Clinical and Population Studies

Association of SCARB1 Variants With Subclinical Atherosclerosis and Incident Cardiovascular Disease
The Multi-Ethnic Study of Atherosclerosis


Objective—We previously reported a statistically significant association of SCARB1 intronic single nucleotide polymorphism (SNP) rs10846744 with common carotid intimal-medial artery thickness in each of the 4 Multi-Ethnic Study of Atherosclerosis racial/ethnic groups (white, Chinese, black, and Hispanic).

Methods and Results—Using an expanded sample of 7936 Multi-Ethnic Study of Atherosclerosis participants, phenotyped for measures of subclinical atherosclerosis, incident myocardial infarction, and cardiovascular disease, and genotyped through the SNP Health Association Resource project, we have now examined the genetic association of these phenotypes with 126 genotyped and imputed SCARB1 SNPs. We also performed stratified analyses to examine whether SCARB1 SNP effects differed by sex. Our analysis of the full Multi-Ethnic Study of Atherosclerosis cohort provides strong evidence for the association of rs10846744 with common carotid intimal-medial thickness (P=1.04E-4 in combined analysis of all 4 Multi-Ethnic Study of Atherosclerosis racial/ethnic groups). In sex-stratified analysis, we observed statistically significant association of rs10846744 with incident cardiovascular disease events in males (P=0.01). Examining analytical results from the Myocardial Infarction Genetics Consortium for replication, we observed further support for the association of rs10846744 with myocardial infarction.


Key Words: cardiovascular disease • genetics • genomics • lipids

The SCARB1 gene encodes scavenger receptor class B type 1 (SR-BI) protein, characterized as a high-density lipoprotein (HDL) receptor.1 In humans, multiple studies have reported association of common SCARB1 single nucleotide polymorphisms (SNPs) with HDL cholesterol levels.2-5 Existing studies also indicate that the effects of SCARB1 variants on HDL levels may vary by sex.6,7,6 Specifically, the earlier work by Acton et al6 showed variability in associations of low-density lipoprotein (LDL) and HDL to SCARB1 SNPs based on sex.

In mice, SR-BI gene manipulation studies have demonstrated its antiatherogenic properties, with SR-BI/apolipoprotein-E double knockout mice developing complex coronary artery disease, myocardial infarction (MI), and heart failure.8 In humans, there have been reports of association of SCARB1 variants with measures of subclinical atherosclerosis (SCA),9,10 as well as with coronary heart disease.11 After identifying association of a SCARB1 SNP (rs838880) with HDL, Teslovich et al1 further examined whether the same SNP was associated with coronary artery disease, but found no evidence of association. We recently took a different approach, directly examining association of SCARB1 SNPs with measures of SCA (coronary artery calcification [CAC], common carotid intimal-medial artery thickness [CCIMT], and internal carotid intimal-medial thickness [ICIMT]) in the Multi-Ethnic Study of Atherosclerosis (MESA), using a subset of 2757 participants typed for selected candidate gene SNPs.9 We found that carriers of the G allele of SNP rs10846744 had significantly lower CCIMT when compared with carriers of the C allele in all MESA racial/ethnic groups (white P=0.05, Chinese P=0.02, black P=0.03, and Hispanic P=0.03), with strong evidence for association in pooled analysis combining all racial/ethnic groups (P=0.0002). The significant association of rs10846744 with SCA was not influenced by traditional atherosclerosis risk factors, such as lipids (including HDL-C), hypertension, body mass index (BMI), and fasting glucose levels.

To replicate these associations and better characterize the relationship between SCARB1 variants with SCA and incident

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cardiovascular disease (CVD) events, we analyzed SCARB1 genotypes available through genome-wide association scan data on 8224 consenting MESA participants, obtained through the National Heart, Lung, and Blood Institute SNP Health Association Resource (SHARe) project. We began by examining the association of the SCARB1 SNP rs10846744 with measures of SCA in the full MESA cohort, and then went on to assess association of 126 (genotyped and imputed) SCARB1 SNPs in MESA participants with the same measures of SCA. The sample for which we report on genetic associations in the current study includes, but is substantially larger than, the study population reported in our previous manuscript. Furthermore, we extended our earlier investigation to include clinical outcomes of incident MI and CVD events. We also performed sex-stratified analyses to permit differential SCARB1 SNP effects by sex. Finally, we performed in silico replication using published results from the Myocardial Infarction Genetics (MIGen) Consortium.

Methods

Study Design

MESA is a longitudinal study of subclinical CVD and risk factors that predict progression to clinically overt CVD or progression of the subclinical disease. The first clinic visits occurred in 2000 to 2002 in 6814 participants recruited from 6 field centers across the United States, and all participants were free of CVD at the baseline examination. Approximately 38% of the recruited participants are white, 28% black, 22% Hispanic, and 12% Asian, predominantly of Chinese descent. MESA has been enhanced by many ancillary studies focused on specific phenotypic and exposure domains.

