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Candidate Gene Association Study of Coronary Artery Calcification in Chronic Kidney Disease

Findings From the CRIC Study (Chronic Renal Insufficiency Cohort)

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Objectives This study sought to identify loci for coronary artery calcification (CAC) in patients with chronic kidney disease (CKD).

Background CKD is associated with increased CAC and subsequent coronary heart disease (CHD), but the mechanisms remain poorly defined. Genetic studies of CAC in CKD may provide a useful strategy for identifying novel pathways in CHD.

Methods We performed a candidate gene study (~2,100 genes; ~50,000 single nucleotide polymorphisms [SNPs]) of CAC within the CRIC (Chronic Renal Insufficiency Cohort) study (N = 1,509; 57% European, 43% African ancestry). SNPs with preliminary evidence of association with CAC in CRIC were examined for association with CAC in the PennCAC (Penn Coronary Artery Calcification) (N = 2,560) and AFCS (Amish Family Calcification Study) (N = 784) samples. SNPs with suggestive replication were further analyzed for association with myocardial infarction (MI) in the PROMIS (Pakistan Risk of Myocardial Infarction Study) (N = 14,885).

Results Of 268 SNPs reaching $p < 5 \times 10^{-4}$ for CAC in CRIC, 28 SNPs in 23 loci had nominal support ($p < 0.05$ and in same direction) for CAC in PennCAC or AFCS. Besides *chr9p21* and *COL4A1*, known loci for CHD, these included SNPs having reported genome-wide association study association with hypertension (e.g., *ATP2B1*). In PROMIS, 4 of the 23 suggestive CAC loci (*chr9p21*, *COL4A1*, *ATP2B1*, and *ABCA4*) had significant associations with MI, consistent with their direction of effect on CAC.

Conclusions We identified several loci associated with CAC in CKD that also relate to MI in a general population sample. CKD imparts a high risk of CHD and may provide a useful setting for discovery of novel CHD genes and pathways. (J Am Coll Cardiol 2013;62:789–98) © 2013 by the American College of Cardiology Foundation

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Abbreviations and Acronyms

AA = African ancestry
CAC = coronary artery calcification
CHD = coronary heart disease
CKD = chronic kidney disease
EA = European ancestry
eGFR = estimated glomerular filtration rate
GWAS = genome-wide association study/studies
HDL = high-density lipoprotein cholesterol
HTN = hypertension
LD = linkage disequilibrium
LDL = low-density lipoprotein
MAF = minor allele frequency
MI = myocardial infarction
OR = odds ratio
PC = principal component
SNP = single nucleotide polymorphism

Atherosclerotic coronary heart disease (CHD) is a major heritable cause of death and morbidity worldwide. Recent genome-wide association studies (GWAS) have provided novel insights into the genetic basis of CHD (1–3). However, these discoveries explain only a small proportion of disease heritability, suggesting that further clinical and genomic strategies are required to explore the genetic basis of the disease and to advance clinical translation.

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One strategy to enhance genetic discovery in CHD is to focus efforts on unique clinical populations at an increased risk of disease. Patients with chronic kidney disease (CKD), representing over 20 million Americans (4), are at high risk for CHD. Although both traditional and nontraditional CHD risk factors are common in patients with CKD (5), the mechanistic basis for the observed accelerated atherosclerosis and CHD (6,7) remains poorly defined. Thus, genetic studies of atherosclerosis in CKD provide a strategy for identification of novel CHD loci that may also be relevant to the general population.

Noninvasive measurement of coronary artery calcification (CAC) is an indicator of subclinical coronary atherosclerosis before emergence of clinically evident CHD in persons with CKD (8,9), and is one of the few identifiable CHD predictors after controlling for traditional risk factors and Framingham risk score. A recent GWAS of CAC scores in community-based cohort studies of European ancestry (EA) identified 2 CAC loci, *9p21* and *PHACTR1* (10), also known for their association with CAD and myocardial infarction (MI) (3,11). Because the burden of CAC is increased substantially in persons with CKD, this patient population may provide specific insights into mechanisms of atherosclerosis and vascular diseases (12,13).

We performed the first systematic study to examine candidate genes for CAC in persons with CKD enrolled in the CRIC (Chronic Renal Insufficiency Cohort) study. Initial validation of CRIC findings was accomplished in 2 general population cohorts with CAC data. SNPs with suggestive replication were further analyzed for association with MI in the PROMIS (Pakistan Risk of Myocardial Infarction Study).

Methods

Study samples. DISCOVERY SAMPLE: THE CRIC STUDY. Our CKD study sample was derived from the CRIC study (N = 3,939), a multicenter, prospective, observational cohort study of renal and cardiovascular outcomes in patients with moderate CKD (14). Ethnically diverse adults (21 to 74 years; 46% female; 45% European ancestry [EA], 46% African ancestry [AA], 5% Hispanic, 4% Asian/Pacific Islander/Native American; ~50% with diabetes mellitus) with mild to moderate CKD (target estimated glomerular filtration rate [eGFR] 20 to 70 ml/min/1.73 m^2) were enrolled from 7 clinical centers in the United States between 2003 and 2006 (14,15). In-person follow-up visits are conducted annually. A nonrandom sample of 2,026 underwent computed tomography for quantification of CAC. This

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paper focuses on genetic associations with CAC in the CRIC EA and AA subsample in which CAC data and consent for genetic studies were available (N = 1,509). The CRIC study protocol was approved by the institutional review boards of all participating institutions, and study participants provided written informed consent. Multiple clinical, biochemical, and imaging variables were assessed on an annual basis as described in the Online Appendix, Feldman et al. (14), and Lash et al. (15).

