Multiethnic Exome-Wide Association Study of Subclinical Atherosclerosis

Pradeep Natarajan, MD, MMSc*; Joshua C. Bis, PhD*; Lawrence F. Bielak, DDS, MPH; Amanda J. Cox, PhD; Marcus Dörr, MD; Mary F. Feitosa, PhD; Nora Franceschini, MD, MPH; Xiuqing Guo, PhD; Shih-Jen Hwang, PhD; Aaron Isaacs, PhD; Min A Jhung, PhD; Maryam Kavousi, MD, PhD; Ruifang Li-Gao, MSc; Leo-Pekka Lyytikäinen, MD; Riccardo E. Marioni, PhD; Ulf Schminke, MD; Nathan O. Stützfel, MD, PhD; Hayato Tada, MD; Jessica van Setten, PhD; Albert V. Smith, PhD; Dina Vojinovic, MD, MSc; Lisa R. Yanek, MPH; Jie Yao, MD, MS; Laura M. Yerges-Armstrong, PhD; Najaf Amin, PhD; Usman Baber, MD; Ingrid B. Borecki, PhD; J. Jeffrey Carr, MD, MSc; Yee-Der Ida Chen, PhD; L. Adrienne Cupples, PhD; Pim A. de Jong, MD, PhD; Harry de Koning, PhD; Bob D. de Vos, MSc; Ayse Demirkan, PhD; Valentin Fuster, MD, PhD; Oscar H. Franco, MD, PhD; Mark O. Goodarzi, MD, PhD; Tamara B. Harris, MD; Susan R. Heckbert, MD, PhD; Gerardo Heiss, MD, PhD; Udo Hoffmann, MD, PhD; Albert Hofman, MD, PhD; Ivana Işgum, PhD; J. Wouter Jukema, MD, PhD; Mika Kähönen, MD, PhD; Sharon L.R. Kardia, PhD; Brian G. Kral, MD, MPH; Lenore J. Launer, PhD; Joe Massaro, PhD; Roxana Mehran, MD, MPH; Braxton D. Mitchell, Ph.D, MPH; Thomas H. Mosley, Jr, PhD; Renée de Mutsert, PhD; Anne B. Newman, MD; Khanh-dung Nguyen, PhD; Kari E. North, PhD; Jeffrey R. O’Connell, PhD; Matthijs Oudkerk, MD; James S. Pankow, PhD, MPH; Gina M. Peloso, PhD; Wendy Post, MD, MS; Michael A. Province, PhD; Laura M. Raffield, PhD; Olli T. Raitakari, MD, PhD; Dermot F. Reilly, PhD; Fernando Rivadeneira, MD, PhD; Frits Rosendaal, MD, PhD; Samantha Sartori, PhD; Kent D. Taylor, PhD; Alexander Teumer, PhD; Stella Trompet, PhD; Stephen T. Turner, MD; Andre G. Uitterlinden, PhD; Dhananjay Vaidya, PhD, MPH, MBBS; Aad van der Lugt, MD, PhD; Uwe Völker, PhD; Joanna M. Wardlaw, MD; Christina L. Wassel, PhD, MS; Stefan Weiss, PhD; Mary K. Wojczynski, PhD; Diane M. Becker, ScD, PhD; Lewis C. Becker, MD; Eric Boerwinkle, PhD; Donald W. Bowden, PhD; Ian J. Deary, PhD; Abbas Dehghan, MD, PhD; Stephan B. Felix, MD; Vilmundur Gudnason, MD, PhD; Terho Lehtimäki, PhD; Rasika Mathias, ScD; Dennis O. Mook-Kanamori, MD, PhD; Bruce M. Psaty, MD; Daniel J. Rader, MD; Jerome I. Rotter, MD; James G. Wilson, MD; Cornelia M. van Duijn, PhD; Henry Völzke, MD; Sekar Kathiresan, MD; Patricia A. Peyser, PhD; Christopher J. O’Donnell, MD, MPH; CHARGE Consortium

Background—The burden of subclinical atherosclerosis in asymptomatic individuals is heritable and associated with elevated risk of developing clinical coronary heart disease. We sought to identify genetic variants in protein-coding regions associated with subclinical atherosclerosis and the risk of subsequent coronary heart disease.

Methods and Results—We studied a total of 25,109 European ancestry and African ancestry participants with coronary artery calcification (CAC) measured by cardiac computed tomography and 52,869 participants with common carotid intima–media thickness measured by ultrasonography within the CHARGE Consortium (Cohorts for Heart and Aging Research in Genomic
Coronary heart disease (CHD) remains the leading cause of death and infirmity in developed countries. Atherosclerosis is the underlying pathology of CHD. The presence of atherosclerosis in individuals without clinical CHD, termed subclinical atherosclerosis, is associated with increased risk of developing clinical CHD independent of traditional risk factors prior to the onset of symptoms. Subclinical atherosclerosis is a heritable clinical phenotype that can be ascertained noninvasively as coronary artery calcification (CAC) by cardiac computed tomography and common carotid intima–media thickness (CIMT) by carotid ultrasound.

Clinical Perspective on p 520

Genome-wide association studies within the CHARGE Consortium (Cohorts for Heart and Aging Research in Genomic Epidemiology) have discovered sites of common noncoding genetic variation associated with both CAC and CIMT, among those of European ancestry. Noncoding single nucleotide polymorphisms at the 9p21 and 6p24 regions, near the CDKN2A and PHACTR1 genes, respectively, are strongly associated with both CAC burden and myocardial infarction. The 8q24 (ZH2X), 19q13 (APOC1), and 8q23 (PINX1) loci are strongly associated with CIMT. Observed associations for subclinical atherosclerosis among individuals of European ancestry, however, have not been replicated in those of African ancestry. Furthermore, because the biological implications of noncoding variation are not as readily interpreted as with coding variation, the roles of such variants in human atherosclerosis remain unclear. Protein-coding variation tends to be infrequently observed and is often inadequately catalogued on earlier genome-wide association study arrays. Rare genomic variation is not well imputed, and exome sequencing to detect such uncommon variation across large populations remains a costly endeavor. Here, we leverage the Illumina HumanExome BeadChip array, enriched for protein-coding variation. We investigated whether there is evidence for associations of protein-coding variation with 2 measures of subclinical atherosclerosis across individuals of European and African ancestry. And we further determine whether such DNA sequence variations may influence CHD risk.

Study Populations
The Illumina HumanExome Beadchip v1.0 or v1.1 (also known as the exome chip) was used to genotype participants across 19 cohorts of the CHARGE Consortium (Data Supplement). Participants with a diagnosis of CHD at the time of CAC phenotyping were excluded from CAC analysis. Participants who underwent carotid endarterectomy prior to CIMT phenotyping were excluded from CIMT analysis. 25,109 participants had CAC measured, and 52,869 participants had CIMT measured. Each study received institutional review board approval, participants provided written informed consent, and respective governing ethics committees approved each study.

Measures

CAC Measurement
Cohorts used different computed tomography scanners to ascertain CAC scoring (Table I in the Data Supplement). CAC scorings by multidetector computed tomography and by electron beam computed tomography have been previously described to be highly concordant and are both recognized as valid tools to estimate CAC score.

Total CAC score was quantified by the sum of CAC area weighted by density within individual coronary arteries by the Agatston method, and the continuous score was used for analysis.

CIMT Measurement
Common CIMT was derived by bilateral longitudinal common carotid artery analysis (imaging and measurement methods are described in the Table II in the Data Supplement). The mean of the maximum thickness for each common carotid artery was the analytic variable.

Statistical Analyses
According to prespecified analysis plans, association analyses and meta-analyses were performed using the seqMeta package (http://cran.r-project.org/web/packages/seqMeta/index.html) in the R statistical software as has previously been performed for exome chip–based analyses. To reduce skewness, CAC was natural log transformed after adding 1 and CIMT was natural log transformed. Each cohort performed an analysis for each genomic variant, with the trait of interest independently and separately for individuals of European and African ancestry to minimize population biases. Covariates in the models included age, sex, and principal components of ancestry derived using EIGENSTRAT. For studies with related samples, the pairwise kinship matrix was computed and accounted for in the regression model. Score statistics and genotypic covariance matrices were computed for each cohort and used for additive single variant and gene-based analyses, respectively.

For our primary analyses, we tested the association of each genomic variant with CAC and with CIMT across all samples by
meta-analysis that included all cohorts, irrespective of ancestry. We performed single-variant analyses on variants that had a minor allele count of at least 20 and gene-based analyses for genes with combined minor allele frequency (MAF) of nonsynonymous variants at least 0.2% to reduce the likelihood of false-positive results. We also performed 2 gene-based tests: (1) T1, where nonsynonymous variants with MAF <1% were collapsed into a gene-based statistic, and (2) sequence kernel association test with MAF <5% for nonsynonymous variants to better account for collapsed variants with bidirectional phenotypic consequences. Regional association plots were generated using LocusZoom. For our secondary analyses, we tested the association of each genomic variant with CAC and CIMT by meta-analysis separately among cohorts of European and African ancestry.

Given the 238,065 variants on the array that passed quality control, the Bonferroni-adjusted level of significance for single variant tests was 0.05/238,065=2.10×10⁻². Given the 17,574 genes with nonsynonymous variants on the array, the Bonferroni-adjusted level of significance for gene-based tests was 0.05/17,574=2.85×10⁻⁴. For CAC, we had >90% power to detect a variant (MAF <1%) with effect size 0.31 standard deviations or a gene (combined MAF <1%) with effect size 0.28 standard deviations at a sample size of 25,000. For CIMT, we had >90% power to detect a variant (MAF <1%) with effect size 0.21 standard deviations or a gene (combined MAF <1%) with effect size 0.20 standard deviations with a sample size of 52,000. Power calculations were performed using the Genetic Power Calculator.

Methods for the secondary analyses are presented in the Data Supplement.

Results

Study Participants

Nineteen cohorts participated in the meta-analyses of these 2 subclinical atherosclerotic traits, and the clinical characteristics are summarized in Tables I and II in the Data Supplement. A total of 25,109 participants were genotyped with the array and had CAC assessed; of these participants, 19,980 were of European ancestry and 5129 were of African ancestry. 52,869 participants were genotyped and had CIMT assessed; 44,963 were of European ancestry and 7906 were of African ancestry. 22,2701 (93.5%) of the 238,065 variants were polymorphic in the CAC meta-analysis; of polymorphic variants, 193,373 (97.1%) were annotated as nonsynonymous or splice-site variants. Similarly, 227,344 (95.5%) of array variants were polymorphic in the CIMT meta-analysis, and of these, 217,235 (95.6%) were nonsynonymous or splice-site variants.

CAC Association

Figure 1 plots the meta-analysis CAC association P value by genomic locus for each variant. The top loci with lead variants associated with CAC among all participants are listed in Table I. No systematic association inflation was observed across the set of statistical tests performed (Figure I in the Data Supplement).

We identified previously described common noncoding variant associations at the 9p21 and 6p24 loci. A 9p21 haplotype marked by lead single nucleotide polymorphism rs10757278-G (MAF 43%), an intergenic variant, was replicated and associated with increased CAC quantity (23.4%; 95% confidence interval [CI], 18.6%–28.3%; P=2×10⁻²⁷). Similarly, rs9349379-G (MAF 34%), an intronic variant within PHACTR1, was associated with increased CAC quantity (20.9%; 95% CI, 16.3%–25.8%; P=5×10⁻²⁰). Although these associations were robust for those of European ancestry, there was no apparent evidence for association in those of African ancestry (Figures II and III in the Data Supplement). Both loci display locus heterogeneity, or multiple independent associations, for CAC in those of European ancestry (Table 1). We did not discover noncoding variants at other loci on the exome chip that met our stringent Bonferroni alpha threshold. Previously, rs3809346, an intronic variant of COL4A2, had a suggestive association with CAC, but now in our European ancestry sample size that is twice as large, genome-wide significant association was not observed (P=2×10⁻³).

Among functional variants, a nonsynonymous APOB (rs5742904-T; MAF 0.2%; NM_000384.2:c.10580G>A; NP_000375.2:p.Arg3527Gln) variant was found to be significantly associated with CAC. Carriers of the rare APOB missense variant had markedly increased CAC (4.1-fold; 95% CI, 2.6- to 6.4-fold; P=3×10⁻¹⁰). In our meta-analysis, the Old Order Amish cohort primarily accounted for the strong association, and the variant was extremely rarely observed within other cohorts. Furthermore, the variant was

![Figure 1](https://example.com/figure1.png)

**Figure 1.** Association of each genotyped variant with coronary artery calcification (CAC) quantity. Plot of −log₁₀(\(P\)) for association of genotyped variants by chromosomal position for all autosomal polymorphisms analyzed in the age-, sex-, and principal components–adjusted model of coronary artery calcification quantity in the meta-analysis. The genes associated with the top associated variants are displayed.
Table 1. Top Meta-Analysis Variant Associations for Coronary Artery Calcification Quantity

<table>
<thead>
<tr>
<th>Variant</th>
<th>Consequence</th>
<th>Nearest Gene*</th>
<th>Chrom:Pos</th>
<th>Minor Allele</th>
<th>MAF</th>
<th>Beta‡</th>
<th>SE</th>
<th>P Value</th>
<th>SE</th>
<th>P Value</th>
<th>MAF</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs10757278§</td>
<td>Intergenic</td>
<td>(CDKN2B)</td>
<td>9:22124477</td>
<td>G</td>
<td>0.43</td>
<td>0.21</td>
<td>0.020</td>
<td>3.14×10⁻⁸</td>
<td>0.48</td>
<td>2.9×10⁻¹⁵</td>
<td>0.21</td>
<td>0.20</td>
</tr>
<tr>
<td>rs9349379¶</td>
<td>Intron</td>
<td>APOE</td>
<td>19:45140279</td>
<td>T</td>
<td>0.081</td>
<td>0.25</td>
<td>0.036</td>
<td>1.19×10⁻⁵</td>
<td>0.074</td>
<td>4.43×10⁻¹⁰</td>
<td>0.11</td>
<td>5.36×10⁻¹⁰</td>
</tr>
<tr>
<td>rs7412¶</td>
<td>Missense</td>
<td>PHACTR1</td>
<td>6:12903961</td>
<td>C</td>
<td>0.34</td>
<td>0.14</td>
<td>0.021</td>
<td>1.56×10⁻¹⁰</td>
<td>0.41</td>
<td>5.58×10⁻¹²</td>
<td>0.072</td>
<td>0.84</td>
</tr>
<tr>
<td>rs1412829§</td>
<td>Intron</td>
<td>CDKN2B</td>
<td>9:22043926</td>
<td>C</td>
<td>0.34</td>
<td>0.14</td>
<td>0.021</td>
<td>1.56×10⁻¹⁰</td>
<td>0.41</td>
<td>5.58×10⁻¹²</td>
<td>0.072</td>
<td>0.84</td>
</tr>
<tr>
<td>rs5742904#</td>
<td>Missense</td>
<td>APOB</td>
<td>2:21229160</td>
<td>T</td>
<td>2.1×10⁻⁴</td>
<td>1.41</td>
<td>0.22</td>
<td>2.93×10⁻¹⁰</td>
<td>2.7×10⁻³</td>
<td>2.93×10⁻¹⁰</td>
<td>0</td>
<td>NA</td>
</tr>
<tr>
<td>rs9366400</td>
<td>Intron</td>
<td>APOE</td>
<td>6:12901441</td>
<td>A</td>
<td>0.43</td>
<td>0.11</td>
<td>0.019</td>
<td>4.91×10⁻⁶</td>
<td>0.38</td>
<td>5.04×10⁻⁹</td>
<td>0.36</td>
<td>0.71</td>
</tr>
<tr>
<td>rs769449¶</td>
<td>Missense</td>
<td>CDKN2B</td>
<td>19:45140002</td>
<td>A</td>
<td>0.10</td>
<td>0.14</td>
<td>0.032</td>
<td>7.93×10⁻⁴</td>
<td>0.11</td>
<td>1.86×10⁻⁶</td>
<td>0.024</td>
<td>0.19</td>
</tr>
<tr>
<td>rs1801696#</td>
<td>Missense</td>
<td>APOB</td>
<td>2:21232044</td>
<td>T</td>
<td>4.6×10⁻⁴</td>
<td>0.63</td>
<td>0.14</td>
<td>1.44×10⁻⁴</td>
<td>5.7×10⁻⁴</td>
<td>9.77×10⁻⁴</td>
<td>0</td>
<td>NA</td>
</tr>
</tbody>
</table>

AA indicates African ancestry; CAC, coronary artery calcification; Chrom:Pos, hg19 chromosome:position; EA, European ancestry; MAF, minor allele frequency; SE, standard error; and SNP, single nucleotide polymorphisms.

