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Common Genetic Variants and Subclinical Atherosclerosis: The Multi-Ethnic Study of Atherosclerosis (MESA)

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Abstract

Background and Aims—Subclinical atherosclerosis (sCVD), measured by coronary artery calcium (CAC) and carotid intima media thickness (CIMT) is associated with cardiovascular disease (CVD). Genome Wide Association Studies (GWAS) of CIMT and CVD have focused primarily on Caucasian populations. We hypothesized that these associations may differ in populations from distinct genetic backgrounds.

Methods—The associations between sCVD and 66 single nucleotide polymorphisms (SNPs) from published GWAS of sCVD and CVD were tested in 8224 Multi-Ethnic Study of Atherosclerosis (MESA) and MESA Family participants [2329 Caucasians (EUA), 691 Chinese (CHN), 2482 African Americans (AFA), and 2012 Hispanic (HIS)] using an additive model adjusting for CVD risk factors, with SNP significance defined by a Bonferroni-corrected $p < 7.6 \times 10^{-4}$ (0.05/66).

Results—In EUA there were significant associations for CAC with SNPs in 9p21 (rs1333049, $P=2 \times 10^{-9}$; rs4977574, $P= 4 \times 10^{-9}$), *COL4A1* (rs9515203, $P=9 \times 10^{-6}$), and *PHACTR1* (rs9349379, $P= 4 \times 10^{-4}$). In HIS, CAC was associated with SNPs in 9p21 (rs1333049, P=8 ×

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DISCLOSURES

None.

10^{-5}; rs4977574, P=5 × 10^{-5}), *APOA5* (rs964184, P=2 × 10^{-4}), and *ADAMTS7* (rs7173743, P=4 × 10^{-4}). There were no associations with the 9p21 region for AFA and CHN. Fine mapping of the 9p21 region revealed SNPs with robust associations with CAC in EUA and HIS but no significant associations in AFA and CHN.

Conclusion—Our results suggest some shared genetic architecture for sCVD across ethnic groups, while also underscoring the possibility of novel variants and/or pathways in risk of CVD in ethnically diverse populations.

Keywords

Genetics; coronary calcium; single nucleotide polymorphism; intima-medial thickness

Introduction

Cardiovascular disease (CVD) is among the leading causes of death worldwide.[1] Recent technological advances have made possible the identification of CVD before it becomes clinically apparent.[2] Subclinical atherosclerosis (sCVD) is common[3] and can be measured non-invasively through imaging techniques such as coronary artery calcium (CAC)[4] and carotid intima media thickness (CIMT)[5] thereby providing a non-invasive way to risk stratify patients.

Genome-wide association studies (GWAS) have been instrumental in advancing our understanding of the genetic basis of CVD and sCVD by identifying novel loci associated with the development of atherosclerosis (see Table 2 in ref[6]).[7–22] Many of these associations are not explained by conventional CVD risk factors, consistent with the possibility that these loci represent novel atherosclerotic pathways. The most widely replicated of these GWAS results that are associated with CVD and sCVD is the 9p21 locus.[16,17,23] In addition to CVD and sCVD, the 9p21 locus has also been associated with risk of abdominal and intracranial aneurysms[24], peripheral arterial disease[24], heart failure[25], sudden cardiac death[26] and stroke.[27] Nonetheless, association of 9p21 with markers of the early stages of atherosclerosis has not been found, including arterial elasticity and retinal microvascular abnormalities.[28]

The number of GWAS loci identified with complex human phenotypes has increased exponentially since the completion of the human genome project, yet the majority of GWAS have been performed on populations of European descent.[29] Moreover, relatively few GWAS of CVD and/or sCVD have focused on non-European populations.[14,21] Small candidate gene studies have complemented these GWAS but are few in number.[30,31] This apparent disparity has raised questions regarding the relevance of GWAS findings to populations of different genetic backgrounds.[32] This issue is particularly relevant to CVD and sCVD given the varying prevalence of these phenotypes in different ethnicities.[33]

In the present study, we use the Multi-ethnic Study of Atherosclerosis (MESA) to investigate whether significant associations found in recent GWAS of CVD and sCVD are also associated with measures of sCVD (CAC and CIMT) in a genetically diverse cohort of African, Hispanic and Asian ancestry.