CAC REPLICATION AND EXTENSION SAMPLES. We selected all SNPs associated with CAC score in CRIC at a threshold of $p < 5 \times 10^{-4}$ (as a suggestive first-stage threshold, given the modest size of our discovery sample) and examined their associations with CAC phenotypes in the PennCAC (Penn Coronary Artery Calcification) sample (EA, n = 2,058; and AA, n = 502) and the AFCS (Amish Family Calcification Study) (n = 784), as described in the Online Appendix, Post et al. (16), and Shen et al. (17). The PROMIS (Pakistan Risk of Myocardial Infarction Study) is a case-control study of acute MI in South Asians as described in the Online Appendix and in Saleheen et al. (18). In support of our use of PROMIS, genetic variants found in association with major lipids and CHD risk in Europeans have been previously replicated in PROMIS (19).

Genotyping. Genotyping (see also the Online Appendix) in the CRIC, PennCAC, and AFCS studies was performed using the HumanCVD BeadChip v2 ITMAT/Broad/CARe (IBC) array (Illumina, San Diego, California). This gene-centric SNP array includes ~50,000 SNPs in ~2,100 candidate genes, and was specifically designed to cover genes for cardiovascular, metabolic, and inflammatory diseases (20). Genotypes were called using Birdseed v2 as described (21). Samples from the CRIC study were excluded if any of the following were present: 1) a sample call rate <0.97; 2) reduced or excess heterozygosity (inbreeding, |F| <0.2); or 3) cryptic relatedness (PI_HAT identity-by-descent <0.2). SNPs were excluded within each race separately if the call rate was <90%; minor allele frequency (MAF) was <1%; or Hardy-Weinberg equilibrium p value was <0.0001. As described (17,22), sample and SNP filtering criteria were similar in PennCAC and AFCS. Genotyping in PROMIS was conducted on the Illumina 660Quad platform.

Statistical analysis. Data are reported as mean ± SD for continuous variables and as proportions for categorical variables. All analyses were conducted stratified by race. A principal component (PC) analysis plot for EA and AA samples in the CRIC study is provided in Online Figure 1. In CRIC, CAC was analyzed using several CAC traits and associated modeling techniques, as per published literature (8), including: 1) CACRes: linear regression of inverse normally transformed CAC residuals, where residuals were generated by a) stratifying by sex, b) regressing log (CAC+1) on age, c) calculating the residuals as the difference between the observed and predicted values, and d) combining the residuals across sexes; 2) LogCAC: linear regression of logCAC for individuals with CAC >0; and 3) separate logistic regressions for each of 3 clinically relevant CAC cut points (>0, CAC0; >100, CAC100; and >300, CAC300). Due to the exploratory nature of our analyses, we did not use a Bonferroni adjustment for all models tested. In all models, we adjusted for CRIC study site and the first 10 PCs derived using all available SNPs, to account for population substructure, whereas for #2 and #3, we additionally adjusted for age, age^2, sex, and interactions for age-by-sex and age^2-by-sex. Separate models were fit for each SNP, and tests of association were based on the Wald test. First, in order to assess the generalizability of the CRIC sample, we examined associations with top established CAD and CAC loci (3,10), using a nominal significance threshold of $p < 0.05$. All SNPs having suggestive signal (2-sided Wald test $p < 5 \times 10^{-4}$) with CAC in CRIC were interrogated for association with CAC phenotypes in PennCAC and AFCS.

In PennCAC, CAC phenotypes were defined and modeled in an identical manner to CRIC with the exception of a term for study site. In the family based AFCS, the Mixed Model Analysis for Pedigree software (17) was used to estimate the effects of genotype on CAC score for age and sex. The score was defined as log(CAC+1) and logCAC (for individuals with CAC >0), and the model also included an additional random polygenic component to account for relatedness in the sample. The lambda statistic of genomic control inflation (23) was calculated in all models for CRIC-EA (1.00 to 1.04), CRIC-AA (1.04 to 1.09), PennCAC-EA (1.04 to 1.07), PennCAC-AA (0.96 to 1.03), and AFCS-EA (1.04 to 1.05) (Online Figs. 2A to 2E). A SNP was considered suggestive if the associated p value corresponding to a test of no association, versus the 1-sided alternative that the corresponding coefficient is different than 0 and in the same direction as observed in CRIC, was >0.05. Top CAC-associated SNPs, or best proxies if SNP data were not available (linkage disequilibrium [LD] $r^2 > 0.6$ using SNAP version 2.2) (24), were analyzed for their association with MI in PROMIS using logistic regression models that included age, sex, and the first 10 PCs. Because MI is a different trait from CAC, a 2-sided p value <0.05 was considered statistically meaningful.

