

Indexes of Kidney Function and Coronary Artery and Abdominal Aortic Calcium (from the Framingham Offspring Study)

Nisha I. Parikh, MD, MPH^{a,b}, Shih-Jen Hwang, PhD^{a,f}, Martin G. Larson, ScD^a, Udo Hoffmann, MD, MPH^d, Daniel Levy, MD^{a,f}, James B. Meigs, MD, MPH^e, Christopher J. O'Donnell, MD, MPH^{a,d,f}, and Caroline S. Fox, MD, MPH^{a,c,f,*}

It is uncertain whether moderate chronic kidney disease (CKD) or measures of kidney function are associated with subclinical atherosclerosis as represented by coronary artery calcium (CAC) or abdominal aortic calcium (AAC). We used logistic and linear regression analyses to relate CKD (glomerular filtration rate <60 ml/min/1.73 m²), cystatin C (cysC), and microalbuminuria (MA) with CAC and AAC obtained using multidetector computed tomography in Framingham Heart Study Offspring participants (mean age 59 years, 55.3% women). Increased CAC and AAC were defined as ≥ 90 th percentile age- and gender-specific cutpoints based on a healthy referent sample. Major cardiovascular disease risk factors were accounted for in multivariable models. Of 1,179 participants, 1,174 had AAC measurements and 1,147 had CAC measurements, 6.3% had CKD, and 8.3% had MA. CKD was not associated with CAC (multivariable-adjusted odds ratio [OR] for CKD 1.18, 95% confidence interval 0.59 to 2.36, $p = 0.63$) or AAC (multivariable-adjusted OR for CKD 1.11, 95% confidence interval 0.61 to 2.04, $p = 0.73$). CysC was associated with CAC in age- and gender-adjusted but not in multivariable models (age- and gender-adjusted OR for log cysC per SD increment and CAC 1.19, 95% confidence interval 1.01 to 1.41, $p = 0.04$; multivariable-adjusted OR 1.14, 95% confidence interval 0.95 to 1.38, $p = 0.15$). MA was not associated with CAC (OR 0.81, 95% confidence interval 0.41 to 1.61, $p = 0.54$). Neither cysC nor MA was significantly associated with AAC in age- and gender- or multivariable-adjusted models. In conclusion, CKD, cysC, and MA are not associated with CAC or AAC when accounting for cardiovascular disease risk factors. © 2008 Elsevier Inc. All rights reserved. (Am J Cardiol 2008;102:440–443)

The availability of 3 separate measurements of kidney function and measurements of coronary artery calcium (CAC) and abdominal aortic calcium (AAC) by multidetector computed tomography in the Framingham Heart Study offspring cohort provides an opportunity to examine the association between kidney function and subclinical atherosclerosis in a community-based sample not selected for chronic kidney disease (CKD).

^aNational Heart, Lung, and Blood Institute's Framingham Heart Study, Framingham, and ^bBeth Israel Deaconess Medical Center, ^cDepartment of Endocrinology, Diabetes, and Hypertension, Brigham and Women's Hospital, Harvard Medical School, and ^dCardiology Division, Department of Medicine, and ^eDivision of General Internal Medicine, Massachusetts General Hospital and Harvard Medical School, Boston, Massachusetts; and ^fNational Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, Maryland. Manuscript received December 19, 2007; revised manuscript received and accepted April 2, 2008.

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*Corresponding author: Tel: 508-935-3447; fax: 508-626-1262.

E-mail address: foxca@nhlbi.nih.gov (C.S. Fox).

Methods

The Framingham Heart Study is a community-based cohort study that began in 1948, consisting of 5,209 men and women in the original cohort.¹ In 1971, 5,124 subjects enrolled in the Framingham Heart Study offspring cohort, including the children and spouses of the children of the original cohort, and attended examinations quadrennially.² The Framingham Offspring Study protocol was reviewed by the Boston University Medical Center institutional review board and all participants signed the informed consent.

