Published in final edited form as:
J Am Coll Cardiol. 2013 August 27; 62(9): 789–798. doi:10.1016/j.jacc.2013.01.103.

Candidate Gene Association Study of Coronary Artery Calcification in Chronic Kidney Disease: Findings from the Chronic Renal Insufficiency Cohort Study

Jane F Ferguson, PhD[1], **Gregory J Matthews, PhD**[2], **Raymond R Townsend, MD**[3,†], **Dominic S Raj, MD**[4], **Peter A. Kanetsky, PhD MPH**[5], **Matthew Budoff, MD**[6], **Michael J Fischer, MD MSPH**[7], **Sylvia E Rosas, MD MSCE**[3], **Radhika Kanthety, MD MSHS**[8], **Mahboob Rahman, MD MS**[8,†], **Stephen R Master, MD PhD**[9], **Atif Qasim, MD MSCE**[1], **Mingyao Li, PhD**[5], **Nehal N. Mehta, MD MSCE**[1], **Haiqing Shen, PhD**[10], **Braxton D Mitchell, MPH PhD**[10], **Jeffrey R O'Connell, PhD**[10], **Alan R Shuldiner, MD**[10,11], **Weang Kee Ho, PhD**[12], **Robin Young, PhD**[12], **Asif Rasheed, MD**[13], **John Danesh, MBChB PhD**[12], **Jiang He, MD PhD**[14,†], **John W Kusek, PhD**[15,†], **Akinlolu O Ojo, MD PhD**[16,†], **John Flack, MD MPH**[17], **Alan S Go, MD**[18,†], **Crystal A Gadegbeku, MD**[19], **Jackson T Wright, MD PhD**[20], **Danish Saleheen, PhD**[1,12,13], **Harold I Feldman, MD MSCE**[3,5,†], **Daniel J Rader, MD**[1], **Andrea S Foulkes, PhD**[2], **Muredach P Reilly, MBChB MSCE**[1], and **the Chronic Renal Insufficiency Cohort (CRIC) Study Investigators**[†]

[1]Cardiovascular Institute, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, PA

[2]School of Public Health and Health Sciences, University of Massachusetts Amherst

[3]Renal, Electrolyte and Hypertension Division, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, PA

[4]MFA – The George Washington University, Washington DC

[5]Center for Clinical Epidemiology and Biostatistics, and Department of Biostatistics and Epidemiology, Perelman School of Medicine at the University of Pennsylvania, Philadelphia

[6]Los Angeles Biomedical Research Institute, Torrance CA

[7]Medicine, Jesse Brown VA Medical Center and University of Illinois Hospital and Health Sciences System, Chicago, and Center for Management of Complex Chronic Care, Edward Hines Jr., VA Hospital, Hines, IL

[8]Department of Nephrology and Hypertension, Case Western Reserve University, Cleveland, OH

[9]Department of Pathology and Laboratory Medicine, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, PA

[10]Department of Medicine, University of Maryland School of Medicine, Baltimore, MD

Corresponding Author: Muredach P Reilly, Perelman School of Medicine at the University of Pennsylvania, 11-136 Translational Research Building, 3400 Civic Center Boulevard, Building 421, Philadelphia, PA 19104.Tel: 215573-1214. muredach@mail.med.upenn.edu.

[†]Chronic Renal Insufficiency Cohort (CRIC) Study Investigators Lawrence J. Appel, MD, MPH, Harold I. Feldman, MD, MSCE, Alan S. Go, MD, Jiang He, MD, PhD, John W. Kusek, PhD, James P. Lash, MD, Akinlolu Ojo, MD, PhD, Mahboob Rahman, MD, Raymond R. Townsend, MD

[11]Geriatric Research and Education Clinical Center, Veterans Administration Medical Center, Baltimore, MD

[12]Department of Public Health and Primary Care, University of Cambridge

[13]Center for Non-Communicable Diseases, Pakistan

[14]Department of Epidemiology, Tulane University School of Public Health and Tropical Medicine, New Orleans, LA

[15]National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK)

[16]University of Michigan School of Medicine, Ann Arbor, MI

[17]Department of Medicine, Wayne State University School of Medicine, Detroit, MI

[18]Division of Research, Kaiser Permanente of Northern California, Oakland, CA

[19]Temple University School of Medicine, Department of Medicine, Section of Nephrology, Philadelphia, PA

[20]Department of Medicine, Case Western Reserve University, Cleveland, OH

Abstract

Objectives—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 SNPs) of CAC within the Chronic Renal Insufficiency Cohort (CRIC) 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 PennCAC (n=2,560) and Amish Family Calcification Study (AFCS; n=784) samples. SNPs with suggestive replication were further analyzed for association with myocardial infarction (MI) in the Pakistan Risk of Myocardial Infarction study (PROMIS) (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 GWAS association with hypertension (e.g., *ATP2B1*). In PROMIS, four 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.