Genotype Data

Participants recruited by the original MESA cohort (6814), and MESA Family Study (2128 from 528 families) and MESA Air (5479 from MESA, 257 external cohort, and 490 from MESA Family Study) were genotyped in 2009 using the Affymetrix Human SNP array 6.0. SNPs were filtered for SNP level call rate <95% and individual level call rate <95%, and monomorphic SNPs were removed. Examining the distribution of heterozygosity rates across all genotyped SNPs, we observed a generally uniform distribution between 0% and 53%, with <0.01% of SNPs having heterozygosity >53%. Thus, we removed all SNPs with heterozygosity >53%. The cleaned genotypic data were deposited with MESA phenotypic data into dbGaP as the MESA SHARe project (study accession phs000209, http://www.ncbi.nlm.nih.gov/projects/gap/cgi-bin/study.cgi?study_id=phs000209.v7.p2) for 8224 consenting individuals (2685 white, 777 Chinese, 2588 non-Hispanic black, and 2174 Hispanic) with 897 981 SNPs passing study-specific quality control.

Principal Component Analysis

Before population structure analysis, we first constructed subsets of typed SNPs, thinned for linkage disequilibrium (LD) within each of the 4 MESA racial/ethnic groups, to prevent the principal components (PCs) from being dominated by regions of known long-range LD, we first removed from consideration regions of known long-range LD Caucasians, including the human leukocyte antigen region, a chromosome 8 inversion, and a region on chromosome 11. We then thinned for local LD within an unrelated subset of the MESA Hispanic cohort using the PLINK option -indep-pairwise to create a subset of typed SNPs thinned for pairwise \( R^2 > 0.2 \) no more than 0.2 in a 100 SNP window, moving the windows 25 SNPs at a time. LD thinning was performed separately within each racial/ethnic group, resulting in a subset of 114 035 SNPs for white; 98 353 SNPs for Chinese; 99 716 SNPs for black; and 61 194 for Hispanics.

Using our LD-thinned subsets constructed separately for each of the 4 MESAracial/ethnic groups, we performed principal component analysis as implemented in the program SMARTPCA. From the software package EIGENSTRAT to compute PCs of ancestry for unrelated subsets of individuals, constructed by removing inferred first-degree relatives from the analysis. In computing the PCs, we performed additional LD correction by using results of regression on the previous 5 SNPs as input to the principal component analysis (SMARTPCA option nsnpdregress) and performed 5 iterations of outlier removal in which we removed individuals with computed values >10 SDs from mean along the top 6 PCs of ancestry. We constructed histograms and Q–Q plots to assess symmetry and normality of the distribution of loadings for each of the resulting PCs to determine the optimal number of PCs to include in genetic association analysis.

Selection of SNPs for Genetic Association Analysis

To perform genetic association analysis for SNPs in the SCARB1 gene region, we began by selecting SNPs of interest in the region. We combined all SCARB1 SNPs investigated in our previous publication with SNPs reported for the SCARB1 gene region in dbSNP to identify a total of 1215 SNPs in the SCARB1 gene region. Of these 1215 SNPs, 49 were genotyped on Affy 6.0 and passed genotype quality control. IMPUTE version 2.1.0 was used to perform imputation for the MESA SHARe participants (chromosomes 1–22) using HapMap Phase I and II. CEU+YRI+CHB+JPT as the reference panel (release #22, National Center for Biotechnology Information Build 36 [dbSNP b126]) (only the CEU reference panel was used for imputation in white participants), and we could impute another 108 SCARB1 SNPs in MESA white, 80 SNPs in Chinese, 80 SNPs in black, and 80 SNPs in Hispanics. 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 analysis. 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 >10\(^{-5}\). After applying these filters, we had 90, 99, 111, and 107 SNPs remaining for genetic association analysis in MESA white, Chinese, black, and Hispanics, respectively, and a total of 126 unique SNPs across all 4 racial/ethnic groups.

Phenotyping of MESA Participants

Measures of SCA examined included the presence or absence of CAC as a binary marker of SCA and ultrasound measurements of intimal-medial thickness in millimeters (mm) for CCIMT and ICIMT. Cardiovascular events were adjudicated by a MESA committee, which included cardiologists, physician epidemiologists, and neurologists. A detailed description of the cardiovascular event adjudication process has already been published. In MESA, we define CVD events to include incident MI, definite angina, probable angina (if followed by coronary artery bypass grafting and percutaneous coronary intervention), resuscitated cardiac arrest, stroke, stroke death, coronary heart disease death, or other CVD death. For the purposes of this study, we examined probable or confirmed CVD events (CVD-AII), confirmed CVD events (CVD-Hard), and incident MI.

Genetic Association Analysis in MESA

To select individuals to be included in analysis, we began with the full MESA cohort. We stratified by racial/ethnic group and eliminated those individuals with top PCs of ancestry >3.5 SD from the mean within any racial/ethnic group. To allow study site to be included as a covariate in genetic association analysis within each racial/ethnic group, we restricted the data set to individuals from study sites with data available for at least 20 individuals of that racial/ethnic group. For each of the phenotypic analyses, we then restricted the data set to
individuals with data available for the particular phenotype of interest. For quantitative traits, outliers were defined as individuals with phenotypic values >3.5 SD from the mean, with the mean and SD calculated separately for each of the stratified analyses performed. Analyses of CCIMT and ICIMT were performed on the log-scale, which yielded approximately normal phenotypic distributions.