Materials and Methods

Study Design

The MESA study has been previously described and it was designed to investigate the impact of sCVD and CVD risk factors on the development of clinically overt CVD.[34] Approximately 38% of the recruited participants are Caucasians (EUA), 12% Chinese (CHN), 28% African American (AFA) and 22% Hispanic (HIS).

Genotype Data

The 66 single nucleotide polymorphisms (SNPs) included in this study (See Table 2 in ref[6]) were obtained from Affymetrix 6.0 GWAS dataset (MESA and MESA family data) on 8,224 consenting MESA participants (2329 EUA, 691 CHN, 2482 AFA, and 2012 HIS) from the National Heart, Lung, and Blood Institute SNP Health Association Resource (SHARe) project. Absent SNPs were imputed using IMPUTE v2.2.2 to the 1000 genomes cosmopolitan Phase 1 v3 as a reference. Genotypes were filtered for SNP level call rate <95% and individual level call rate <95%, and monomorphic SNPs as well as SNPs with heterozygosity >53% were removed. Allele frequencies were calculated separately within each racial/ethnic group, and only those SNPs with minor allele frequencies >0.01 were included in genetic association analyses. We further filtered imputed SNPs based on imputation quality >0.5, using the observed versus expected variance quality metric, and filtered genotyped SNPs for Hardy-Weinberg equilibrium P-value $\geq 10^{-5}$.

SCVD Measurement

The imaging outcomes in the present study are coronary artery calcium [CAC, measured as a continuous variable as the raw Agatston CAC score plus one (CAC-c) or as a dichotomous variable (CAC-d) with CAC>0] and carotid artery intima-media thickness [CIMT; internal carotid intima media thickness (CIMT-i), common carotid intima media thickness (CIMT-c)].

CAC was measured by either electron-beam tomography or multi-detector computed tomography, as described previously.[4] All scans were read at the Los Angeles Biomedical Research Institute at Harbor-UCLA Medical Center. Measurements of CAC were adjusted between the different field centers and imaging machines by using a standard calcium phantom of known density, which was scanned with each participant and CAC calculated as described previously[35] and the mean value from two scans used for analysis.

CIMT measurements were performed by B-mode ultrasonography of the right and left, near and far walls, and images were recorded using a Logiq 700 ultrasound device (General Electric Medical Systems, Waukesha, WI). Maximal CIMT-i and CIMT-c was measured as the mean of the maximum values of the near and far wall of the right and left sides at a central ultrasound reading center (Department of Radiology, New England Medical Center, Boston, MA) as described previously.[36]

Statistical Analyses

Given skewed distributions, the common (CIMT-c) and internal (CIMT-i) IMT values were log normalized. CAC was analyzed as a continuous variable by obtaining the log of the raw CAC score plus one (CAC-c) or as a dichotomous variable (CAC-d) with CAC > 0. Analyses were first performed stratified within each racial/ethnic group. For analysis involving EUA and CHN, an unrelated subset of individuals was constructed by selecting at most one individual from each pedigree. For analysis of phenotypes with a substantial familial component, among AFA and HIS, the analysis was performed using a linear mixed-effects model (continuous variables) and by generalized estimating equations (dichotomous variables). Associations between each SNP and each individual phenotype was determined using separate multiple linear regressions (continuous variables) or logistic regressions (dichotomous variables) assuming an additive model. Two models were used to analyze the data. Model 1 accounted for age, sex, site of ascertainment, and principal components. Model 2 included Model 1 plus HDL cholesterol (HDL-C), LDL cholesterol (LDL-C), triglycerides, body mass index (BMI), hypertension status (self-report of physician-diagnosed hypertension along with use of antihypertensive medication or systolic blood pressure of 140 mm Hg or greater and/or diastolic blood pressure of 90 mm Hg or greater), diabetes status (fasting blood glucose was 126 mg/dL or greater or use of diabetes medications), and current smoking use (self-reported current smoking use within the past 30 days). Fixed effect meta-analysis was used to combine results across all four race/ethnic groups, as implemented in METAL.