Keywords

Coronary artery calcification (CAC); chronic kidney disease (CKD); Chronic Renal Insufficiency Cohort Study (CRIC); myocardial infarction (MI); risk factors; candidate genes; single nucleotide polymorphisms (SNPs)

INTRODUCTION

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.

One strategy to enhance genetic discovery in CHD is to focus efforts on unique clinical populations at 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 non-traditional 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.

Non-invasive 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 two CAC loci, *9p21* and *PHACTR1* (10), also known for their association with CAD and myocardial infarction (MI) (3,11). As 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 Chronic Renal Insufficiency Cohort (CRIC) Study. Initial validation of CRIC findings was accomplished in two general population cohorts with CAC data. SNPs with suggestive replication were further analyzed for association with MI in the Pakistan Risk of Myocardial Infarction study (PROMIS).

METHODS

Study Samples

Discovery Sample: The CRIC Study—Our CKD study sample was derived from the CRIC Study (n=3,939), a multi-center prospective observational cohort study of renal and cardiovascular outcomes in patients with moderate CKD (14). Ethnically diverse adults (21–74 years; 46% female; 45% European- (EA), 46% African-ancestry (AA), 5% Hispanic, 4% Asian/ Pacific Islander/ Native American; ~50% 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 seven clinical centers in the US between 2003 and 2006 (14,15). In-person follow-up visits are conducted annually. A non-random sample of 2026 underwent computed tomography (CT) for quantification of CAC. This manuscript focuses on genetic associations with CAC in the CRIC EA and AA sub-sample 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 (IRB) 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 supplement and (14,15).

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 1st stage threshold given

the modest size of our discovery sample) and examined their associations with CAC phenotypes in the Penn Coronary Artery Calcification sample (PennCAC, EA n=2,058 and AA n=502) and the Amish Family Calcification Study (AFCS) (n=784) as described in the supplement and in (16,17). The Pakistan Risk of Myocardial Infarction study (PROMIS) is a case-control study of acute MI in South Asians as described in the supplement and in (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 Supplementary Methods) in the CRIC, PennCAC and AFCS studies was performed using the HumanCVD BeadChip V2 IBC ITMAT/Broad/CARE (IBC) Array (Illumina, Inc.). 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 as described (20). Samples from the CRIC Study were excluded if: sample call rate <0.97; reduced or excess heterozygosity (Inbreeding | F | <0.2); or cryptic relatedness (PI_HAT identity-by-descent <0.2). SNPs were excluded within each race separately if the call rate <90%; minor allele frequency (MAF) <1%; Hardy-Weinberg equilibrium (HWE) P <0.0001. As described (17,21), 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 means ± standard deviations (SD) for continuous variables and as proportions for categorical variables. All analyses were conducted stratified by race. A principal component analysis plot for EA and AA samples in CRIC is provided in Supplementary 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 gender, (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 genders; (2) LogCAC: linear regression of logCAC for individuals with CAC >0; and (3) separate logistic regressions for each of three 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 principal components (PCs) derived using all available SNPs, to account for population substructure while for (2) and (3), we additionally adjusted for age, age^2, gender and interactions for age-by-gender and age^2-by-gender. 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. Then, all SNPs having suggestive signal (two-sided Wald Test P <5×10^{-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 (22) was calculated in all models for CRIC-EA (1.00 – 1.04), CRIC-AA (1.04 – 1.09), PennCAC-EA (1.04 – 1.07), PennCAC-AA (0.96 – 1.03) and AFCS-EA (1.04 – 1.05) (see

also Supplementary Figures 2 A–E). A SNP was considered suggestive if the associated *P*-value corresponding to a test of no association versus the one-sided alternative that the corresponding coefficient is different than 0 and in the same direction as observed in CRIC, was greater than 0.05. Top CAC associated SNPs, or best proxies if SNP data were not available (LD r^2 >0.6 using SNAP; http://www.broadinstitute.org/mpg/snap/ldsearch.php), were analyzed for their association with MI in PROMIS using logistic regression models that included age, gender and the first 10 PCs. Because MI is a different trait to CAC a two-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 (23) (http://www.sph.umich.edu/csg/abecasis/Metal) as we have described (1,21). All analysis, with the exception of the pedigree analysis for AFCS, was performed using PLINK version 1.06 or R version 2.14.1.