*Genes for SNPs that are outside the transcript boundary of the protein-coding gene are shown in parentheses; for example, (CDKN2B).

†Genomic positions correspond to GRCh37.p13 reference, forward strand.

‡β coefficients are estimated for natural log transformation of total Agatston CAC score +1.

§Lead variants show modest correlation among EA (r²=0.24) and no correlation among AA.

¶Lead variants are not observed to be correlated.

‖Lead variants show minimal correlation among EA (r²=0.01) and no correlation among AA.

We noted that in addition to diminished CAC, the rs7412-T APOE ε2 allele was associated with diminished CIMT (−1.4%; 95% CI, −1.8% to −1.0%; P=4×10⁻¹⁴). There was consistency of association across European and African ancestry cohorts (Figure 4; Figure VIII in the Data Supplement). There was no significant heterogeneity among the cohorts for this association (P heterogeneity =0.23).

There were 2 additional independent suggestive associations at 19q13 at noncoding variants. A variant 5 kb upstream of LDLR (rs11668477) was associated with diminished CIMT (P=5×10⁻⁸) primarily among those of European ancestry. This variant has previously been associated with reduced low-density lipoprotein (LDL) cholesterol. The nearby rs7188-G variant (MAF 33% European ancestry and 7.9% African ancestry) within the 3’ untranslated region region of KANK2 was associated with CIMT in those of European ancestry (P=1×10⁻⁸). Additionally, a rare missense variant (rs43873045-A; MAF 0.5% African ancestry; NM_001136191.2: c.1274G>T; NP_001129663.1:p.Ser425Leu) in KANK2 only observed in individuals of African ancestry showed suggestive association with increased CIMT (P=4×10⁻⁸). Finally, in gene-based analyses, collapsing nonsynonymous variants within a gene did not yield significant associations (Figure IX in the Data Supplement).

**APOE ε2’s Effect Conditional on LDL Cholesterol**

We sought to determine whether LDL cholesterol concentration accounted for the observed ε2 association with CAC. First, when restricting the original analysis only to participants with LDL cholesterol measurements (n=20,527), ε2 remained significantly associated with reduced CAC quantity (−22.3%; 95% CI, −25.1% to −19.3%; P=2×10⁻¹ⁱ; Table III in the Data Supplement). When further adjusting for medication-adjusted LDL cholesterol, the effect estimate was diminished, yet the association remained genome-wide significant (−17.0%; 95% CI, −19.7% to −14.2%; P=2×10⁻⁸).
Natarajan et al. Exome Association With Subclinical Atherosclerosis

APOE ε2’s Effect Conditional on ε3 and ε4
Given the absence of ε4 from the array, we sought to determine whether ε2’s apparent effect on reduced CAC quantity was because of a referent that includes a previously described risk allele (ε3+ε4). Five thousand eight hundred seventy-two participants had CAC and the major APOE genotypes assessed by polymerase chain reaction. Each APOE genotype’s association with CAC (to the ε3/ε3 referent) was performed by cohort and ethnicity and subsequently meta-analyzed with fixed effects. ε2/ε3 was associated with 10.8% reduced CAC (95% CI, −19.6% to −0.01%; \(P=0.03\)) and ε2/ε2 with 27.4% reduced CAC (95% CI, −45.2% to −0.04%; \(P=0.03\); Figure X in the Data Supplement).

Concordance of CHD Variants With Subclinical Atherosclerosis Associations
Of the 57 loci previously associated with CHD mainly in individuals of European or South Asian descent, 40 published variants were on the array and available for analysis. Thirty-two of the 40 variants have the same effect direction for CAC and CHD (\(P=1.8\times10^{-4}\)), whereas only 23 variants were concordant for CIMT (\(P=0.43\)) in European ancestry participants (Table IV in the Data Supplement). When restricting the analysis to variants with at least nominal association (\(P<0.05\)) with CAC, all 17 variants had concordant effect directions (\(P=4.8\times10^{-7}\)). A similar analysis with variants at least nominally associated with CIMT showed that 6 of 11 variants had concordant effect directions for CHD (\(P=0.56\)).

Replication of Convergent Subclinical Atherosclerosis Finding With CHD
21,182 individuals of European ancestry, independent of the sample for subclinical atherosclerosis investigations, were genotyped by the Illumina HumanExome BeadChip array, of whom 9,472 had CHD. In cross-sectional analyses, meta-analysis of rs7412-T confirmed a significantly lower odds of CHD (odds ratio 0.77; 95% CI, 0.71–0.84; \(P=1.47\times10^{-10}\)).

Discussion
In our exome-wide association analysis for subclinical atherosclerosis in 2 distinct ethnicities, we find that protein-coding mutations in APOB and APOE are associated with subclinical atherosclerosis. Although the association for APOB was driven by a founder mutation in the Amish, a missense mutation in APOE (ε2) was associated with both reduced CAC and CIMT in individuals of European ancestry and African ancestry, even when adjusting for LDL cholesterol concentration.
Furthermore, carriers of the ε2 allele had a reduced risk of CHD. Here, we provide evidence for the first exome-wide association across multiple subclinical atherosclerosis traits and multiple ethnicities for APOE ε2.

Both CAC and CIMT have been proposed as proximal clinical phenotypes of atherosclerosis that may identify individuals at high risk for developing clinical CHD. However, we see that alleles that associate with increased CHD risk also seem to largely result in increased CAC, which is less consistently observed with CIMT. This is concordant with the prior observation that CAC outperforms CIMT in predicting cardiovascular events.5,29 Recently, post hoc analyses in statin trials to prevent cardiovascular disease observed that those with a higher burden of CHD-predisposing alleles are more likely to derive clinical benefit from preventive statin therapy.30

The APOB p.Arg3527Gln (also known as p.Arg3500Gln) has been previously shown to lead to increased concentrations of LDL cholesterol and premature CHD.31 Our association signal for this variant was nearly exclusively driven by the Old Order Amish, where it is known to be a founder mutation (MAF 12%) predisposing to increased LDL cholesterol concentrations and CAC quantity through disruption of the LDL receptor–binding domain.32 We also observed a distinct APOB missense mutation, p.Glu2566Lys, with borderline association with increased CAC quantity. Unlike p.Arg3527Gln, p.Glu2566Lys does not occur within the LDL receptor–binding domain but occurs within a conserved amphipathic motif of the β2 domain predicted to influence the conversion of very low–density lipoprotein to LDL.33

Furthermore, we demonstrated that APOE p.Arg176Cys (ε2 allele) was associated with reduced CAC and reduced CIMT in both individuals of European and African ancestry. APOE is an essential mediator of the catabolism and clearance of triglyceride-rich and cholesterol-rich lipoproteins. The major alleles, ε2, ε3, and ε4, have been previously linked to cardiovascular disease, from the candidate gene era, and ε2 is the least common allele.34,35 Previously, CHD risk predisposition from ε4 was primarily thought to be mediated by LDL cholesterol–raising effects, but observations with ε2 have been mixed.35 Similarly, ε4, unlike ε2, has been generally linked to ischemic stroke risk.36 Major reasons for the lack of association of the major APOE alleles with cardiovascular traits in prior genome-wide association studies include the notable absence of rs7412 and rs429358 on population-based genotyping arrays, as well as poor imputation of these

Table 2. Top Meta-Analysis Variant Associations for Carotid Intima–Media Thickness

<table>
<thead>
<tr>
<th>Variant</th>
<th>Consequence</th>
<th>Nearest Gene*</th>
<th>Chrom:Pos†</th>
<th>Minor Allele</th>
<th>All</th>
<th>EA</th>
<th>AA</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs7412</td>
<td>Missense</td>
<td>APOE</td>
<td>19:45412079 T</td>
<td>0.083</td>
<td>-0.014</td>
<td>0.0022</td>
<td>3.79×10⁻¹⁴</td>
</tr>
<tr>
<td>rs11668477</td>
<td>Intergenic</td>
<td>(LDLR)</td>
<td>19:11195030 G</td>
<td>0.27</td>
<td>-0.0064</td>
<td>0.0016</td>
<td>4.69×10⁻⁷</td>
</tr>
<tr>
<td>rs7188</td>
<td>3′ UTR</td>
<td>(KANK2)</td>
<td>19:11275139 G</td>
<td>0.39</td>
<td>0.054</td>
<td>0.0011</td>
<td>3.23×10⁻⁵</td>
</tr>
<tr>
<td>rs1712790</td>
<td>Intergenic</td>
<td>(FAM55B)</td>
<td>11:114621469 C</td>
<td>0.47</td>
<td>-0.0048</td>
<td>0.0011</td>
<td>5.93×10⁻⁶</td>
</tr>
<tr>
<td>rs2298375</td>
<td>Missense</td>
<td>C2orf15</td>
<td>22:24106448 A</td>
<td>0.086</td>
<td>0.0082</td>
<td>0.0019</td>
<td>9.51×10⁻⁹</td>
</tr>
<tr>
<td>rs174547</td>
<td>Intronic</td>
<td>(FADS1)</td>
<td>11:61570783 C</td>
<td>0.30</td>
<td>-0.0049</td>
<td>0.0011</td>
<td>1.07×10⁻⁵</td>
</tr>
</tbody>
</table>

AA indicates African ancestry; Chrom:Pos, hg19 build chromosome:position; CIMT, carotid intima–media thickness; EA, European ancestry; MAF, minor allele frequency; SE, standard error; SNP, single nucleotide polymorphisms; and UTR, untranslated region.

*Genes for SNPs that are outside the transcript boundary of the protein-coding gene are shown in parentheses; for example, (LDLR).
†Genomic positions correspond to GRCh37.p13 reference, forward strand.
‡β coefficients are estimated for natural log transformation of CIMT.

Figure 3. Association of each genotyped variant with carotid intima–media thickness (CIMT). Plot of −log₁₀(P) for association of genotyped variants by chromosomal position for all autosomal polymorphisms analyzed in the age-, sex-, and principal components–adjusted model of carotid intima–media thickness in the meta-analysis. The genes associated with the top associated variants are displayed.
variants. Similarly, rs429358 is not included on the array used for this study.

ApoE is a major ligand of LDL receptor and a key mediator of remnant lipoprotein particle clearance.40–42 The ε2 allele is believed to result in less efficient LDL receptor binding by altering the positive potential.39 Using publicly available data, ε2 does not impact expression of nearby genes in Genotype-Tissue Expression project nor does it demonstrate enhancer or promoter chromatin marks in Encyclopedia of DNA Elements HepG2 liver cells supporting ε2’s direct impact on ApoE itself. ApoE ε2 can alternatively clear lipoproteins via cell-surface heparan sulfate proteoglycan and LDL receptor-related protein.40–42 ApoE ε2 transgenic mice crossbred with apolipoprotein B transgenic mice have lower LDL cholesterol.42 Furthermore, ApoE ε2 transgenic mice lacking LDL receptor still had lower LDL cholesterol, suggesting that hypocholesterolemia appears independent of ε2’s effects on LDL receptor.55,47 ApoE ε2 impairs lipoprotein lipase-mediated metabolism of very low–density lipoprotein to LDL potentially through the displacement of apolipoprotein C-II, an activator of lipoprotein lipase.43 The consequent diminished hepatic cholesterol may subsequently increase LDL receptors for apolipoprotein B–containing lipoproteins like LDL.

Interestingly, despite accounting for LDL cholesterol, we observe that ε2 still is highly associated with reduced CAC quantity. It is likely that a single cross-sectional of lipoproteins, although correlates with, does not fully account for lifelong lipoprotein exposures. ApoE ε2 homozygotes who develop type III hyperlipoproteinemia have a marked increase in remnant lipoprotein particles unlike heterozygotes. Analogously, ApoE ε2-overexpressing mice have increased hepatic very low–density lipoprotein production.42 Thus, although ApoE ε2 heterozygotes may have an increase in very low–density lipoprotein production and decreased triglyceride catabolism via lipoprotein lipase, the observation of similar triglyceride levels compared with noncarriers suggests preservation of, or enhanced, clearance of remnant lipoprotein particles. We hypothesize that ApoE ε2’s association with reduced subclinical atherosclerosis may be because of increased clearance of both atherogenic LDL and remnant lipoprotein particles through LDL receptor–dependent and –independent pathways. Further work is needed to test this hypothesis.

