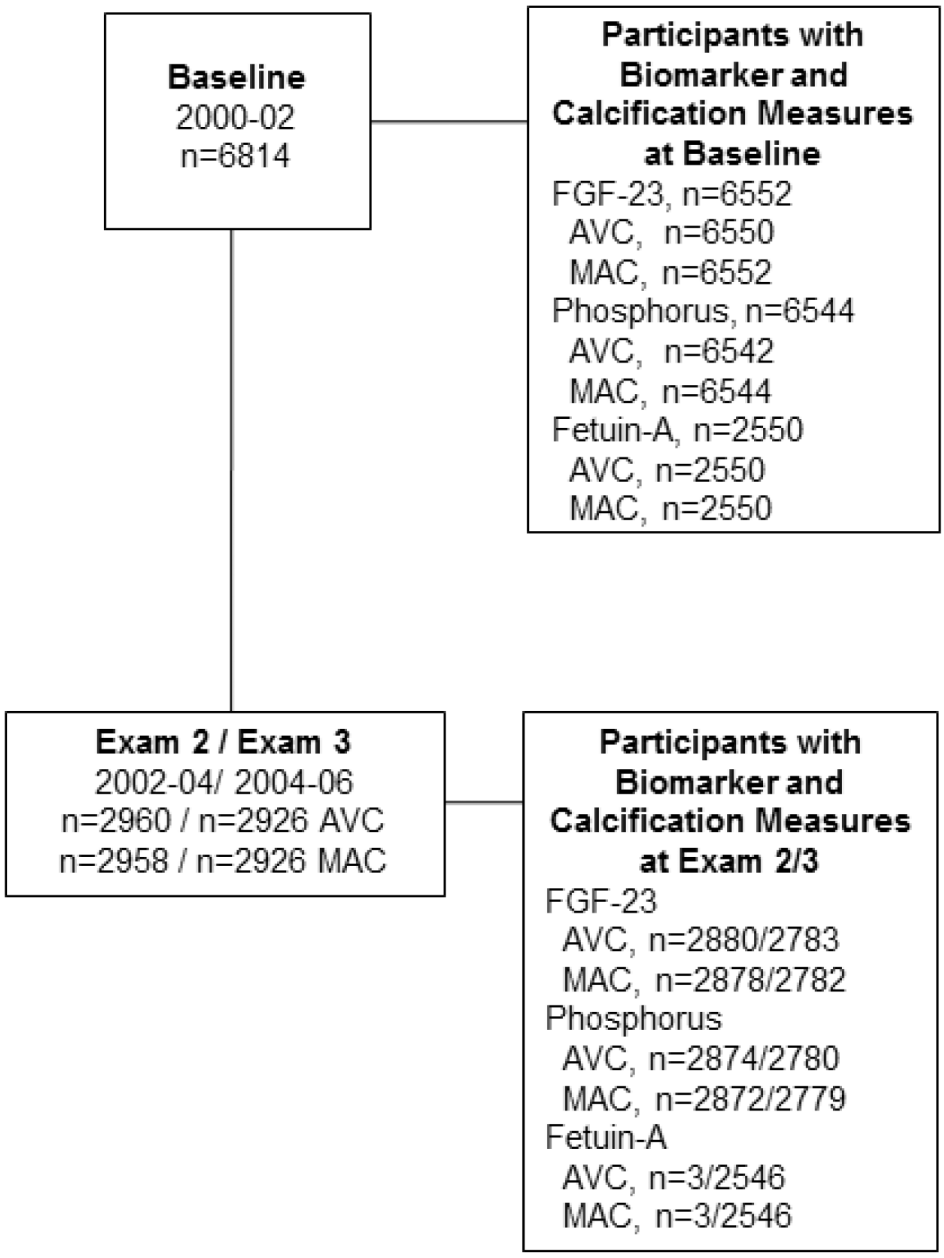

Figure 1.
Participants with calcification and biomarker measurements at different vists.