Fine mapping of the 9p21 region (100 kb upstream or downstream from SNPs rs1333049, rs4977574, and rs16905644) was performed for each ethnic group by selecting all SNPs on the chromosome 9 imputation set (NCBI Build 37) between positions 21997022-22225503. A total of 3,282 SNPs were identified (598, 631, 1256 and 797 SNPs in EUA, CHN, AFA and HIS, respectively). This list of SNPs was supplemented by adding novel SNPs identified by deep sequencing efforts in this region.[37,38] Given that each ethnicity has its own LD structure, to account for multiple comparisons in each of the race/ethnic-specific analyses, we use an eigen-decomposition to estimate the effective number of independent SNPs in each race/ethnic group.

Significance was defined by Bonferroni correction by dividing an alpha of 0.05 by the number of SNPs tested ($p < 7.6 \times 10^{-4}$ given 66 SNPs tested (0.05/66) for the initial analysis, with greater number of SNPs used for the correction for the fine mapping effort). To assess genetic heterogeneity seen in stratified analyses of the four MESA race/ethnic groups, we used the I^2 heterogeneity metric to quantify the proportion of total variation across studies attributable to heterogeneity rather than chance.[39]

Results

Study Sample Characteristics

Table 1 in ref[6] illustrates the baseline demographic characteristics of the MESA participants included in this study as well as their CVD risk factors and measures of sCVD. Sample sizes reflect the inclusion of individuals from the original MESA cohort and the ancillary MESA

Family cohort. The samples sizes are similar in the different ethnicities with the exception of CHN, which had a relatively lower number of study participants (n=691). There is a lower BMI in the CHN (median 23.7), higher prevalence of hypertension (60%) and smoking (19.3%) in the AFA, and higher prevalence of CAC (56.9%) and median CAC score (115.7) in the EUA populations. There were no differences across ethnicities for CIMT.

Association of SNPs with sCVD

The association of previously identified SNPs associated with CVD and sCVD with CAC-c in the four MESA ethnicities is shown in figure 1 in ref.[6] After Bonferroni correction, multiple SNPs were associated with CAC-c in EUA and HIS, one in AFA, and no SNP significantly associated with CAC-c in CHN. The significant SNPs in EUA, HIS and AFA, and the closest gene in the locus, *P*-values and effect sizes, are shown in Table 1. In EUA there were significant associations with CAC-c in 9p21 (rs1333049, $P=2 \times 10^{-9}$; rs4977574, $P= 4 \times 10^{-9}$), *COL4A1* (rs9515203, $P=9 \times 10^{-6}$), and *PHACTR1* (rs9349379, $P= 4 \times 10^{-4}$). All of these associations, except for *COL4A1*, have effects in the same direction as shown in the initial discoveries. The difference in the direction of effect of rs9515203 in *COL4A1* is not expected, given that the alleles in this study are the same and the minor allele frequencies are equivalent as those previously published. In HIS, SNPs were associated with CAC-c in 9p21 (rs1333049, $P=8 \times 10^{-5}$; rs4977574, $P=5 \times 10^{-5}$), *APOA5* (rs964184, $P=2 \times 10^{-4}$), and *ADAMTS7* (rs7173743, $P=4 \times 10^{-4}$). There were no associations with the 9p21 region in AFA and CHN. The only significant association in AFA with CAC-c was in the *LPA* locus (rs10455872, $P=5.66\times10^{-4}$).

