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Meta-analysis of genome-wide association studies from the CHARGE consortium identifies common variants associated with carotid intima media thickness and plaque

Carotid intima media thickness (cIMT) and plaque determined by ultrasonography are established measures of subclinical atherosclerosis that each predicts future cardiovascular disease events. We conducted a meta-analysis of genome-wide association data in 31,211 participants of European ancestry from nine large studies in the setting of the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) Consortium. We then sought additional evidence to support our findings among 11,273 individuals using data from seven additional studies. In the combined meta-analysis, we identified three genomic regions associated with common carotid intima media thickness and two different regions associated with the presence of carotid plaque ($P < 5 \times 10^{-8}$). The associated SNPs mapped in or near genes related to cellular signaling, lipid metabolism and blood pressure homeostasis, and two of the regions were associated with coronary artery disease ($P < 0.006$) in the Coronary Artery Disease Genome-Wide Replication and Meta-Analysis (CARDIoGRAM) consortium. Our findings may provide new insight into pathways leading to subclinical atherosclerosis and subsequent cardiovascular events.

Coronary heart disease and stroke rank among the leading causes of death in the industrialized world[1], and a substantial genetic component underlies both of these outcomes. These clinical events are often preceded by the development of subclinical atherosclerosis, typically a thickening of the artery wall caused by deposition of cholesterol-rich material in the arteries that supply blood to the major organs[2]. Generalized atherosclerosis results from endothelial dysfunction, inflammation, abnormalities in lipoprotein metabolism[3], coagulation and fibrinolysis[4].

Measures of subclinical atherosclerosis, which is disease that occurs before symptoms are noted, are predictive of incident clinical events and can be detected non-invasively and with reasonable precision in population samples using high-resolution ultrasound techniques. Both carotid intima media thickness (cIMT) and plaque, which reflect a thickening of the carotid artery wall or the presence of large irregular arterial wall deposits, respectively, are established measures of subclinical atherosclerotic disease. Although there may be variation in carotid ultrasound measurement techniques, multiple independent studies have established consistent association of carotid phenotypes with coronary events and stroke in prospective studies of young, middle-aged and older adults[5,6], and recent consensus prevention guidelines cite cIMT as a potentially useful measure for prediction of these events[7]. Although there is a correlation between common cIMT and carotid plaque, common cIMT reflects carotid artery wall thickening that may result from multiple vascular etiologies including hypertension and atherosclerosis, whereas carotid plaque is an indicator of the discrete occurrence of carotid atherosclerosis. Several recent studies have provided evidence that carotid plaque is a better predictor of future cardiovascular disease risk than common cIMT[8–10].

Numerous family studies have established consistent evidence for moderate heritabilities for common cIMT, internal cIMT and carotid plaque (**Supplementary Table 1**). However, candidate gene studies have not found consistent associations between SNPs and cIMT[11], and genome-wide linkage scans completed to date have revealed only suggestive regions for common cIMT[12,13]. We performed a genome-wide association study (GWAS) of three measures of subclinical carotid atherosclerosis—common cIMT, internal cIMT and plaque—in a sample of up to 31,211 participants from nine population-based studies that performed genome-wide genotyping with commercial SNP arrays and imputed the samples to the approximately 2.5 million autosomal SNPs in the phase II HapMap European CEU reference panel. In addition, we followed up our discovery findings in a second stage that included 11,273 participants from seven independent studies.

RESULTS

The cross-sectional discovery genome-wide analysis of carotid artery phenotypes included 31,211 participants from nine community-based studies whose mean age ranged from 44–76 years. Characteristics of the samples are presented in the **Supplementary Note**. In the studies in which all three carotid measures were available, the correlations between common cIMT and plaque ranged from 0.27 to 0.39, and the correlations between common cIMT and internal cIMT ranged from 0.36 to 0.67 (**Supplementary Table 2**).

The *a priori* threshold for genome-wide significance used was $P = 5 \times 10^{-8}$, and $5 \times 10^{-8} < P < 4 \times 10^{-7}$, corresponding to not more than one expected false positive finding over 2.5 million tests, was considered suggestive evidence for association in our analyses.

A full list of authors and affiliations appears at the end of the paper.