We began with stratified analyses within each racial/ethnic group. For analysis of white and Chinese, and where there were insufficient families with phenotypes among black and Hispanics, we first constructed an unrelated subset of individuals by selecting at most 1 individual from each pedigree and performed linear regression of quantitative phenotypes or logistic regression of dichotomous phenotypes in R. For analysis of phenotypes with a substantial familial component among black and Hispanic cohorts, we performed analysis using an additive model with a linear mixed-effects model for quantitative traits or generalized estimating equations for dichotomous traits, to account for familial relationships as implemented in the package R/GWAF.21

In all analyses, we began with a basic model (Model 1) including age, sex, study site, and PCs of ancestry. (Based on our examination of PCs within each racial/ethnic group, as described above, we used 3 PCs for analysis of white, 1 PC for Chinese, 1 PC for black, and 3 PCs for Hispanics.) To examine sensitivity of our results to other known risk factors for atherosclerosis, we also performed genetic association analysis under a second model (Model 2) including all covariates from Model 1, with additional adjustment for HDL cholesterol (HDL-C), LDL cholesterol (LDL-C), triglycerides, BMI, fasting glucose, and hypertension status. To account for multiple comparisons in each of the stratified analyses, we used a strict Bonferroni correction for the number of SNPs remaining in analysis after filtering on minor allele frequencies >0.01, imputation quality >0.5, and Hardy-Weinberg Equilibrium $P$ value $\geq 10^{-3}$ in each racial/ethnic group.

After stratified analyses, we performed meta-analysis to combine results across all 4 racial/ethnic groups. We performed fixed effect meta-analysis to combine estimated effects and standard errors from stratified analyses, as implemented in METAL.22 To account for multiple comparisons, we used a strict Bonferroni correction for each phenotype based on the total number of SNPs included in meta-analysis of that trait.

In addition to the main analyses, we further performed sex-stratified analysis. The sex-stratified association analysis followed the basic procedure used for our main analyses, beginning with sex-specific stratified analyses for each racial/ethnic group, and combining the sex-specific results by meta-analysis. As for pooled analysis, we performed sex-stratified analyses using 2 statistical models where Model 1 included the basic covariates age, study site, and PCs of ancestry, whereas Model 2 included all covariates from Model 1, with additional adjustment for HDL-C, LDL-C, triglycerides, BMI, fasting glucose, and hypertension status.

To assess genetic heterogeneity seen in stratified analysis of the 4 MESA racial/ethnic groups, we tested a heterogeneity using Cochran’s Q and also examined the inconsistency metric $I^2$ that quantifies the proportion of total variation across studies attributable to heterogeneity rather than chance.23 We also used Cochran’s Q to examine heterogeneity in estimated genetic effects by sex.

### Linkage Disequilibrium in the SCARB1 Gene Region

To examine LD in the SCARB1 gene region, we made use of all genotyped SNPs on the Affy 6.0 array available through MESA SHARE (see Selection of SNPs for Genetic Association Analysis). For each of the 4 MESA racial/ethnic groups, we produced a graphical display of LD among these genotyped SNPs using the software Haploview24 (Figures I–IV in the online-only Data Supplement), and LD is reported using $r^2$ for each pair of SNPs.

### Results

#### Characteristics of the Study Sample

Baseline characteristics, atherosclerosis risk factors, and primary phenotypes for our investigation, including measures of SCA (CCIMT, ICIMT, and CAC [present/absent]) and counts of clinical events (CVD-All, CVD-Hard, and MI), are summarized separately for each racial/ethnic group in Table 1. Sample sizes reflect the inclusion of individuals from the original MESA cohort, combined with those from the ancillary MESA Family and MESA Air studies (described in Methods). Counts of participants with data available are reported separately for each category of phenotypic observations (participant characteristics, lipid levels, SCA, or clinical events). The relatively lower numbers of black and Hispanic samples with data available for clinical events reflect the fact that these data were recorded for the original MESA cohort only and are not available for individuals recruited through the MESA Family and MESA Air ancillary studies.

In examining baseline characteristics across MESA racial/ethnic groups, we observed lower BMI among Chinese participants (median 23.8 kg/m$^2$) compared with white (median 27.1 kg/m$^2$), black (median 29.5 kg/m$^2$), and Hispanic participants (median 28.6 kg/m$^2$). We further observed a higher rate of hypertension among black participants (60.1%) compared with white (38.6%), Chinese (37.8%), and Hispanic participants (41.3%).

Rates of clinical events (CVD-All, CVD-Hard, and MI) were higher among white (6.9%, 4.6%, and 2.5%, respectively) and Hispanics (6.4%, 4.7%, and 2.5%, respectively) compared with black (5.8%, 4.0%, and 1.4%, respectively) and Chinese (4.3%, 2.5%, and 1.3%, respectively). Within each of the MESA racial/ethnic groups, we also observed rates of clinical events consistently higher in males than females. For example, rates of CVD-Hard in MESA white, Chinese, black, and Hispanic male participants were 5.2%, 2.9%, 4.7%, and 6.3%, respectively, compared with 3.9%, 2.0%, 3.3%, and 3.2%, respectively, in females. Rates of MI across the 4 MESA racial/ethnic groups followed the same trend with respect to sex (3.3%, 1.6%, 2.3%, and 3.7%, respectively, in males versus 1.7%, 1.0%, 0.006%, and 1.3%, respectively, in females).