Results from meta-analysis across ethnicities (see figure 2 in ref[6]) show the associations between previously identified CVD and sCVD SNPs and the four sCVD phenotypes in MESA. After Bonferroni correction, there were four significant associations with CAC-c and CAC-d (Table 2). There was evidence of significant heterogeneity based upon inconsistent direction of association in different ethnicities, elevated I^2 heterogeneity, and low heterogeneity p-values. This increased heterogeneity may reflect different directions of association in the AFA population. There was also increased heterogeneity observed with respect to the *COL4A1* and CAC-c association. In contrast, the associations between *PHACTR1* and *ADAMTS7* showed significant p-values as well as low heterogeneity metrics in the context of CAC-d and CAC-c respectively. There was one significant association with CIMT-c and two significant associations with CIMT-i The *SMG6* locus was significantly associated with CIMT-c but there were increased heterogeneity. The *LPA* and *TRIB1* loci were significantly associated with CIMT-i with low heterogeneity indices, indicating a consistent association across ethnicities.

Functional Annotation

To assess the functional significance of the SNPs with significant associations, we used the publically available ENCODE Project Consortium and HaploRegv2 for functional annotation of selected SNPs.[40] The 9p21 locus SNP rs1333049 is in a heterochromatin protein 1 (HP1 site), a protein known to be important in gene expression regulation. rs4977574, also in the 9p21 locus, is located in an enhancer in various types of cells and is also in a DNase site (in LNCaP cells). rs9515203 in the *COL4A1* locus is located in an

enhancer in various types of cells and binds RNA polymerase II (POL2). The *PHACTR1* SNP rs9349379 is in a site for myocyte enhancer factor 2 (Mef2), a family of transcription factors important in cellular differentiation and embryonic development. rs10455872 in the *LPA* locus is a modulator of FOXO, a family of transcription factors involved in cellular metabolism and proliferation. The *APOA5* SNP rs964184 and the *ADAMTS7* SNP rs7173743 are both located in DNase sites in various types of cells. Another SNP in *ADAMTS7*, rs1994016, is in the binding site of the transcription factor activator protein 1 (AP-1) which is important in regulating cellular proliferation, differentiation and apoptosis. The *SMG6* SNP rs216172 is in enhancer and DNase sites in numerous cell types.

Fine Mapping of 9p21 Region

Given the inconsistent association across ethnicities between measures of CAC and the 9p21 region, fine mapping of this region was performed in an attempt to uncover as yet unidentified associations. Figure 1 presents the associations between 9p21 SNPs (598 SNPs in EUA, 631 SNPs in CHN, 1256 SNPs in AFA, and 797 SNPs in HIS) and CAC-c demonstrating robust associations in EUA and HIS but no significant associations in AFA and CHN. Table 3 in ref[6] lists the significant SNPs in EUA and HIS along with their location, effect size and p-values. Figure 2 shows the regional association plots of meta-analyses across ethnicities illustrating the associations between 9p21 SNPs and the four sCVD phenotypes included in this study. There were no significant associations between 9p21 SNPs and CIMT-c and CIMT-i. There was a similar pattern of associations between these SNPs and CAC-d and CAC-c although there were more significant associations with CAC-c. However, as shown in Table 4 in ref[6], although these associations are statistically significant, there was inconsistent direction of associations across ethnicities, particularly with respect to AFA, and increased heterogeneity indices for some SNPs.

DISCUSSION

The association of SNPs identified in GWAS of CVD and sCVD has been observed in populations of mostly European descent and information regarding these associations in ethnically diverse cohorts is lacking. In this study, we evaluate the association of those SNPs with sCVD (CAC and CIMT) in the MESA cohort. Our findings reveal previously unknown associations between various SNPs and sCVD. We also explored whether these associations were present in different ethnicities.

The vast majority of SNPs tested in this candidate gene study had been previously associated with CVD, while a handful had been associated with sCVD (see Table 2 in ref[6]). We have confirmed the previously reported associations between CAC and SNPs in 9p21, *PHACTR1* and *COL4A1*,[16,41] while reporting associations between CAC and SNPs in *ADAMTS7*. The *ADAMTS7* locus has been previously associated with CAD, but not with CAC. We also report associations between CIMT and loci previously associated with CAD (*SMG6, LPA* and *TRIB1*). We found no association between the 9p21 locus and CIMT in any of the ethnicities tested, as has been previously reported,[42] suggesting a mechanism for CVD risk mediation independent of CIMT. Although our power calculations (see tables 5 and 6 in ref[6]) revealed greater than greater 90% power for alleles with minor allele