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 1A. The average age was 57 years, and did not vary significantly by ancestry or sex; 43% were AA, 47% were female and 40% had diabetes. The median eGFR was 48 ml/min/1.73 m^2. Compared to expectations for similar age distributions in the general population, the CRIC Study sample was more likely to be overweight and have HTN, increased levels of TG, fibrinogen, and CRP and a high proportion had cardiovascular diseases at enrollment. Mean CAC scores and the distribution by three cutpoints (>0, >100, >300) in each of the ancestry and gender groups are presented in Table 1B. In agreement with prior reports in the CRIC Study (12) and the general population (24), CAC score tended to be higher in males 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 to the general population, this CRIC Study sample displayed increased prevalence of traditional and non-traditional 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 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) (3,25) and *PCSK9* (rs11591147/R46L) (26) 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 (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, Supplementary Table 1 shows those SNPs (n=268), that were associated at $P < 5\times10^{-4}$ 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 ($P < 3\times10^{-6}$) (27). Regional plots including recombination rate, linkage disequilibrium, and *P* values for SNPs at selective top CAC loci in CRIC are presented in Supplementary Figures 3 A–D.

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 2 for the subset of SNPs that had nominal evidence (effect in the same direction, one 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*) (3,10,28), known HTN and diabetes loci not previously associated with coronary atherosclerosis (*HNF4A*, *ATP2B1*, *ADIPOR2* (29–31), as well as several loci not previously reported to be associated with CAC, CAD or CHD risk factors. In exploratory meta-analyses of CRIC and PennCAC data for these 28 SNPs (Supplementary Table 2), SNPs at two loci (*ABCA4* and *HNF4A*) reached $P < 2.38\times10^{-5}$, the Bonferroni-corrected threshold for the number of genes tested. No locus met the more stringent IBC array SNP-wide Bonferroni correction ($P < 3\times10^{-6}$)(27).

Association of suggestive CAC loci with MI in the PROMIS sample

For SNPs with suggestive CAC association, we observed directionally consistent associations (allele associated with greater CAC also increased odds of MI with 2-sided $P < 0.05$) with MI in PROMIS for four of 23 (17.4%) independent loci (exact binomial test p=0.026; null proportion=0.05 versus one-sided alternative that proportion is greater than 0.05) (Table 3). Not surprisingly, the strongest signal was for SNPs at the 9p21 locus (most significant SNP rs4977574; $P=5.67\times10^{-12}$). Three additional loci including *ATP2B1* (rs11105354; 3.3×10^{-5}), *COL4A1* (rs13260; $P=9.6\times10^{-4}$) and *ABCA4* (rs3789422; 1.7×10^{-2}) were associated with MI. Associations at both *ATP2B1* and *COL4A* exceeded P-value Bonferroni adjustment for multiple testing of suggestive CAC SNPs ($P < 0.0022$; 0.05/23 independent loci).

DISCUSSION

We sought to identify genes for CAC in patients with CKD, a population at 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 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 HDL-C and diabetes) (29,32), previously identified by GWAS to be associated with CHD risk factors. Besides *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 one 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 European and Asian ancestry (30,33). *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 (34) 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 2.8×10^{-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 (30). This same SNP is in strong LD (r2=0.9) with the strongest *ATP2B1* variant for HTN in Japanese (33). Furthermore, the *ATP2B1* SNP related to lower CAC also had lower odds of MI (OR=0.9, P $=3.3\times10^{-5}$) 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 (35) 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=5.68×10^{-10}) (36). 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). However, there is high correlation between these 2 SNPs in Asian samples (r^2=0.963 in CHBJPT) (LD estimates from 1000 Genomes Pilot 1 using SNAP http://www.broadinstitute.org/mpg/snap/ldsearchpw.php) suggesting that the CAC and HTN association for the *ATP2B1* locus in EA populations overlaps with 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 (37), metabolic dyslipidemia (38) and type 2 diabetes (T2DM) in multi-ethnic populations (29,39). *HNF4A* encodes a nuclear transcription factor that regulates development and function of the liver, kidney, pancreas and intestines (40) and modulates hepatic lipogenesis as well as apoC-III and VLDL secretion (41). Mutations in *HNF4A* affect insulin secretion and have been linked to maturity onset diabetes of the young (MODY-1) (42). In 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 sub-sample while cardio-metabolic 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* (31), *PPARGC1* which regulates *PPARG*, adipose and lipids (43), *FOXO3*, a longevity gene linked to insulin pathway signaling (44), *ACSL5* which regulates fatty acid metabolism, *BCL2* (45) 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 (46). Whether any of these loci have causal roles in atherosclerosis and CHD remains to be determined. These results do suggest, however, that loci modulating cardio-metabolic 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 CRIC. The CRIC Study is a rigorously designed, multi-center, NIH-sponsored cohort study of CKD that includes almost equal numbers EA and AA individuals and generates resources for sub-clinical 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.

