Lack of Associations of Ten Candidate Coronary Heart Disease Risk Genetic Variants and Subclinical Atherosclerosis in Four U.S. Populations: the Population Architecture using Genomics and Epidemiology (PAGE) Study

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Abstract

Background—A number of genetic variants have been discovered by recent genome-wide association studies for their associations with clinical coronary heart disease (CHD). However, it is unclear whether these variants are also associated with the development of CHD as measured by subclinical atherosclerosis phenotypes, ankle brachial index (ABI), carotid artery intima-media thickness (cIMT) and carotid plaque.

Methods—Ten CHD risk single nucleotide polymorphisms (SNPs) were genotyped in individuals of European American (EA), African American (AA), American Indian (AI), and Mexican American (MA) ancestry in the Population Architecture using Genomics and Epidemiology (PAGE) study. In each individual study, we performed linear or logistic regression

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to examine population-specific associations between SNPs and ABI, common and internal cIMT, and plaque. The results from individual studies were meta-analyzed using a fixed effect inverse variance weighted model.

Results—None of the ten SNPs was significantly associated with ABI and common or internal cIMT, after Bonferroni correction. In the sample of 13,337 EA, 3,809 AA, and 5,353 AI individuals with carotid plaque measurement, the *GCKR* SNP rs780094 was significantly associated with the presence of plaque in AI only (OR = 1.32, 95% confidence interval: 1.17, 1.49, $P = 1.08 \times 10^{-5}$), but not in the other populations ($P = 0.90$ in EA and $P = 0.99$ in AA). A 9p21 region SNP, rs1333049, was nominally associated with plaque in EA (OR = 1.07, $P = 0.02$) and in AI (OR = 1.10, P = 0.05).

Conclusions—We identified a significant association between rs780094 and plaque in AI populations, which needs to be replicated in future studies. There was little evidence that the index CHD risk variants identified through genome-wide association studies in EA influence the development of CHD through subclinical atherosclerosis as assessed by cIMT and ABI across ancestries.

Keywords

ankle brachial index; carotid artery intima-media thickness; carotid plaque; coronary heart disease; genetic association study; multiethnic populations; subclinical atherosclerosis

Introduction

Coronary heart disease (CHD) is the leading cause of death and a major health burden worldwide[1]. Subclinical atherosclerosis phenotypes, including carotid artery intima-media thickness (cIMT), carotid plaque, and resting ankle-brachial index (ABI), reflect the degree of atherosclerosis in coronary arteries and peripheral arteries. The associations between cIMT and plaque and the subsequent development of incident CHD, ischemic stroke, and heart failure have been well established in multiple epidemiologic studies[2–8]. ABI is an assessment of the patency of the lower extremity arteries as an indication of the presence of peripheral artery disease, and has also been associated with multiple clinical cardiovascular outcomes[9–11].

Recent genome-wide association studies (GWAS), mostly conducted in populations of European ancestry, have identified a number of single nucleotide polymorphisms (SNPs) associated with CHD[12–18]. Particularly, SNPs in the chromosome 9p21 region (e.g. rs1333049) have been consistently associated with clinical CHD and related conditions in multiple studies[12–18]. However, in many cases, the biologic mechanisms underlying these associations remain unknown. Determination of whether these variants are associated with intermediate cardiovascular phenotypes, such as subclinical atherosclerosis, may help further our understanding of pathogenic mechanisms for CHD. To date, there have been six GWAS of subclinical atherosclerosis in EA[18–23]. Two studies identified several genome-wide significant loci for common cIMT and plaque which have not been associated with CHD previously[20]. A GWAS study of ABI identified associations with rs10757269, a SNP in high LD with rs1333049 ($r^2 > 0.8$) at the 9p21 region.[21]

Several other smaller studies that have examined the associations between CHD risk SNPs and subclinical atherosclerosis have shown mixed results.[22–26] Two candidate gene studies in European descent individuals reported associations of rs1333049 with prevalent ($P = 0.004$ to 0.006 in different samples) and incident (P < 0.0001) carotid plaque[24], and ABI ($P = 1.3 \times 10^{-4}$)[25]. However, other studies in European populations (sample sizes ranging from 854 to 2277) showed a lack of association between CHD risk SNPs and common cIMT[26–28].

Overall, most studies were conducted in populations of European descent, had relatively small sample sizes, and not always analyzed carotid artery plaque burden. Therefore, we conducted the current study, as a part of the Population Architecture using Genomics and Epidemiology (PAGE) Study[29], to investigate whether genetic variants reported to be associated with CHD in individuals of European ancestry are associated with ABI, common and internal cIMT, and carotid artery plaque in individuals of European American (EA), as well as African American (AA), American Indian (AI) and Mexican American (MA).

Methods and Materials

Study population

Data from five population-based studies in the PAGE study were included: the Atherosclerosis Risk In Communities (ARIC) Study[30], the Cardiovascular Health Study (CHS)[31], the Strong Heart Study (SHS)[32], the Strong Heart Family Study (SHFS)[33], and the National Health and Nutrition Examination Surveys (NHANES 1999–2002), the latter of which is accessed by Epidemiologic Architecture for Genes Linked to Environment (EAGLE) investigators[34]. Study designs of these five studies are summarized in Supplemental Table 1. Participants in all five studies provided written informed consents. All studies were approved by the institutional review boards of the participating institutions.

Participants were excluded if they did not self-identify themselves as one of the following four populations: EA, AA, AI, or MA, or if they did not consent to genetic research. Participants without genotype information or missing information on phenotypes were further excluded from analyses. Participants with ABI > 1.4 were excluded from the analyses of ABI. Total sample sizes for analyses of ABI were 15,113 in EA, 4,472 in AA, 1,984 in AI and 944 in MA. The sample sizes for analyses of cIMT were 13,002 in EA, 3,615 in AA and 5,315 in AI. The sample sizes for analyses of plaque were 13,330 in EA, 3,807 in AA and 5,318 in AI.