#### Genetic Association of Common Carotid Intimal-Medial Thickness With SNP rs10846744

SNP rs10846744 was previously reported as the SCARB1 SNP most statistically significantly associated with CCIMT in our previous analysis of a subset of individuals from MESA.9 In the current analysis, rs10846744 was again statistically significantly associated with log CCIMT in meta-analysis ($P=1.07E-04$), including adjustment for age, sex, study site, and PCs of ancestry within each ethnic group (Model 1), even after strict Bonferroni correction for the number of SNPs under consideration ($\alpha=0.05/126=0.0004$) (Table 2, Figure 1A). The strength of association of rs10846744 with log CCIMT decreased only slightly in meta-analysis ($P=5.61E-04$) after additional adjustment for known risk factors of atherosclerosis (Model 2). In stratified analysis, this association was strongest in Hispanics (nominal $P=0.005$).
We examined evidence for heterogeneity in genetic effects of rs10846744 on log CCIMT across the 4 MESA racial/ethnic groups. The rs10846744 allele G was associated with lower levels of CCIMT in all 4 racial/ethnic groups (Table 2, Figure 1B), with little evidence for heterogeneity performed in METAL23 under the base model (Model 1, heterogeneity \( P = 0.727 \), heterogeneity \( I^2 = 0 \)).

Many of the individuals included in our current MESA sample were also included in the MESA Candidate Gene cohort (the original report of association of rs10846744 with CCIMT).9 We therefore examined the association of rs10846744 with CCIMT in the subset of individuals \( n=4994 \) who were not part of the original MESA Candidate Gene cohort (Table I in the online-only Data Supplement). A stratified analysis was conducted for each racial/ethnic group, as well as meta-analysis combining all racial/ethnic groups, using the same regression models applied for association analysis of the complete MESA cohort (Table 2). For each of the 4 racial/ethnic groups, the estimated effect for the G allele of rs10846744 was in the same direction as reported in our analysis of the MESA Candidate Gene cohort. 9 In meta-analysis, the direction of effect was also in agreement with our previous

### Table 1. Characteristics of Study Population

<table>
<thead>
<tr>
<th>Group</th>
<th>White</th>
<th>Chinese</th>
<th>Black</th>
<th>Hispanic</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of subjects</td>
<td>2526</td>
<td>775</td>
<td>2529</td>
<td>2106</td>
</tr>
<tr>
<td>Women</td>
<td>1320 (52.3)</td>
<td>394 (50.8)</td>
<td>1419 (56.1)</td>
<td>1137 (51.8)</td>
</tr>
<tr>
<td>Age, y</td>
<td>63 (54, 71)</td>
<td>62 (53, 71)</td>
<td>60 (53, 68)</td>
<td>60 (52, 68)</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>27.1 (24.2, 30.4)</td>
<td>23.8 (21.8, 26.0)</td>
<td>29.5 (26.1, 33.8)</td>
<td>28.6 (25.9, 32.0)</td>
</tr>
<tr>
<td>Fasting glucose, mmol/L</td>
<td>4.8 (4.5, 5.3)</td>
<td>5.1 (4.8, 5.6)</td>
<td>5.1 (4.7, 5.7)</td>
<td>5.2 (4.7, 5.8)</td>
</tr>
<tr>
<td>Hypertension</td>
<td>975 (38.6)</td>
<td>292 (37.8)</td>
<td>1519 (60.1)</td>
<td>870 (41.3)</td>
</tr>
</tbody>
</table>

### Lipid levels*

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of subjects</th>
<th>White</th>
<th>Chinese</th>
<th>Black</th>
<th>Hispanic</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of subjects</td>
<td>2520</td>
<td>772</td>
<td>2518</td>
<td>2098</td>
<td></td>
</tr>
<tr>
<td>HDL-C, mmol/L</td>
<td>1.3 (1.1, 1.6)</td>
<td>1.2 (1.0, 1.5)</td>
<td>1.3 (1.1, 1.6)</td>
<td>1.2 (1.0, 1.4)</td>
<td></td>
</tr>
<tr>
<td>LDL-C, mmol/L</td>
<td>3.0 (2.5, 3.5)</td>
<td>3.0 (2.5, 3.4)</td>
<td>3.0 (2.5, 3.5)</td>
<td>3.1 (2.5, 3.6)</td>
<td></td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>5.0 (4.5, 5.6)</td>
<td>4.9 (4.4, 5.4)</td>
<td>4.9 (4.3, 5.5)</td>
<td>5.1 (4.5, 5.7)</td>
<td></td>
</tr>
<tr>
<td>Triglycerides, mmol/L</td>
<td>1.3 (0.9, 1.9)</td>
<td>1.4 (1.0, 2.0)</td>
<td>1.0 (0.7, 1.4)</td>
<td>1.5 (1.1, 2.2)</td>
<td></td>
</tr>
</tbody>
</table>

### Subclinical atherosclerosis

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of subjects</th>
<th>White</th>
<th>Chinese</th>
<th>Black</th>
<th>Hispanic</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of subjects</td>
<td>2526</td>
<td>773</td>
<td>2529</td>
<td>2099</td>
<td></td>
</tr>
<tr>
<td>Common IMT, mm</td>
<td>0.84 (0.73, 0.97)</td>
<td>0.81 (0.71, 0.92)</td>
<td>0.86 (0.75, 0.99)</td>
<td>0.81 (0.71, 0.93)</td>
<td></td>
</tr>
<tr>
<td>Internal IMT, mm</td>
<td>0.89 (0.71, 1.38)</td>
<td>0.73 (0.60, 0.94)</td>
<td>0.91 (0.70, 1.30)</td>
<td>0.84 (0.68, 1.19)</td>
<td></td>
</tr>
<tr>
<td>CAC present/absent</td>
<td>1433 (56.7)</td>
<td>392 (50.7)</td>
<td>1085 (42.9)</td>
<td>962 (45.8)</td>
<td></td>
</tr>
</tbody>
</table>