frequency of 10%, this lack of association could be due to relatively limited power particularly for SNPs with lesser minor allele frequencies and/or effect sizes. However, the fact that some loci are associated with CAD and CAC/CIMT while others are not raises the intriguing possibility that not all genetic mechanisms for CAD are mediated via sCVD as currently characterized by the imaging techniques in this study.[16] Functional annotation of the SNPs in these loci reveals that they likely mediate the development of CVD and/or sCVD by means of regulating gene expression and cellular proliferation.

There were notable differences in sCVD associations based on ethnicity. The 9p21 locus was only significantly associated with CAC in EUA and HIS ancestry populations. The *COL4A1* and *PHACTR1* SNPs were only associated with CAC in the group of EUA ancestry. The *LPA* SNP was only associated with CAC in AFA ancestry and the *APOA5* and *ADAMTS7* SNPs only achieved significance in the HIS group. Of note, SNPs previously associated with atherosclerosis in Han Chinese[14] were not associated with sCVD in CHN in this study. It is possible that a greater sample size would uncover associations in CHN not seen in the current study. These findings could also be explained by ethnic differences in the heritability of the sCVD trait. For example, the heritability of CAC in AFA has been estimated at 30% which is lower than the 50% estimated for EUA, which suggests the possibility of ethnic differences in the impact of genetic variation on sCVD.[43] These differences in heritability could reflect a greater impact of environmental factors on sCVD or a potential greater role for gene-environment interactions. Furthermore, the prevalence of sCVD has been shown to vary by ethnic group while controlling for traditional CVD risk factors[44,45] and this difference in disease prevalence could explain some of our findings.

Differences in linkage disequilibrium (LD) structure in different ethnicities[46] could explain the different genetic associations with sCVD. It is possible that SNPs used in this study are not adequate markers of the functional SNPs responsible for a potential genetic effect on sCVD. As such, lower LD between the marker SNPs and functional SNPs could decrease the effect size and therefore the power for detection. We attempted to overcome this limitation for one of the loci included in the present study. The 9p21 locus is one of the most consistently associated loci to emerge from modern genetic analysis of CVD susceptibility. There were no associations between the 9p21 SNPs in this study and sCVD in AFA and CHN and our meta-analysis across ethnicities shows increased heterogeneity in this region. Prior studies have also failed to show association with 9p21 and sCVD and CVD in AFA.[21,23,25] In order to uncover a potentially as-yet-unmeasured functional SNP in this locus we performed fine mapping in this region but again found no significant associations in AFA and CHN despite robust associations in EUA and HIS. It is possible that different biological pathways in the development of sCVD and CVD are at play in different ethnicities. This observation is supported by the fact that greater European admixture in AFA populations has been previously associated with higher CAC[47]. However, it is important to note that although certainly related, sCVD and CVD represent different phenotypic endpoints with potentially different pathophysiology depending on ethnicity. For example, AFA have been shown to have lower CAC scores but higher CVD as compared to other ethnicities.[45,48]

There are important limitations in the current study. First, although our power calculation shows adequate power for the minor allele frequencies (MAF) and effect sizes of the SNPs included in this study, the GWAS studies from which these SNPs were selected had much larger sample sizes and power than the current study. Furthermore, inter-ethnic differences in MAFs could also limit the power to detect associations. Second, ethnic differences in linkage disequilibrium between tested SNPs and putative functional loci could also limit our ability to detect associations. Although fine mapping of the 9p21 region allowed us to examine distinct patterns of association across race/ethnic groups, comprehensive fine mapping of all loci under investigation was beyond the scope of the current manuscript. Lastly, the SNPs included in this study were mostly originally associated with CVD, which is a different, albeit related, phenotypic entity than sCVD. Examining the associations between these SNPs and CVD was beyond the scope of the current study. Soft plaque characteristics predictive of CVD, e.g. positive remodeling and low attenuation, were not available for analysis as only non-contrast CT was performed in the current study.[49] Moreover, incorporating novel biomarkers predictive of CVD, such as high sensitivity troponins, into the analysis could help unmask new associations.[50] Replication of the current observations in larger data sets is imperative.