Meta-analysis of summary statistics across race or study in CRIC and PennCAC applied a weighted *z*-score method using METAL (25), as we have previously described (1,22). All analysis, with the exception of the pedigree analysis for AFCS, was performed using PLINK version 1.06 or R version 2.14.1 (26).

Results

Baseline characteristics of the CRIC sample. Baseline clinical and demographic characteristics of the CRIC study CAC genetic subsample by ancestry and sex are presented in Table 1. The average age was 57 years, and did not vary significantly by ancestry or sex; 43% were AA, 47% were

Table 1 Baseline Characteristics of the CRIC Genetic Sample With CAC Data

	European Ancestry		African Ancestry	
	Male (n = 469)	Female (n = 387)	Male (n = 324)	Female (n = 329)
Age, yrs	57.9 ± 11.3	57.5 ± 11.3	56.3 ± 11.2	58 ± 10.6
Tobacco and alcohol use				
Current smoker	40 (8.5)	29 (7.5)	58 (17.9)	51 (15.5)
Never smoked*	210 (44.8)	207 (53.5)	133 (41.0)	174 (52.9)
Alcohol	376 (80.2)	296 (76.5)	213 (65.7)	170 (51.7)
Cardiovascular disease				
Myocardial infarction or coronary revascularization	69 (14.7)	33 (8.5)	39 (12.0)	39 (11.9)
Stroke	34 (7.2)	21 (5.4)	42 (13.0)	44 (13.4)
PAD	23 (4.9)	11 (2.8)	23 (7.1)	19 (5.8)
BP variables				
Hypertension†	378 (80.6)	267 (69.0)	298 (92.0)	305 (92.7)
Systolic BP, mm Hg	121.3 ± 17.1	118.4 ± 18.4	132.2 ± 22.9	131.3 ± 21.4
Diastolic BP, mm Hg	71.4 ± 11	67.1 ± 10.7	77.4 ± 14.6	73 ± 12.3
Lipoprotein and blood variables				
Hypercholesterolemia‡	401 (86.0)	265 (69.0)	268 (83.0)	241 (73.0)
Lipid-lowering medication	295 (63.0)	199 (51.0)	182 (57.0)	169 (52.0)
LDL cholesterol, mg/dl	97.1 ± 31.4	107.3 ± 32	104.4 ± 37.2	113.3 ± 37.9
HDL cholesterol, mg/dl	42.7 ± 12	56 ± 16.8	45 ± 13.3	54.3 ± 17.3
Total cholesterol, mg/dl	175.9 ± 39.8	191.3 ± 39.2	181 ± 45	195 ± 48.6
Triglycerides, mg/dl	135 (90.5–202.5)	119 (84–167)	114 (78–170)	105 (78–148)
Adjusted serum calcium, mg/dl§	9.1 ± 0.4	9.2 ± 0.4	9.3 ± 0.4	9.4 ± 0.5
Serum phosphate, mg/dl	3.4 ± 0.6	3.7 ± 0.6	3.6 ± 0.7	3.8 ± 0.6
C-reactive protein, mg/l	1.6 (0.8–3.9)	2.2 (0.9–5.4)	2.1 (1.01–4.97)	4.4 (1.6–9.0)
Fibrinogen, g/l	3.7 ± 1	3.8 ± 0.9	4 ± 1.1	4.5 ± 1.2
Metabolic variables				
BMI, kg/m^2	30 ± 5.1	30.5 ± 7.8	31.4 ± 5.6	33.9 ± 7.3
Waist circumference, cm	104.7 ± 13.6	101 ± 18.3	105.5 ± 14.8	108 ± 17.4
Diabetes	186 (39.7)	117 (30.2)	150 (46.3)	155 (47.1)
Metabolic syndrome‖	274 (58.4)	188 (48.6)	192 (59.3)	237 (72)
Blood glucose, mg/dl	109.7 ± 49.7	104.9 ± 44.4	112.4 ± 54.4	112.1 ± 44.5
Hemoglobin A_{1c}, %	6.3 ± 1.4	6.1 ± 1.3	6.7 ± 1.6	6.8 ± 1.7
Kidney function				
Adjusted serum creatinine, mg/dl¶	1.6 ± 0.4	1.3 ± 0.4	1.9 ± 0.6	1.6 ± 0.6
eGFR, ml/min/1.73 m^2#	51.1 ± 16	51.3 ± 19	49 ± 17	46.2 ± 17
Cystatin-C, mg/l	1.3 ± 0.4	1.3 ± 0.5	1.4 ± 0.5	1.4 ± 0.5

Values are mean ± SD, n (%), or median (interquartile range). *Never smoking was defined as <100 cigarettes over lifetime. †Hypertension was defined as systolic/diastolic blood pressure ≥140 mm Hg or ≥90 mm Hg, or use of antihypertensive medications. ‡Hypercholesterolemia was defined as use of cholesterol-lowering medications or total serum cholesterol >200 mg/dl. §Adjusted serum calcium (mg/dl) (calcium adjusted for hypoalbuminemia) = serum calcium (mg/dl) + 0.8 × (4 − serum albumin [g/dl]). ‖Metabolic syndrome was defined using National Cholesterol Education Program Adult Treatment Panel III criteria. ¶Serum creatinine was calibrated to the laboratory measurement. #The estimated GFR (eGFR) was calculated using the CRIC study–specific estimating equation that was derived in its iothalamate glomerular filtration rate subcohort.