### Clinical events

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of subjects</th>
<th>White</th>
<th>Chinese</th>
<th>Black</th>
<th>Hispanic</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of subjects</td>
<td>2523</td>
<td>773</td>
<td>1610</td>
<td>1445</td>
<td></td>
</tr>
<tr>
<td>Cardiovascular disease-all</td>
<td>173 (6.9)</td>
<td>33 (4.3)</td>
<td>94 (5.8)</td>
<td>92 (6.4)</td>
<td></td>
</tr>
<tr>
<td>Cardiovascular disease-hard</td>
<td>115 (4.6)</td>
<td>19 (2.5)</td>
<td>64 (4.0)</td>
<td>68 (4.7)</td>
<td></td>
</tr>
<tr>
<td>Myocardial infarction</td>
<td>63 (2.5)</td>
<td>10 (1.3)</td>
<td>22 (1.4)</td>
<td>36 (2.5)</td>
<td></td>
</tr>
</tbody>
</table>

Our examination of evidence for heterogeneity in genetic effects of rs10846744 on log CCIMT across the 4 MESA racial/ethnic groups. The rs10846744 allele G was associated with lower levels of CCIMT in all 4 racial/ethnic groups (Table 1), with little evidence for heterogeneity performed in METAL23 under the base model (Model 1, heterogeneity \( P = 0.727 \), heterogeneity \( I^2 = 0 \)).

Many of the individuals included in our current MESA sample were also included in the MESA Candidate Gene cohort (the original report of association of rs10846744 with CCIMT).9 We therefore examined the association of rs10846744 with CCIMT in the subset of individuals \( n=4994 \) who were not part of the original MESA Candidate Gene cohort (Table I in the online-only Data Supplement). A stratified analysis was conducted for each racial/ethnic group, as well as meta-analysis combining all racial/ethnic groups, using the same regression models applied for association analysis of the complete MESA cohort (Table 2). For each of the 4 racial/ethnic groups, the estimated effect for the G allele of rs10846744 was in the same direction as reported in our analysis of the MESA Candidate Gene cohort.9 In meta-analysis, the direction of effect was also in agreement with our previous

### Table 2. Linear Regression Summary Statistics for the Association of SCARB1 SNP rs10846744 and log CCIMT by MESA Racial/Ethnic Group and in Meta-Analysis, Under Multiple Models of Adjustment

<table>
<thead>
<tr>
<th>Group</th>
<th>Allele Frequency</th>
<th>Model 1</th>
<th>n</th>
<th>Beta</th>
<th>SE</th>
<th>( P ) value</th>
<th>Model 2</th>
<th>n</th>
<th>Beta</th>
<th>SE</th>
<th>( P ) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>White</td>
<td>0.82</td>
<td>2405</td>
<td>−0.011</td>
<td>0.007</td>
<td>0.092</td>
<td>2367</td>
<td>−0.010</td>
<td>0.007</td>
<td>0.115</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chinese</td>
<td>0.43</td>
<td>701</td>
<td>−0.017</td>
<td>0.010</td>
<td>0.098</td>
<td>687</td>
<td>−0.019</td>
<td>0.010</td>
<td>0.056</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Black</td>
<td>0.38</td>
<td>2457</td>
<td>−0.009</td>
<td>0.005</td>
<td>0.102</td>
<td>2425</td>
<td>−0.007</td>
<td>0.005</td>
<td>0.206</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hispanic</td>
<td>0.65</td>
<td>2050</td>
<td>−0.018</td>
<td>0.006</td>
<td>0.005</td>
<td>2001</td>
<td>−0.015</td>
<td>0.006</td>
<td>0.016</td>
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</tr>
<tr>
<td>Meta-analysis</td>
<td>−0.013</td>
<td>0.003</td>
<td>1.07E-04</td>
<td>0.011</td>
<td>0.003</td>
<td>5.61E-04</td>
<td></td>
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</tr>
</tbody>
</table>

BMI indicates body mass index; CCIMT, common carotid intimal-medial artery thickness; HDL, high-density lipoprotein; IMT, intimal-medial thickness; LDL, low-density lipoprotein; MESA, Multi-Ethnic Study of Atherosclerosis; SNP, single nucleotide polymorphism.

Allele frequencies and estimated effects are reported for the effect allele G (versus the reference allele C). Model 1 includes adjustment for age, sex, study site, and principal components of ancestry. Model 2 includes all covariates in Model 1, with the addition of HDL-C, LDL-C, triglycerides, BMI, fasting glucose, and hypertension status. Estimated effect sizes are presented based on an additive genetic model with 1 degree of freedom, for the log-transformed phenotype common IMT (mm).
report, and the overall association under Model 1 was nominally significant for the single candidate SNP ($P=0.034$).