In conclusion, this is the first study testing the association with sCVD of previously identified SNPs in GWAS of CVD and sCVD across three different ethnicities of non-European descent. Although our study is limited by power to detect associations compared to the larger power in the original GWAS describing the associations, we describe several associations with sCVD. Furthermore, we found no association between 9p21 and CAC in AFA despite a fine mapping effort in this region suggesting a lack of signal in this region in AFA. This lack of association is particularly intriguing given the consistent association between this region and CVD in cohorts of European descent. Our results suggest some shared genetic architecture for sCVD across ethnic groups, while also underscoring the possibility of novel variants and/or pathways in risk of CVD in ethnically diverse populations.

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- Genetic studies of atherosclerosis have focused on Caucasian populations.
- Genetic associations were tested in the Multi-Ethnic Study of Atherosclerosis.
- There was replication of associations but also notable differences by ethnicity.

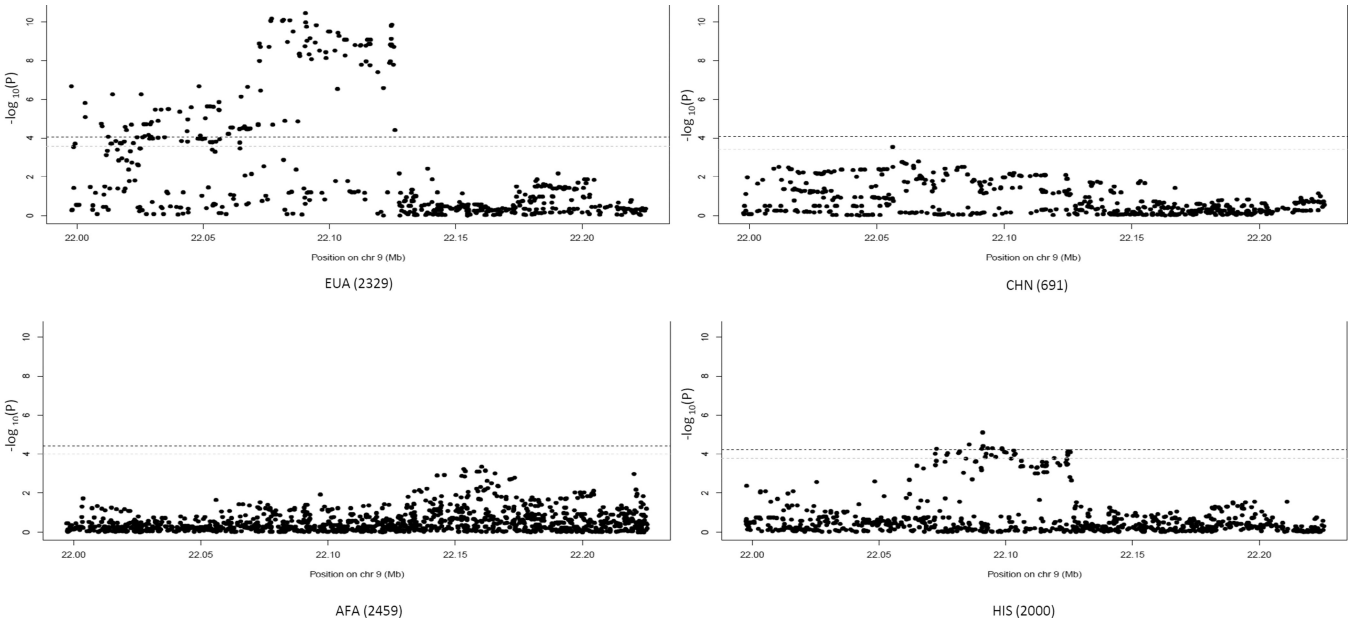

Figure 1.