Our study has several strengths. First, we perform a genetic association meta-analysis across the largest set of individuals to date for subclinical atherosclerosis in 2 distinct ancestries. Second, we characterize the association

<table>
<thead>
<tr>
<th>Ethnicity</th>
<th>Cohort</th>
<th>Relative CIMT</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>European</td>
<td>AGES-Reykjavik</td>
<td>0.987</td>
<td>(0.973,1.001)</td>
</tr>
<tr>
<td></td>
<td>ARIC</td>
<td>0.982</td>
<td>(0.971,0.993)</td>
</tr>
<tr>
<td></td>
<td>BioImage</td>
<td>0.987</td>
<td>(0.973,1.000)</td>
</tr>
<tr>
<td></td>
<td>Cardiovascular Health</td>
<td>0.981</td>
<td>(0.967,0.994)</td>
</tr>
<tr>
<td></td>
<td>Diabetes Heart</td>
<td>0.997</td>
<td>(0.985,1.010)</td>
</tr>
<tr>
<td></td>
<td>Erasmus Rucphen Family</td>
<td>0.997</td>
<td>(0.985,1.031)</td>
</tr>
<tr>
<td></td>
<td>Framingham Heart</td>
<td>0.984</td>
<td>(0.987,0.991)</td>
</tr>
<tr>
<td></td>
<td>GeneSTAR</td>
<td>0.997</td>
<td>(0.986,1.029)</td>
</tr>
<tr>
<td></td>
<td>LBC1936</td>
<td>0.976</td>
<td>(0.937,1.017)</td>
</tr>
<tr>
<td></td>
<td>MESA</td>
<td>1.003</td>
<td>(0.985,1.023)</td>
</tr>
<tr>
<td></td>
<td>NEO</td>
<td>0.997</td>
<td>(0.986,1.008)</td>
</tr>
<tr>
<td></td>
<td>Rotterdam</td>
<td>0.975</td>
<td>(0.958,0.992)</td>
</tr>
<tr>
<td></td>
<td>SHIP</td>
<td>0.977</td>
<td>(0.963,0.990)</td>
</tr>
<tr>
<td></td>
<td>SHIP Trend</td>
<td>0.981</td>
<td>(0.968,0.995)</td>
</tr>
<tr>
<td>African</td>
<td>ARIC</td>
<td>0.980</td>
<td>(0.953,0.997)</td>
</tr>
<tr>
<td></td>
<td>BioImage</td>
<td>0.995</td>
<td>(0.985,1.025)</td>
</tr>
<tr>
<td></td>
<td>Cardiovascular Health</td>
<td>0.969</td>
<td>(0.942,0.996)</td>
</tr>
<tr>
<td></td>
<td>GeneSTAR</td>
<td>0.981</td>
<td>(0.929,1.036)</td>
</tr>
<tr>
<td></td>
<td>Jackson Heart</td>
<td>0.985</td>
<td>(0.965,1.006)</td>
</tr>
<tr>
<td></td>
<td>MESA</td>
<td>0.966</td>
<td>(0.948,0.989)</td>
</tr>
<tr>
<td>ALL</td>
<td></td>
<td>0.979</td>
<td>(0.969,0.988)</td>
</tr>
</tbody>
</table>

Figure 4. Forest plot of relative carotid intima–media thickness (CIMT) for APOE ε2 carriers. CIMT for APOE ε2 carriers relative to non-carriers is displayed for all cohorts stratified by European and African ancestries to demonstrate consistency across diverse cohorts and ethnicities. AGES indicates Age, Gene/Environment Susceptibility; ARIC, Atherosclerosis Risk in Communities; CI, confidence interval; GeneSTAR, Gene Study of Atherosclerosis Risk in Families; LBC1936, Lothian Birth Cohort 1936; MESA, MultiEthnic Study of Atherosclerosis; NEO, Netherlands Epidemiology of Obesity Study; and SHIP, Study of Health in Pomerania.
of protein-coding genomic variation, which has not been well studied at the population level, with subclinical atherosclerosis. Third, we explore mechanisms of association through lipoprotein-mediation analyses. Fourth, we provide novel insights with both cross-ethnicity and cross-atherosclerosis trait observations. Fifth, we relate the associations of these subclinical atherosclerosis genetic variants on risk for CHD.

Although our study has several strengths, we note some key limitations. First, not all protein-coding variation is catalogued on the exome chip. Because of purifying selection, disruptive protein-coding variation is rare. By potentially not accounting for the totality of disruptive variation not on the array, variance is increased and power is not optimized for gene-based analyses. Whole exome sequencing can better address this limitation because such technologies continue to become more cost-effective for large-scale experiments. Second, our analyses of prior associations at noncoding sites are restricted to sites on the exome chip. We were able to robustly replicate prior noncoding association analyses for CAC at 9p21 and 6p24.12 A prior meta-analysis for CIMT genome-wide association discovered one genome-wide association, an intergenic common variant (rs11781551-A) 385 kb from ZHX2 at 8q24.4 No variant with modest linkage disequilibrium with this variant was present on the exome chip, thereby, limiting ability for replication. An intronic variant in PINX1 at 8q23 and intergenic variant 2.3 kb from APOC1 at 19q13 previously had suggestive association, but no suitable proxies to replicate association were available on the exome chip. Third, our analysis still demonstrates a paucity of genome-wide associations for these quantitative atherosclerotic traits and highlights an important challenge to ongoing CAC association analyses.

Genetic determinants of CHD have been characterized among individuals of European ancestry, but the strongest association signals have not replicated in those of African ancestry, which may be because of smaller sample sizes hindering statistical power or different key genetic drivers. But now we demonstrate a cardioprotective genetic mechanism in those of European ancestry and African ancestry through the reduction of subclinical atherosclerosis. We propose potential mechanisms and call for renewed attention to APoE e2 in the genesis of atherosclerosis underlying clinical cardiovascular disease. Finally, given the strong concordance of subclinical atherosclerosis measures and clinical CHD, our findings support a future study of genotypes, subclinical atherosclerosis, and incident CHD.

Sources of Funding

Dr Natarajan is supported by the John S. LaDue Memorial Fellowship in Cardiology, Harvard Medical School. Dr Kavousi is supported by the NWO VENI grant (VENI, 91616079). Infrastructure for the CHARGE Consortium (Cohorts for Heart and Aging Research in Genomic Epidemiology) is supported in part by the National Heart, Lung, and Blood Institute grant R01HL105756. Funding support for the CHARGE Consortium Exome Chip analyses is provided in part by the National Heart, Lung, and Blood Institute grant R01HL120393. Please refer to the Data Supplement regarding additional sources of funding.

Disclosures

Dr Natarajan reports grant support from Amarin Corporation. Dr Borecki is the Executive Director of Analytic Genetics at Regeneron Pharmaceuticals Inc. Dr Isgum reports research grants from Pié Medical Imaging BV and the Netherlands Organisation for Health Research and Development (ZonMw). Dr Franco is employed by ErasmusAGE, a center for aging research across the life course funded by Nestlé Nutrition (Nestec Ltd.), Metagenics Inc, and AXA. Dr Borecki is employed by Regeneron Pharmaceuticals Inc. Dr Reilly is employed by Merck. Dr Nguyen is employed by Biogen. The other authors report no conflicts.

Appendix

From the Center for Human Genetic Research and Cardiovascular Research Center (P.N., G.M.P., S.K.) and Department of Radiology (U.H.), Massachusetts General Hospital, Boston; Program in Medical and Population Genetics, Broad Institute of Harvard and MIT, Cambridge, MA (P.N., G.M.P., S.K.); Cardiovascular Health Research Unit, Departments of Medicine (J.C.B., B.M.P.), Epidemiology (S.R.H., B.M.P.), and Health Services (B.M.P.), University of Washington, Seattle; Group Health Research Institute, Group Health, Seattle, WA (B.M.P.); Department of Epidemiology, School of Public Health, University of Michigan, Ann Arbor (L.F.B., M.A.J., S.L.R.K., P.A.P.); Diabetes Heart Study, Wake Forest Health Sciences, Winston-Salem, NC (A.J.C., L.M.R., D.W.B.); Department of Internal Medicine B (M.D., S.B.F.). Department of Neurology (U.S.), Institute for Community Medicine (A.T., H.V.), and Interfaculty Institute of Genetics and Functional Genomics (U.V., S.W.), University of Medicine Greifswald; DZHK (German Center for Cardiovascular Research), partner Site Greifswald, Germany (M.D., A.T., U.V., S.B.F., H.V.); Division of Statistical Genetics, Department of Genetics (M.F.F., N.O.S., M.A.P., M.K.W.), Cardiovascular Division, Department of Medicine (N.O.S.), and McDonnell Genome Institute (N.O.S.), Washington University School of Medicine, St Louis, MO; Epidemiology, Gilling School of Global Public Health, University of North Carolina, Chapel Hill (N.F., G.H., K.E.N.); Institute for Translational Genomics and Population Sciences, Los Angeles Biomedical Research Institute and Department of Pediatrics at Harbor-UCLA Medical Center, Torrance, CA (X.G., J.Y., Y.-D.C., K.D.T., J.R.B.); Framingham Heart Study, National Heart Lung and Blood Institute/National Institutes of Health, MA (S.-J.H.); Department of Epidemiology (M. Kavousi, D. Vojinovic, N.A., A. Demirkan, O.H.F., A.H., F. Rivadeneira, A.G.U., A. Dehghan, C.M.v.d.), Internal Medicine (F. Rivadeneira, A.G.U.), and Radiology (A.v.d.L.), Erasmus MC, University Medical Center Rotterdam; Department of Epidemiology, Harvard T.H. Chan School of Public Health, Boston, MA (A.H.); MRC-PHE Centre for Environment & Health, Imperial College London (A. Dehghan); Maastricht Centre for Systems Biology (MaCSBio), Maastricht University (A.J.), Departments of Clinical Epidemiology (R.L.-G., R.d.M., F. Rosendaal, D.O.M.-K.), Cardiology (J.W.J., S.T.), and Public Health and Primary Care (D.O.M.-K.), Leiden University Medical Center, Netherlands; Department of Clinical Chemistry, Fimlab Laboratories (L.-P.L., T.L.); Departments of Clinical Chemistry (L.-P.L., T.L.) and Clinical Physiology (M. Kähönen), University of Tampere School of Medicine, Finland; Center for Cognitive Ageing & Cognitive Epidemiology (R.E.M., J.M.W., I.J.D.), Center for Genomic & Experimental Medicine, Institute of Genetics & Molecular Medicine (R.E.M.), Division of Neuroimaging Sciences & Brain Research Imaging Center, Center for Clinical Brain Sciences (J.M.W.), and Department of Psychology (I.J.D.), University of Edinburgh, United Kingdom; Queensland Brain Institute, University of Queensland, Brisbane, Australia (R.E.M.); Division of Cardiovascular Medicine, Kanazawa University Graduate School of Medicine, Japan (H.T.); Cardiology (J.V.S.), Department of Radiology (P.A.D.), and Image Sciences Institute (B.D.d.V., I.L.), University Medical Center Utrecht, Netherlands; Icelandic Heart Association, Kopavogur, Iceland (A.V.S., V.G.); Faculty of Medicine, University of Iceland, Reykjavik (A.V.S., V.G.); Department of Medicine, Johns Hopkins University School of Medicine (L.R.Y., B.G.K., W.P., D. Vaidya,
References


**CLINICAL PERSPECTIVE**

Atherosclerosis is the underlying pathological substrate for coronary heart disease. The presence of atherosclerosis, termed subclinical atherosclerosis, is associated with an increased risk of developing clinical coronary heart disease. Genetic factors influence the development of subclinical atherosclerosis. Prior analyses of single nucleotide variants (SNVs) across the genome have identified SNVs associated with both coronary artery calcification and carotid intima–media thickness. However, such variants reside within noncoding portions of the genome, limiting the interpretation of the biological role of these SNVs. Therefore, we used a novel genotyping platform focused on densely cataloguing protein-coding SNVs across the genome. We associated these protein-coding SNVs with coronary artery calcification and carotid intima–media thickness in 25,109 and 52,869 individuals, respectively, of European or African ancestry. We discovered that APOE p.Arg176Cys (APOE e2 allele) is associated with reduced coronary artery calcification and carotid intima–media thickness in carriers compared with noncarriers. For the first time, we observed these associations in both individuals of European ancestry and of African ancestry. The association of the variant with coronary artery calcification is preserved even after accounting for its effects on low-density lipoprotein cholesterol. Finally, carriers are at reduced risk for developing clinical coronary heart disease. These observations represent novel insights about the genetic determinants of atherosclerosis in a multiethnic sample.
Multiethnic Exome-Wide Association Study of Subclinical Atherosclerosis

CHARGE Consortium

Circ Cardiovasc Genet. 2016;9:511-520; originally published online November 21, 2016; doi: 10.1161/CIRCGENETICS.116.001572
Circulation: Cardiovascular Genetics is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2016 American Heart Association, Inc. All rights reserved.
Print ISSN: 1942-325X. Online ISSN: 1942-3268
SUPPLEMENTAL MATERIAL

Supplementary Text
Cohort descriptions
Genotyping methods
Secondary Statistical Analyses
Acknowledgements

Supplemental Figures
Figure S1. Quantile-quantile plot of coronary artery calcification quantity single variant association.
Figure S2. Regional association plots of coronary artery calcification quantity at 9p21 (CDKN2B).
Figure S3. Regional association plots of coronary artery calcification quantity at 6p24 (PHACTRI).
Figure S4. Regional association plots of coronary artery calcification quantity at 2p24 (APOB).
Figure S5. Regional association plots of coronary artery calcification quantity at 19q13 (APOE).
Figure S6. Quantile-quantile plots of coronary artery calcification quantity gene-based association.
Figure S7. Quantile-quantile plot of carotid intima media thickness single variant association.
Figure S8. Regional association plots of carotid intima media thickness at 19q13 (APOE).
Figure S9. Quantile-quantile plot of carotid intima media thickness gene-based association.
Figure S10. Association of major APOE genotypes with CAC.

Supplemental Tables
Table S1. Demographics of Participants by Cohort in CAC Analysis.
Table S2. Demographics of Participants by Cohort in CIMT Analysis.
Table S3. APOE ε2 association with CAC adjusted for blood lipids.
Table S4. CHD-associated variants and association with subclinical atherosclerosis

Supplemental References
Cohort descriptions

**Cardiovascular Health Study (CHS)**
The CHS is a population-based cohort study of risk factors for CHD and stroke in adults ≥65 years conducted across four field centers in the United States. The original predominantly European-ancestry cohort of 5201 persons was recruited in 1989-1990 from a random sample of people on Medicare eligibility lists and an additional 687 African-Americans were enrolled subsequently for a total sample of 5888. Blood samples were drawn from all participants at their baseline examination and DNA was subsequently extracted from available samples. Genotyping was performed at the General Clinical Research Center’s Phenotyping/Genotyping Laboratory at Cedars-Sinai among CHS participants who consented to genetic testing and had DNA available using the Illumina HumanExome v.1.0 BeadChip array. Genotypes were jointly called at The University of Texas Health Science Center at Houston as part of approximately 62,000 ethnically-diverse samples from the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) Consortium. Analyses were performed separately for individuals of European and African ancestries.

**Diabetes Heart Study (DHS)**
The Diabetes Heart Study (DHS) is a family-based observational cohort study of cardiovascular disease from a single research center in the United States. The original predominantly (85%) European-ancestry cohort of 1443 persons was recruited in 1997-2005 from a random from families with at least two type 2 diabetes affected siblings and, if possible, a non-diabetic sibling. Blood samples were drawn from all participants at their baseline examination and DNA was subsequently extracted from available samples. Genotyping was performed on the Illumina HumanExome v.1.0 BeadChip array at the Center for Genomics and Personalized Medicine Research at Wake Forest University School of Medicine. Genotypes were called using ZCall and QC was performed at Wake Forest University Center for Genomics and Personalized Medicine Research.

**Jackson Heart Study (JHS)**
The JHS aimed at enrolling representative, population-based cohort of self-defined African-American persons aged 35–84 years, with an embedded collection of families for genetic study. Participants were enrolled from the three counties that make up the Jackson, Mississippi metropolitan area. Relatives of selected participants were recruited to develop a large, nested family cohort. Participants provided extensive medical and social history, had an array of physical and biochemical measurements and diagnostic procedures, and provided genomic DNA. Data and biologic materials have been collected from 5302 adult African Americans, including 1499 members of 291 families. Participants have a high prevalence of diabetes, hypertension, obesity, and related disorders. Genotyping was performed among JHS participants who consented to genetic testing and had DNA available using the Illumina HumanExome v.1.0 BeadChip array. Genotypes were jointly called at The University of Texas Health Science Center at Houston as part of approximately 62,000 ethnically-diverse samples from the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) Consortium. Individuals that overlapped with ARIC were removed.