BMI = body mass index; BP = blood pressure; CAC = coronary artery calcium; CRIC = Chronic Renal Insufficiency Cohort study; HDL = high-density lipoprotein cholesterol; LDL = low-density lipoprotein cholesterol; PAD = peripheral artery disease.

female, and 40% had diabetes. The median eGFR was 48 ml/min/1.73 m^2. Compared with expectations for similar age distributions in the general population, the CRIC study sample was more likely to be overweight and have hypertension (HTN), increased levels of triglycerides, fibrinogen, and C-reactive protein, and a high proportion had cardiovascular diseases at enrollment. Mean CAC scores and the distribution by 3 cut points (>0, >100, >300) in each of the ancestry and sex groups are presented in Table 2. In agreement with prior reports in the CRIC study (12) and the general population (27), CAC scores tended to be higher in men and EA. Median CAC scores and the prevalence of CAC >0 (66%), >100 (39%), and >300 (25%) were substantially higher than reported for population samples of similar age and ethnicity (8). Thus, compared with the general population, this CRIC study sample displayed increased prevalence of traditional and nontraditional CHD risk factors as well as a greater burden of subclinical and clinical atherosclerosis.

Association of established CAC and CAD loci with CAC in CRIC. The top published GWAS *9p21* allele (rs1333049C) for CAC (10) was associated in the same direction with CAC traits in CRIC (e.g., $z = 2.81$, $p = 0.005$ for CAC residual in meta-analysis of AA and

Table 2 Coronary Artery Calcification (CAC) Scores* in the CRIC Genetic Subsample

	European Ancestry		African Ancestry	
	Male (n = 469)	Female (n = 387)	Male (n = 324)	Female (n = 329)
Mean ± SD	531 ± 931	222.3 ± 552	350 ± 821	234 ± 568
Median (IQR)	123 (3.44–642.79)	4.2 (0.0–138.7)	20 (0.0–280.0)	12 (0.0–186.0)
CAC >0	368 (79)	215 (56)	218 (67)	199 (61)
CAC >100	245 (52)	113 (29)	128 (39)	98 (30)
CAC >300	178 (38)	67 (17)	77 (24)	59 (18)

Values are mean ± SD, median (interquartile range), or n (%). *Coronary artery calcification was estimated using Agatston scoring. IQR = interquartile range.

EA; p = 0.03 in EA, p = 0.07 in AA). Similarly, rs4977574G, a top *9p21* GWAS allele for CAD (3), was also associated in the same direction with CAC in CRIC (e.g., z = 3.18, p = 0.001 for CAC residual in meta-analysis of AA and EA; p = 0.04 in EA, p = 0.01 in AA).

Rare variants in *LPA* (rs3798220) (3,28) and *PCSK9* (rs11591147/R46L) (29) are associated with CHD risk. The IBC array included these variants or proxies (LD $r^2 > 0.6$). Despite limited power to detect associations with SNPs of low frequency, there was suggestive evidence of CAC associations with these rare variants in the expected direction of effect. These findings were generally consistent across CAC traits; the strongest association for rs3798220 in *LPA* was with LogCAC in EA (beta = 1.1, p = 0.02, MAF = 0.02) and for rs11591147 in *PCSK9* was with CAC0 in EA (odds ratio [OR]: 3.6, p = 0.14, MAF = 0.009).

Strongest IBC loci for CAC in CRIC. In order to maximize identification of candidate loci for CAC in the CRIC sample, we tested IBC SNP associations across multiple CAC trait definitions within each race separately as well as in a race-combined meta-analysis. For ~45,000 SNPs examined, Online Table 1 shows those SNPs (n = 268) that were associated at $p < 5 \times 10^{-4}$ with any CAC trait within either race or in their meta-analysis. As might be expected with our relatively small sample size, none of these SNPs reached the Bonferroni-corrected threshold for the estimated number of independent SNPs tested on the IBC array ($p < 3 \times 10^{-6}$) (30). Regional plots including recombination rate, LD, and p values for SNPs at selective top CAC loci in CRIC are presented in Online Figures 3A to 3D.

Next, we examined these top CRIC CAC SNPs for their association with CAC in PennCAC interrogating the same CAC trait and race (or meta-analysis) combinations evaluated in the CRIC sample. We also tested these SNPs for their associations in AFCS, but in this case, the strongest available CAC phenotype association is presented because the AFCS family structure and analysis did not permit an interrogation of the identical CAC traits and race as those in CRIC. Summary data for each study are shown in Table 3 for the subset of SNPs that had nominal evidence (effect in the same direction, 1-sided $p < 0.05$) for similar effects in PennCAC or AFCS. Overall, 28 SNPs representing 23 independent loci met these suggestive replication criteria and included known CAD and CAC loci (*9p21* and *COL4A1*) (3,10,31), known HTN and diabetes loci not previously associated with coronary atherosclerosis (*HNF4A*, *ATP2B1*, *ADIPOR2* [32–34]), as well as several loci not previously reported to be associated with CAC, CAD, or CHD risk factors. In exploratory meta-analyses of the CRIC and PennCAC data for these 28 SNPs (Online Table 2), SNPs at 2 loci (*ABCA4* and *HNF4A*) reached $p < 2.38 \times 10^{-5}$, the Bonferroni-corrected threshold for the number of genes tested. No locus met the more stringent IBC array SNP-wide Bonferroni correction ($p < 3 \times 10^{-6}$) (30).