SNP genotyping

Using GWAS results that were published as of January 2009, we selected the following ten candidate SNPs: rs2144300 (*GALNT2*)[16], rs599839 (*CELSR2/PSRC1/SORT1*)[14], rs780094 (*GCKR*)[16], rs499818 (6p24.1)[18], rs10757278 (9p21)[13], rs1333049 (9p21)[12, 14], rs2383206 (9p21)[17], rs2383207 (9p21)[13], rs17228212 (*SMAD3*)[14], and rs2549513 (16q23.1)[18].

For the majority of the SNPs, the CALiCo Core Genotyping Laboratory at Human Genetics Center at University of Texas (Houston, TX) genotyped samples from ARIC, CHS, SHS and SHFS using Taqman assays (Applied Biosystems by Life Technology, Carlsbad, CA). For some of the SNPs in both the ARIC study and the CHS, GWAS data (Affymetrix 6.0 for ARIC and Illumina Human 370CNV BeadChip for CHS)[35] were used: rs2549513 in ARIC and rs2383207, rs2549513, rs780094 and rs499818 in CHS were genotyped in their respective GWAS arrays. In addition, rs2383207 and rs499818 in ARIC were derived from imputation of the GWAS panel with around 2.5 million SNPs using the phase II HapMap European CEU reference panel. In EAGLE, NHANES 1999–2002 genotyping was performed by the Vanderbilt University DNA Resources Core using the Life Technologies OpenArray (rs599839) and the Illumina BeadXpress (rs2383206 and rs17228212). Genotyping in EAGLE was also performed in the laboratory of Dr. Jonathan Haines for rs2144300, rs780094, rs10757278, and rs2383207 using the iPLEX® Gold assay (Sequenom®, Inc, San Diego, CA). Quality control assessments included sample call rates (> 90%), concordance of blinded replicates (> 98%) and deviation from Hardy-Weinberg equilibrium among controls within self-reported racial groups (P 0.01), except for rs10757278 in ARIC AA (excess in heterozygotes, $P = 0.001$). For imputed SNPs, the

posterior probability of the most likely genotype (r^2) was 0.97 for rs2383207 and 0.91 for rs499818.

Outcome measurement—Outcomes were taken from Visit 1 of the ARIC study (1987–89), Year 5 of the CHS (1994–95), Phase III of the SHS (1998–99), Phase IV of the SHFS (2001–03) and NHANES 1999–2002. All studies defined ABI as the ratio of the mean systolic blood pressure of both ankles to the brachial systolic blood pressure of the right arm, except for the ARIC study, where the systolic blood pressure from one randomly selected ankle was used. In NHANES, only adults aged 40 years and older at the study interview were measured for ABI.

In all studies except for NHANES, cIMT and plaque were measured by trained and certified sonographers using standardized protocols and equipment. In NHANES, cIMT and plaque were not measured. The study protocols and descriptive and quality control statistics have been previously published[36–40]. All studies used high-resolution B-mode real-time ultrasound to bilaterally assess the presence of discrete plaque and cIMT in the common, bifurcation and internal carotid artery except for the SHS and the SHFS, where internal cIMT was not measured. At each arterial segment, multiple measurements of the far wall of the cIMT were taken, and the derived mean cIMT of multiple measurements of left and right sides was used in the current analysis. Each study considered the presence of plaques differently when measuring cIMT. In the ARIC study, cIMT was measured regardless of the presence of plaque. In the CHS, cIMT measurement was centered at the site of plaque. In the SHS and the SHFS, cIMT was never obtained at the level of a discrete plaque. Each study also defined carotid plaque differently. In the SHS and the SHFS, plaque was defined as focal thickening of > 50% of the surrounding wall at any available arterial segments. In the ARIC study, plaque was defined if two of the following three criteria were met: 1) abnormal arterial wall thickness (defined as cIMT > 1.5mm); 2) abnormal shape (protrusion into the lumen, loss of alignment with adjacent arterial wall boundary); 3) abnormal wall texture (brighter echoes than adjacent boundaries). In the CHS, plaque was defined as stenosis degree less than 25% because most plaques are non-obstructive[36, 40, 41]. All phenotypes were carefully harmonized across studies for the purpose of meta-analysis[29].

Statistical Methods

All study-specific association analyses were stratified by self-reported race/ethnicity (EA, AA, AI and MA). Natural log transformation of cIMT measurements was performed. An additive genetic model was assumed for all SNPs. The associations between SNPs and ABI, common cIMT and internal cIMT were examined using multivariate linear regression adjusting for sex, age (at baseline), and center if applicable. Logistic regression was used for the analyses of plaque adjusting for sex, age (at baseline), and center if applicable. In the SHFS, a variance components-based biometrical model with random effects was used to account for kinship[42]. The population-specific beta coefficients or log odds ratios (ORs) were meta-analyzed across studies using a fixed effect inverse variance weighted model to obtain joint estimates. To reduce type I error in results of associations, the Bonferroni correction was applied (alpha = 0.05/10 = 0.005).

Exploratory analyses were performed to examine potential effect modification by CHD risk factors (e.g. type 2 diabetes, hypertension). In each study, we stratified the population-specific samples into subgroups (type 2 diabetes: yes/no; hypertension: yes/no) and further evaluated the heterogeneity of associations of SNPs with outcomes of interest between subgroups. We used Cochran's Q test to assess heterogeneity across studies and across subgroups[43]. A Q statistic with P for heterogeneity (P_{het}) < 0.05 indicated significant heterogeneity.

Besides the simple model with adjustment of sex, age and center (if applicable), two sets of sensitivity analyses were performed: 1) in all five studies, a complex model was performed adjusting for sex, age, center if applicable, body mass index (BMI), smoking (current/former/never smoker), hypertension, type 2 diabetes, low density lipoprotein (LDL) cholesterol, high density lipoprotein (HDL) cholesterol and triglyceride levels. All covariates were from the visit when the outcomes were derived; 2) to examine the potential confounding effect of population stratification, in the ARIC study, we adjusted for principal components (PCs) that were derived from GWAS data[44] and were associated with the outcomes at alpha = 0.05. In the SHFS, to test for population stratification, we compared the likelihood of a model in which the association parameters, βb (between family genotype score) and βw (within family genotype score), were estimated, to the likelihood of a model in which they were constrained to be equal, as would be the case in the absence of population stratification[45, 46]. Analyses were performed with either Stata 11 (College Station, Texas) or SAS V9.2 (Cary, NC) in all studies except SHFS. Analyses in the SHFS were implemented in the SOLAR (Sequential Oligogenic Linkage Analysis Routines, San Antonio, Texas) software package (version 4.4)[46, 47]. Meta-analyses were conducted using "metan" module in Stata 11. Aggregate statistics related to this work will be available via dbGaP as part of the PAGE study.