**Genetic Association Analysis of Subclinical Atherosclerosis Traits for SCARB1 SNPs**

We conducted analysis of 126 genotyped or imputed SCARB1 SNPs in MESA SHARe for CAC (presence/absence), CCIMT, and ICIMT. Given the strength of association for rs10846744 with CCIMT, we first determined whether other SCARB1 SNPs demonstrated stronger association with CCIMT and whether the association profile varied by racial/ethnic group (Figure 1). Our results support rs10846744 as providing the strongest evidence for association in meta-analysis of common carotid intimal-medial thickness. No other SCARB1 SNPs reached the Bonferroni threshold for common carotid intimal-medial thickness, either in meta-analysis or in analyses stratified by racial/ethnic group (Tables II–VI in the online-only Data Supplement). We performed analyses stratified by sex...
for each of the 4 racial/ethnic groups, as well as combined by meta-analysis. We did not observe any additional SNPs exhibiting statistically significant association with CCIMT after multiple testing correction.

We further examined association of SCARB1 SNPs with CAC and ICIMT, in analyses stratified by racial/ethnic group (Tables II–V in the online-only Data Supplement) and by meta-analysis to combine the results of these stratified analyses (Table VI in the online-only Data Supplement). We did not observe any statistically significant results after correction for multiple testing. We also failed to observe a significant association of additional SCARB1 SNPs in sex-stratified analyses.

Genetic Association of Incident MI and CVD Events With rs10846744

As a prospective cohort study designed to investigate the prevalence and progression of subclinical CVD, individuals with previous evidence of CVD were excluded at the time of MESA enrollment beginning in July 2000. As a result, the number of clinical CVD events reported for the MESA cohort was too small to warrant formal analysis of incident CVD at that time. In the current analyses, there is a larger number of genotyped individuals with longer follow-up time (increased number of incident CVD events) to permit examination of the association of rs10846744 with incident CVD. In meta-analysis of all individuals, there were no statistically significant associations with any clinical CVD events (Table 3). In stratified analysis by sex using the baseline model (Model 1), there were statistically significant associations in males with MI ($\beta$=−0.410, SE=0.180, P=0.023) and CVD-Hard ($\beta$=−0.354, SE=0.138, P=0.010). These results correspond to odds ratios for MI of $0.664 (95\% \text{ CI} 0.466–0.944)$ and for CVD-Hard of $0.702 (95\% \text{ CI} 0.536–0.920)$ per copy of the effect allele G versus the reference allele C. Results were very similar under the model with additional adjustment for known risk factors of atherosclerosis (Model 2), with slightly stronger statistical significance and estimated effect sizes. There were no nominally significant results for females. We did not observe statistically significant heterogeneity in genetic effects of rs10846744 in males as compared with females for incident MI (Model 1, heterogeneity P=0.166) or for CVD-Hard (Model 1, heterogeneity P=0.067).

The estimated effect of rs10846744 on the risk of MI is stronger in black males ($\beta$=−0.664, SE=0.442), Chinese males ($\beta$=−0.618, SE=0.726), and white males ($\beta$=−0.555, SE=0.266) compared with Hispanic males ($\beta$=0.011, SE=0.330) (Table 4). The estimated effect of rs10846744 on CVD-Hard was strongest in black ($\beta$=−0.509, SE=0.284) and white males ($\beta$=−0.422, SE=0.223) compared with Chinese ($\beta$=−0.193, SE=0.511) and Hispanic males ($\beta$=−0.187, SE=0.250).

We further investigated the full set of 126 SNPs in the SCARB1 region to determine whether any SNPs showed strong association with incident CVD in meta-analysis across all 4 MESA racial/ethnic groups (Table VI in the online-only Data Supplement). We did not identify SNPs surpassing the Bonferroni threshold for statistical significance ($\alpha=0.05/126=0.0004$) in meta-analysis of all individuals or in sex-stratified analyses.

Replication Through the MIGen

After observing statistically significant association of rs10846744 with incident MI and CVD in MESA, we sought in silico replication through the MIGen Consortium. The MIGen consortium recently completed genome-wide association study of 2967 cases of early-onset MI (in men ≤50 years old or women ≤60 years old) and 3075 age- and sex-matched controls free of MI, all of European ancestry, with genetic association results for ~2.5 million directly genotyped and imputed SNPs. From these results, we examined association of the SCARB1 SNP rs10846744 with early-onset MI. We observed that the G allele of rs10846744 was associated with reduced risk of premature MI (odds ratio=0.910; 95% CI 0.833–0.993, P=0.043).

Table 3. Summary Statistics From Meta-Analysis to Combine All 4 MESA Racial/Ethnic Groups in Association Analysis of the SCARB1 SNP rs10846744 and 3 Clinical Outcomes of Interest (MI, CVD-Hard, and CVD-All) Under Multiple Models of Adjustment