Association of suggestive CAC loci with MI in the PROMIS sample. For SNPs with suggestive CAC association, we observed directionally consistent associations (alleles associated with greater CAC also increased odds of MI with 2-sided $p < 0.05$) with MI in PROMIS for 4 of 23 (17.4%) independent loci (exact binomial test p = 0.026; null proportion = 0.05 vs. 1-sided alternative that proportion is >0.05) (Table 4). Not surprisingly, the strongest signal was for SNPs at the 9p21 locus (most significant SNP rs4977574; $p = 5.67 \times 10^{-12}$). Three additional loci, *ATP2B1* (rs11105354; $p = 3.3 \times 10^{-5}$), *COL4A1* (rs13260; $p = 9.6 \times 10^{-4}$), and *ABCA4* (rs3789422; $p = 1.7 \times 10^{-2}$), were associated with MI. Associations at both *ATP2B1* and *COL4A* exceeded the p value Bonferroni adjustment for multiple testing of suggestive CAC SNPs ($p < 0.0022$; 0.05 of 23 independent loci).

Discussion

We sought to identify genes for CAC in patients with CKD, a population at an increased risk of CHD. We found that previously identified loci for CAC and CAD in non-CKD populations had the expected pattern of associations with CAC in the CRIC. We also identified a group of suggestive loci for CAC in the CRIC study sample, for which associations were similar in PennCAC or AFCS datasets. In addition to *chr9p21* and *COL4A1*, previously shown by GWAS to be associated with CAC and CAD (3,10), we identified *ATP2B1* (a locus for HTN) (30) and *HNF4A* (a locus for high-density lipoprotein cholesterol [HDL-C] and diabetes) (32,35), previously identified by GWAS to be associated with CHD risk factors. Besides

Table 3 Top Associations With CAC Traits in CRIC, With Directionally Consistent Associations in PennCAC or AFCS

SNP	Trait	Race*	Chr	Locus	Effect Allele	Effect Allele Frequency (AA/EA)	CRIC (N = 1,509) β (OR)†	CRIC SE	CRIC p Value	PennCAC (N = 2,563) β (OR)†	PennCAC SE	PennCAC p Value‡	AFCS§ (N = 784) β	AFCS SE	AFCS p Value‡
SNPs with CAC association in either AA or EA															
rs3766332	CAC100	AA	1	*PTGFR*	A	0.15/0.06	0.67 (1.96)	0.19	3.30E−04	0.38 (1.46)	0.27	0.08	1.33	0.62	0.01
rs1436606‖	LogCAC	AA	8	*SDC2*	A	0.92/0.999	1.04	0.29	3.90E−04	0.68	0.36	0.03	NA	NA	NA
rs11236998	CACRes	AA	11	*PHCA*	A	0.07/0.06	−0.39	0.11	3.60E−04	−0.01	0.15	0.48	−0.29	0.13	0.01
rs7134070	CAC0	AA	12	*ADIPOR2*	A	0.85/0.99	0.69 (1.99)	0.19	3.20E−04	0.33 (1.39)	0.19	0.04	0.50	1.14	0.33
rs4456611	CAC100	AA	18	*BCL2*	A	0.49/0.50	−0.48 (0.62)	0.14	4.10E−04	−0.19 (0.83)	0.18	0.15	−0.31	0.13	0.01
rs3943258	CAC100	AA	18	*BCL2*	A	0.49/0.46	0.49 (1.63)	0.13	2.80E−04	0.10 (1.11)	0.20	0.30	0.31	0.13	0.01
rs2868095‖	CAC0	AA	20	*HNF4A*	A	0.11/NA	0.96 (2.61)	0.24	7.60E−05	0.43 (1.54)	0.22	0.02	0.12	0.08	0.06
rs3789422	CAC0	EA	1	*ABCA4*	A	0.05/0.04	1.82 (6.18)	0.46	8.50E−05	0.52 (1.68)	0.21	0.005	0.19	0.27	0.23
rs12613413	CAC300	EA	2	*MAP3K2*	A	0.82/0.80	−0.54 (0.58)	0.15	3.60E−04	−0.10 (0.90)	0.12	0.19	−0.31	0.19	0.04
rs13386681	LogCAC	EA	2	*ATOH8*	A	0.09/0.07	−0.80	0.21	1.60E−04	−0.27	0.13	0.01	−0.10	0.35	0.38
rs4946932	CAC0	EA	6	*FOXO3*	A	0.75/0.29	−0.56 (0.57)	0.14	6.70E−05	−0.01 (0.99)	0.16	0.48	−0.32	0.16	0.02
rs10499276	CACRes	EA	6	N/A	A	0.09/0.13	0.29	0.07	6.90E−05	0.08	0.05	0.05	0.10	0.13	0.21
rs7904918	CAC100	EA	10	*ACSL5*	A	0.52/0.71	0.44 (1.56)	0.13	3.90E−04	0.17 (1.19)	0.09	0.03	0.30	0.17	0.04
rs11105354‖	CAC100	EA	12	*ATP2B1*	A	0.90/0.82	−0.63 (0.53)	0.15	2.80E−05	−0.10 (0.90)	0.11	0.17	−0.30	0.19	0.05
rs13260	CAC0	EA	13	*COL4A1*	A	0.23/0.1	−0.80 (0.45)	0.20	8.70E−05	−0.10 (0.90)	0.12	0.19	−1.20	0.67	0.03