This present investigation is comprised of offspring cohort participants who also participated in the multidetector computed tomographic substudy (June 2002 to April 2005). Participants ($n = 1,422$) underwent multidetector computed tomographic assessment of CAC and AAC. Substudy inclusion was weighted toward participants from larger Framingham Heart Study families and those residing in/near New England. Men were ≥ 35 years of age, women were ≥ 40 years of age and not pregnant, and all participants had to weigh <150 kg due to scanner limitations. Of 1,422 substudy participants, 1,303 had interpretable multidetector computed tomographic scans for CAC analysis. Then further hierarchical exclusions were made: missing glomerular filtration rate ($n = 15$), missing cystatin C (cysC) measurements ($n = 29$), and prevalent diagnosis of cardiovascular disease ($n = 112$), resulting in a final sample of 1,147 for CAC analysis. Of the 1,422 substudy participants, 1,384 had

interpretable AAC measurements. We further excluded those with missing glomerular filtration rate ($n = 17$), missing cysC ($n = 32$), and prevalent cardiovascular disease ($n = 161$), leaving a final sample of 1,174 for AAC analysis. Because not all participants had chest and abdominal scans, there was a total of 1,179 participants in our study with CAC and/or AAC measurements.

The urinary albumin-to-creatinine ratio was measured on a subset of participants ($n = 2,966$) at the sixth offspring examination cycle (1995 to 1998).³ Of those with urinary albumin-to-creatinine ratio measurements, 930 were also participants in the multidetector computed tomographic study and were therefore included in the analysis of microalbuminuria (MA), CAC, and AAC.

Kidney function was estimated by glomerular filtration rate, calculated using the simplified Modification of Diet in Renal Disease (MDRD) study equation.⁴ Our CKD definition was based on the National Kidney Foundation Disease Outcome Quality Initiative definition of kidney disease, which defines CKD as a glomerular filtration rate <60 ml/min/1.73 m².⁵

Serum creatinine was measured using the modified Jaffe method and was calibrated using a 2-step process. First, National Health and Nutritional Examination Survey III creatinine values were calibrated to the Cleveland Clinic Laboratory, requiring a correction factor of 0.23 mg/dl (20.3 μ mol/L).⁶ Then age-specific (20 to 39, 40 to 59, 60 to 69, ≥ 70 years of age) and gender-specific creatinine values were aligned to the corresponding corrected National Health and Nutrition Examination Survey III age- and gender-specific means.⁷

Urinary albumin-to-creatinine ratio was measured on previously frozen 3-ml spot urine samples that were kept at -20°C until quantification. Urinary creatinine concentration was measured using the modified Jaffe method; the intra-assay coefficient of variation was 1.7% to 3.8%. Urine albumin concentration was assessed using immunoturbimetry (Tina-quant Albumin assay; Roche Diagnostics, Indianapolis, Indiana). Differences in urine concentrations were accounted for by taking the ratio of urinary albumin to urinary creatinine and were reported as milligrams per gram. The urinary albumin-to-creatinine ratio is correlated with albumin excretion rates determined using a 24-hour urine collection.^{8,9} MA was defined as a urinary albumin-to-creatinine ratio ≥ 30 mg/g.⁵

CysC concentrations were measured on previously frozen (for ~ 4 to 7 years) blood (serum) samples (stored at -80°C) by nephelometry (Dade Behring Diagnostic, Marburg, Germany) and were reported as milligrams per liter. Intra- and interassay coefficients of variation were 2.4% and 3.3%, respectively. Range of detection is 0.29 to 7.22 mg/L. Samples underwent 1 previous freeze-thaw cycle, which is not reported to affect accuracy of cysC levels.¹⁰

Subjects underwent noncontrast-enhanced 8-slice multidetector computed tomographic scanning of the heart and abdomen (LightSpeed Ultra, General Electric, Milwaukee, Wisconsin) using electrocardiographic triggering. An average of 48 contiguous 2.5-mm slices of the chest was taken (120 kVp, 400 mA, gantry rotation time 500 ms, table feed 3:1). For abdominal computed tomographic imaging 25 contiguous 5-mm-thick slices covering 125 mm above the

Table 1

Characteristics for 1,179 offspring participants with coronary artery calcium or abdominal aortic calcium measurements for association with renal function

Age (yrs)	59.1 \pm 9
CAC	25.5 (0.0–191.4)
AAC	444.7 (10.9–2079.87)
Glomerular filtration rate (ml/min/1.73 m ²)*	86 \pm 17
Mean cysC (mg/L)	0.93 \pm 0.18
Body mass index (kg/m ²)	28.2 \pm 5.1
Systolic blood pressure (mm Hg)	125 \pm 18
Total cholesterol (mg/dl)	202 \pm 35
High-density lipoprotein cholesterol (mg/dl)	54 \pm 16
Women	652 (55.3%)
CKD	74 (6.3%)
MA†	79 (8.3%)
Hypertension treatment	311 (26.4%)
Diabetes mellitus	102 (8.7%)
Lipid treatment	178 (15.1%)
Smoking	113 (9.6%)

Values are means \pm SDs, medians (interquartile ranges), or numbers of patients (percentages).