<table>
<thead>
<tr>
<th>Trait</th>
<th>Analysis</th>
<th>Model 1</th>
<th></th>
<th>Model 2</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Beta</td>
<td>SE</td>
<td>P value</td>
<td>Beta</td>
<td>SE</td>
</tr>
<tr>
<td>Myocardial infarction</td>
<td>All</td>
<td>−0.247</td>
<td>0.153</td>
<td>0.106</td>
<td>−0.227</td>
</tr>
<tr>
<td></td>
<td>Sex-stratified: male only</td>
<td>−0.408</td>
<td>0.180</td>
<td>0.024</td>
<td>−0.420</td>
</tr>
<tr>
<td></td>
<td>Sex-stratified: female only</td>
<td>0.087</td>
<td>0.319</td>
<td>0.785</td>
<td>0.107</td>
</tr>
<tr>
<td>CVD-Hard</td>
<td>All</td>
<td>−0.164</td>
<td>0.106</td>
<td>0.123</td>
<td>−0.125</td>
</tr>
<tr>
<td></td>
<td>Sex-stratified: Male only</td>
<td>−0.358</td>
<td>0.138</td>
<td>0.010</td>
<td>−0.318</td>
</tr>
<tr>
<td></td>
<td>Sex-stratified: female only</td>
<td>0.052</td>
<td>0.174</td>
<td>0.764</td>
<td>0.110</td>
</tr>
<tr>
<td>CVD-All</td>
<td>All</td>
<td>−0.119</td>
<td>0.089</td>
<td>0.179</td>
<td>−0.080</td>
</tr>
<tr>
<td></td>
<td>Sex-stratified: male only</td>
<td>−0.206</td>
<td>0.113</td>
<td>0.068</td>
<td>−0.187</td>
</tr>
<tr>
<td></td>
<td>Sex-stratified: female only</td>
<td>−0.014</td>
<td>0.148</td>
<td>0.924</td>
<td>0.028</td>
</tr>
</tbody>
</table>

CVD indicates cardiovascular disease; MESA, Multi-Ethnic Study of Atherosclerosis; MI, myocardial infarction; SNP, single nucleotide polymorphism. Results are presented for pooled analysis of males and females, as well as sex-stratified analysis. Estimated effects are presented for an additive genetic association model on rs10846744, coded as the number of copies of the effect allele G (versus the reference allele C). Model 1 includes adjustment for age, sex, study site, and principal components of ancestry for pooled analysis of males and females; sex is not included as a covariate in sex-stratified analyses. Model 2 includes all covariates in Model 1, with the addition of HDL-C, LDL-C, triglycerides, BMI, fasting glucose, and hypertension status.
We previously performed an association study of 43 SCARB1 SNPs with measures of SCA in 2757 individuals from the MESA cohort, detecting a significant association of SCARB1 variation with CCIMT. Here, we extend our previous work by (1) using an expanded set of 7936 phenotyped individuals from the MESA study with genotypes available for 126 SNPs available to us through dense genome-wide genotyping, as well as imputation of additional SNPs based on HapMap Phase I and II data. Our results are consistent with those recently reported by Grallert et al., whereby these investigators found a significant association of rs10846744 with prevalent coronary heart disease/coronary artery disease in the CARDIoGRAM consortium. Our results, and those of Grallert et al., offer strong evidence that rs10846744 is either in strong LD with the causal variant (Figures I–IV in the online-only Data Supplement) or is itself a causal variant underlying these important associations.

The rs10846744 SNP is located within intron 1 of the SCARB1 gene (chromosome 12, position 125312425, dbSNP). This SNP is not located at a traditional alternative splice site. Results from our (A. Rodriguez, unpublished data) laboratory confirm the absence of alternative mRNA transcripts in carriers of the G allele. A search of publically available microRNA databases also failed to identify this SNP as either a seed or target of microRNA regulation (A. Rodriguez, unpublished data). A bioinformatics screen using University of California, Santa Cruz Genome Bioinformatics web site (http://genome.ucsc.edu) revealed that the region contains DNase I hypersensitivity clusters and enhancer-promoter histone markers, suggestive of a region that might exert cis or trans regulatory effects.

A mechanism by which rs10846744 could affect atherosclerosis is by acting as an enhancer within intron 1. As an enhancer, it could possibly affect the expression of SR-BI or alter expression of a distant gene. No correlation between rs10846744 genotype and levels of SR-BI RNA or protein in macrophages isolated from subjects with hyperalphalipoproteinemia has been identified (A. Rodriguez, unpublished), suggesting that this SNP is not directly regulating activity of the SCARB1 promoter. Based on the work by Suchindran et al., it might be possible that rs10846744 is an enhancer that exerts a distal effect on expression of Lp-PLA2. These investigators examined genetic determinants that were significantly associated with either Lp-PLA2 activity or mass and identified rs10846744 as being negatively correlated with Lp-PLA2.