This work also has 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 non-conservative 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 cardio-metabolic 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 (47,48)) risk biomarkers for CHD in CKD. Future studies will focus on determining the role of intermediate factors in the genetic associations with CAC ion 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 (49) and is a useful predictor of CHD including in patients with CKD (8,9).

In conclusion, 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.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We acknowledge the time and commitment of the participants, investigators and staff of the CRIC study. Full acknowledgements and funding details are found in the Supplement.

Relationship with Industry: None relevant. M.P.R. has received research grant support from GlaxoSmithKline and Merck Research Laboratories.

Abbreviations

SNP	single nucleotide polymorphism
CAC	coronary artery calcification
CHD	coronary heart disease
CRP	C-reactive protein
HDL	high-density lipoprotein
LDL	low-density lipoprotein

References

1. Reilly MP, Li M, He J, et al. Identification of ADAMTS7 as a novel locus for coronary atherosclerosis and association of ABO with myocardial infarction in the presence of coronary atherosclerosis: two genome-wide association studies. Lancet. 377:383–392. [PubMed: 21239051]
2. C4D. A genome-wide association study in Europeans and South Asians identifies five new loci for coronary artery disease. Nat Genet. 2011; 43:339–344. [PubMed: 21378988]
3. Schunkert H, Konig IR, Kathiresan S, et al. Large-scale association analysis identifies 13 new susceptibility loci for coronary artery disease. Nat Genet. 2011; 43:333–338. [PubMed: 21378990]
4. Coresh J, Selvin E, Stevens LA, et al. Prevalence of chronic kidney disease in the United States. Jama. 2007; 298:2038–2047. [PubMed: 17986697]
5. Muntner P, He J, Astor BC, Folsom AR, Coresh J. Traditional and nontraditional risk factors predict coronary heart disease in chronic kidney disease: results from the atherosclerosis risk in communities study. J Am Soc Nephrol. 2005; 16:529–538. [PubMed: 15625072]
6. Go AS, Chertow GM, Fan D, McCulloch CE, Hsu CY. Chronic kidney disease and the risks of death, cardiovascular events, and hospitalization. N Engl J Med. 2004; 351:1296–1305. [PubMed: 15385656]
7. Weiner DE, Tighiouart H, Griffith JL, et al. Kidney disease, Framingham risk scores, and cardiac and mortality outcomes. Am J Med. 2007; 120:552. e1-8. [PubMed: 17524759]
8. Detrano R, Guerci AD, Carr JJ, et al. Coronary calcium as a predictor of coronary events in four racial or ethnic groups. N Engl J Med. 2008; 358:1336–1345. [PubMed: 18367736]
9. Nakamura S, Ishibashi-Ueda H, Niizuma S, Yoshihara F, Horio T, Kawano Y. Coronary calcification in patients with chronic kidney disease and coronary artery disease. Clin J Am Soc Nephrol. 2009; 4:1892–1900. [PubMed: 19833908]
10. O'Donnell CJ, Kavousi M, Smith AV, et al. Genome-Wide Association Study for Coronary Artery Calcification With Follow-Up in Myocardial Infarction. Circulation. 2011; 124:2855–2864. [PubMed: 22144573]
11. Kathiresan S, Voight BF, Purcell S, et al. Genome-wide association of early-onset myocardial infarction with single nucleotide polymorphisms and copy number variants. Nat Genet. 2009; 41:334–341. [PubMed: 19198609]