**Family Heart Study (FamHS)**
The FamHS (https://dsgweb.wustl.edu/fhsc/) began in 1992 with the ascertainment of 1,200 families, half randomly sampled and half selected because of an excess of CHD or risk factor abnormalities as compared with age- and sex-specific population rates. The families, with approximately 6,000 subjects, were sampled from four population-based parent studies: the Framingham Heart Study, the Utah Family Tree Study, and two centers for the Atherosclerosis Risk in Communities study (ARIC: Minneapolis, and Forsyth County, NC). These individuals attended a clinic visit between the years 1994-1996 and a broad range of phenotypes were assessed in the general domains of CHD, atherosclerosis, cardiac and vascular function, inflammation and hemostasis, lipids and lipoproteins, blood pressure, diabetes and insulin resistance, pulmonary function, diet, habitual physical activity, anthropometry, medical history and medication use. Approximately 8 years later, study participants belonging to the largest pedigrees were invited for a second clinical exam (2002-04) and computed tomography scan measurements were assessed, among many other phenotypes. A total of 2,756 European American (EA) subjects in 510 extended random and high CHD risk families were studied. A total of 1,865 key EA subjects within this group of families were genotyped using the Illumina Infinium HumanExome v1.0 BeadChip. In addition, 633 African American (AA) subjects were recruited at an additional ARIC field center at the University of Alabama in Birmingham. A total of 608 AA subjects were genotyped using the Illumina Infinium HumanExome v1.0 BeadChip. Informed consent was obtained from all participants, and this project was approved by the Institutional Review Boards of all participating institutions.

**Lothian Birth Cohort 1936 (LBC1936)**
The LBC1936 consists of 1,091 relatively healthy individuals assessed on cognitive and medical traits at about 70 years of age. They were all born in 1936 and most took part in the Scottish Mental Survey of 1947. At baseline the sample of 548 men and 543 women had a mean age 69.6 years (SD = 0.8). They were all Caucasian, community-dwelling, and almost all lived in the Lothian region (Edinburgh city and surrounding area) of Scotland. A full description of participant recruitment and testing can be found elsewhere. 988 individuals were genotyped using the Illumina HumanExome BeadChip at the Wellcome Trust Clinical Research Facility, Edinburgh.

**SHIP / SHIP-Trend**
SHIP is a population-based project in West Pomerania, a region in the northeast of Germany, that consists of two independent prospectively collected cohorts (SHIP and SHIP-TREND) assessing the prevalence and incidence of common population-based diseases and their risk factors. The study design has been previously described in detail. Briefly, a sample from the population aged 20 to 79 years was drawn from population registries. First, the three cities of the region (with 17,076 to 65,977 inhabitants) and the 12 towns (with 1,516 to 3,044 inhabitants) were selected, and then 17 out of 97 smaller towns (with less than 1,500 inhabitants), were drawn at random. Second, from each of the selected communities, subjects were drawn at random, proportional to the population size of each community and stratified by age and gender. Only individuals with German citizenship and main residency in the study area were included.

For SHIP, baseline examinations were carried out from 1997 until 2001, and the sample finally comprised 4,308 participants. Baseline examinations for SHIP-TREND were carried out between 2008 and 2012, finally comprising 4420 participants. Analyses were performed separately for individuals of both cohorts.
**Genetic Study of Atherosclerosis Risk (GeneSTAR)**

GeneSTAR is a family-based prospective study of risk factors, occult disease, and incident cardiovascular disease in siblings, later extended to offspring and whole pedigrees. European- and African American families were identified from probands with early-onset (< age 60) coronary artery disease (CAD) hospitalized in any of 10 Baltimore hospitals between 1983 and 2007. Siblings completed baseline screening between 1983 and 2007, while offspring, co-parents of the offspring, and additional siblings completed baseline screening between 2003 and 2007. Siblings were followed every 5 years for cardiovascular and other comorbid incident events. DNA was collected at baseline (1991-2007) or follow-up (for those whose baseline visit was pre-1991). Probands were not eligible if they had CAD associated with calcific aortic stenosis or chronic glucocorticosteroid therapy, following organ transplantation or post intensive chest radiation, had an autoimmune diseases like systemic lupus, or if they had a cocaine-induced myocardial infarction. Participants younger than age 21 or older than age 80, who had known CAD or an autoimmune disease such as systemic lupus, were taking chronic glucocorticosteroids, had undergone any organ transplantation, or had major comorbidity that had a life expectancy under 5 years of age were excluded. The full sample includes 4423 participants, 51% female/49% male, and 38% African American/61% European American/1% other American. Genotyping was performed by the Northwest Genomics Center at the University of Washington through the RS&G service using the Illumina HumanExome v.1.2 BeadChip array. Analyses were performed separately for European and African Americans.

**ImaGene**

Project ‘Cardiovascular phenotype-genotype analysis with a CT based lung cancer screening trial’ within the Population Imaging Genetics (ImaGene) study was set up to investigate the relationship between genetics and image characteristics related to cardiovascular disease in an at-risk population of current or former heavy smokers between 50 and 75 years of age. 905 participants of the Dutch-Belgian lung cancer screening trial (NELSON) were successfully genotyped using the Illumina HumanExome v1.1 BeadChip array. Low-dose, non-ECG synchronized, non-contrast enhanced baseline chest CTs from the NELSON study were available for all participants. CTs were acquired on a 16 detector-row scanner (Mx8000 IDT, Somatom Sensation 16, or Brilliance 16P, Philips Medical Systems, Cleveland, OH, USA) in spiral mode with 16 × 0.75 mm collimation. Axial images of 1.0 mm thickness at 0.7 mm increment were reconstructed with a moderately soft kernel (Philips “B”). The peak voltage was 120–140 kVp depending on patient weight, with a tube current of 30 mAs.

**Old Order Amish**

The Amish Family Calcification Study (AFCS) was initiated in 2001 to identify the determinants of vascular calcification and to evaluate the relationship between calcification of bone and vascular tissue among members of the Old Order Amish community in Lancaster County. For this study relatively healthy subjects and their family members were recruited from the Amish community between 2001 and 2006 for vascular imaging by electron beam CT scan. The final sample size comprised 1,075 subjects. Blood samples were drawn for biochemical assays and DNA analysis.

**Framingham Heart Study (FHS)**
Framingham Heart Study (FHS) is a community-based prospective study designed to investigate the incidence of cardiovascular diseases and factors related to its development. Study population was composed of three cohorts with European ancestry, original (5,209 participants ascertained systematically from two-thirds of the households in the town of Framingham, MA, beginning in 1948), Offspring (5,124 children of the original cohort, and spouses of those children, beginning in 1972), and the Third Generation (4,095 children of the Offspring cohort, beginning in 2002). In addition to the initial examination and face-to-face interview, participants were invited to follow-up examinations at the study clinics that were accomplished every two years for the original cohort or every four years for the Offspring and Gen3 cohorts. An unrelated spouse cohort was formed in 2004 include 103 spouses of the offspring participants. Genotyping for more than 276,000 variants on the basis of the Illumina Infinium Human Exome Array v1.0 or v1.1 was conducted in 8351 FHS samples. Quality control of the genotye was accomplished through jointly called at The University of Texas Health Science Center at Houston as part of approximately 62,000 ethnically-diverse samples from the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) Consortium.

BioImage – High Risk Plaque
The BioImage study (NCT00738725) is a multi-ethnic, observational study aimed at characterizing subclinical atherosclerosis in 6,699 US adults (55-80 years at baseline, 2008-2009) at risk for, but without, clinical ASCVD. Participants were genotyped on the Illumina Infinium Human Exome Array v1.1.

Multi-Ethnic Study of Atherosclerosis (MESA)
MESA is an NHLBI-sponsored population-based, prospective, multi-center cohort study including participants recruited from six field sites in the United States – Forsyth County, NC (Wake Forest University), Northern Manhattan/Bronx, NY (Columbia University), Baltimore/Baltimore County, MD (Johns Hopkins University), St. Paul, MN (University of Minnesota, Twin Cities), Chicago, IL (Northwestern University), and Los Angeles County, CA (UCLA). Details of recruitment and study design have been previously published elsewhere. Briefly, the MESA cohort comprises 6,814 men and women of diverse ethnic background who were 45 to 84 years old at the baseline exam and free of clinically overt cardiovascular disease (CVD) who were recruited to elucidate the determinants and natural history of subclinical CVD, study progression of subclinical CVD, and its impact on incident clinical CVD. The cohort is 53% women with an ethnic composition of approximately 38% Caucasian, 28% African American, 22% Hispanic and 12% Asian, primarily of Chinese descent. Five clinical exams have taken place (2000-02, 2002-04, 2004-05, 2005-07, 2010-12), with follow-up every 9 to 12 months for events. The MESA was approved by the Institutional Review Board of all participating field sites and reading centers and participants gave informed consent for participation and use of DNA specimens. The current study includes data from African-Americans and Caucasians with available exome chip data, coronary artery calcium (CAC), and carotid intima media thickness (IMT) at the baseline exam (2000-02). Joint genotype calling for the exome chip V1.0 was performed at UT-Houston for all CHARGE samples, including for MESA. Additional QC was performed at Cedars-Sinai and University of Virginia. After QC, 238,895 SNPs remained for analysis.

Genetic Epidemiology Network of Arteriopathy (GENOA)
GENOA is one of four networks in the NHLBI Family-Blood Pressure Program (FBPP).\textsuperscript{14} GENOA’s long-term objective is to elucidate the genetics of target organ complications of hypertension, including both atherosclerotic and arteriolosclerotic complications involving the heart, brain, kidneys, and peripheral arteries. The longitudinal GENOA Study recruited European-American and African-American sibships with at least 2 individuals with clinically diagnosed essential hypertension before age 60 years. All other members of the sibship were invited to participate regardless of their hypertension status. Participants were diagnosed with hypertension if they had either 1) a previous clinical diagnosis of hypertension by a physician with current anti-hypertensive treatment, or 2) an average systolic blood pressure \( \geq 140 \text{ mm Hg} \) or diastolic blood pressure \( \geq 90 \text{ mm Hg} \) based on the second and third readings at the time of their clinic visit. Exclusion criteria were secondary hypertension, alcoholism or drug abuse, pregnancy, insulin-dependent diabetes mellitus, or active malignancy. During the first exam (1995-2000), 1,583 European Americans from Rochester, MN and 1,854 African Americans from Jackson, MS were examined. Between 2000 and 2005, 1,241 of the European Americans returned for an examination that included a CT scan of the heart for coronary artery calcification. Between 2009 and 2011, 657 of the African Americans returned for an examination that included a CT scan of the heart for coronary artery calcification. Because African-American probands for GENOA were recruited through the Atherosclerosis Risk in Communities (ARIC) Jackson field center participants, we excluded ARIC participants from analyses. Written informed consent was obtained from all participants, and this project was approved by the Institutional Review Boards of all participating institutions. Both European Americans and African Americans were genotyped using the Illumina Infinium HumanExome v1.0 BeadChip. Genotyping and genotype calling for European Americans was performed at the University of Texas Health Sciences Center. Genotyping and genotype calling for African Americans was performed at the Center for Inherited Disease Research (CIDR).

**AGES Reykjavik**

The Reykjavik Study cohort originally comprised a random sample of 30,795 men and women born in 1907–1935 and living in Reykjavik in 1967.\textsuperscript{2} A total of 19381 attended, resulting in 71% recruitment rate. The study sample was divided into six groups by birth year and birth date within month. One group was designated for longitudinal follow-up and was examined in all stages. One group was designated a control group and was not included in examinations until 1991. Other groups were invited to participate in specific stages of the study. Between 2002 and 2006, the AGES-Reykjavik study re-examined 5764 survivors of the original cohort who had participated before in the Reykjavik Study. The AGES Reykjavik Study GWAS was approved by the National Bioethics Committee (00-063- V8+1) and the Data Protection Authority. DNA was genotyped using the Illumina HumanExome v1.0 BeadChip array. Samples were excluded from the dataset based on sample failure, genotype mismatch with reference panel, and sex mismatch, resulting in clean genotype data on 2,983 individuals. Standard protocols for working with Illumina data were followed, with clustering score greater than 0.4. SNPs were excluded using filters based on call rate (<97%), Hardy-Weinberg Equilibrium (< \( 1 \times 10^{-6} \)) 238,015 SNPs passing all QC (of 247,039 prior to cleaning steps).

**Atherosclerosis Risk in Communities (ARIC)**

ARIC is a population-based prospective study of men and women aged 45-64 years at baseline, which recruited 15,792 African American and White individuals from 4 U.S. communities to study the etiology and natural history of subclinical and clinical atherosclerosis. Participants
were examined at baseline (1987-1989) and 4 follow-up clinic visits (1990-1992, 1993-1995, 1996-1998, 2011-2013), at which a rich array of health assessments have been made, with over 25 years of follow-up through annual phone updates for hospital admissions, medical history, and events.\textsuperscript{15} DNA has been collected, with extensive genome-wide array and genetic sequencing data available.

**Rotterdam Study (RS)**
The Rotterdam Study (RS) is a prospective population-based cohort study among persons aged 55 years or older in the Ommoord district of Rotterdam, the Netherlands. The rationale and design of the Rotterdam Study have been described elsewhere.\textsuperscript{16} The cohort started in 1990 and has been extended twice. The present study used data for carotid intima media thickness from the baseline examination of the original cohort (RS-I, visit 1: 1990-1993) and for coronary calcification from the third examination of the original cohort (RS-I, visit 3: 1997-1999). The RS participants are interviewed and have an extensive set of examinations every 3-4 years and have been followed-up for a variety of diseases. The Rotterdam Study has been approved by the Medical Ethics Committee of the Erasmus MC and by the Ministry of Health, Welfare and Sport of the Netherlands, implementing the Wet Bevolkingsonderzoek: ERGO (Population Studies Act: Rotterdam Study). All participants provided written informed consent to participate in the study and to obtain information from their treating physicians. The approval has been renewed every 5 years, as well as with the introduction of major new elements in the study. The methods regarding the Exome Chip Array has been described in detail elsewhere.\textsuperscript{1}

**Erasmus Rucphen Family Study (ERF)**
Erasmus Rucphen Family study (ERF) is a family based study conducted in a genetically isolated population in the South-West of the Netherlands, studied as part of the Genetic Research in Isolated Population (GRIP) program.\textsuperscript{17, 18} The aim of this study is to identify genetic risk factors of complex diseases and genetic associations to complex traits. Study population includes approximately 3,000 participants who are descendants of a limited number of founders living in the 19th century. All data were collected between 2002 and 2005. All participants gave written informed consent and the Medical Ethics Committee at Erasmus MC University Medical Center approved the study. Study participants from the ERF cohort (N = 1,527) were genotyped on the Illumina Infinium HumanExome BeadChip, version 1.1. Calling was performed with GenomeStudio and the ZCall variant calling tool (Broad Institute).\textsuperscript{3}

**Netherland Epidemiology of Obesity Study (NEO)**
The NEO was designed for extensive phenotyping to investigate pathways that lead to obesity-related diseases. The NEO study is a population-based, prospective cohort study that includes 6,671 individuals aged between 45-65 years, with an oversampling of individuals with overweight or obesity. At baseline, information on demography, lifestyle, and medical history have been collected by questionnaires. In addition, samples of 24-h urine, fasting and postprandial blood plasma and serum, and DNA were collected. Genotyping was performed using the Illumina HumanCoreExome chip, which was subsequently imputed to the 1000 genome reference panel. Participants underwent an extensive physical examination, including anthropometry, electrocardiography, spirometry, and measurement of the carotid artery intima-media thickness by ultrasonography. In random subsamples of participants, magnetic resonance imaging of abdominal fat, pulse wave velocity of the aorta, heart, and brain, magnetic resonance
spectroscopy of the liver, indirect calorimetry, dual energy X-ray absorptiometry, or accelerometry measurements were performed. The collection of data started in September 2008 and completed at the end of September 2012. Participants were currently being followed for the incidence of obesity-related diseases and mortality.  