<table>
<thead>
<tr>
<th>Trait</th>
<th>Group</th>
<th>n</th>
<th>Beta</th>
<th>SE</th>
<th>P value</th>
<th>n</th>
<th>Beta</th>
<th>SE</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myocardial infarct</td>
<td>White</td>
<td>1172</td>
<td>-0.555</td>
<td>0.266</td>
<td>0.037</td>
<td>1152</td>
<td>-0.577</td>
<td>0.270</td>
<td>0.033</td>
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<td></td>
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<td>350</td>
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<td>0.726</td>
<td>0.394</td>
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<td>-0.773</td>
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<td>0.369</td>
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<tr>
<td></td>
<td>Black</td>
<td>732</td>
<td>-0.664</td>
<td>0.422</td>
<td>0.116</td>
<td>723</td>
<td>-0.618</td>
<td>0.427</td>
<td>0.148</td>
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<td></td>
<td>Hispanic</td>
<td>641</td>
<td>0.011</td>
<td>0.330</td>
<td>0.973</td>
<td>626</td>
<td>0.012</td>
<td>0.341</td>
<td>0.971</td>
</tr>
<tr>
<td>CVD-Hard</td>
<td>White</td>
<td>1172</td>
<td>-0.422</td>
<td>0.223</td>
<td>0.058</td>
<td>1152</td>
<td>-0.449</td>
<td>0.232</td>
<td>0.053</td>
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<tr>
<td></td>
<td>Chinese</td>
<td>350</td>
<td>-0.193</td>
<td>0.511</td>
<td>0.706</td>
<td>342</td>
<td>-0.360</td>
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<tr>
<td></td>
<td>Black</td>
<td>732</td>
<td>-0.509</td>
<td>0.284</td>
<td>0.073</td>
<td>723</td>
<td>-0.513</td>
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<tr>
<td></td>
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<td>641</td>
<td>-0.187</td>
<td>0.250</td>
<td>0.455</td>
<td>626</td>
<td>-0.189</td>
<td>0.264</td>
<td>0.474</td>
</tr>
</tbody>
</table>

BMI indicates body mass index; CVD, cardiovascular disease; HDL, high-density lipoprotein; LDL, low-density lipoprotein; MI, myocardial infarction; SNP, single nucleotide polymorphism.

Estimated effects are presented for an additive genetic association model on rs10846744, coded as the number of copies of the effect allele G (versus the reference allele C). Model 1 includes adjustment for age, study site, and principal components of ancestry. Model 2 includes all covariates in Model 1, with the addition of HDL-C, LDL-C, triglycerides, BMI, fasting glucose, and hypertension status.
activity and mass. As we observed here and previously for CCIMT, the effect of rs10846744 on Lp-PLA2 activity and mass is independent of lipids, including HDL-C levels. Thus, while SR-BI exerts an independent effect on HDL-C levels in humans, the effect of the rs10846744 variant on SCA and incident CVD is likely mediated via nonlipid pathways and through other SCARB1-dependent processes, such as affecting endothelial function or inflammatory pathways.

The risk of premature death as a result of coronary artery disease has been shown in SR-BI null mice on an apoE knockout background. While there are now a number of epidemiological studies showing a significant association of SCARB1 variants with lipid levels, only the recent publication by Grallert et al and our current study have shown an association between rs10846744 variants and clinical CVD. We previously showed a significant association between rs10846744 and SCA in a smaller cohort of MESA participants. We have now replicated this earlier study, again observing that rs10846744 is significantly associated with lower CCIMT in carriers of the G allele.

In conclusion, SCARB1 exerts an important influence on cardiovascular health in humans. The mechanism by which the intrinsic rs10846744 SCARB1 SNP affects carotid intimal-medial thickness and clinical cardiovascular events is not yet established and warrants further study.

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Disclosures
None.

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Association of SCARBJ Variants With Subclinical Atherosclerosis and Incident Cardiovascular Disease: The Multi-Ethnic Study of Atherosclerosis
Ani Manichaikul, Adam C. Naj, David Herrington, Wendy Post, Stephen S. Rich and Annabelle Rodriguez

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Supplementary Material for

“Association of SCARB1 variants with subclinical atherosclerosis and incident cardiovascular disease: The Multi-Ethnic Study of Atherosclerosis”

Supplementary Table I: Analysis of a subset of individuals who were not part of the original MESA Candidate Gene cohort. Linear regression summary statistics are presented for the association of *SCARBI* SNP rs10846744 and log CCIMT by MESA racial/ethnic group and in meta-analysis, under multiple models of adjustment.

<table>
<thead>
<tr>
<th>Group</th>
<th>Allele frequency</th>
<th>Model 1</th>
<th>Model 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>N</td>
<td>Beta</td>
</tr>
<tr>
<td>Caucasian</td>
<td>0.82</td>
<td>1741</td>
<td>-0.006</td>
</tr>
<tr>
<td>Chinese</td>
<td>0.43</td>
<td>57</td>
<td>-0.030</td>
</tr>
<tr>
<td>African American</td>
<td>0.38</td>
<td>1820</td>
<td>-0.007</td>
</tr>
<tr>
<td>Hispanic</td>
<td>0.65</td>
<td>1376</td>
<td>-0.014</td>
</tr>
<tr>
<td>Meta-analysis</td>
<td></td>
<td>-0.009</td>
<td>0.004</td>
</tr>
</tbody>
</table>

Allele frequencies and estimated effects are reported for the effect allele G (versus the reference allele C). Model 1 includes adjustment for age, sex, study site, and principal components of ancestry. Model 2 includes all covariates in Model 1, with the addition of HDL-C, LDL-C, triglycerides, BMI, fasting glucose, and hypertension status. Estimated effect sizes are presented based on an additive genetic model with 1 degree of freedom, for log CCIMT (mm).
Supplementary Figure I: Linkage disequilibrium among genotyped *SCARBI* gene region SNPs for MESA Caucasians.
Supplementary Figure II: Linkage disequilibrium among genotyped *SCARBI* gene region SNPs for MESA Chinese.
Supplementary Figure III: Linkage disequilibrium among genotyped *SCARBI* gene region SNPs for MESA African Americans.
Supplementary Figure IV: Linkage disequilibrium among genotyped *SCARBI* gene region SNPs for MESA Hispanics.