Fine mapping of 9p21 region. SNPs were selected 100 kb upstream/downstream from SNPs rs1333049, rs4977574, and rs16905644. A total of 3282 SNPs were identified (598, 631, 1256 and 797 SNPs in EUA, CHN, AFA and HIS, respectively). Association of CAC-c with CVD and sCVD SNPs by ethnicity. Results from a linear regression assuming an additive model and controlling for age, gender, site of ascertainment, principal components, HDL cholesterol (HDL-C), LDL cholesterol (LDL-C), triglycerides, BMI, hypertension status, diabetes status and current smoking. The y-axis represents the – log10 of the p-value and the upper dotted line the Bonferroni corrected significance threshold.

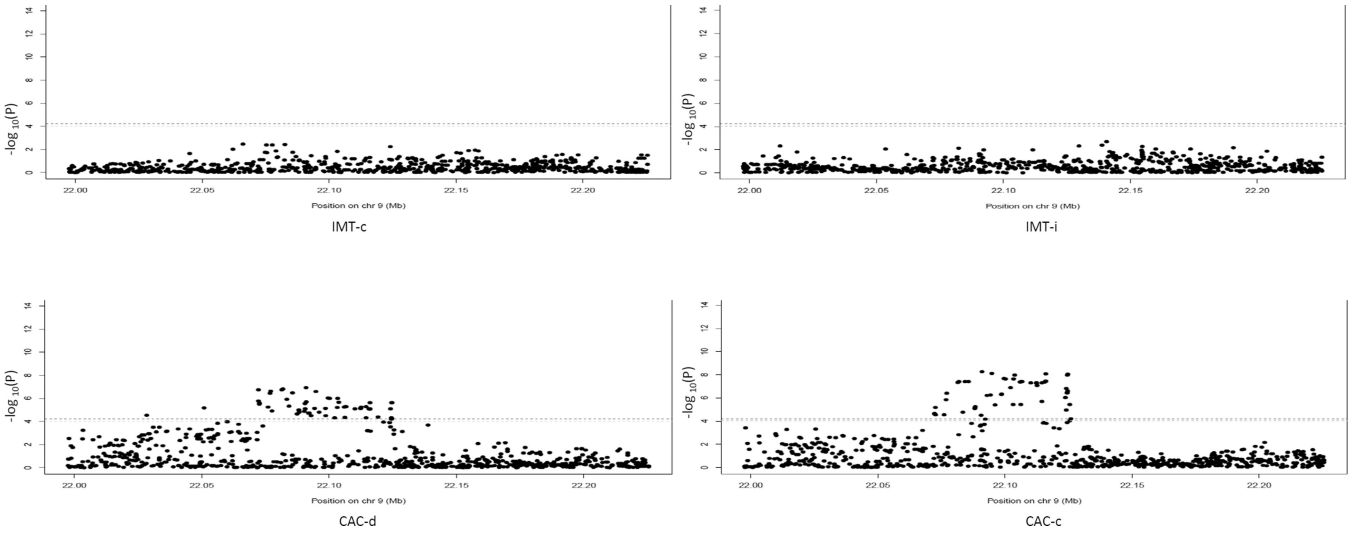

Figure 2.

Meta-analysis across ethnicities of the association of sCVD measures and SNPs in the 9p21 region. A linear regression assuming an additive model and controlling for age, gender, site of ascertainment, principal components, HDL cholesterol (HDL-C), LDL cholesterol (LDL-C), triglycerides, BMI, hypertension status, diabetes status and current smoking was performed in each ethnic group as described above. The program METAL was used to conduct a fixed effect meta-analysis to combine estimated effects and standard errors from stratified analyses. The y-axis represents the –log10 of the p-value and the dotted line the Bonferroni corrected significance threshold.

Table 1

Significant associations between CAC-c and CVD and sCVD SNPs by ethnicity. Results from a linear regression assuming an additive model and controlling for age, gender, site of ascertainment, principal components, HDL cholesterol (HDL-C), LDL cholesterol (LDL-C), triglycerides, BMI, hypertension status, diabetes status and current smoking. Chr=Chromosone, Gene=Closest gene, Beta=published beta (ethnicity), MAF=Minor allele frequency. N/A=SNP not available. P-values meeting Bonferroni correction are highlighted.