SNP	Trait	Race*	Chr	Locus	Effect Allele	Effect Allele Frequency (AA/EA)	z-Score	p Value	z-Score	p Value	β	SE	p Value
Additional SNPs with CAC association in meta-analysis of AA and EA data													
rs12132247	CAC300	Both	1	*GNG12*	A	0.36/0.32	−4.06	5.00E−05	−0.43	0.38	−0.23	0.13	0.03
rs1635502	CAC300	Both	1	*EXO1*	A	0.32/0.44	3.63	2.80E−04	0.41	0.34	0.13	0.07	0.03
rs6667260	CACRes	Both	1	*ITPKB*	A	0.41/0.51	3.94	8.00E−05	0.26	0.39	0.18	0.11	0.04
rs3768991	LogCAC	Both	2	*NPAS2*	A	0.79/0.48	−3.56	3.70E−04	−1.09	0.14	−0.24	0.14	0.04
rs12374310	CAC100	Both	4	*PPARGC1A*	C	0.82/0.60	−3.63	2.80E−04	−1.53	0.06	−0.25	0.13	0.03
rs17056112	CAC100	Both	8	*ADRA1A*	A	0.01/0.03	3.59	3.30E−04	1.70	0.04	NA	NA	NA
rs4977574	CAC100	Both	9	*9p21*	A	0.83/0.49	−3.92	9.00E−05	−0.89	0.18	−0.36	0.13	4.00E-04
rs2891168	CAC100	Both	9	*9p21*	A	0.81/0.49	−3.76	1.70E−04	−0.86	0.19	−0.38	0.10	1.60E-04
rs10757278	CAC100	Both	9	*9p21*	A	0.82/0.49	−3.57	3.60E−04	−0.99	0.16	−0.34	0.11	0.001
rs10757274	CAC100	Both	9	*9p21*	A	0.80/0.49	−3.70	2.20E−04	−0.86	0.19	−0.36	0.11	4.00E-04
rs10757272	CAC100	Both	9	*9p21*	A	0.20/0.51	3.58	3.50E−04	0.85	0.20	0.36	0.11	4.00E-04
rs7964239	CAC0	Both	12	*BCAT1*	A	0.78/0.92	−3.65	2.60E−04	−0.84	0.20	−0.37	0.16	0.01
rs2834669	CAC300	Both	21	*RUNX1*	A	0.92/0.92	3.55	3.90E−04	1.96	0.02	0.42	0.23	0.03

*Both = European and African ancestry. †For continuous variables (LogCAC and CACRes [CAC residual]), the effect is presented as beta (β); for CAC cut points (>0, >100, >300), the effect is additionally presented as the odds ratio (OR). ‡A 1-sided p value is presented for PennCAC and AFCS, corresponding to a test of no association versus the 1-sided alternative that the corresponding coefficient is different than 0 and in the same direction as observed in CRIC. §Best AFCS p value from LogCAC or LogCAC+1 analyses. ‖SNP also reached $p < 5 \times 10^{-4}$ in meta-analysis across race in CRIC.

AFCS = Amish Family Calcification Study; Chr = chromosome; PennCAC = Penn Coronary Artery Calcification; SNP = single nucleotide polymorphism; other abbreviations as in Table 1.