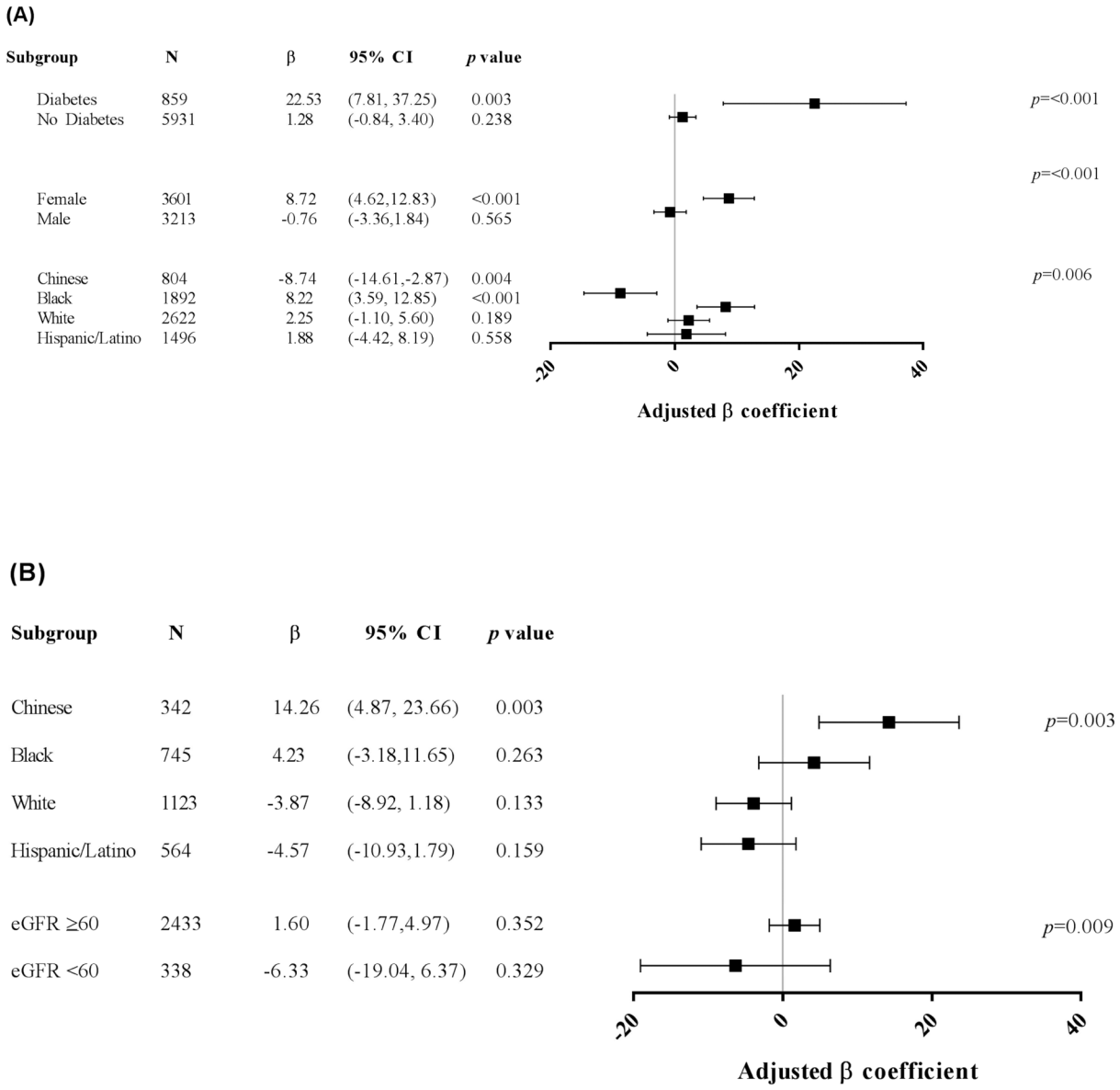

Figure 2.

Stratified analyses for interactions at the $p<0.01$.

(A) FGF-23 and (B) fetuin-A with MAC. β coefficients were adjusted for covariates in Model 2 (age, sex, race-ethnicity, CT-type, education, BMI, systolic blood pressure, antihypertensive medication, diabetes, smoking, LDL, HDL,TG, statin, eGFR, and UACR) and indicate the annual acceleration in Agatston units per SD increment in the biomarker. For FGF-23, SD=18.46 pg/mL; and for fetuin-A, SD=0.105 g/L.

Table 1.

Baseline (Exam 1) demographics and biomarkers in study participants with and without prevalent aortic valve (AVC) or mitral annular calcification (MAC) in the Multi-Ethnic Study of Atherosclerosis.