12. Budoff MJ, Rader DJ, Reilly MP, et al. Relationship of estimated GFR and coronary artery calcification in the CRIC (Chronic Renal Insufficiency Cohort) Study. Am J Kidney Dis. 58:519–526. [PubMed: 21783289]
13. Wade AN, Reilly MP. Coronary calcification in chronic kidney disease: morphology, mechanisms and mortality. Clin J Am Soc Nephrol. 2009; 4:1883–1885. [PubMed: 19965543]
14. Feldman HI, Appel LJ, Chertow GM, et al. The Chronic Renal Insufficiency Cohort (CRIC) Study: Design and Methods. J Am Soc Nephrol. 2003; 14:S148–S153. [PubMed: 12819321]
15. Lash JP, Go AS, Appel LJ, et al. Chronic Renal Insufficiency Cohort (CRIC) Study: baseline characteristics and associations with kidney function. Clin J Am Soc Nephrol. 2009; 4:1302–1311. [PubMed: 19541818]
16. Post W, Bielak LF, Ryan KA, et al. Determinants of coronary artery and aortic calcification in the Old Order Amish. Circulation. 2007; 115:717–724. [PubMed: 17261661]
17. Shen H, Bielak LF, Ferguson JF, et al. Association of the vitamin D metabolism gene CYP24A1 with coronary artery calcification. Arterioscler Thromb Vasc Biol. 2010; 30:2648–2654. [PubMed: 20847308]
18. Saleheen D, Zaidi M, Rasheed A, et al. The Pakistan Risk of Myocardial Infarction Study: a resource for the study of genetic, lifestyle and other determinants of myocardial infarction in South Asia. Eur J Epidemiol. 2009; 24:329–338. [PubMed: 19404752]
19. Saleheen D, Soranzo N, Rasheed A, et al. Genetic determinants of major blood lipids in Pakistanis compared with Europeans. Circ Cardiovasc Genet. 3:348–357. [PubMed: 20570915]
20. Keating BJ, Tischfield S, Murray SS, et al. Concept, design and implementation of a cardiovascular gene-centric 50 k SNP array for large-scale genomic association studies. PLoS ONE. 2008; 3:e3583. [PubMed: 18974833]
21. Ferguson JF, Hinkle CC, Mehta NN, et al. Translational studies of lipoprotein-associated phospholipase a(2) in inflammation and atherosclerosis. J Am Coll Cardiol. 2012; 59:764–772. [PubMed: 22340269]
22. Devlin B, Roeder K. Genomic control for association studies. Biometrics. 1999; 55:997–1004. [PubMed: 11315092]
23. Willer CJ, Li Y, Abecasis GR. METAL: fast and efficient meta-analysis of genomewide association scans. Bioinformatics. 26:2190–2191. [PubMed: 20616382]
24. Coylewright M, Rice K, Budoff MJ, et al. Differentiation of severe coronary artery calcification in the Multi-Ethnic Study of Atherosclerosis. Atherosclerosis. 219:616–622. [PubMed: 21930271]
25. Clarke R, Peden JF, Hopewell JC, et al. Genetic variants associated with Lp(a) lipoprotein level and coronary disease. N Engl J Med. 2009; 361:2518–2528. [PubMed: 20032323]
26. Cohen JC, Boerwinkle E, Mosley TH Jr, Hobbs HH. Sequence variations in PCSK9, low LDL, protection against coronary heart disease. N Engl J Med. 2006; 354:1264–1272. [PubMed: 16554528]
27. IBC K. Large-scale gene-centric analysis identifies novel variants for coronary artery disease. PLoS Genet. 2011; 7:e1002260. [PubMed: 21966275]
28. Samani NJ, Erdmann J, Hall AS, et al. Genomewide association analysis of coronary artery disease. N Engl J Med. 2007; 357:443–453. [PubMed: 17634449]
29. Kooner JS, Saleheen D, Sim X, et al. Genome-wide association study in individuals of South Asian ancestry identifies six new type 2 diabetes susceptibility loci. Nat Genet. 43:984–989. [PubMed: 21874001]
30. Levy D, Ehret GB, Rice K, et al. Genome-wide association study of blood pressure and hypertension. Nat Genet. 2009; 41:677–687. [PubMed: 19430479]
31. Vaxillaire M, Dechaume A, Vasseur-Delannoy V, et al. Genetic analysis of ADIPOR1 and ADIPOR2 candidate polymorphisms for type 2 diabetes in the Caucasian population. Diabetes. 2006; 55:856–861. [PubMed: 16505255]
32. Kathiresan S, Willer CJ, Peloso GM, et al. Common variants at 30 loci contribute to polygenic dyslipidemia. Nat Genet. 2009; 41:56–65. [PubMed: 19060906]
33. Tabara Y, Kohara K, Kita Y, et al. Common variants in the ATP2B1 gene are associated with susceptibility to hypertension: the Japanese Millennium Genome Project. Hypertension. 56:973–980. [PubMed: 20921432]