We performed power calculations assuming an additive genetic effect, risk allele frequencies of the target populations, a two-sided alpha = 0.005, using continuous phenotype distributions or population baseline prevalence from the target populations (Quanto V. 1.2.4)[48].

Results

Baseline characteristics of the study participants by population are summarized in Table 1. The average ABIs in EA, AA, AI, and MA were 1.11, 1.09, 1.12 and 1.11, respectively. In the analyses of cIMT and plaque, because of the inclusion of the SHFS, the AI study population was larger (n = 5,353) and younger (mean age = 50.8 years) compared to the samples used for the ABI analyses (n = 1,984, mean age = 63.3 years). The mean of common cIMT was very similar in the EA (0.77mm) and AA (0.78mm) populations, both of which were higher than the mean of common cIMT in AI (0.70mm). On the other hand, the prevalence of plaque was much higher in AI (45.0%), compared to 32.9% in EA and 31.6% in AA.

Associations between each of the ten SNPs and subclinical atherosclerosis phenotypes from the simple model are presented in Figure 1 and Tables 2–5. In meta-analyses, there was no evidence for heterogeneity of associations across studies ($P_{het} > 0.05$, Tables 2–5). For ABI, common and internal cIMT, there was no significant association of any of the ten SNPs at the 0.005 level. However, there was a significant association between rs780094 (minor allele frequency = 0.20) and the presence of plaque in the AI population (age-, sex-adjusted OR = 1.32, 95% confidence interval (CI): 1.17, 1.49, P = 1.08×10^{-5}). The association remained significant in the complex model with adjustment of age, sex, BMI, smoking, hypertension, type 2 diabetes, LDL cholesterol, HDL cholesterol and triglyceride levels (OR = 1.34, 95% CI: 1.18, 1.53, P = 6.97×10^{-6}). In addition, the associations between rs780094 and plaque were consistent across the three study centers of the SHS and the three centers of the SHFS (test for heterogeneity: $P_{het} = 0.45$) (Figure 2). However, SNP rs780094 was not associated with plaque in the other populations (OR = 0.99, P = 0.90 in EA; OR = 1.00, P = 0.99 in AA).

Since rs1333049 was previously associated with ABI and plaque in EA[24, 25], we specifically examined these associations in a larger number of multiethnic individuals; however, no

association was observed with ABI in any of our populations (beta coefficient (β) = −0.0014, P = 0.37 in EA; β = −0.0013, P = 0.73 in AA; β = 0.0026, P = 0.54 in AI; β = −0.0050, P = 0.39 in MA). In EA and AI, the association between rs1333049 and plaque appeared to be direction-consistent with the original report[12] (OR = 1.07, P = 0.02 in EA; OR = 1.10, P = 0.05 in AI), but the results were not statistically significant after Bonferroni correction. In the AA population, rs1333049 was not associated with plaque (OR = 0.90, P = 0.08).

The exploratory analyses examining potential heterogeneity of gene-phenotype associations by hypertension and by type 2 diabetes status showed that very few SNPs had $P_{het} < 0.05$ between subgroups (Supplemental Tables 2–7). Moreover, none of these heterogeneous associations were observed across all populations.

In the sensitivity analysis, results from the complex model remained unchanged compare to the simple model. In the ARIC study, the association results obtained from models with and without adjusting for PCs did not differ and the causal inference was not changed in EA and AA. In the SHFS, as implemented in SOLAR, the tests for population stratification for rs780094 were not significant ($P > 0.05$)[49].

Discussion

In populations of EA, AA, AI and MA ancestry from the PAGE study, we found little evidence for the associations between ten candidate SNPs for clinical CHD and measures of subclinical atherosclerosis, including ABI, common cIMT, and internal cIMT. The *GCKR* SNP rs780094 was significantly associated with presence of plaque in AI. Each copy of the A allele in rs780094 was associated with a 1.32-fold increase in odds of carotid plaque (95% CI: 1.17, 1.49, $P = 1.08 \times 10^{-5}$). This finding will need to be replicated in additional samples of AIs and other populations.

These findings are important for several reasons. First, our analysis consisted of a large sample of EA from population-based settings than previous candidate gene studies that examined the association between CHD risk SNPs and subclinical atherosclerosis[24–28]. With our samples, we had 80% power to detect a β of 0.007 or larger in the analysis of ABI, a β of 0.008 or larger in the analysis of cIMT, and an OR of 1.12 or larger in the analysis of plaque in the EA population. More importantly, this is the first investigation of these candidate SNPs with subclinical atherosclerosis phenotypes in large and reasonably powered samples of major U.S. populations other than European descendents: AA, AI and MA. In the AA population, we had 80% power to detect a β of 0.016 for ABI, a β of 0.019 for cIMT, and an OR of 1.31 for plaque. In the AI population, we had 80% power to detect a β of 0.02 for ABI, a β of 0.014 for cIMT, and an OR of 1.19 for plaque. In the MA population, we had 80% power to detect a β of 0.026 in the ABI analysis. Lastly, we were able to assess associations between CHD risk SNPs and carotid plaque, which is a more specific atherosclerosis phenotype and a better predictor of clinical CHD than cIMT[50], but has not been frequently examined in previous studies[27, 28]. We found no association between CHD risk SNPs and ABI, common and internal cIMTs. SNP rs780094 was highly significantly associated with plaque in AI, but not in EA and AA populations. Though previous studies suggested that the association between 9p21 locus rs1333049 and ABI and plaque may exist[24, 25], we were not able to replicate these associations, likely due to differences in study populations and designs.