* Mean glomerular filtration rate in subjects with CKD was 52 ml/min/1.73 m².

† MA was defined as a urinary albumin-to-creatinine ratio ≥ 30 mg/g and median urinary albumin-to-creatinine ratio in those with MA was 48 mg/g.

level of S₁ were acquired. Calcium quantification was performed on an offline workstation (Acquarius, Terarecon, San Mateo, California) by 4 experienced observers.¹¹ A calcified lesion in the coronary arteries or aorta was defined as an area of ≥ 3 connected pixels with computed tomographic attenuation >130 HU using 3-dimensional connectivity criteria (6 points). Each scan was evaluated for the presence of CAC and AAC and a modified Agatston score was determined as previously described.¹² Briefly, this score is based on the area and density of calcified plaques within major coronary arteries and was originally devised for electron beam computed tomography.

The presence of CAC and AAC was defined as ≥ 90 th percentile age-, gender-, and cohort-specific cutpoints based on a healthy referent sample (free of hyperlipidemia, diabetes, hypertension, smoking, and prevalent cardiovascular disease).

At each examination cycle, all participants underwent a routine physical examination, anthropometry, and laboratory assessment of vascular risk factors as previously described.¹³ Prevalent cardiovascular disease was defined as coronary heart disease, stroke, transient ischemic attack, or intermittent claudication.

We used multivariable logistic regression analysis to study the association between exposure variables CKD, cysC, and MA and dependent variables CAC and AAC. CysC was treated continuously (with natural-logarithmic transformation to normalize the positively skewed distribution). CAC and AAC were treated dichotomously using 90th percentile age- and gender-specific cutpoints as described earlier. Covariates for multivariable adjustment included systolic blood pressure, hypertension treatment, diabetes mellitus, body mass index, total/high-density lipoprotein cholesterol, lipid treatment, and cigarette smoking. All analyses were performed using SAS 8.1 (SAS Institute, Cary, North Caro-

Table 2

Chronic kidney disease, cystatin C, and microalbuminuria and coronary artery calcium and abdominal aortic calcium

	CAC*		AAC*	
	(n = 1,147)		(n = 1,174)	
	OR (95% CI)	p Value	OR (95% CI)	p Value
CKD				
Age/gender	1.24 (0.64–2.41)	0.52	1.22 (0.69–2.16)	0.49
Multivariable	1.18 (0.59–2.36)	0.63	1.11 (0.61–2.04)	0.73
Log cysC (per SD)				
Age/gender	1.19 (1.01–1.41)	0.04	0.95 (0.81–1.12)	0.55
Multivariable	1.14 (0.95–1.38)	0.15	0.85 (0.71–1.01)	0.06
MA [†]				
Age/gender	1.16 (0.61–2.19)	0.64	1.53 (0.90–2.62)	0.12
Multivariable	0.81 (0.41–1.61)	0.54	1.10 (0.61–1.99)	0.74

Multivariable models were adjusted for age, gender, systolic blood pressure, hypertension treatment, diabetes, body mass index, total/high-density lipoprotein cholesterol, lipid treatment, and smoking.

* Defined as ≥ 90 th percentile age- and gender-specific cutpoints.

[†] Defined as a urinary albumin-to-creatinine ratio ≥ 30 mg/g.

CI = confidence interval.

lina). A 2-tailed p value < 0.05 was considered statistically significant.

Results

Baseline characteristics of our study sample are listed in Table 1. Mean age of the study sample was 59 years; 55.3% were women. Mean glomerular filtration rate in those with CKD was 52 ml/min/1.73 m², and median urinary albumin-to-creatinine ratio in those with MA was 48 mg/g. Prevalence of CAC ≥ 90 % healthy reference value was 15.7% (180 of 1,147), and that of AAC ≥ 90 % healthy reference value was 20.0% (229 of 1,174).