34. Kobayashi Y, Hirawa N, Tabara Y, et al. Mice Lacking Hypertension Candidate Gene ATP2B1 in Vascular Smooth Muscle Cells Show Significant Blood Pressure Elevation. Hypertension. 2012; 59:854–860. [PubMed: 22311909]
35. Shanahan CM, Crouthamel MH, Kapustin A, Giachelli CM. Arterial calcification in chronic kidney disease: key roles for calcium and phosphate. Circ Res. 2011; 109:697–711. [PubMed: 21885837]
36. Lu X, Wang L, Chen S, et al. Genome-wide association study in Han Chinese identifies four new susceptibility loci for coronary artery disease. Nat Genet. 2012
37. Teslovich TM, Musunuru K, Smith AV, et al. Biological, clinical and population relevance of 95 loci for blood lipids. Nature. 466:707–713. [PubMed: 20686565]
38. Suviolahti E, Lilja HE, Pajukanta P. Unraveling the complex genetics of familial combined hyperlipidemia. Ann Med. 2006; 38:337–351. [PubMed: 16938803]
39. Jafar-Mohammadi B, Groves CJ, Gjesing AP, et al. A role for coding functional variants in HNF4A in type 2 diabetes susceptibility. Diabetologia. 54:111–119. [PubMed: 20878384]
40. Maestro MA, Cardalda C, Boj SF, Luco RF, Servitja JM, Ferrer J. Distinct roles of HNF1beta, HNF1alpha, and HNF4alpha in regulating pancreas development, beta-cell function and growth. Endocr Dev. 2007; 12:33–45. [PubMed: 17923767]
41. Yin L, Ma H, Ge X, Edwards PA, Zhang Y. Hepatic hepatocyte nuclear factor 4alpha is essential for maintaining triglyceride and cholesterol homeostasis. Arterioscler Thromb Vasc Biol. 31:328–336. [PubMed: 21071704]
42. Pearson ER, Pruhova S, Tack CJ, et al. Molecular genetics and phenotypic characteristics of MODY caused by hepatocyte nuclear factor 4alpha mutations in a large European collection. Diabetologia. 2005; 48:878–885. [PubMed: 15830177]
43. Liu C, Li S, Liu T, Borjigin J, Lin JD. Transcriptional coactivator PGC-1alpha integrates the mammalian clock and energy metabolism. Nature. 2007; 447:477–481. [PubMed: 17476214]
44. Banasik K, Ribel-Madsen R, Gjesing AP, et al. The FOXO3A rs2802292 G-allele associates with improved peripheral and hepatic insulin sensitivity and increased skeletal muscle-FOXO3A mRNA expression in twins. J Clin Endocrinol Metab. 96:E119–E124. [PubMed: 20881262]
45. Saxena R, Elbers CC, Guo Y, et al. Large-Scale Gene-Centric Meta-Analysis across 39 studies Identifies Type 2 Diabetes Loci. Am J Hum Genet.
46. Kaess BM, Tomaszewski M, Braund PS, et al. Large-scale candidate gene analysis of HDL particle features. PLoS ONE. 6:e14529. [PubMed: 21283740]
47. Palmer SC, Hayen A, Macaskill P, et al. Serum levels of phosphorus, parathyroid hormone, and calcium and risks of death and cardiovascular disease in individuals with chronic kidney disease: a systematic review and meta-analysis. Jama. 2011; 305:1119–1127. [PubMed: 21406649]
48. Park KS, Chang JW, Kim TY, et al. Lower concentrations of serum phosphorus within the normal range could be associated with less calcification of the coronary artery in Koreans with normal renal function. Am J Clin Nutr. 2011; 94:1465–1470. [PubMed: 22030227]
49. Rumberger JA, Schwartz RS, Simons DB, Sheedy PF 3rd, Edwards WD, Fitzpatrick LA. Relation of coronary calcium determined by electron beam computed tomography and lumen narrowing determined by autopsy. Am J Cardiol. 1994; 73:1169–1173. [PubMed: 8203333]