Our most significant finding was the association between the A allele in rs780094 and the presence of plaque in AI (OR = 1.32, 95% CI: 1.17, 1.49, P = 1.08×10^{-5}). However, this association was not observed in either the EA or AA populations despite the fact that this

study had at least 90% power to detect an OR of 1.32 in our samples. SNP rs780094 is located in an intron of Glucokinase Regulatory Protein gene (*GCKR*, OMIM: 600842, 2p23.3) and has been associated with fasting glucose, triglycerides, LDL cholesterol, C-reactive protein, serum uric acid, serum phospholipid and metabolic traits[16,51–56]. In EA and Asian, rs780094 is in tight LD with rs1260326 (r^2 = 0.93 and 0.90, respectively), which encodes a common missense variant in GCKR. In AA there is some LD among these SNPs (r2 = 0.47), but the LD in AI is unknown. In both ARIC and CHS, the association between the risk allele (A) of rs780094 and higher levels of fasting serum triacylglycerol, total cholesterol, and serum uric acid but lower levels of fasting blood glucose in EA populations were previously reported[57, 56, 58] and consistent with other studies. However, despite these associations, this SNP was not associated with subclinical CHD outcomes in these two studies. In our sample of AI, this association was independent of lipid levels and type 2 diabetes (OR = 1.34, 95% CI: 1.18, 1.53, P = 6.97×10^{-6}) after adjusting for sex, age, centers, BMI, smoking, hypertension, type 2 diabetes, LDL cholesterol, HDL cholesterol and triglyceride levels. There have been no previous reports of the association of rs780094 with plaque, but it has been shown to be associated with common cIMT in 455 Caucasians with metabolic syndrome (P < 0.05)[59]. However, we were not able to replicate this association in our samples of EA, which may be due to differences in the study populations since we have population-based individuals, while the previous study focused on a small number of individuals with metabolic syndrome. On the other hand, the association between rs780094 and plaque may be a false positive. However, family studies are robust to population stratification. In our prior evaluation of population stratification in the SHFS using quantitative trait linkage disequilibrium tests[49], we found no evidence population substructure for this SNP. The A allele is more common in the EA population (40% in EA vs 20% in AI), whereas the prevalence of plaque was lower (32.9% in EA vs 45.0% in AI). Similarly, in the AA, the frequency of the A allele in rs780094 is slightly lower (0.18 in AA vs 0.20 in AI), whereas the prevalence of plaque is much lower in AA (31.6% in AA vs 45.0% in AI). Given that the A allele is associated with an increased risk of plaque, it does not appear that confounding by EA or AA admixture in the AI population leads to the observed association in AI. In summary, the association between rs780094 and plaque in the AI population needs to be replicated in future studies.

Another suggestive finding is the association between rs1333049 on chromosome 9p21 and prevalence of plaque. SNPs in the chromosome 9p21 region (close to the *CDKN2A/2B* gene) have been consistently associated with clinical CHD, incident CHD, heart failure, myocardial infarction, coronary artery calcification, ischemic stroke and abdominal aortic aneurysm in populations of European ancestry[12–18]. However, associations between these SNPs and subclinical atherosclerosis phenotypes have been conflicting[22–26]. While some studies reported associations of rs1333049 with plaque and ABI[24, 25], three other candidate studies failed to detect associations between 9p21 SNPs (rs1333049, rs7044859, rs496892, rs7865618, rs4977574) and cIMT[26–28]. In the current analyses, we observed direction-consistent associations between the C allele in rs1333049 and decreased ABI, increased cIMT and increased risk of plaque in our EA population, as in previous findings with clinical CHD and with subclinical atherosclerosis. In a recent meta-analysis of 21 GWAS, rs10757269, in high LD with rs1333049 (r^2 = 0.87 in CEU from HapMap III), was associated with ABI (β = −0.006, P = 2.46×10^{-8})[21]. In a previous PAGE analysis[60] of 6,626 individuals with CHD, rs10757278, in high LD with rs1333049 (r^2 = 0.9 in EA) in the 9p21 region, was significantly associated with incident CHD (hazard ratio = 1.19, P = 4.7×10^{-41})[60]. The lack of significant associations in our study is likely due to the modest effect size (β = −0.0014 in ABI analyses) and relatively small sample size compared to previous studies.

There are several aspects of the study that strengthened our findings. First, the current study examined associations between SNPs for clinical CHD and subclinical atherosclerosis intermediate phenotypes, which have been thought to be a way to increase statistical power for finding genes for complex traits[61]. The "upstream" intermediate phenotypes (e.g. plaque), which are more proximal to the genes on the pathogenic pathway, should be more strongly associated with the genetic variants than the "downstream" clinical traits (CHD)[62]. Such association studies may theoretically elucidate how these CHD genetic variants act on the pathogenesis of CHD through the development and progression of atherosclerosis. Second, our analyses included large studies from multiple racial/ethnic backgrounds with well-characterized and harmonized phenotypes. Despite the lack of power to detect the modest associations with ABI, common cIMT and internal cIMT, we have identified a highly significant association in the analyses of plaque.

We also note the limitations of our study. Although phenotypes were carefully harmonized, due to the complexity of these phenotypes and differences in study protocols, some unresolved heterogeneity in the measurement of phenotypes may still exist limiting our power to detect associations. Second, two imputed SNPs with relatively high imputation scores (r^2 is 0.97 for rs2383207 and 0.91 for rs499818) were used in some of our analyses. However, similar inferences were made when these genotypes were not included in the analyses. Lastly, in the present study, we simply evaluated the published index variant identified from previous GWASs, which were primarily found in populations of European ancestry; therefore, differences in linkage disequilibrium between the available tested SNP and the "causative" variant in different populations were not accounted for in our study and may have obscure true associations in these regions.