Table 4 Association of Top CAC SNPs* With MI in PROMIS

SNP	Race†	Chr	Locus	Effect Allele	PROMIS (N = 14,885) OR	95% CI	p Value‡
SNPs with effect estimates in same direction for CAC and MI							
rs4977574	**Both**	**9**	***9p21***	**A**	**0.85**	**0.81–0.89**	**5.70E−12**
rs2891168	**Both**	**9**	***9p21***	**A**	**0.85**	**0.81–0.89**	**7.70E−12**
rs10757278	**Both**	**9**	***9p21***	**A**	**0.85**	**0.82–0.89**	**2.20E−11**
rs10757272	**Both**	**9**	***9p21***	**A**	**1.17**	**1.11–1.22**	**8.10E−11**
rs10757274	**Both**	**9**	***9p21***	**A**	**0.82**	**0.76–0.88**	**6.80E−08**
rs11105354	**EA**	**12**	***ATP2B1***	**A**	**0.90**	**0.86–0.95**	**3.30E−05**
rs13260	**EA**	**13**	***COL4A1***	**A**	**0.87**	**0.80–0.94**	**9.70E−04**
rs3789422	**EA**	**1**	***ABCA4***	**A**	**1.20**	**1.03–1.40**	**0.02**
rs4946932	EA	6	*FOXO3*	A	0.96	0.92–1.01	0.09
rs7904918	EA	10	*ACSL5*	A	1.04	0.99–1.09	0.15
rs2868095	AA	20	*HNF4A*	A	1.03	0.98–1.09	0.23
rs11236998	AA	11	*PHCA*	A	0.96	0.87–1.06	0.46
rs2834669	Both	21	*RUNX1*	A	1.04	0.94–1.15	0.46
rs12613413	EA	2	*MAP3K2*	A	0.99	0.94–1.03	0.56
rs7964239	Both	12	*BCAT1*	A	0.99	0.94–1.04	0.70
rs4456611	AA	18	*BCL2*	A	0.99	0.95–1.04	0.82
rs1635502	Both	1	*EXO1*	A	1.00	0.95–1.05	0.90
SNPs with effect estimates in opposite direction for CAC and MI							
rs7134070	AA	12	*ADIPOR2*	A	0.92	0.83–1.01	0.09
rs3766332	AA	1	*PTGFR*	A	0.95	0.90–1.01	0.11
rs12374310	Both	4	*PPARGC1A*	C	1.05	0.98–1.13	0.16
rs10499276	Both	6	*LOC729635*	A	0.93	0.83–1.03	0.18
rs17056112	Both	8	*ADRA1A*	A	0.94	0.84–1.04	0.22
rs13386681	EA	2	*ATOH8*	A	1.02	0.97–1.08	0.44
rs6667260	Both	1	*ITPKB*	A	0.99	0.94–1.03	0.58
rs12132247	Both	1	*GNG12*	A	1.01	0.95–1.06	0.82
rs3768991	Both	2	*NPAS2*	A	1.00	0.96–1.05	0.86
rs3943258	AA	18	*BCL2*	A	1.00	0.95–1.04	0.90

*SNPs with suggestive association with CAC in CRIC as per Table 3. Data not available for rs1436606. †Both = European and African ancestry. ‡Two-sided p value. Values in **bold** are statistically significant. MI = myocardial infarction; PROMIS = Pakistan Risk of Myocardial Infarction Study; other abbreviations as in Tables 1 and 3.

*9p21*and *COL4A*, the suggestive CAC loci, *ATP2B1* and *ABCA4*, were associated with MI in the PROMIS sample further supporting their potential importance in CHD.

CKD imparts a substantial increase in CHD risk (9) although the mechanisms remain incompletely understood. The CKD milieu might provide a discovery opportunity for CHD genes and pathways. Indeed, we found that SNPs at the *9p21* locus, the top GWAS signal for CAC and CHD in the general population, had the expected pattern of association with CAC in the CRIC study sample. Further, despite modest sample size, we identified trends for low frequency and rare CHD variants in *LPA* and *PCSK9* (25,26), with the expected direction and magnitude of effect, on CAC in the CRIC study. These findings support our search for CAC loci in CKD patients and suggest that this may be 1 strategy to enhance discovery of novel genes for heart disease. Our top findings in the CRIC sample provide preliminary support for the concept that genes identified for CAC in CKD may have relevance to CHD risk in the general population. In addition to SNPs at *9p21* and *COL4A1*, top SNPs for CAC in the CRIC sample reside in loci (e.g., *ATP2B1*, *HNF4A*, and *ABCA4*) that have established genetic associations with CHD risk factors.

Several GWAS have identified *ATP2B1* as a locus for HTN and blood pressure in samples of EA and AA (33,36). *ATP2B1* encodes a plasma membrane calcium-transporting ATPase that plays a critical role in intracellular calcium homeostasis by removing bivalent calcium ions from eukaryotic cells. This suggests a potential role in regulation of arterial tone and vascular calcification. Indeed, mice lacking *Atp2b1* in vascular smooth muscle cells had elevated blood pressure (37) suggesting a protective role in HTN and CHD. In the CRIC sample, the *ATP2B1* rs11105354A allele that is associated with lower CAC (e.g., OR: 0.53; $p = 2.8 \times 10^{-5}$ for CAC100) is also the most significant *ATP2B1* allele for lower blood pressure and reduced HTN in a large meta-analysis of EA individuals (33). This same SNP is in strong LD ($r^2 = 0.9$) with the strongest *ATP2B1* variant for HTN in Japanese (36). Furthermore, the *ATP2B1* SNP related to lower CAC also had lower odds of MI (OR: 0.9, $p = 3.3 \times 10^{-5}$) in the PROMIS sample. Thus, our findings provide strong support for a role for *ATP2B1* in coronary atherosclerosis and CHD.

Given the established GWAS associations with HTN, the *ATP2B1* locus effect on CAC may be mediated through regulation of vasomotor tone and blood pressure. *ATP2B1* might also play a specific role in regulating arterial calcification in the setting of disordered calcium and phosphate metabolism that is characteristic of progressive CKD (38) although this has yet to be established. Using cross-sectional CRIC baseline data, we found a weak association of the *ATP2B1* rs11105354A allele with increased serum calcium ($p = 0.02$), but no association with serum phosphorous, baseline blood pressure traits, or eGFR (data not shown).