**Young Finns Study (YFS)**

The Cardiovascular Risk in Young Finns Study (YFS) is a population-based follow up-study started in 1980. The main aim of the YFS is to determine the contribution made by childhood lifestyle, biological and psychological measures to the risk of cardiovascular diseases in adulthood. In 1980, over 3,500 children and adolescents all around Finland participated in the baseline study. The follow-up studies have been conducted mainly with 3-year intervals. The latest 30-year follow-up study was conducted in 2010-11 (ages 33-49 years) with 2,063 participants. The study was approved by the local ethics committees (University Hospitals of Helsinki, Turku, Tampere, Kuopio and Oulu) and was conducted following the guidelines of the Declaration of Helsinki. All participants gave their written informed consent. 

---

19. 

20.
Genotyping methods

Genotypes from the Age, Gene/Environment, Susceptibility-Reykjavik (AGES) Study, Cardiovascular Health Study (CHS), Rotterdam Study (RS), Framingham Heart Study (FHS), Family Heart Study (FamHS), and Jackson Heart Study (JHS) were jointly called.\(^1\) High Risk Plaque-Bioimage (Bioimage), genotyped with v1.1 of the Beadchip, was called in GenomeStudio (Illumina) using GenCall and low frequency variant calling was further refined using zCall.\(^3\) The Diabetes Heart Study (DHS), Genetic Epidemiology Network of Arteriopathy Study (GENOA), ImaGene, Young Finns Study (YFS), Gene Study of Atherosclerosis Risk in Families (GeneSTAR), Old Order Amish Study (Amish), Netherlands Epidemiology of Obesity Study (NEO), Erasmus Ruchphen Family Study, Lothian Birth Cohort, Study of Health in Pomerania (SHIP), and SHIP-TREND were called in GenomeStudio (Illumina) using the joint calling cluster file and used zCall.

It is estimated that 95 % of European ancestry and 85% of African ancestry variation with minor allele frequency (MAF) > 0.1% is captured by the content of the Illumina HumanExome Beadchip array.\(^21\)

Variant quality control was performed jointly\(^1\) and by individual cohorts. Low-performing variants from joint-calling were excluded from analysis.\(^1\) 238,065 variants were included in analysis. Samples were excluded by each cohort if genotypes had poor concordance with prior genome-wide array-based genotypes when available, >5% of genotypes missing, they were population clustering outliers, had high inbreeding coefficients or heterozygosity rates, phenotypic and genotypic sex discordance, one from each duplicate (or monozygotic twin pairing), and high degrees of cryptic relatedness in studies without families was observed.\(^21\) All variants were coded in an additive fashion. Variants were annotated using dbNSFP v2.0.\(^22\)
Secondary Statistical Analyses

**APOE ε2 Association Conditional on LDL cholesterol**
To determine whether low-density lipoprotein (LDL) cholesterol is an important intermediary in the association between rs7412-T and CAC, we tested the association further adjusting for LDL cholesterol. In studies with available measures, fasting LDL cholesterol was obtained and/or calculated based on the Friedewald calculation when triglycerides were less than 400 mg/dL and directly measured when triglycerides were greater than 400 mg/dL. To account for the effect of lipid-lowering therapy, we adjusted LDL cholesterol to reflect the observation that statins, on average, reduce LDL cholesterol by 30% prior to conditioning. Association analyses were performed within each cohort and meta-analyzed as described above with fasting LDL cholesterol as an additional covariate.

**APOE ε2 Association Conditional on Other APOE Genotypes**
The major APOE polymorphisms at 19q13.2 are ε2, ε3, and ε4. The ε2 allele is determined by rs7412-T and the ε4 allele by rs429358-C, while the referent ε3 genotype has the rs7412-C / rs429358-T haplotype. Given the absence of rs429358 on the exome chip and prior genome-wide genotyping arrays, we obtained directly genotyped ε2, ε3, and ε4 by polymerase chain reaction in 5,872 participants from FHS, AGES, GENOA, CHS, and DHS. Our hypothesis was that the effect of the ε2 allele on subclinical atherosclerosis was independent of ε4 status. We tested the association of the APOE non-reference genotypes (ε3/ε3 referent) with CAC stratified for ethnicity and sex while accounting for age and principal components within each cohort. We then performed a fixed-effects meta-analysis weighted by the inverse of variances to summarize the association across cohorts.

**Association with CHD**
To test for association of rs7412-T with prevalent CHD, we obtained summary results from an association analysis led by the Myocardial Infarction Genetics (MIGen) Consortium. 21,182 individuals of European ancestry, independent of the subclinical atherosclerosis analyses, were genotyped with the Illumina HumanExome Beadchip v1.0 at the Broad Institute (Cambridge, MA) and 9,472 of these individuals were classified as having CHD. We obtained the single variant association results for rs7412-T accounting for age, sex, ethnicity, and principal components of ancestry.

We also determined the concordance of subclinical atherosclerosis genetic architecture with that of CHD. We focused on the concordance of effect direction of variants previously associated with CHD at a genome-wide level for individuals of European ancestry. We selected the top associated CHD variant for each locus genotyped on the exome chip. Separately for CAC and CIMT, we determined the probability of a CHD variant having a concordant effect direction with each subclinical atherosclerotic trait. We determined the likelihood of effect observed concordance compared to chance accounting for each variant’s risk allele’s frequency with an exact binomial test.
Acknowledgements / Funding

Cardiovascular Health Study (CHS)
This Cardiovascular Health Study (CHS) research was supported by NHLBI contracts HHSN268201200036C, HHSN268200800007C, N01HC55222, N01HC85079, N01HC85080, N01HC85081, N01HC85082, N01HC85083, N01HC85086, N01HC85085, N01HC45133; and NHLBI grants U01HL080295, R01HL087652, R01HL105756, R01HL103612, R01HL120393, R01HL130114, and R01HL068986 with additional contribution from the National Institute of Neurological Disorders and Stroke (NINDS). Additional support was provided through R01AG023629 from the National Institute on Aging (NIA). A full list of principal CHS investigators and institutions can be found at CHS-NHLBI.org. The provision of genotyping data was supported in part by the National Center for Advancing Translational Sciences, CTSI grant UL1TR000124, and the National Institute of Diabetes and Digestive and Kidney Disease Diabetes Research Center (DRC) grant DK063491 to the Southern California Diabetes Endocrinology Research Center. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

Diabetes Heart Study (DHS)
The Diabetes Heart Study (DHS) research reported in this article was supported in part by R01 HL67348, R01 HL092301, R01 NS058700 and the General Clinical Research Centre of the Wake Forest School of Medicine (M01 RR07122, F32 HL085989). The authors thank the other investigators, the staff, and the participants of the DHS study for their valuable contributions.

Jackson Heart Study (JHS)
We thank the Jackson Heart Study (JHS) participants and staff for their contributions to this work. The JHS is supported by contracts HHSN268201300046C, HHSN268201300047C, HHSN268201300048C, HHSN268201300049C, HHSN268201300050C from the National Heart, Lung, and Blood Institute and the National Institute on Minority Health and Health Disparities.

Family Heart Study (FamHS)
This Family Heart Study (FamHS) research was supported by NIH grants R01-HL-117078, R01-HL-087700 and R01-HL-088215 from NHLBI; and R01-DK-089256 from NIDDK.

Lothian Birth Cohort 1936 (LBC1936)
We thank the cohort participants and team members who contributed to this study. Genotyping was supported by Centre for Cognitive Ageing and Cognitive Epidemiology (Pilot Fund award), Age UK and the Royal Society of Edinburgh and was conducted at the Wellcome Trust Clinical Research Facility Genetics Core at Western General Hospital, Edinburgh, UK. Phenotype collection was supported by Age UK (The Disconnected Mind project). The work was undertaken by The University of Edinburgh Centre for Cognitive Ageing and Cognitive Epidemiology, part of the cross council Lifelong Health and Wellbeing Initiative (MR/K026992/1). Funding from the BBSRC and MRC is gratefully acknowledged.

SHIP / SHIP-Trend
SHIP is part of the Community Medicine Research net of the University of Greifswald, Germany, which is funded by the Federal Ministry of Education and Research (grants no. 01ZZ9603, 01ZZ0103, and 01ZZ0403), the Ministry of Cultural Affairs as well as the Social Ministry of the Federal State of Mecklenburg-West Pomerania, and the network ‘Greifswald Approach to Individualized Medicine (GANI_MED)’ funded by the Federal Ministry of Education and Research (grant 03IS2061A). ExomeChip data have been supported by the Federal Ministry of Education and Research (grant no. 03Z1CN22) and the Federal State of Mecklenburg-West Pomerania. The University of Greifswald is a member of the Caché Campus program of the InterSystems GmbH.

Genetic Study of Atherosclerosis Risk (GeneSTAR)
GeneSTAR was supported by grants from the National Institutes of Health/National Heart, Lung, and Blood Institute (HL112064, U01 HL72518, HL097698, HL59684, HL071025, HL092165, HL099747, and K23HL105897) and by a grant from the National Institutes of Health/National Center for Research Resources (M01-RR00052) to the Johns Hopkins General Clinical Research Center. Genotyping services were provided through the RS&G Service by the Northwest Genomics Center at the University of Washington, Department of Genome Sciences, under U.S. Federal Government contract number HHSN268201100037C from the National Heart, Lung, and Blood Institute.

ImaGene
The ImaGene study was financially supported by the Netherlands Organization for Scientific Research (NWO)/Foundation for Technological Sciences (STW); Project 12726. CT scans were acquired within the NELSON study.

Old Order Amish
Supported by NIH grants: R01 HL69313, R01 HL088119, and P30 DK072488.

Framingham Heart Study (FHS)
The National Heart, Lung and Blood Institute’s Framingham Heart Study is supported by contract N01-HC-25195

BioImage – High Risk Plaque
The High Risk Plaque (HRP) Initiative encompassing the BioImage Study is a precompetitive industry collaboration funded by Abbott, Abbvie, AstraZeneca, BG Medicine, Merck, Philips, and Takeda. HRP Joint Steering Committee: Pieter Muntendam, MD (BG Medicine); Aram Adourian (BG Medicine); Michael Klimas, PhD (Merck); Joel Raichlen, MD (AstraZeneca); Oliver Steinbach (Philips); James Becket (Philips); Ramon Espaillot (Abbvie); Michael Jarvis (Abbvie) and Tomoyuki Nishimoto (Takeda). The sponsor had no role in the study design; in the collection, analysis, and interpretation of the data; in the writing of this report; or in the decision to submit the paper for publication.

Multi-Ethnic Study of Atherosclerosis (MESA)
MESA and the MESA SHARE project are conducted and supported by the National Heart, Lung, and Blood Institute (NHLBI) in collaboration with MESA investigators. Support for MESA is provided by contracts HHSN268201500031I, N01-HC-95159, N01-HC-95160, N01-HC-95161,
N01-HC-95162, N01-HC-95163, N01-HC-95164, N01-HC-95165, N01-HC-95166, N01-HC-95167, N01-HC-95168, N01-HC-95169, UL1-TR-001079, UL1-TR-000040, and DK063491. MESA Family is conducted and supported by the National Heart, Lung, and Blood Institute (NHLBI) in collaboration with MESA investigators. Support is provided by grants and contracts R01HL071051, R01HL071205, R01HL071250, R01HL071251, R01HL071258, R01HL071259, by the National Center for Research Resources, Grant UL1RR033176, and the National Center for Advancing Translational Sciences, Grant UL1TR000124. Funding for SHARE genotyping was provided by NHLBI Contract N02-HL-64278.

**Genetic Epidemiology Network of Arteriopathy (GENOA)**

Support for GENOA was provided by the National Heart, Lung and Blood Institute (HL119443, HL118305, HL054464, HL054457, HL054481, HL071917, HL085571, HL086694, and HL87660) of the National Institutes of Health. Genotyping was performed at the University of Texas Health Sciences Center (Eric Boerwinkle, Megan Grove-Gaona) and the Center for Inherited Disease Research (CIDR). We would like to thank the families that participated in the GENOA study.

**AGES Reykjavik**

This study has been funded by NIH contracts N01-AG-1-2100 and 271201200022C, the NIA Intramural Research Program, Hjartavernd (the Icelandic Heart Association), and the Althingi (the Icelandic Parliament). The study is approved by the Icelandic National Bioethics Committee, VSN: 00-063. The researchers are indebted to the participants for their willingness to participate in the study.

**Atherosclerosis Risk in Communities (ARIC)**

The Atherosclerosis Risk in Communities Study is carried out as a collaborative study supported by National Heart, Lung, and Blood Institute contracts (HHSN268201100005C, HHSN268201100006C, HHSN268201100007C, HHSN268201100008C, HHSN268201100009C, HHSN268201100010C, HHSN268201100011C, and HHSN268201100012C), R01HL087641, R01HL59367 and R01HL086694; National Human Genome Research Institute contract U01HG004402; and National Institutes of Health contract HHSN268200625226C.

**Rotterdam Study (RS)**

The generation and management of the Illumina exome chip v1.0 array data for the Rotterdam Study (RS-I) was executed by the Human Genotyping Facility of the Genetic Laboratory of the Department of Internal Medicine, Erasmus MC, Rotterdam, The Netherlands. The Exome chip array data set was funded by the Genetic Laboratory of the Department of Internal Medicine, Erasmus MC, from the Netherlands Genomics Initiative (NGI)/Netherlands Organisation for Scientific Research (NWO)-sponsored Netherlands Consortium for Healthy Aging (NCHA; project nr. 050-060-810); the Netherlands Organization for Scientific Research (NWO; project number 184021007) and by the Rainbow Project (RP10; Netherlands Exome Chip Project) of the Biobanking and Biomolecular Research Infrastructure Netherlands (BBMRI-NL; www.bbmri.nl ) . We thank Ms. Mila Jhamai, Ms. Sarah Higgins, and Mr. Marijn Verkerk for their help in creating the exome chip database, and Carolina Medina-Gomez, MSc, Lennard Karsten, MSc, and Linda Broer PhD for QC and variant calling. Variants were called using the
best practice protocol developed by Grove et al. as part of the CHARGE consortium exome chip central calling effort [PMID:23874508]. The Rotterdam Study is funded by Erasmus Medical Center and Erasmus University, Rotterdam, Netherlands Organization for the Health Research and Development (ZonMw), the Research Institute for Diseases in the Elderly (RI), the Ministry of Education, Culture and Science, the Ministry for Health, Welfare and Sports, the European Commission (DG XII), and the Municipality of Rotterdam. The authors are grateful to the study participants, the staff from the Rotterdam Study and the participating general practitioners and pharmacists.