In conclusion, using the diverse populations from the PAGE study, we found that rs780094 (*GCKR*) was significantly associated with the presence of carotid plaque in American Indian populations. This association appears to be population-specific and will need to be replicated in future studies. In addition, there was little evidence that CHD susceptibility variants identified through GWAS influence the development of CHD through subclinical atherosclerosis as assessed by cIMT and ABI.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Highlights

- We examine the associations between CAD risk SNPs and subclinical atherosclerosis

- We include large samples of African Americans, Mexican Americans, and American Indians

- SNP rs780094 is significantly associated with plaque in American Indians

- Lack of association between CAD risk SNPs with ABI and cIMT
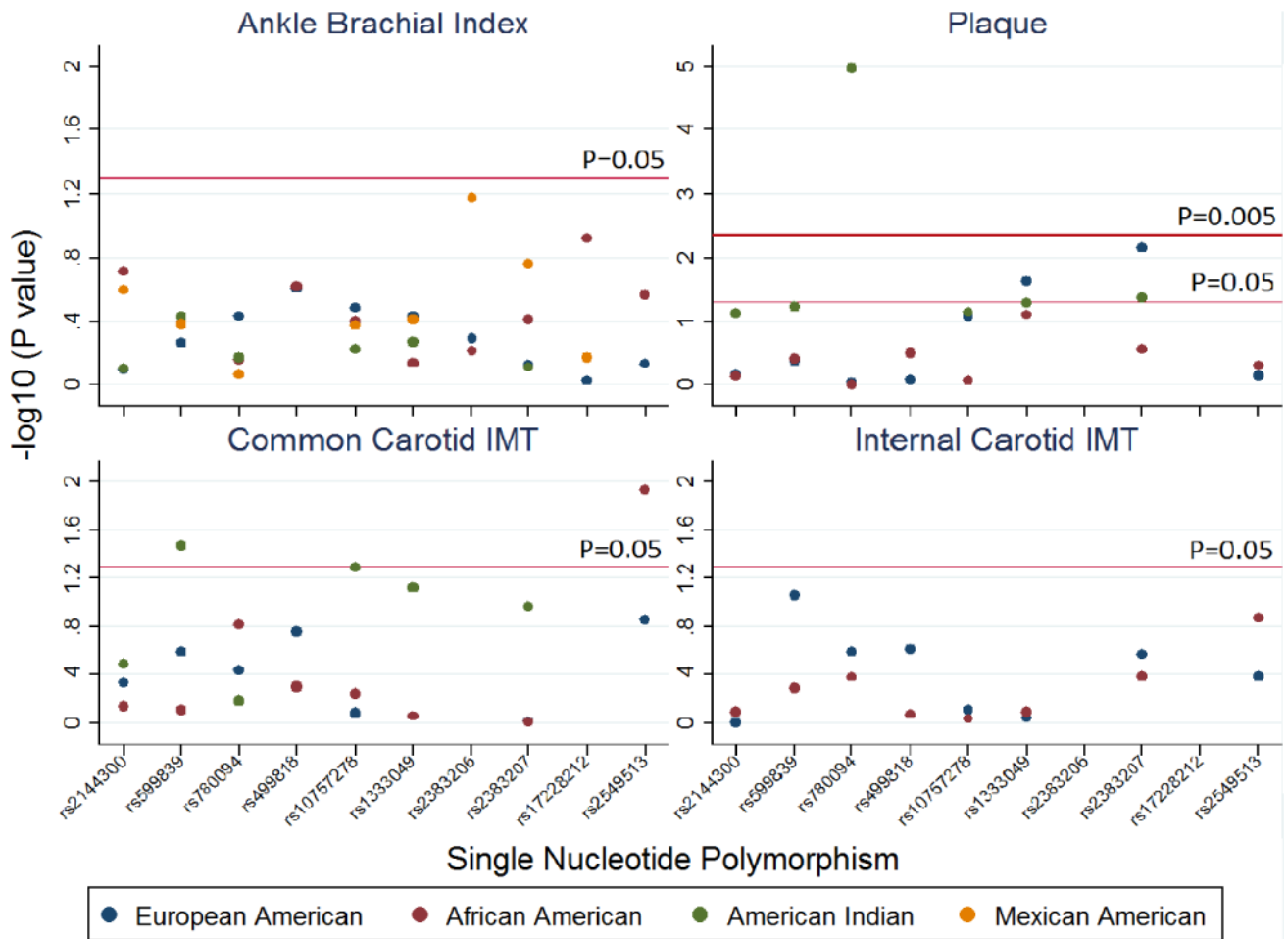
Figure 1.
Associations of Single Nucleotide Polymorphisms with Ankle Brachial Index, Plaque, Common Carotid and Internal Carotid Artery Intima-media Thickness (IMT) in the Population Architecture Using Genetics and Epidemiology (PAGE) study, by Populations.
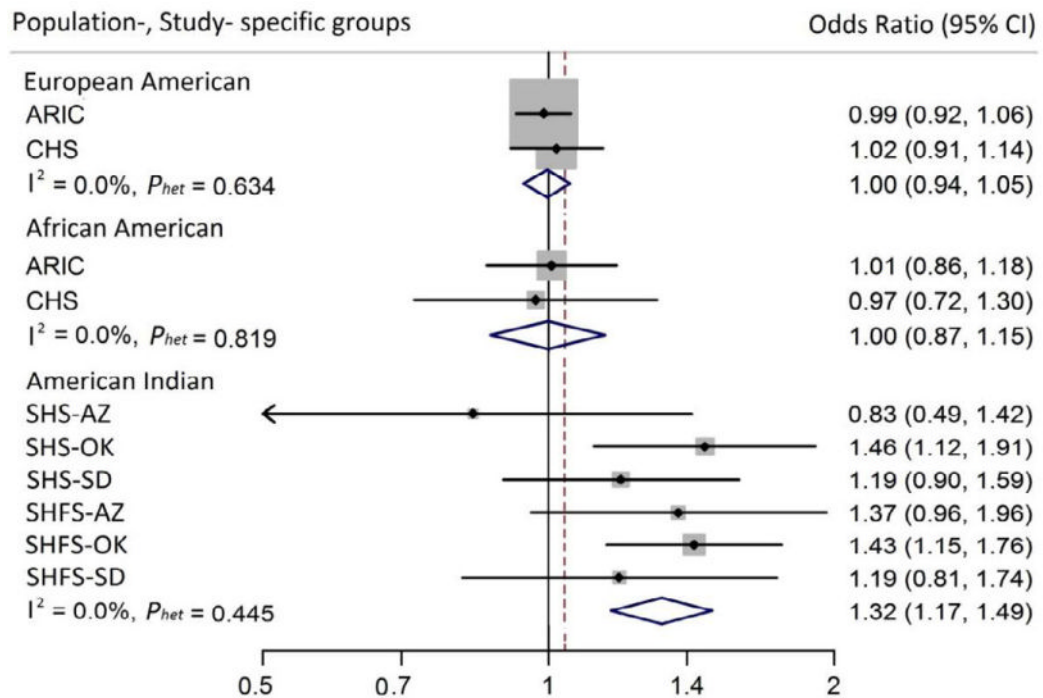
Figure 2.
Population- and study-specific and pooled associations between rs780094 and plaque in the Population Architecture Using Genetics and Epidemiology (PAGE) study, by populations. Odd ratio and 95% confidence intervals (95%CI) are obtained from logistic regression model adjusting for sex, age and center if applicable. I^2 and P_{het} are obtained from Cochran's Q test using a fixed effect model. The size of each square is proportional to the percent weight that each population-, study-specific group contributed in the overall association. Diamond represents the pooled odds ratio for each population. Bars represent the 95% confidence intervals for the odds ratios.