			EUA			CHN			AFA			HIS		
SNP	**Chr**	**Gene**	**Beta**	**P-value**	**MAF**	**Beta**	**P-value**	**MAF**	**Beta**	**P-value**	**MAF**	**Beta**	**P-value**	**MAF**
rs1333049	9	CDKN2A	0.371	2.00E-09	0.493	0.191	8.81E-02	0.494	−0.036	6.16E-01	0.257	0.274	7.87E-05	0.437
rs4977574	9	CDKN2A	0.366	3.84E-09	0.489	0.229	4.07E-02	0.463	0.029	7.20E-01	0.197	0.283	5.24E-05	0.412
rs9515203	13	COL4A1	−0.310	9.18E-06	0.256	0.029	8.76E-01	0.111	−0.16	2.14E-02	0.273	0.020	7.94E-01	0.295
rs12526453	6	PHACTR1	0.237	3.65E-04	0.429	N/A	N/A	0.004	−0.086	3.10E-01	0.165	−0.106	1.42E-01	0.328
rs9349379	6	PHACTR1	0.237	3.65E-04	0.429	0.326	5.96E-02	0.285	0.329	1.62E-03	0.124	0.181	2.25E-02	0.333
rs10455872	6	LPA	0.042	7.97E-01	0.053	N/A	N/A	0.002	0.985	5.66E-04	0.020	−0.023	9.20E-01	0.033
rs964184	11	APOA5	0.003	9.74E-01	0.142	0.051	7.13E-01	0.226	0.027	7.23E-01	0.210	−0.285	1.68E-04	0.291
rs7173743	15	ADAMTS7	0.002	9.68E-01	0.462	−0.03	7.97E-01	0.489	−0.119	6.46E-02	0.440	−0.242	3.80E-04	0.470

Table 2

SNPs with significant associations with sCVD in meta-analysis across ethnicities. Chr=Chromosone, Gene=Closest gene, P. Beta=published beta (ethnicity), H. P-value=Heterogeneity p-value. I2=Heterogeneity metric. The direction of effect in each ethnicity is also illustrated. P-values meeting Bonferroni correction are highlighted.

SNP	Chr	Gene	P. Beta	EUA	CHN	AFA	HIS	Beta	P-value	I^2	H. P-value
CAC-D											
rs1333049	9	CDKN2A	0.302 (EUA)	−	−	+	−	−0.189	3.83E-06	81.2	1.17E-03
rs4977574	9	CDKN2A	0.254 (EUA)	+	+	−	+	0.212	5.33E-07	71.2	1.52E-02
rs9349379	6	PHACTR1	0.200 (EUA) 0.140 (CHN)	+	+	+	+	0.250	4.53E-07	0.0	7.52E-01
rs1994016	15	ADAMTS7	0.174 (EUA)	+	+	+	+	0.186	8.44E-05	0.0	7.46E-01
CAC-C											
rs1333049	9	CDKN2A	0.302 (EUA)	−	−	+	−	−0.218	2.42E-09	19.5	2.18E-04
rs4977574	9	CDKN2A	0.254 (EUA)	+	+	+	+	0.252	2.55E-11	11.2	1.05E-02
rs9515203	13	COL4A1	0.077 (EUA)	−	+	−	+	−0.149	2.07E-04	73.7	9.79E-03
rs9349379	6	PHACTR1	0.200 (EUA) 0.140 (CHN)	+	+	+	+	0.242	4.45E-08	1.5	6.73E-01
IMT-C											
rs216172	17	SMG6	0.068 (EUA)	+	−	+	+	0.012	8.58E-05	23.4	2.71E-01
IMT-I											
rs10455872	6	LPA	0.225 (EUA)	+	+	+	+	0.102	1.48E-05	0.0	3.13E-01
rs2954029	8	TRIB1	0.049 (EUA)	−	−	−	−	−0.029	4.03E-05	0.0	9.57E-01