**Erasmus Rucphen Family Study (ERF)**
The ERF study as a part of EUROSPAN (European Special Populations Research Network) was supported by European Commission FP6 STRP grant number 018947 (LSHG-CT-2006-01947) and also received funding from the European Community's Seventh Framework Programme (FP7/2007-2013)/grant agreement HEALTH-F4-2007-201413 by the European Commission under the programme "Quality of Life and Management of the Living Resources" of 5th Framework Programme (no. QLG2-CT-2002-01254). The ERF study was further supported by ENGAGE consortium and CMSB. High-throughput analysis of the ERF data was supported by joint grant from Netherlands Organisation for Scientific Research and the Russian Foundation for Basic Research (NWO-RFBR 047.017.043). Exome sequencing in ERF was supported by the ZonMw grant (project 91111025). Exome-chip genotyping was supported by BBMRI-NL, a Research Infrastructure financed by the Dutch Government (NWO 184.021.007). We are grateful to all study participants and their relatives, general practitioners and neurologists for their contributions and to P. Veraart for her help in genealogy, J. Vergeer for the supervision of the laboratory work and P. Snijders for his help in data collection.

**Netherlands Epidemiology of Obesity Study (NEO)**
The authors of the NEO study thank all individuals who participated in the Netherlands Epidemiology of Obesity study, all participating general practitioners for inviting eligible participants and all research nurses for collection of the data. We thank the NEO study group, Pat van Beelen, Petra Noordijk and Ingeborg de Jonge for the coordination, lab and data management of the NEO study. The genotyping in the NEO study was supported by the Centre National de Génomique (Paris, France), headed by Jean-Francois Deleuze. The NEO study is supported by the participating Departments, the Division and the Board of Directors of the Leiden University Medical Center, and by the Leiden University, Research Profile Area Vascular and Regenerative Medicine. Dennis O. Mook-Kanamori is supported by Dutch Science Organization (ZonMW-VENI Grant 916.14.023)

**Young Finns Study (YFS)**
The Young Finns Study has been financially supported by the Academy of Finland: grants 286284, 134309 (Eye), 126925, 121584, 124282, 129378 (Salve), 117787 (Gendi), and 41071 (Skidi); the Social Insurance Institution of Finland; Kuopio, Tampere and Turku University Hospital Medical Funds (grant X51001); Juho Vainio Foundation; Paavo Nurmi Foundation; Finnish Foundation of Cardiovascular Research; Finnish Cultural Foundation; Tampere Tuberculosis Foundation; Emil Aaltonen Foundation; and Yrjö Jahnsson Foundation. The expert technical assistance in the statistical analyses by Ville Aalto and Irina Lisinen is gratefully acknowledged.
The expected association p-values for the distribution of genotyped versus the observed
distribution of p-values for CAC quantity association is displayed. Significant systemic inflation
is not observed ($\lambda_{GC} = 1.057$). The dotted red line represents the pre-specified threshold for
statistical significance accounting for the number of individual variants tested for association
(0.05 / 238,065 = 2.10 x 10^{-7}). Lead variants of novel significant associated loci (APOE p.R176C
and APOB p.R3527Q) are highlighted in red.
Figure S2. Regional association plot of coronary artery calcification quantity at 9p21.

A.

B.
The CDKN2B gene resides in the 9p21 genomic region. These regional association plots demonstrate the strength of association, by \(-\log_{10}(p\text{-value})\), for the lead 9p21 variant, rs10757278, and each genotyped variant within +/- 400 kilobases. Color-coding based on ethnic-specific correlation observed in the 1000 Genomes Project is demonstrated. Dot sizes are scaled to the number genotyped for a given variant. Plots are separately shown for participants of: A. European ancestry and B. African ancestry.
Figure S3. Regional association plots of coronary artery calcification quantity at 6p24.

A.

Plotted SNPs

B.
The *PHACTRI* gene resides in the 6p24 genomic region. These regional association plots demonstrate the strength of association, by $-\log_{10}(p$-value), for the lead 6p24 variant, rs9349379, and other genotyped variants within +/- 400 kilobases. Color-coding based on ethnic-specific correlation observed in the 1000 Genomes Project is demonstrated. Dot sizes are scaled to the number genotyped for a given variant. Plots are separately shown for participants of: A. European ancestry and B. African ancestry.
Figure S4. Regional association plots of coronary artery calcification quantity at 2p24.

A.

B.
The *APOB* gene resides in the 2p24 genomic region. These regional association plots demonstrate the strength of association, by \(-\log_{10}(p\text{-value})\), for the lead 2p24 variant, rs5742904, and each genotyped variant within +/- 400 kilobases. Color-coding based on ethnic-specific correlation observed in the 1000 Genomes Project is demonstrated. Dot sizes are scaled to the number genotyped for a given variant. Plots are separately shown for participants of: A. European ancestry and B. African ancestry.
Figure S5. Regional association plots of coronary artery calcification quantity at 19q13.

A. Plotted SNPs

B. Plotted SNPs
The *APOE* gene resides in the 19q13 genomic region. These regional association plots demonstrate the strength of association, by $-\log_{10}(p\text{-value})$, for the lead 19q13 variant, rs7412, and each genotyped variant within $\pm 400$ kilobases. Color-coding based on ethnic-specific correlation observed in the 1000 Genomes Project is demonstrated. Dot sizes are scaled to the number genotyped for a given variant. Plots are separately shown for participants of: A. European ancestry and B. African ancestry.
Figure S6. Quantile-quantile plots of coronary artery calcification quantity gene-based association.

A.

B.
The expected associations p-values for the distribution of genotyped versus the observed distributions of p-values for CAC quantity gene-based association is displayed. Systemic inflation is not observed (T1 $\lambda_{GC} = 1.026$, SKAT $\lambda_{GC} = 1.107$). Significant observed deviations from the expected distribution are not noted. Association analyses are displayed for: A. T1 and B. SKAT.
The expected association p-values for the distribution of genotyped versus the observed distribution of p-values for CIMT association is displayed. Systemic inflation is not observed ($\lambda_{GC} = 1.080$). The dotted red line represents the prespecified threshold for statistical significance accounting for the number of individual variants tested for association ($0.05 / 238,065 = 2.1 \times 10^{-7}$). The lead variant of the novel significant associated locus ($APOE$ p.R176C) is highlighted in red.
Figure S8. Regional association plots of carotid intima media thickness at 19q13 (APOE).

A.

B.
The *APOE* gene resides in the 19q13 genomic region. These regional association plots demonstrate the strength of association, by $-\log_{10}(p\text{-value})$, for the lead 19q13 variant, rs7412, and each genotyped variant within +/- 400 kilobases. Color-coding based on ethnic-specific correlation observed in the 1000 Genomes Project is demonstrated. Dot sizes are scaled to the number genotyped for a given variant. Plots are separately shown for participants of: A. European ancestry and B. African ancestry.
Figure S9. Quantile-quantile plot of carotid intima media thickness gene-based association.

A.

B.
The expected associations p-values for the distribution of genotyped versus the observed distributions of p-values for CIMT gene-based association is displayed. Significant systemic inflation is not observed (T1 $\lambda_{GC} = 1.043$, SKAT $\lambda_{GC} = 1.114$). Significant observed deviations from the expected distribution are not noted. Association analyses are displayed for: A. T1 and B. SKAT.
Figure S10. Association of major APOE genotypes with CAC.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Frequency</th>
<th>Relative CAC</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>ε2/ε2</td>
<td>0.01</td>
<td>0.73</td>
<td>(0.55, 0.96)</td>
</tr>
<tr>
<td>ε2/ε3</td>
<td>0.12</td>
<td>0.89</td>
<td>(0.80, 0.99)</td>
</tr>
<tr>
<td>ε3/ε3</td>
<td>0.62</td>
<td>1.00</td>
<td>(1.00, 1.00)</td>
</tr>
<tr>
<td>ε4/ε3</td>
<td>0.21</td>
<td>1.04</td>
<td>(0.96, 1.13)</td>
</tr>
<tr>
<td>ε4/ε4</td>
<td>0.02</td>
<td>1.13</td>
<td>(0.87, 1.45)</td>
</tr>
<tr>
<td>ε4/ε2</td>
<td>0.03</td>
<td>1.21</td>
<td>(1.02, 1.43)</td>
</tr>
</tbody>
</table>

Major APOE genotypes ascertained by PCR were obtained in 5,872 individuals and were associated with log-transformed CAC quantity relative to the ε3/ε3 referent genotype within six cohorts accounting for age, sex, and ethnicity. Effect estimates were combined using fixed-effects meta-analysis and are displayed.
<table>
<thead>
<tr>
<th>Cohort</th>
<th>Ethnicity</th>
<th>Years measured</th>
<th>N</th>
<th>Age, y (sd)</th>
<th>Women, n (%)</th>
<th>CAC Score, median (IQR)</th>
<th>CAC&gt;0, n (%)</th>
<th>CAC&gt;100, n (%)</th>
<th>Hypertension, n (%)</th>
<th>Hypercholesterolemia, n (%)</th>
<th>Diabetes mellitus, n (%)</th>
<th>Recent cigarette smoker, n (%)</th>
<th>Device</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cardiovascular Health Study</td>
<td>EA</td>
<td>Baseline 1989-90</td>
<td>339</td>
<td>70.6 (3.8)</td>
<td>210 (62%)</td>
<td>368 (74.15, 826.65)</td>
<td>311 (92%)</td>
<td>237 (70%)</td>
<td>147 (43%)</td>
<td>103 (30%)</td>
<td>103 (30%)</td>
<td>27 (8%)</td>
<td>39 (12%)</td>
<td>Imatron C-150</td>
</tr>
<tr>
<td></td>
<td>AA</td>
<td>Baseline 1989-90</td>
<td>104</td>
<td>70.9 (4.0)</td>
<td>59 (57%)</td>
<td>124.85 (7.15, 499.02)</td>
<td>88 (85%)</td>
<td>55 (53%)</td>
<td>70 (67%)</td>
<td>21 (19%)</td>
<td>21 (19%)</td>
<td>Imatron C-150</td>
<td></td>
<td>27</td>
</tr>
<tr>
<td></td>
<td>EA</td>
<td>Baseline 1998-2006</td>
<td>667</td>
<td>60.9 (9.5)</td>
<td>416 (62%)</td>
<td>102.5 (12.0, 673.0)</td>
<td>326 (91%)</td>
<td>208 (58%)</td>
<td>544 (81.56 %)</td>
<td>290 (43.67%)</td>
<td>527 (79%)</td>
<td>110 (17%)</td>
<td>GE LightSpeed QX16 Pro, Philips/Marconi MX 8000</td>
<td>28</td>
</tr>
<tr>
<td></td>
<td>AA</td>
<td>Baseline 2005-08</td>
<td>1355</td>
<td>59.4 (10.9)</td>
<td>448 (33%)</td>
<td>0.0 (0.0, 65.62)</td>
<td>619 (46%)</td>
<td>283 (21%)</td>
<td>801 (59%)</td>
<td>233 (17%)</td>
<td>178 (13%)</td>
<td>402 (30%)</td>
<td>GE LightSpeed Plus, Siemens Volume Zoom, Philips/Marconi MX 8000</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td>EA</td>
<td>Baseline 2002-04</td>
<td>2370</td>
<td>55.6 (13.1)</td>
<td>1388 (59%)</td>
<td>0.5 (0.0, 78.0)</td>
<td>1304 (55%)</td>
<td>540 (23%)</td>
<td>855 (36%)</td>
<td>652 (28%)</td>
<td>258 (11%)</td>
<td>234 (10%)</td>
<td>GE LightSpeed Plus, Siemens Volume Zoom, Philips/Marconi MX 8000</td>
<td>5, 30</td>
</tr>
<tr>
<td></td>
<td>AA</td>
<td>Baseline 2002-04</td>
<td>537</td>
<td>52.4 (10.7)</td>
<td>363 (68%)</td>
<td>0 (0.00,28 .00)</td>
<td>266 (50%)</td>
<td>78 (15%)</td>
<td>385 (72%)</td>
<td>117 (22%)</td>
<td>120 (23%)</td>
<td>118 (22%)</td>
<td>GE LightSpeed Plus, Siemens Volume Zoom MDCT, 2008-12</td>
<td>51</td>
</tr>
<tr>
<td></td>
<td>EA</td>
<td>Baseline 2001, 2003-07, 2008-12</td>
<td>488</td>
<td>49.4 (10.3)</td>
<td>255 (52%)</td>
<td>0 (0.0, 29.7)</td>
<td>205 (42%)</td>
<td>78 (16%)</td>
<td>187 (38%)</td>
<td>172 (35%)</td>
<td>42 (9%)</td>
<td>83 (17%)</td>
<td>Siemens SOMATOM</td>
<td>31</td>
</tr>
<tr>
<td></td>
<td>AA Baseline or Follow up</td>
<td>2001-03-07, 2008-12</td>
<td>290</td>
<td>50.0 (10.3)</td>
<td>194 (67%)</td>
<td>0 (0, 2.6)</td>
<td>94 (32%)</td>
<td>25 (9%)</td>
<td>163 (56%)</td>
<td>74 (26%)</td>
<td>49 (17%)</td>
<td>81 (28%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>----------------</td>
<td>--------------------------</td>
<td>---------------------</td>
<td>-----</td>
<td>-------------</td>
<td>-----------</td>
<td>------------</td>
<td>----------</td>
<td>--------</td>
<td>-----------</td>
<td>---------</td>
<td>---------</td>
<td>--------</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NELSON</td>
<td>AA Baseline</td>
<td>2003-06</td>
<td>905</td>
<td>62.0 (6.0)</td>
<td>6 (1%)</td>
<td>97.41 (1.97, 654.47)</td>
<td>706 (78%)</td>
<td>452 (50%)</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Old Order Amish</td>
<td>EA Baseline</td>
<td>2001-06</td>
<td>1075</td>
<td>56.6 (13.1)</td>
<td>591 (55%)</td>
<td>2.40 (0, 144.86)</td>
<td>568 (53%)</td>
<td>304 (26%)</td>
<td>198 (18%)</td>
<td>453 (42%)</td>
<td>45 (4%)</td>
<td>84 (8%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>AA Follow up</td>
<td>2005</td>
<td>3184</td>
<td>52.2 (11.6)</td>
<td>1543 (48%)</td>
<td>0 (0, 45.26)</td>
<td>1204 (38%)</td>
<td>598 (19%)</td>
<td>905 (28%)</td>
<td>722 (23%)</td>
<td>183 (6%)</td>
<td>413 (13%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>EA Baseline</td>
<td>2008-09</td>
<td>4182</td>
<td>69.1 (6.0)</td>
<td>2357 (56%)</td>
<td>57.0 (0.0, 274.0)</td>
<td>2983 (71%)</td>
<td>1783 (43%)</td>
<td>2944 (70%)</td>
<td>2713 (65%)</td>
<td>686 (16%)</td>
<td>357 (9%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>AA Baseline</td>
<td>2008-09</td>
<td>843</td>
<td>67.9 (5.7)</td>
<td>501 (59%)</td>
<td>2.0 (0.0, 77.5)</td>
<td>443 (53%)</td>
<td>179 (21%)</td>
<td>708 (84%)</td>
<td>545 (65%)</td>
<td>250 (30%)</td>
<td>80 (9%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>EA Baseline</td>
<td>2000-02</td>
<td>2526</td>
<td>62.7 (10.2)</td>
<td>1320 (52%)</td>
<td>9.35 (0, 6452.61)</td>
<td>1438 (57%)</td>
<td>780 (31%)</td>
<td>975 (39%)</td>
<td>707 (28%)</td>
<td>151 (6%)</td>
<td>287 (11%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>AA Baseline</td>
<td>2000-02</td>
<td>1611</td>
<td>62.3 (10.1)</td>
<td>868 (54%)</td>
<td>0 (0, 5599.45)</td>
<td>721 (45%)</td>
<td>311 (19%)</td>
<td>959 (59.53%)</td>
<td>367 (23%)</td>
<td>279 (17%)</td>
<td>194 (13%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Definition Flash dual-source 256 MDCT
2001-07: Siemens Volume Zoom multidetector row computed tomograph;
2008-12: Siemens SOMATOM Definition Flash dual-source 256 multi-detector scanner