Table 1

A. Baseline Characteristics of the CRIC Genetic Sample with Coronary Artery Calcium Data

	European Ancestry		African Ancestry	
	Male N=469	**Female N=387**	**Male N=324**	**Female N=329**
Age in years; mean (SD)	57.9 (11.3)	57.5 (11.3)	56.3 (11.2)	58 (10.6)
Tobacco and Alcohol use; N (%)				
Current smoker	40 (8.5)	29 (7.5)	58 (17.9)	51 (15.5)
Never smoked[1]	210 (44.8)	207 (53.5)	133 (41)	174 (52.9)
Alcohol	376 (80.2)	296 (76.5)	213 (65.7)	170 (51.7)
Cardiovascular disease; N (%)				
Myocardial infarction or coronary revascularization	69 (14.7)	33 (8.5)	39 (12)	39 (11.9)
Stroke	34 (7.2)	21 (5.4)	42 (13)	44 (13.4)
Peripheral Arterial Disease (PAD)	23 (4.9)	11 (2.8)	23 (7.1)	19 (5.8)
Blood Pressure (BP) variables				
Hypertension; N (%)[2]	378 (80.6)	267 (69)	298 (92)	305 (92.7)
Systolic BP, mmHg; mean (SD)	121.3 (17.1)	118.4 (18.4)	132.2 (22.9)	131.3 (21.4)
Diastolic BP, mmHg; mean (SD)	71.4 (11)	67.1 (10.7)	77.4 (14.6)	73 (12.3)
Lipoprotein and Blood Variables				
Hypercholesterolemia N (%)[3]	401 (86)	265 (69)	268 (83)	241 (73)
Lipid lowering medication, N (%)	295 (63)	199 (51)	182 (57)	169 (52)
LDL cholesterol, mg/dl; mean (SD)	97.1 (31.4)	107.3 (32)	104.4 (37.2)	113.3 (37.9)
HDL cholesterol, mg/dl; mean (SD)	42.7 (12)	56 (16.8)	45 (13.3)	54.3 (17.3)
Total cholesterol, mg/dl; mean (SD)	175.9 (39.8)	191.3 (39.2)	181 (45)	195 (48.6)
Triglycerides mg/dl, median (IQR)	135 (111)	119 (83)	114 (92)	105 (70)
Adj Serum calcium[4] (mg/dl) mean (SD)	9.1 (0.4)	9.2 (0.4)	9.3 (0.4)	9.4 (0.5)
Serum Phosphate, mg/dl; mean (SD)	3.4 (0.6)	3.7 (0.6)	3.6 (0.7)	3.8 (0.6)
C-reactive protein (mg/l), median (IQR)	1.6 (3)	2.2 (4.5)	2.1 (4)	4.4 (7.4))
Fibrinogen (G/L), mean (SD)	3.7 (1)	3.8 (0.9)	4 (1.1)	4.5 (1.2)
Metabolic Variables				
BMI (kg/m2); mean (SD)	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; N (%)	186 (39.7)	117 (30.2)	150 (46.3)	155 (47.1)
Metabolic syndrome; N (%)[5]	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 A1c (%)	6.3 (1.4)	6.1 (1.3)	6.7 (1.6)	6.8 (1.7)
Kidney function; mean (SD)				
Adjusted serum creatinine[6] (mg/dL)	1.6 (0.4)	1.3 (0.4)	1.9 (0.6)	1.6 (0.6)
eGFR[7] (ml/min/1.73 m2)	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)

B. Coronary Artery Calcification Scores in the CRIC Genetic Sub-sample

Coronary Calcium[1]	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 (640)	4.2 (139)	20 (280)	12 (186)
CAC > N (% of individuals)	368 (79)	215 (56)	218 (67)	199 (61)
CAC >100 N (% of individuals)	245 (52)	113 (29)	128 (39)	98 (30)
CAC >300 N (% of individuals)	178 (38)	67 (17)	77 (24)	59 (18)

[1] Never smoking defined as <100 cigarettes over lifetime.

[2] Hypertension was defined as Systolic/Diastolic Blood Pressure ≥140 mm/hg or ≥90 mm/Hg; or use of anti-hypertensive medications.

[3] Hypercholesterolemia defined as use of cholesterol-lowering medications or total serum cholesterol >200 mg/dL

[4] Calcium adjusted for hypoalbuminemia: Adj Calcium (mg/dL)=serum calcium (mg/dL) + 0.8[4 – serum albumin (g/dL)].