Characteristic	AVC			MAC		
	No (n=5899)	Yes (n=913)	*p* Value	No (n=6170)	Yes (n=644)	*p* Value
Agastston score	0	59.6 (19.6, 148.6)	--	0	87.5 (24.3, 325.25)	--
Age, years	61±10	70±8	<0.001	61±10	72±8	<0.001
Women, n (%)	3236 (54.9)	364 (39.9)	0.001	3215 (52.1)	386 (59.9)	0.001
Race n (%)						
White	2206 (37.4)	414 (45.4)	0.001	2307 (37.4)	315 (48.9)	0.001
Chinese	737 (12.5)	67 (7.3)		767 (12.4)	37 (5.8)	
Black	1660 (28.1)	232 (25.4)		1751 (28.4)	141 (21.9)	
Hispanic/Latino	1296 (22.0)	200 (21.9)		1345 (21.8)	151 (23.5)	
Education, n (%)						
<High school	1015 (17.3)	210 (23.1)	0.001	1079 (17.5)	146 (22.8)	0.001
High school	1053 (17.9)	182 (20.0)		1093 (17.8)	143 (22.3)	
Some college	961 (16.3)	148 (16.3)		1010 (16.4)	99 (15.5)	
College graduate	2852 (48.5)	368 (40.5)		2969 (48.3)	252 (39.4)	
BMI, kg/m^2	28.3±5.56	28.5±5.0	0.320	28.3±5.5	29.0±5.7	0.003
SBP, mmHg	125±21	135±22	<0.001	126±21	135±23	<0.001
Antihypertensive medication, n (%)	2030 (34.4)	505 (55.4)	0.001	2185 (35.4)	351 (54.5)	0.001
Warfarin, n (%)	17 (0.3)	7 (0.8)	0.023	17 (0.3)	7 (1.1)	0.001
Diabetes, n (%)	677 (11.5)	181 (19.8)	0.001	733 (11.9)	126 (19.6)	0.001
Smoking, n (%)						
Never	3021 (51.4)	396 (43.6)	0.001	3090 (50.2)	328 (51.3)	0.008
Former	2070 (35.2)	417 (45.9)		2234 (36.3)	253 (39.5)	
Current	790 (13.4)	96 (10.6)		828 (13.5)	59 (9.2)	
LDL, mg/dL	117±31	119±34	0.170	117±31	115±33	0.094
HDL, mg/dL	51±15	49±14	<0.001	51±15	52±15	0.085
Triglycerides, mg/dL	110 (77,159)	120 (84, 172)	<0.001	111 (78,161)	113 (80,161)	0.361
Statin use, n (%)	796 (13.5)	213 (23.4)	0.001	859 (14.0)	151 (23.5)	0.001
eGFR, ml/min/1.73 m^2	78.9±16.0	70.5±16.4	<0.001	78.6±16.0	69.5±16.8	<0.001
Urine albumin-creatinine ratio, mg/g	5.1 (3.2-10.3)	7.1 (4.0-17.8)	<0.001	5.1 (3.2-10.3)	8.2 (4.7-18.2)	<0.001
EBCT scan type, n (%)	3149 (53.4)	436 (47.8)	0.002	3289 (53.3)	296 (46.0)	0.001
Calcium, mg/dL	9.1±0.5	9.1±0.5	0.349	9.1±0.51	9.1±0.56	0.334
25-OH vitamin D, ng/mL	25.3±11.4	26.3±12.0	0.014	25.3±11.4	26.7±12.1	0.003
Phosphate, mg/dL	3.7±0.5	3.7±0.5	0.949	3.7±0.5	3.8±0.5	<0.001
PTH, pg/mL	44.3±20.5	47.3±28.7	0.020	44.4±20.9	47.7±29.1	0.099
Fetuin-A, g/L	0.48±0.10	0.46±0.10	<0.001	0.48±0.10	0.46±0.11	0.017

Characteristic	AVC			MAC		
	No (n=5899)	Yes (n=913)	*p* Value	No (n=6170)	Yes (n=644)	*p* Value
FGF-23, pg/mL	39.9±18.2	43.0±19.5	<0.001	40.0±18.4	43.6±18.1	<0.001

BMI= body mass index, eGFR= estimated glomerular filtration rate, EBCT= electron beam computerized tomography, FGF=fibroblast growth factor, HDL=high density lipoprotein, OH=hydroxy, LDL=low density lipoprotein, PTH= parathyroid hormone, pM=picomoles/L, SBP=systolic blood pressure.

Table 2.

Associations of biomarkers of mineral metabolism with progression or prevalent aortic valve (AVC) or mitral annular calcification (MAC).

Biomarker	AVC	MAC
	β_3 coefficient* (95% CI), *p* value	β_3 coefficient* (95% CI), *p* value
Progression of calcification		
FGF-23		
Model 1	0.92, (0.002,1.84), 0.049	2.94, (0.61, 5.27), 0.013
Model 2	0.82, (−0.13, 1.78), 0.091	2.83, (0.49, 5.17), 0.018
Model 3	0.85, (−0.10, 1.81), 0.079	2.88 (0.50, 5.26), 0.018
Phosphate		
Model 1	0.05 (−0.86, 0.96), 0.915	1.36, (−0.95, 3.67), 0.247
Model 2	0.08 (−0.87, 1.03), 0.876	1.31, (−1.02, 3.65), 0.270
Model 3	−0.02 (−0.97, 0.93), 0.969	1.31, (−1.07, 3.68), 0.281
Fetuin-A		
Model 1	−0.74 (−1.95, 0.48), 0.235	1.07, (−2.33, 4.46), 0.538
Model 2	−0.85 (−2.11, 0.41), 0.186	0.52, (−2.83, 3.86), 0.762
Model 3	−0.91, (−2.20, 0.38), 0.168	0.63, (−2.85, 4.10), 0.723

*Indicating annual acceleration in Agatston units per SD increment in the biomarker.

For FGF-23, SD=18.46 pg/mL; for phosphate, SD=0.519 mg/dL; for fetuin-A, SD=0.105 g/L. Model 1: age, sex, race-ethnicity, CT-type. Model 2: Model 1 + education, BMI, systolic blood pressure, antihypertensive medication, diabetes, smoking, LDL, HDL,(and for MAC: TG), statin, eGFR, and UACR. Model 3: Model 2 + for FGF-23: calcium, phosphate, and 25-hydroxy vitamin D; for phosphate: calcium, 25-hydroxy vitamin D, and FGF-23; for fetuin-A: calcium and phosphate.