Mx8000 IDT, Somatom Sensation 16, Brilliance 16P
Imatron C-150
LightSpeed Ultra (GE)
Philips Brilliance 64-slice MDCT (Philips Healthcare, Andover, Massachusetts)

Wake Forest: Light Speed Plus, Light Speed QX/I; Columbia, Northwestern, UCLA: Imatron C-150; Johns Hopkins and
<table>
<thead>
<tr>
<th>Study</th>
<th>Group</th>
<th>Year</th>
<th>Follow up</th>
<th>n</th>
<th>BMI</th>
<th>WC</th>
<th>WC (80% CI)</th>
<th>WC (60% CI)</th>
<th>WC (40% CI)</th>
<th>WC (20% CI)</th>
<th>WC (10% CI)</th>
<th>Letters</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Minnesota:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GENOA</td>
<td>EA</td>
<td>Follow up</td>
<td>2000 - 04</td>
<td>1033</td>
<td>58.0</td>
<td>615</td>
<td>(10.1)</td>
<td>(59%)</td>
<td>30.8 (141.86)</td>
<td>680 (66%)</td>
<td>298 (29%)</td>
<td>727 (70%)</td>
</tr>
<tr>
<td></td>
<td>AA</td>
<td>Follow up</td>
<td>2009 - 11</td>
<td>354</td>
<td>67.3</td>
<td>257</td>
<td>(8.4)</td>
<td>(73%)</td>
<td>196.27 (11.09)</td>
<td>213 (60%)</td>
<td>114 (32%)</td>
<td>293 (83%)</td>
</tr>
<tr>
<td><strong>AGES Reykjavik</strong></td>
<td>EA</td>
<td>Baseline</td>
<td>2002-04</td>
<td>2286</td>
<td>76.2</td>
<td>1457</td>
<td>(5.5)</td>
<td>(63.8%)</td>
<td>53.76 (22.59,6)</td>
<td>1948 (85%)</td>
<td>1377 (60%)</td>
<td>1763 (77%)</td>
</tr>
<tr>
<td><strong>Rotterdam Study</strong></td>
<td>EA</td>
<td>Follow up</td>
<td>1997-99</td>
<td>785</td>
<td>71.8</td>
<td>353</td>
<td>(5.9)</td>
<td>(45%)</td>
<td>539.09 (146.47)</td>
<td>734 (94%)</td>
<td>443 (56%)</td>
<td>502 (64%)</td>
</tr>
<tr>
<td><strong>Young Finns Study</strong></td>
<td>EA</td>
<td>Follow up</td>
<td>2007</td>
<td>499</td>
<td>41.7</td>
<td>284</td>
<td>(2.6)</td>
<td>(57%)</td>
<td>0.00 (0.00,0.00)</td>
<td>96 (19%)</td>
<td>13 (3%)</td>
<td>136 (27%)</td>
</tr>
</tbody>
</table>
slice CT
(Siemens Healthcare, Erlangen, Germany)
(Kuopio)
<table>
<thead>
<tr>
<th>Cohort</th>
<th>Ethnicity</th>
<th>Years measured</th>
<th>N</th>
<th>Age, y mean(sd)</th>
<th>Women, n (%)</th>
<th>CIMT, mm median (IQR)</th>
<th>Carotid plaque, n (%)</th>
<th>Hypertension, n (%)</th>
<th>Hypercholesterolemia, n (%)</th>
<th>Diabetes mellitus, n (%)</th>
<th>Recent cigarette smoker, n (%)</th>
<th>Device</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cardiovascular Health Study</td>
<td>EA</td>
<td>Baseline 1989-90</td>
<td>4040</td>
<td>72.8(5.6)</td>
<td>2276 (56%)</td>
<td>1.015 (0.91, 1.14)</td>
<td>2722 (70%)</td>
<td>2234 (55%)</td>
<td>1033 (26%)</td>
<td>557 (14%)</td>
<td>426 (11%)</td>
<td>B-mode US (model SSA-270A; Toshiba)</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td>AA</td>
<td>Baseline 1989-90, 1992-93</td>
<td>738</td>
<td>72.8(5.7)</td>
<td>461 (62%)</td>
<td>1.09 (0.97, 1.22)</td>
<td>552 (75%)</td>
<td>536 (73%)</td>
<td>174 (24%)</td>
<td>166 (23%)</td>
<td>122 (17%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetes Heart Study</td>
<td>EA</td>
<td>Baseline 1998-2006</td>
<td>647</td>
<td>60.5(9.7)</td>
<td>413 (64%)</td>
<td>0.631 (0.567, 0.706)</td>
<td>NA</td>
<td>527 (81%)</td>
<td>272 (42%)</td>
<td>510 (79%)</td>
<td>113 (18%)</td>
<td>B-mode US (model AU5; Biosound Esaote)</td>
<td>41</td>
</tr>
<tr>
<td>Jackson Heart Study</td>
<td>AA</td>
<td>Baseline 2000-04</td>
<td>1979</td>
<td>60.0(10.5)</td>
<td>678 (34%)</td>
<td>0.70 (0.60, 0.82)</td>
<td>NA</td>
<td>1209 (61%)</td>
<td>348 (18%)</td>
<td>285 (14%)</td>
<td>621 (31%)</td>
<td>B-mode US (Hewlett Packard Sonos 4500)</td>
<td>42</td>
</tr>
<tr>
<td>Lothian Birth Cohort 1936</td>
<td>EA</td>
<td>Follow up 2007-10</td>
<td>746</td>
<td>72.8(0.8)</td>
<td>346 (48%)</td>
<td>0.85 (0.70, 0.95)</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>B-mode US</td>
<td>6</td>
</tr>
<tr>
<td>SHIP</td>
<td>EA</td>
<td>Baseline 1997-2001</td>
<td>3504</td>
<td>52.8(13.5)</td>
<td>1742 (50%)</td>
<td>0.81 (0.24)</td>
<td>1864 (53%)</td>
<td>1891 (54%)</td>
<td>NA</td>
<td>403 (12%)</td>
<td>985 (28%)</td>
<td>B-Mode US (Diasonics VST Gateway, Santa Clara, California, USA)</td>
<td>7, 43</td>
</tr>
<tr>
<td>SHIP-Trend</td>
<td>EA</td>
<td>Baseline 2008-12</td>
<td>3461</td>
<td>52.1(15.3)</td>
<td>1770 (51%)</td>
<td>0.72 (0.24)</td>
<td>1425 (41%)</td>
<td>1646 (48%)</td>
<td>NA</td>
<td>433 (13%)</td>
<td>868 (25%)</td>
<td>B-Mode US (vivid-i, GE Medical Systems, Waukesha, Wisconsin, WI, USA)</td>
<td>7, 43</td>
</tr>
<tr>
<td>GeneSTAR</td>
<td>EA</td>
<td>Follow 2009-12</td>
<td>441</td>
<td>50.0(11.3)</td>
<td>236</td>
<td>0.679</td>
<td>NA</td>
<td>147 (33%)</td>
<td>148 (34%)</td>
<td>37 (8%)</td>
<td>74 (17%)</td>
<td>B-mode US</td>
<td>31, 44</td>
</tr>
<tr>
<td>Study</td>
<td>Group</td>
<td>Baseline</td>
<td>Right</td>
<td>Left</td>
<td>Age</td>
<td>Follow up</td>
<td>Age</td>
<td>Follow up</td>
<td>Age</td>
<td>Follow up</td>
<td>Age</td>
<td>Follow up</td>
<td></td>
</tr>
<tr>
<td>---------------------</td>
<td>-------</td>
<td>----------</td>
<td>-------</td>
<td>------</td>
<td>-----</td>
<td>----------</td>
<td>-----</td>
<td>----------</td>
<td>-----</td>
<td>----------</td>
<td>-----</td>
<td>----------</td>
<td></td>
</tr>
<tr>
<td>BioImage</td>
<td>AA</td>
<td>Baseline</td>
<td>2008-09</td>
<td>4378</td>
<td>69.1(6.0)</td>
<td>2449</td>
<td>0.73 (0.65, 0.84)</td>
<td>3487</td>
<td>0.73 (0.65, 0.84)</td>
<td>748</td>
<td>0.73 (0.65, 0.84)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MESA</td>
<td>EA</td>
<td>Baseline</td>
<td>2000-02</td>
<td>2501</td>
<td>62.6(10.3)</td>
<td>1307</td>
<td>0.84 (0.47, 2.45)</td>
<td>NA</td>
<td>0.84 (0.47, 2.45)</td>
<td>963</td>
<td>0.84 (0.47, 2.45)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>AA</td>
<td>Baseline</td>
<td>2000-02</td>
<td>1573</td>
<td>62.2(10.3)</td>
<td>848</td>
<td>0.89(0.4, 0.2, 39)</td>
<td>NA</td>
<td>0.89(0.4, 0.2, 39)</td>
<td>928</td>
<td>0.89(0.4, 0.2, 39)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AGES Reykjavik</td>
<td>EA</td>
<td>Baseline</td>
<td>2002-04</td>
<td>2835</td>
<td>73.6(5.5)</td>
<td>1629</td>
<td>1.13 (1.02, 1.24)</td>
<td>NA</td>
<td>1.13 (1.02, 1.24)</td>
<td>2288</td>
<td>1.13 (1.02, 1.24)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ARIC</td>
<td>EA</td>
<td>Baseline</td>
<td>1987-89</td>
<td>8668</td>
<td>54.3(5.7)</td>
<td>4594</td>
<td>0.76 (0.65, 0.85)</td>
<td>1628</td>
<td>0.76 (0.65, 0.85)</td>
<td>2301</td>
<td>0.76 (0.65, 0.85)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>AA</td>
<td>Baseline</td>
<td>1987-89</td>
<td>2662</td>
<td>53.9(5.9)</td>
<td>1647</td>
<td>0.78 (0.69, 0.90)</td>
<td>457</td>
<td>0.78 (0.69, 0.90)</td>
<td>1493</td>
<td>0.78 (0.69, 0.90)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rotterdam Study</td>
<td>EA</td>
<td>Baseline</td>
<td>1990-93</td>
<td>2500</td>
<td>69.5(8.4)</td>
<td>1341</td>
<td>0.99 (0.88, 1.13)</td>
<td>1451</td>
<td>0.99 (0.88, 1.13)</td>
<td>1332</td>
<td>0.99 (0.88, 1.13)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Model Information:**
- Toshiba SSH-140A imaging unit with a 7.0 MHz transducer
- Philips iU22 US systems
- Philips Healthcare, Bothell, Washington
- B-mode US (Logiq 700 ultrasound device (GE Med Systems, Waukesha, WI))
- 7.5 MHz linear array

---

1. MESA: Baseline 2000-02, 2501, 62.6(10.3), 1307 (53%), 0.84 (0.47, 2.45), NA, 963 (39%), 698 (28%), 150 (6%), 282 (11%)
2. AGES Reykjavik: Baseline 2002-04, 2835, 73.6(5.5), 1629 (58%), 1.13 (1.02, 1.24), NA, 2288 (81%), NA, 329 (12%), 357 (13%)
3. ARIC: Baseline 1987-89, 8668, 54.3(5.7), 4594 (47%), 0.76 (0.65, 0.85), 1628 (19%), 2301 (27%), 2216 (26%), 723 (8%), 2155 (25%)
4. Rotterdam Study: Baseline 1990-93, 2500, 69.5(8.4), 1341 (54%), 0.99 (0.88, 1.13), 1451 (58%), 1332 (53%), 1493 (60%), 252 (10%), 607 (24%), 7.5 MHz linear array
<table>
<thead>
<tr>
<th>Study</th>
<th>Transducer Model and Manufacturer</th>
<th>Transducer Model and Manufacturer</th>
<th>Frequencies</th>
<th>Transducer and Mainframe</th>
<th>Technology</th>
<th>Follow-up Period</th>
<th>Baseline Echogenicity</th>
<th>Follow-up Echogenicity</th>
<th>Baseline Echogenicity</th>
<th>Follow-up Echogenicity</th>
</tr>
</thead>
</table>

**Notes:**
- **ERF**
- **NEO**
- **Young Finns Study**

**Follow-up Periods:**
- ERF: 2002-05
- NEO: 2008-12

**Transducer Models:**
- **ERF**
- **NEO**
- **Young Finns Study**
Table S3. *APOE* ε2 association with CAC adjusted for blood lipids.

<table>
<thead>
<tr>
<th>Adjustment</th>
<th>Beta</th>
<th>SE</th>
<th>P</th>
<th>Beta</th>
<th>SE</th>
<th>P</th>
<th>Beta</th>
<th>SE</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Base Model</td>
<td>-0.252</td>
<td>0.0373</td>
<td>1.54 x 10^{-11}</td>
<td>-0.1792</td>
<td>0.0442</td>
<td>4.94 x 10^{-5}</td>
<td>-0.4346</td>
<td>0.0700</td>
<td>5.36 x 10^{-10}</td>
</tr>
<tr>
<td>LDL cholesterol</td>
<td>-0.1862</td>
<td>0.0332</td>
<td>2.08 x 10^{-8}</td>
<td>-0.1748</td>
<td>0.0455</td>
<td>1.23 x 10^{-4}</td>
<td>-0.1993</td>
<td>0.0486</td>
<td>4.14 x 10^{-5}</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>-0.2061</td>
<td>0.0327</td>
<td>2.95 x 10^{-10}</td>
<td>-0.1701</td>
<td>0.0447</td>
<td>1.44 x 10^{-4}</td>
<td>-0.2473</td>
<td>0.0479</td>
<td>2.45 x 10^{-7}</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>-0.2260</td>
<td>0.0326</td>
<td>4.45 x 10^{-12}</td>
<td>-0.1942</td>
<td>0.0448</td>
<td>1.44 x 10^{-5}</td>
<td>-0.2619</td>
<td>0.0477</td>
<td>3.95 x 10^{-8}</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>-0.1851</td>
<td>0.0329</td>
<td>1.78 x 10^{-8}</td>
<td>-0.1630</td>
<td>0.0450</td>
<td>2.91 x 10^{-4}</td>
<td>-0.2103</td>
<td>0.0481</td>
<td>1.26 x 10^{-5}</td>
</tr>
</tbody>
</table>

For individuals where fasting lipids were available, the association of ε2 with CAC was repeated individually adjusting for each of the major blood lipid measures within each cohort and meta-analyzed in a fixed effects model. β-Coefficients are estimated for natural log transformation of total Agatston CAC score+1. The base model was adjusted for age, sex, and principal components of ancestry.