[5] Metabolic Syndrome defined using ATP-III criteria

[6] Serum creatinine calibrated to the measurement laboratory.

[7] eGFR was calculated using the CRIC study-specific estimating equation that was derived in its iothalamate glomerular filtration rate (iGFR) subcohort.

LDL = Low density lipoprotein cholesterol; HDL = high density lipoprotein cholesterol.

[1] Coronary Calcium estimated using Agatston Scoring

Table 2

Top associations with CAC traits in CRIC, with directionally consistent associations in PennCAC or AFCS

SNP	Trait	*Race	Chr	Locus	Effect Allele	Effect Allele Freq (AA/ EA)	CRICN N=1509			PennCAC N=2563			^Amish N=784		
							¥β (OR)	SE	P	¥β (OR)	SE	#P	β	SE	#P
SNPs with CAC association in either African or European ancestry															
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	0.5	1.14	0.33
rs4456611	CAC100	AA	18	BCL2	A	0.49 / 0.5	−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	0.1 (1.11)	0.20	0.3	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 / 0.8	−0.54 (0.58)	0.15	3.60E-04	−0.1 (0.9)	0.12	0.19	−0.31	0.19	0.04
rs13386681	LogCAC	EA	2	ATOH8	A	0.09 / 0.07	−0.8	0.21	1.60E-04	−0.27	0.13	0.01	−0.1	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	0.1	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	0.3	0.17	0.04
rs11105354⊿	CAC100	EA	12	ATP2B1	A	0.9 / 0.82	−0.63 (0.53)	0.15	2.80E-05	−0.1 (0.9)	0.11	0.17	−0.3	0.19	0.05
rs13260	CAC0	EA	13	COL4A1	A	0.23 / 0.1	−0.8 (0.45)	0.20	8.70E-05	−0.1 (0.9)	0.12	0.19	−1.2	0.67	0.03
SNP	**Trait**	***Race**	**Chr**	**Locus**	**Effect Allele**	**Effect Allele Freq (AA / EA)**	**Z**		**P**	**Z**		**P**	**β**	**SE**	**P**
Additional SNPs with CAC association in meta-analysis of African and European ancestry 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 / 0.6	−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	1.7	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	0.8 / 0.49	−3.7	2.20E-04	−0.86	0.19	−0.36	0.11	4.00E-04
rs10757272	CAC100	Both	9	9p21	A	0.2 / 0.51	3.58	3.50E-04	0.85	0.2	0.36	0.11	4.00E-04
rs7964239	CAC0	Both	12	BCAT1	A	0.78 / 0.92	−3.65	2.60E-04	−0.84	0.2	−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

*Race: AA = African Ancestry; EA = European Ancestry; Both = European and Ancestry.

¥For continuous variables (Log CAC and 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 one-sided*P* value is presented for PennCAC and AFCS corresponding to a test of no association versus the one-sided alternative that the corresponding coefficient is different than 0 and in the same direction as observed in CRIC.

^Best Amish P value from LogCAC or LogCAC+1 analyses.

NA = not available.

ΔSNP also reached $P < 5 \times 10^{-4}$ in meta-analysis across race in CRIC.

Table 3

Association of top CAC SNPs[1] with myocardial infarction in PROMIS

SNP	*Race	Chr	Locus	Effect Allele	PROMIS (N=14,885) OR	95% CI	#P
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**	**0.9**	**0.86–0.95**	**3.30E-05**
rs13260	**EA**	**13**	**COL4A1**	**A**	**0.87**	**0.8–0.94**	**9.70E-04**
rs3789422	**EA**	**1**	**ABCA4**	**A**	**1.2**	**1.03–1.4**	**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	0.7
rs4456611	AA	18	BCL2	A	0.99	0.95–1.04	0.82
rs1635502	Both	1	EXO1	A	1	0.95–1.05	0.9
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	0.9–1.01	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

J Am Coll Cardiol. Author manuscript; available in PMC 2014 August 27.

SNP	*Race	Chr	Locus	Effect Allele	PROMIS (N=14,885) OR	95% CI	#P
rs3768991	Both	2	NPAS2	A	1	0.96–1.05	0.86
rs3943258	AA	18	BCL2	A	1	0.95–1.04	0.9

[1] SNPs with suggestive association with CAC in CRIC as per Table 2. Data not available for rs1436606.

* Race: AA = African Ancestry; EA = European Ancestry; Both = European and Ancestry.

Two-sided *P* value.