Abbreviations: ARIC, Atherosclerosis Risk in Communities; AZ, Arizona; CHS, Cardiovascular Health Study; NHANES, National Health and Nutrition Examination Survey; OK, Oklahoma; SD, South Dakota; SHFS, Strong Heart Family Study; SHS, Strong Heart Study.

Table 1

Characteristics of Participants in the Analysis of Ankle Brachial Index and Intima-media thickness/Plaque in the Population Architecture Using Genetics and Epidemiology (PAGE) study, by Populations

|	European Americans (EA)	African Americans (AA)	American Indians (AI)	Mexican Americans (MA)
Participant characteristics-Ankle Brachial Index analysis*	**N = 15,113**	**N = 4,472**	**N = 1,984**	**N = 944**
Ankle brachial index, mg/dL	1.11 (0.14)	1.09 (0.14)	1.12 (0.14)	1.11 (0.13)
Age, years	60.5 (6.3)	57.3 (6.2)	63.3 (7.8)	57.2 (11.9)
Female, %	53.6	60.4	64.4	48.3
Body-mass index, kg/m^2	27.0 (4.8)	29.4 (5.9)	31.2 (6.6)	29.0 (5.3)
Smoking status, %				
Current	20.3	26.8	29.1	20.0
Former	38.1	26.4	34.5	33.1
Never	41.6	46.8	36.3	46.9
Type 2 diabetes, %	10.1	20.0	47.1	15.5
Hypertension, %	39.8	60.5	54.4	41.6
LDL Cholesterol, mg/dL	132.1 (37.2)	134.2 (41.5)	118.0 (32.8)	126.8 (32.6)
HDL Cholesterol, mg/dL	51.3 (16.4)	55.5 (17.4)	42.1 (13.4)	48.5 (13.8)
Triglycerides, mg/dL	144.8 (96.2)	114.8 (76.7)	154.8 (112.7)	186.9 (148.7)
Participant characteristics-Intima-media thickness/plaque analysis#	**N = 13,337**	**N = 3,809**	**N = 5,353**	**NA**
Common Carotid IMT, mm	0.77 (0.16)	0.78 (0.16)	0.70 (0.16)	NA
Internal Carotid IMT, mm	0.93 (0.32)	0.83 (0.23)	NA	NA
Prevalence of Plaque, %	32.9	31.6	45.0	NA
Age, years	60.2 (5.3)	57.5 (5.8)	50.8 (13.9)	NA
Female, %	54.2	62.6	62.0	NA
Body-mass Index, kg/m^2	26.9 (4.8)	29.3 (5.9)	32.0 (7.4)	NA
Smoking status, %				NA
Current	20.2	26.0	32.4	NA
Former	38.4	26.8	29.7	NA
Never	41.5	47.2	37.9	NA
Type 2 diabetes, %	10.6	20.8	34.0	NA
Hypertension, %	37.9	60.3	43.0	NA
LDL Cholesterol, mg/dL	132.6 (37.2)	135.0 (42.4)	106.5 (30.5)	NA
HDL Cholesterol, mg/dL	51.6 (16.4)	55.6 (17.4)	47.6 (14.3)	NA
Triglycerides, mg/dL	141.0 (91.9)	113.9 (74.5)	161.3 (112.6)	NA

Abbreviations: N/A, not applicable.

[a]Data are mean (standard deviation).

[b]Ankle Brachial Index was measured in participants of the ARIC study, CHS, NHANES 1999–2002, and SHS. Participants were excluded if they did not self identify as any of above populations, or did not consent to genetic study, or missed Ankle Brachial Index measurement or ABI > 1.4. SNP rs17228212 and rs2383206 were not genotyped in the CHS and SHS cohort component, so sample sizes for the analysis of these two SNPs were 11,522 in EA, 3,714 in AA and 944 in MA. SNP rs2549513 and rs499818 were not genotyped in the NHANES 1999–2002 and SHS cohort component, so sample sizes for the analysis of these two SNPs were 12,739 in EA and 3,776 in AA.

[c]Intima-media thickness and plaque were measured in participants of the ARIC study, CHS, SHS and SHFS. Participants were excluded if they did not self identify as any of above populations, or did not consent to genetic study, or not measured for carotid sonogram. SNP rs2549513 and rs499818 were not genotyped in the SHS, so sample sizes for the analysis of these two SNPs associated with IMT/plaque were 13,337 in EA and 3,809 in AA.

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Table 2

Associations of Single Nucleotide Polymorphisms with Ankle Brachial Index in the Population Architecture Using Genetics and Epidemiology (PAGE) study, by Populations.