AA = African ancestry; CAC = coronary artery calcification; EA = European ancestry; HDL = high density lipoprotein; LDL = low-density lipoprotein; SE = standard error
<table>
<thead>
<tr>
<th>Chr</th>
<th>Pos</th>
<th>Gene</th>
<th>SNP</th>
<th>Effect Allele</th>
<th>Effect Allele Frequency</th>
<th>OR</th>
<th>P</th>
<th>CAC</th>
<th>Beta</th>
<th>SE</th>
<th>P</th>
<th>CIMT</th>
<th>Beta</th>
<th>SE</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>55496039</td>
<td>PCSK9</td>
<td>rs11206510</td>
<td>T</td>
<td>0.85</td>
<td>1.08</td>
<td>2.34E-08</td>
<td>-0.0094</td>
<td>0.0273</td>
<td>7.31E-01</td>
<td>-0.0006</td>
<td>0.0014</td>
<td>6.95E-01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>56965664</td>
<td>PPA2B</td>
<td>rs9970807</td>
<td>C</td>
<td>0.92</td>
<td>1.13</td>
<td>5.00E-14</td>
<td>0.0700</td>
<td>0.0353</td>
<td>4.75E-02</td>
<td>0.0033</td>
<td>0.0019</td>
<td>8.09E-02</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>109818530</td>
<td>SORT1</td>
<td>rs646776</td>
<td>T</td>
<td>0.75</td>
<td>1.11</td>
<td>9.01E-19</td>
<td>0.0629</td>
<td>0.0250</td>
<td>1.18E-02</td>
<td>0.0030</td>
<td>0.0013</td>
<td>1.84E-02</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>154422067</td>
<td>IL6R</td>
<td>rs4845625</td>
<td>T</td>
<td>0.45</td>
<td>1.05</td>
<td>3.93E-08</td>
<td>0.0655</td>
<td>0.0212</td>
<td>1.97E-03</td>
<td>-0.0006</td>
<td>0.0011</td>
<td>5.83E-01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>222823529</td>
<td>MIA3</td>
<td>rs17465637</td>
<td>C</td>
<td>0.66</td>
<td>1.08</td>
<td>3.52E-12</td>
<td>0.0732</td>
<td>0.0233</td>
<td>1.67E-03</td>
<td>0.0002</td>
<td>0.0012</td>
<td>8.65E-01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>199424737</td>
<td>AK097927</td>
<td>rs16986953</td>
<td>A</td>
<td>0.11</td>
<td>1.09</td>
<td>1.45E-08</td>
<td>0.1468</td>
<td>0.0423</td>
<td>5.15E-04</td>
<td>-0.0009</td>
<td>0.0021</td>
<td>6.60E-01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>21286057</td>
<td>APOB</td>
<td>rs515135</td>
<td>C</td>
<td>0.79</td>
<td>1.07</td>
<td>3.09E-08</td>
<td>0.0272</td>
<td>0.0276</td>
<td>3.24E-01</td>
<td>0.0038</td>
<td>0.0014</td>
<td>9.12E-03</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>44073881</td>
<td>ABCG5-ABCG8</td>
<td>rs6544713</td>
<td>T</td>
<td>0.32</td>
<td>1.05</td>
<td>8.88E-07</td>
<td>0.0240</td>
<td>0.0227</td>
<td>2.90E-01</td>
<td>0.0010</td>
<td>0.0012</td>
<td>3.91E-01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>85809989</td>
<td>VAMP5-VAMP8-GGCX-ZEB2-ACO74093.1</td>
<td>rs1561198</td>
<td>T</td>
<td>0.46</td>
<td>1.06</td>
<td>6.37E-10</td>
<td>0.0433</td>
<td>0.0208</td>
<td>3.78E-02</td>
<td>0.0005</td>
<td>0.0011</td>
<td>6.16E-01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>145801461</td>
<td>MRAS</td>
<td>rs9818870</td>
<td>T</td>
<td>0.14</td>
<td>1.07</td>
<td>2.21E-06</td>
<td>0.0317</td>
<td>0.0210</td>
<td>1.32E-01</td>
<td>0.0001</td>
<td>0.0011</td>
<td>9.35E-01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>156635309</td>
<td>GUCY1A3</td>
<td>rs7692387</td>
<td>G</td>
<td>0.81</td>
<td>1.07</td>
<td>7.35E-09</td>
<td>0.0113</td>
<td>0.0265</td>
<td>6.70E-01</td>
<td>0.0014</td>
<td>0.0014</td>
<td>3.08E-01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>120930957</td>
<td>PHACTR1</td>
<td>rs9349379</td>
<td>G</td>
<td>0.43</td>
<td>1.14</td>
<td>1.81E-42</td>
<td>0.1922</td>
<td>0.0212</td>
<td>1.28E-19</td>
<td>-0.0006</td>
<td>0.0011</td>
<td>6.00E-01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>39174922</td>
<td>KCNK3</td>
<td>rs10947789</td>
<td>T</td>
<td>0.78</td>
<td>1.05</td>
<td>1.63E-06</td>
<td>-0.0126</td>
<td>0.0242</td>
<td>6.04E-01</td>
<td>-0.0004</td>
<td>0.0013</td>
<td>7.25E-01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>160863532</td>
<td>SLC22A3-LPAL2-LPA</td>
<td>rs2048327</td>
<td>C</td>
<td>0.35</td>
<td>1.06</td>
<td>2.46E-09</td>
<td>-0.0041</td>
<td>0.0219</td>
<td>8.52E-01</td>
<td>-0.0014</td>
<td>0.0011</td>
<td>2.20E-01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>161143608</td>
<td>PLG</td>
<td>rs4252120</td>
<td>T</td>
<td>0.74</td>
<td>1.03</td>
<td>3.32E-03</td>
<td>-0.0080</td>
<td>0.0230</td>
<td>7.29E-01</td>
<td>-0.0014</td>
<td>0.0012</td>
<td>2.28E-01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>19036775</td>
<td>HDAC9</td>
<td>rs2023938</td>
<td>C</td>
<td>0.1</td>
<td>1.06</td>
<td>1.36E-04</td>
<td>0.0948</td>
<td>0.0349</td>
<td>6.58E-03</td>
<td>0.0044</td>
<td>0.0018</td>
<td>1.44E-02</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>107244545</td>
<td>7q22</td>
<td>rs10953541</td>
<td>C</td>
<td>0.78</td>
<td>1.05</td>
<td>1.02E-05</td>
<td>-0.0207</td>
<td>0.0243</td>
<td>3.95E-01</td>
<td>-0.0002</td>
<td>0.0013</td>
<td>8.96E-01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>129663496</td>
<td>ZC3HC1</td>
<td>rs11556924</td>
<td>C</td>
<td>0.69</td>
<td>1.08</td>
<td>5.34E-11</td>
<td>0.0377</td>
<td>0.0216</td>
<td>8.17E-02</td>
<td>0.0004</td>
<td>0.0011</td>
<td>7.39E-01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>19813180</td>
<td>LPL</td>
<td>rs264</td>
<td>G</td>
<td>0.85</td>
<td>1.06</td>
<td>1.06E-05</td>
<td>0.0536</td>
<td>0.0300</td>
<td>7.46E-02</td>
<td>0.0024</td>
<td>0.0015</td>
<td>1.13E-01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>126490972</td>
<td>TRIB1</td>
<td>rs2954029</td>
<td>A</td>
<td>0.55</td>
<td>1.04</td>
<td>2.61E-06</td>
<td>0.0011</td>
<td>0.0218</td>
<td>9.60E-01</td>
<td>0.0018</td>
<td>0.0012</td>
<td>1.32E-01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>22098574</td>
<td>9p21</td>
<td>rs9775754</td>
<td>G</td>
<td>0.49</td>
<td>1.21</td>
<td>6.35E-98</td>
<td>0.2111</td>
<td>0.0208</td>
<td>2.88E-24</td>
<td>0.0006</td>
<td>0.0011</td>
<td>5.60E-01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>136154168</td>
<td>ABO</td>
<td>rs579459</td>
<td>C</td>
<td>0.21</td>
<td>1.08</td>
<td>1.14E-10</td>
<td>0.0450</td>
<td>0.0262</td>
<td>8.62E-02</td>
<td>-0.0030</td>
<td>0.0014</td>
<td>2.81E-02</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CHD Risk Variant</td>
<td>Gene</td>
<td>rsID</td>
<td>Minor Allele</td>
<td>Effect Size</td>
<td>95% CI</td>
<td>p-Value</td>
<td>Person</td>
<td>p-Value</td>
<td>z-Score</td>
<td>p-Value</td>
<td>p-Value</td>
<td>p-Value</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-----------------</td>
<td>------</td>
<td>------</td>
<td>--------------</td>
<td>-------------</td>
<td>--------</td>
<td>--------</td>
<td>--------</td>
<td>--------</td>
<td>---------</td>
<td>--------</td>
<td>---------</td>
<td>---------</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>KIAA1462</td>
<td>rs2505083</td>
<td>C</td>
<td>0.40</td>
<td>1.06</td>
<td>1.57E-10</td>
<td>0.0732</td>
<td>0.0211</td>
<td>5.29E-04</td>
<td>0.0033</td>
<td>0.0011</td>
<td>2.43E-03</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>CXCL12</td>
<td>rs501120</td>
<td>T</td>
<td>0.81</td>
<td>1.08</td>
<td>1.39E-11</td>
<td>0.0672</td>
<td>0.0312</td>
<td>3.13E-02</td>
<td>-0.0032</td>
<td>0.0016</td>
<td>4.91E-02</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>LIPA</td>
<td>rs11203042</td>
<td>T</td>
<td>0.45</td>
<td>1.04</td>
<td>1.22E-04</td>
<td>0.0136</td>
<td>0.0210</td>
<td>5.16E-01</td>
<td>-0.0012</td>
<td>0.0011</td>
<td>2.56E-01</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>CYP17A1</td>
<td>rs12413409</td>
<td>G</td>
<td>0.89</td>
<td>1.08</td>
<td>1.07E-07</td>
<td>-0.0296</td>
<td>0.0377</td>
<td>4.31E-01</td>
<td>0.0014</td>
<td>0.0019</td>
<td>4.50E-01</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>PDGF</td>
<td>rs974819</td>
<td>T</td>
<td>0.33</td>
<td>1.07</td>
<td>2.44E-10</td>
<td>0.0818</td>
<td>0.0233</td>
<td>4.56E-04</td>
<td>0.0033</td>
<td>0.0012</td>
<td>7.15E-03</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>AP05-</td>
<td>rs964184</td>
<td>G</td>
<td>0.19</td>
<td>1.05</td>
<td>5.60E-05</td>
<td>0.0288</td>
<td>0.0295</td>
<td>3.30E-01</td>
<td>-0.0010</td>
<td>0.0017</td>
<td>5.45E-01</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>ATP2B1</td>
<td>rs2681472</td>
<td>G</td>
<td>0.20</td>
<td>1.08</td>
<td>6.17E-11</td>
<td>0.0956</td>
<td>0.0279</td>
<td>6.17E-04</td>
<td>-0.0053</td>
<td>0.0015</td>
<td>2.78E-04</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>SH2B3</td>
<td>rs3184504</td>
<td>T</td>
<td>0.42</td>
<td>1.07</td>
<td>1.03E-09</td>
<td>-0.0062</td>
<td>0.0208</td>
<td>7.67E-01</td>
<td>-0.0015</td>
<td>0.0012</td>
<td>1.38E-02</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>FLT1</td>
<td>rs9319428</td>
<td>A</td>
<td>0.31</td>
<td>1.04</td>
<td>7.13E-05</td>
<td>0.0081</td>
<td>0.0224</td>
<td>7.17E-01</td>
<td>-0.0029</td>
<td>0.0012</td>
<td>6.93E-01</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>HHPL1</td>
<td>rs2895811</td>
<td>C</td>
<td>0.41</td>
<td>1.04</td>
<td>1.86E-05</td>
<td>0.0283</td>
<td>0.0212</td>
<td>1.81E-01</td>
<td>0.0004</td>
<td>0.0011</td>
<td>5.16E-01</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>FURIN-FES</td>
<td>rs1751486</td>
<td>A</td>
<td>0.44</td>
<td>1.05</td>
<td>3.10E-07</td>
<td>-0.0410</td>
<td>0.0210</td>
<td>5.08E-02</td>
<td>0.0019</td>
<td>0.0011</td>
<td>8.59E-02</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>SMG6</td>
<td>rs216172</td>
<td>C</td>
<td>0.35</td>
<td>1.05</td>
<td>5.07E-07</td>
<td>0.0461</td>
<td>0.0217</td>
<td>3.36E-02</td>
<td>-0.0031</td>
<td>0.0012</td>
<td>1.05E-02</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>RAI1-PREMTP</td>
<td>rs12936587</td>
<td>G</td>
<td>0.61</td>
<td>1.03</td>
<td>8.24E-04</td>
<td>0.0153</td>
<td>0.0219</td>
<td>4.86E-01</td>
<td>0.0014</td>
<td>0.0011</td>
<td>2.11E-01</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>UBE2Z</td>
<td>rs46522</td>
<td>T</td>
<td>0.51</td>
<td>1.04</td>
<td>1.84E-05</td>
<td>0.0434</td>
<td>0.0208</td>
<td>3.71E-02</td>
<td>0.0001</td>
<td>0.0011</td>
<td>9.27E-01</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>LDLR</td>
<td>rs1122608</td>
<td>G</td>
<td>0.77</td>
<td>1.08</td>
<td>2.75E-11</td>
<td>0.0188</td>
<td>0.0243</td>
<td>4.39E-01</td>
<td>0.0036</td>
<td>0.0013</td>
<td>7.59E-03</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>APOC3</td>
<td>rs2075650</td>
<td>G</td>
<td>0.13</td>
<td>1.07</td>
<td>1.61E-06</td>
<td>0.0822</td>
<td>0.0299</td>
<td>6.06E-03</td>
<td>0.0018</td>
<td>0.0015</td>
<td>2.23E-01</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>KCNE2</td>
<td>rs9982601</td>
<td>T</td>
<td>0.13</td>
<td>1.12</td>
<td>1.33E-13</td>
<td>0.0763</td>
<td>0.0324</td>
<td>1.84E-02</td>
<td>-0.0003</td>
<td>0.0016</td>
<td>8.71E-01</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Variants that have been previously associated with CHD in individuals of European, South Asian, or East Asian ancestry, effect estimates, and risk allele frequencies are displayed. The CAC and CIMT effects estimates and significance for each CHD risk variant among those of European ancestry are also displayed. β-Coefficients for CAC and CIMT are estimated for natural log transformation of total Agatston CAC score+1 and natural log transformation of CIMT, respectively.
CAC = coronary artery calcification; CHD = coronary heart disease; Chr = chromosome; CIMT = carotid intima media thickness; OR = odds ratio; P = p-value; Pos = genomic position (hg19 build); SE = standard error; SNP = single nucleotide polymorphism
Supplemental References