CKD was not associated with CAC or AAC in age- and gender-adjusted or multivariable-adjusted models (Table 2). CysC was associated with CAC in age- and gender-adjusted (odds ratio [OR] for log cysC per SD increment 1.19, 95% confidence interval 1.01 to 1.41, $p = 0.04$) but not multivariable models ($p = 0.15$). CysC was not related to AAC in age- and gender-adjusted or multivariable-adjusted models. MA was not significantly associated with CAC or AAC in age-/gender-adjusted or multivariable models (Table 2).

Discussion

In a cohort of community-dwelling men and women, CKD, cysC, and MA were not associated with CAC or AAC when accounting for classic cardiovascular disease risk factors. CysC and CAC were related in age- and gender-adjusted models but not with multivariable adjustment for cardiovascular disease risk factors.

In a mixed-ethnic sample of subjects 30 to 65 years of age, an association between CKD and CAC was detected only for CAC scores > 400 versus < 10 .¹⁴ More extreme CAC comparisons and different age and ethnic makeups of the study sample could explain the contrasting findings between our study and the previous investigation. Another recent clinical investigation demonstrated a high prevalence of CAC in subjects with stage 3 and 4 CKD and diabetes mellitus.¹⁵ The Multiethnic Study of Atherosclerosis

(MESA) noted that serum creatinine predicted CAC progression (mean scan interval 2.4 years between scans)¹⁶ but not baseline CAC. It is still possible that CKD is a more important predictor of change in CAC rather than prevalent CAC and/or AAC. Although we demonstrated an inverse correlation between glomerular filtration rate and CAC in our previous pilot study, participants in that study were sampled on the basis of quintiles of 10-year Framingham Coronary Heart Disease Risk Score¹⁷ which could have accounted for the differing results between the pilot study and the present study.

Noted associations between end-stage renal disease and vascular calcium¹⁸ could reflect more severe pathologic derangements than observed in moderate CKD including calcium and phosphate derangements,¹⁹ parathyroid hormone/vitamin D imbalance,²⁰ fetuin A deficiency/decreased hydroxyapatite, inhibiton,²¹ and inflammation.²²

Our finding of a positive association between cysC and CAC in age- and gender-adjusted but not in multivariable adjusted models suggests that associations between cysC and atherosclerosis may be mediated through classic cardiovascular disease risk factors. CysC was significantly associated with CAC progression over a 2.5-year period independent of major cardiovascular disease risk factors,²³ suggesting that cysC may be a more important predictor of incident than prevalent CAC and/or AAC.

MA is an independent predictor of cardiovascular disease,²⁴ possibly by pathways related to endothelial dysfunction.²⁵ Our finding that MA was not associated with CAC and AAC in multivariable models suggests that calcium may not be an independent pathway through which MA leads to cardiovascular disease.

Given nonsignificant findings, we assessed our statistical power to detect positive associations for CAC. At an alpha level of 0.05, we had 76% power to detect an OR of 1.75 for CKD and CAC, 80% power to detect an OR of 1.5 for cysC and CAC, and 80% power to detect an OR of 1.75 for MA and CAC.

Study strengths include routine assessment of renal function, cardiovascular disease risk factors, and calcium measurements. Our sample was unselected for kidney disease or diabetes. Several limitations should be acknowledged. Glomerular filtration rate values, estimated by the MDRD equation, could have led to misclassification, driving our association toward the null value. The MDRD equation tends to underestimate glomerular filtration rate especially in healthy subjects compared with patients with CKD.²⁶ Previous evidence has demonstrated that CAC levels²⁷ and presence of CKD²⁸ and albuminuria²⁹ differ across various racial groups and our study sample was largely of white, European descent. Therefore our results may not be generalizable to other ethnic groups. Our multidetector computed tomographic measurements were done a variable amount of time after exposures and covariate data collection, which could have led to misclassification. We had strong statistical power to detect ORs of 1.5 to 1.75, although we may have had more modest power to detect more modest associations between CKD and CAC or AAC. In subjects with CKD, mean glomerular filtration rate was 52 ml/min/1.73 m², which is quite moderate. Thus, our findings may not apply to patients with more severe CKD. Nonetheless, patients with stage 3 CKD comprise the largest proportion of patients with CKD in the United States.³⁰

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