SNP (Gene)	Coded Allele	Populations	Coded Allele Frequency	Sample size	Beta^a	95% Confidence Interval	P value	P_{het}
rs2144300 (GALNT2)	C	EA	0.40	14018	0.0004	-0.003, 0.004	0.80	0.55
		AA	0.87	4094	-0.0060	-0.015, 0.003	0.19	0.05
		AI	0.45	2084	-0.0012	-0.010, 0.007	0.78	0.48
		MA	0.44	943	-0.0066	-0.018, 0.005	0.25	N/A
rs599839 (CELSR2/PSR C1/SORT1)	A	EA	0.77	14140	-0.0012	-0.005, 0.003	0.54	0.16
		AA	0.27	4086	0.0029	-0.004, 0.010	0.41	0.33
		AI	0.79	2084	-0.0046	-0.015, 0.005	0.37	0.48
		MA	0.79	906	-0.0057	-0.020, 0.008	0.42	N/A
rs780094 (GCKR)	A	EA	0.40	13437	0.0015	-0.002, 0.005	0.37	0.61
		AA	0.18	3927	0.0016	-0.006, 0.010	0.69	0.69
		AI	0.20	2096	-0.0022	-0.012, 0.008	0.67	0.43
		MA	0.33	942	0.0011	-0.011, 0.013	0.86	N/A
rs499818 (6p24.1)	A	EA	0.22	11886	0.0023	-0.002, 0.006	0.25	0.36
		AA	0.14	560	0.0190	-0.013, 0.051	0.24	N/A
rs10757278 (9p21)	G	EA	0.49	14758	-0.0015	-0.004, 0.001	0.32	0.15
		AA	0.19	4315	-0.0028	-0.009, 0.004	0.40	0.55
		AI	0.46	2091	0.0023	-0.006, 0.011	0.60	0.31
		MA	0.47	938	-0.0046	-0.016, 0.007	0.42	N/A
rs1333049 (9p21)	C	EA	0.49	14223	-0.0014	-0.005, 0.002	0.37	0.23
		AA	0.23	4127	-0.0013	-0.008, 0.006	0.73	0.97
		AI	0.47	2099	0.0026	-0.006, 0.011	0.54	0.31
		MA	0.46	934	-0.0050	-0.016, 0.006	0.39	N/A
rs2383206 (9p21)	G	EA	0.52	10891	-0.0011	-0.004, 0.002	0.51	0.47
		AA	0.42	3500	0.0017	-0.005, 0.008	0.61	0.70
		MA	0.51	933	-0.0105	-0.022, 0.001	0.07	N/A
rs2383207 (9p21)	G	EA	0.52	14201	-0.0016	-0.004, 0.003	0.31	0.41
		AA	0.89	4068	-0.0039	-0.013, 0.005	0.39	0.30
		AI	0.49	2066	0.0012	-0.007, 0.010	0.77	0.52

SNP (Gene)	Coded Allele	Populations	Coded Allele Frequency	Sample size	Beta[a]	95% Confidence Interval	P value	P_{het}
		MA	0.54	938	−0.0080	−0.019, 0.003	0.17	NA
rs17228212 (*SMAD3*)	C	EA	0.28	11124	0.0002	−0.004, 0.004	0.94	0.22
		AA	0.12	3497	−0.0075	−0.017, 0.002	0.12	0.86
		MA	0.14	936	0.0034	−0.012, 0.019	0.67	N/A
rs2549513 (16q23.1)	C	EA	0.13	11183	−0.0009	−0.006, 0.004	0.73	0.52
		AA	0.25	3252	−0.0043	−0.012, 0.003	0.27	0.23

Abbreviations: AA, African American; AI, American Indian; Beta, beta coefficient; EA, European American; MA, Mexican American; N/A, not applicable; SNP, single nucleotide polymorphism.

*
P value: multivariable linear regression adjusting for sex, age and center if applicable. *P*het: Cochran's Q test using a fixed effect model to assess heterogeneities across studies.

[a]Beta coefficient represents increase in ankle brachial index associated with each copy of the coded allele for the ten candidate SNPs.

Table 3

Associations of Single Nucleotide Polymorphisms with Common Carotid Artery Intima-media Thickness in the Population Architecture Using Genetics and Epidemiology (PAGE) study, by Populations.

SNP (Gene)	Coded Allele	Populations	Coded Allele Frequency	Sample size	Beta[a]	95% Confidence Interval	P value	P_{het}
rs2144300 (*GALNT2*)	C	EA	0.40	8212	−0.0018	−0.007, 0.003	0.47	0.59
		AA	0.87	3269	0.0025	−0.011, 0.016	0.72	0.76
		AI	0.45	5386	0.0038	−0.004, 0.011	0.32	0.14
rs599839 (*CELSR2/PSRC1/SORT1*)	A	EA	0.77	8419	0.0032	−0.002, 0.009	0.26	0.004
		AA	0.27	3291	0.0016	−0.009, 0.012	0.77	0.12
		AI	0.79	5373	0.0100	0.001, 0.019	0.03	0.75
rs780094 (*GCKR*)	A	EA	0.40	8414	−0.0022	−0.007, 0.003	0.36	0.23
		AA	0.18	3085	−0.0091	−0.022, 0.003	0.15	0.85
		AI	0.20	5407	−0.0022	−0.005, 0.001	0.65	0.61
rs499818 (6p24.1)	A	EA	0.22	9238	−0.0037	−0.009, 0.002	0.18	0.96
		AA	0.14	572	0.0102	−0.020, 0.040	0.50	N/A
rs10757278 (9p21)	G	EA	0.49	8958	−0.0005	−0.005, 0.004	0.82	0.33
		AA	0.19	3476	0.0028	−0.007, 0.013	0.58	0.29
		AI	0.46	5393	−0.0076	−0.015, 0.001	0.05	0.23
rs1333049 (9p21)	C	EA	0.49	8418	0.0004	−0.004, 0.005	0.87	0.28
		AA	0.23	3299	−0.0010	−0.012, 0.010	0.86	0.85
		AI	0.47	5411	−0.0070	−0.015, 0.001	0.08	0.23
rs2383207 (9p21)	G	EA	0.52	9210	−0.0002	−0.004, 0.005	0.99	0.94
		AA	0.89	3220	−0.0002	−0.014, 0.014	0.38	0.38
		AI	0.49	5374	−0.0063	−0.014, 0.001	0.11	0.16
rs2549513 (16q23.1)	C	EA	0.13	8568	−0.0052	−0.012, 0.002	0.14	0.51
		AA	0.25	3098	−0.0142	−0.025, −0.003	0.01	0.44

Abbreviations: AA, African American; AI, American Indian; Beta, beta coefficient; EA, European American; MA, Mexican American; N/A, not applicable; SNP, single nucleotide polymorphism.