Since initial submission of our paper, a GWAS in Han Chinese has demonstrated that SNPs at the *ATP2B1* locus have genome-wide significant associations with clinical CAD (e.g., rs7136259; $p = 5.68 \times 10^{-10}$) (39). Although the lead SNP for CAD in the Han has only nominal associations with CAC in our CRIC data (rs7136259; $p = 0.01$ for CAC100 in CRIC EA), this SNP has low LD in EA samples with our top CAC-associated *ATP2B1* SNP (rs11105354; $r^2 = 0.136$ in CEU [Utah residents with ancestry from northern and western Europe]). However, there is high correlation between these 2 SNPs in Asian samples ($r^2 = 0.963$ in CHBJPT [Han Chinese in Beijing, China, and Japanese in Tokyo, Japan]) (LD estimates from 1000 Genomes Project Pilot 1 data using SNAP), suggesting that the CAC and HTN association for the *ATP2B1* locus in EA populations overlaps with the CAD finding in the Han population. This new report reinforces the significance of our findings in CKD and underscores the importance of this locus in CHD.

Through GWAS, the *HNF4A* locus has been associated with HDL-C (40), metabolic dyslipidemia (41), and type 2 diabetes (T2DM) in multiethnic populations (32,42). *HNF4A* encodes a nuclear transcription factor that regulates development and function of the liver, kidney, pancreas, and intestines (43) and modulates hepatic lipogenesis as well as apolipoprotein C-III and very low-density lipoprotein secretion (44). Mutations in *HNF4A* affect insulin secretion and have been linked to maturity onset diabetes in the young (45). In the CRIC, the *HNF4A* SNP that is associated with higher CAC is not in LD with variants that have published associations with lower HDL-C and higher odds of T2DM. This may reflect differences in ethnic LD structure because *HNF4A* SNP associations with CAC in CRIC were detected in the AA subsample, whereas cardiometabolic findings to date for the *HNF4A* locus have been in non-AA samples. However, evidence for *HNF4A* association with clinical CHD, including within PROMIS, is lacking.

Many of the suggestive loci for CAC contain genes with known associations with cardiometabolic traits and pathways. Besides *ATP2B1* and *HNF4A*, these include the adiponectin receptor *ADIPOR2* (34); *PPARGC1*, which regulates *PPARG*, adipose, and lipids (46); *FOXO3*, a longevity gene linked to insulin pathway signaling (47); *ACSL5*, which regulates fatty acid metabolism; *BCL2* (48) and *BCAT2*, recently found to associate with T2DM; and *ABCA4*, a gene for Stargardt retinal disease that also has suggestive association with HDL particle number and size (49). Whether any of these loci have causal roles in atherosclerosis and CHD remains to be determined. These results do suggest, however, that loci modulating cardiometabolic risks that are exacerbated in CKD might be revealed through the study of atherosclerosis in patients with CKD.

Our study has several strengths. This is the first systematic search for candidate genes for a coronary atherosclerosis trait in patients with CKD within the CRIC. The CRIC study is a rigorously designed, multicenter, National Institutes of Health–sponsored cohort study of CKD that includes almost equal numbers of EA and AA individuals and generates resources for subclinical atherosclerosis, multiple biomarkers of CKD and CHD risk, as well as incident CHD events and CKD progression (12,14). The CRIC study genotyped the IBC array in all eligible participants, in part to facilitate comparisons with other existing IBC datasets. Thus, we were able to extend CRIC study findings by leveraging independent resources with IBC CAC datasets, as well as a large GWAS study of MI.

Study limitations. First, the CRIC study sample size is relatively small for genetic studies of complex traits. Second, because of the exploratory focus of our analyses, we applied nonconservative statistical thresholds that did not meet criteria for genome-wide significance, and we did not attempt to perform Bonferroni correction for the full extent of multiple testing. Rather, our approach sought to identify a group of loci with suggestive evidence for CAC and CHD risk that warrant further study. Our initial findings do provide some support for the potential importance of several of these loci in cardiometabolic disease. Because our work tested many different outcome models across race and CAC phenotypes, we did not run additional models adjusting for traditional or novel (e.g., phosphate and FGF23 [50,51]) risk biomarkers for CHD in CKD. Future studies will focus on determining the role of intermediate factors in the genetic associations with CAC in CKD. We acknowledge the need for larger studies and targeted replications. Third, in using CKD as a setting to try to identify CHD loci of broader relevance, our CAC follow-up in PennCAC and AFCS was not CKD focused; the PROMIS study also was not in CKD patients and was focused on South Asians. This heterogeneity may have limited replication. In support of use of the PROMIS sample, however, most CHD loci have consistent associations with MI in European and South Asian samples (2,18). Finally, although not a direct measure, studies have shown that CAC provides a quantitative estimate of coronary atherosclerosis (52) and is a useful predictor of CHD, including in patients with CKD (8,9).

Conclusions

CKD, which imparts a high risk for CHD, may provide a setting for discovery of genes for heart disease. Using

a CRIC study sample of patients with CKD, we identified several loci with suggestive evidence for CAC, some of which are also associated with MI in a general population sample. Our findings support the potential for discovery of novel pathways involved in CHD through focus on atherosclerosis traits in patients with CKD.

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Key Words: candidate genes ▪ chronic kidney disease (CKD) ▪ Chronic Renal Insufficiency Cohort Study (CRIC) ▪ coronary artery calcification (CAC) ▪ myocardial infarction (MI) ▪ risk factors ▪ single nucleotide polymorphisms (SNPs).

APPENDIX

For a supplemental methods section, figures, and tables, please see the online version of this article.