[*] *P* value: multivariable linear regression adjusting for sex, age and center if applicable. P_{het}: Cochran's Q test using a fixed effect model to assess heterogeneities across studies.

[a] Beta coefficient represents increase in common carotid artery intima-media thickness (mm) associated with each copy of the coded allele for the ten candidate SNPs.

Table 4

Associations of Single Nucleotide Polymorphisms with Internal Carotid Artery Intima-media Thickness in the Population Architecture Using Genetics and Epidemiology (PAGE) study, by Populations.

SNP (Gene)	Coded Allele	Populations	Coded Allele Frequency	Sample size	Beta[a]	95% Confidence Interval	P value	P_{het}
rs2144300 (GALNT2)	C	EA	0.40	8212	0	−0.008, 0.008	0.99	0.09
		AA	0.87	3269	0.0023	−0.015, 0.020	0.80	0.36
rs599839 (CELSR2/PSRC1/SORT1)	A	EA	0.77	8419	0.0078	−0.001, 0.017	0.09	0.14
		AA	0.27	3291	−0.0044	−0.018, 0.009	0.51	0.14
rs780094 (GCKR)	A	EA	0.40	8414	0.0045	−0.003, 0.012	0.26	0.14
		AA	0.18	3085	0.0064	−0.009, 0.022	0.42	0.17
rs499818 (6p24.1)	A	EA	0.22	9238	−0.0051	−0.014, 0.003	0.24	0.26
		AA	0.14	572	−0.0057	−0.064, 0.052	0.85	N/A
rs10757278 (9p21)	G	EA	0.49	8958	−0.0010	−0.008, 0.006	0.77	0.03
		AA	0.19	3476	−0.0007	−0.013, 0.012	0.91	0.13
rs1333049 (9p21)	C	EA	0.49	8418	0.0005	−0.007, 0.008	0.90	0.04
		AA	0.23	3299	−0.0017	−0.015, 0.012	0.80	0.21
rs2383207 (9p21)	G	EA	0.52	9210	−0.0014	−0.003, 0.011	0.71	0.25
		AA	0.89	3220	0.0072	−0.010, 0.024	0.41	0.12
rs2549513 (16q23.1)	C	EA	0.13	8568	0.0046	−0.006, 0.016	0.41	0.78
		AA	0.25	3098	−0.0105	−0.024, 0.003	0.13	0.32

Abbreviations: AA, African American; AI, American Indian; Beta, beta coefficient; EA, European American; MA, Mexican American; N/A, not applicable; SNP, single nucleotide polymorphism.

*
P value: multivariable linear regression adjusting for sex, age and center if applicable. P_{het}: Cochran's Q test using a fixed effect model to assess heterogeneities across studies.

[a]
Beta coefficient represents increase in internal carotid artery intima-media thickness (mm) associated with each copy of the coded allele for the ten candidate SNPs.

Table 5

Associations of Single Nucleotide Polymorphisms with Carotid Plaque in the Population Architecture Using Genetics and Epidemiology (PAGE) study, by Populations.

SNP (Gene)	Coded Allele	Populations	Coded Allele Frequency	Sample size	Odds Ratio[a]	95% Confidence Interval	P value	P_{het}
rs2144300 (*GALNT2*)	C	EA	0.40	12199	0.99	0.94, 1.05	0.69	0.05
		AA	0.87	3444	1.03	0.88, 1.20	0.73	0.37
		AI	0.45	5391	0.92	0.84, 0.01	0.08	0.64
rs599839 (*CELSR2/PSRC1/SORT1*)	A	EA	0.77	12430	1.03	0.96, 1.10	0.42	0.91
		AA	0.27	3470	1.05	0.94, 1.19	0.38	0.65
		AI	0.79	5378	1.12	1.00, 1.27	0.06	0.48
rs780094 (*GCKR*)	A	EA	0.40	11575	0.99	0.94, 1.06	0.90	0.63
		AA	0.18	3266	1.00	0.87, 1.15	0.99	0.82
		AI	0.20	5412	1.32	1.17, 1.49	1.08×10^{-5}	0.45
rs499818 (6p24.1)	A	EA	0.22	12428	0.99	0.93, 1.06	0.83	0.86
		AA	0.14	572	0.84	0.59, 1.19	0.32	N/A
rs10757278 (9p21)	G	EA	0.49	12992	1.05	0.95, 1.16	0.08	0.92
		AA	0.19	3660	0.99	0.89, 1.10	0.85	0.19
		AI	0.46	5398	1.09	0.99, 1.20	0.07	0.95
rs1333049 (9p21)	C	EA	0.49	12426	1.07	1.01, 1.13	0.02	0.78
		AA	0.23	3478	0.90	0.79, 1.01	0.08	0.54
		AI	0.47	5416	1.10	1.00, 1.20	0.05	0.97
rs2383207 (9p21)	G	EA	0.52	12405	1.08	0.92, 1.03	0.007	0.61
		AA	0.89	3399	0.91	0.83, 1.13	0.27	0.04
		AI	0.49	5379	1.10	1.00, 1.21	0.04	0.88
rs2549513 (16q23.1)	C	EA	0.13	11691	0.99	0.91, 1.07	0.72	0.64
		AA	0.25	2715	0.96	0.85, 1.08	0.50	0.98

Abbreviations: AA, African American; AI, American Indian; EA, European American; MA, Mexican American; N/A, not applicable; SNP, single nucleotide polymorphism.

*
  P value: multivariable logistic regression adjusting for sex, age and center if applicable. P_{het}: Cochran's Q test using a fixed effect model to assess heterogeneities across studies.

[a]Odds ratio represents increase in odds of carotid plaque associated with each copy of the coded allele for the ten candidate SNPs.