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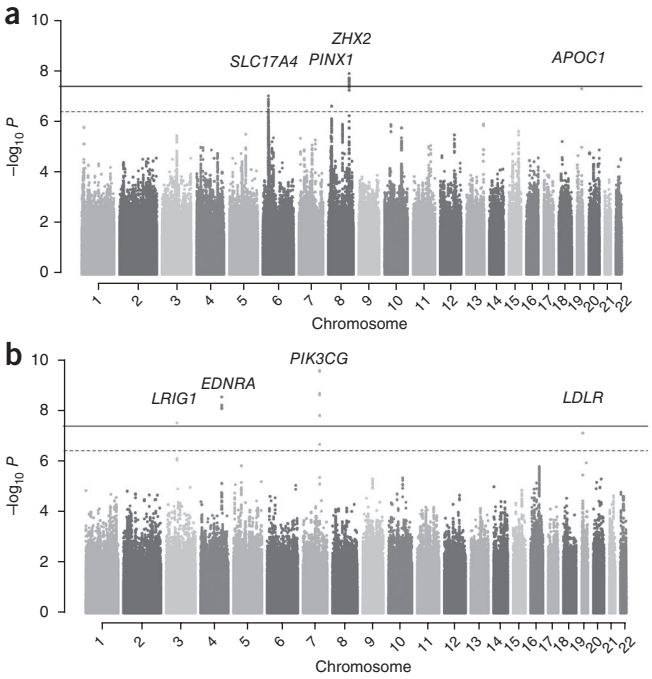

Figure 1 Genome-wide Manhattan plots for common cIMT and plaque. Plots show the individual *P* values (based on the discovery meta-analysis) against their genomic position for common cIMT (**a**) and the presence of plaque (**b**). Within each chromosome, shown on the *x* axis, the results are plotted left to right from the p-terminal end. The dotted lines indicate the threshold for follow up, $P < 4 \times 10^{-7}$, and the solid lines indicate the threshold for genome-wide significance, $P < 5 \times 10^{-8}$. The nearest genes are indicated above points that surpassed our significance threshold for follow-up.

Figure 1a provides a plot of $-\log_{10} P$ for the associations of the approximately 2.5 million SNPs with common cIMT by chromosome and position for the meta-analysis of the nine discovery studies. The *P* values from the meta-analysis of plaque ($n = 25{,}179$ participants) and internal cIMT ($n = 10{,}962$) are presented according to their genomic positions (shown in **Fig. 1b** and **Supplementary Fig. 1**, respectively). Overall, from the discovery meta-analysis of common cIMT and plaque, we carried forward three genome-wide significant SNPs and five suggestive SNPs to the second stage. Our second stage included 11,273 participants from seven community-based studies, six of which provided results for common cIMT (total $N = 10{,}403$) and three of which provided results for plaque ($N = 6{,}013$). Characteristics of the participants in these studies are shown in the **Supplementary Note**.

Table 1 presents the genome-wide significant association results for the discovery, second-stage and combined meta-analyses for common cIMT and plaque. We show the discovery GWAS results for the 100-kb regions surrounding the signal SNPs for common cIMT and plaque along with the recombination rates and the known genes in that region (**Figs. 2** and **3**). We also show the study-specific findings from the combined meta-analyses of common cIMT and plaque (**Figs. 4** and **5**). Results for the suggestive loci in the meta-analyses of common cIMT and plaque are shown in **Supplementary Table 3** and **Supplementary Figures 2–5**.

Common cIMT

For common cIMT, three independent loci achieved our genome-wide significance threshold ($P < 5 \times 10^{-8}$) in the combined meta-analysis. The strongest association was for rs11781551, found on 8q24 approximately 385 kb from *ZHX2*, where the A allele (allele frequency (AF) = 0.48) was associated with lower common cIMT (β, expressing the mean difference in ln(cIMT) per copy of the modeled allele, $= -0.0078$, $P = 2.4 \times 10^{-11}$), meaning there is a 0.8% lower mean common cIMT per copy of the A allele. The second association was for rs445925, located 2.3 kb from *APOC1* on 19q13, a region that also includes *APOE*, *APOC2* and *APOC4*. The G allele (AF = 0.11) was associated with lower common cIMT ($\beta = -0.0156$, $P = 1.7 \times 10^{-8}$). The third association was for rs6601530, located within *PINX1* on 8q23.1. Each copy of the G allele (AF = 0.45) was associated with higher common cIMT ($\beta = 0.0078$, $P = 1.7 \times 10^{-8}$). We also identified a suggestive locus, marked by rs4712972 near *SLC17A4* on 6p22, where the A allele was associated with higher common cIMT ($\beta = 0.0099$, $P = 7.8 \times 10^{-8}$).

Although our genome-wide significant and suggestive SNPs from the combined meta-analyses for common cIMT explained a small proportion of the trait variance (up to 1.1%), we further constructed an additive genetic risk score (0–8 alleles) comprised of the number of common cIMT risk alleles at the four loci. In the discovery samples, the additive risk score showed graded increasing association with common cIMT across all studies, with an average increase of 9.5% in common cIMT from the lowest (0–2) to the highest (6–8) risk category (**Supplementary Fig. 6**).

Plaque

In the analysis of carotid artery plaque, two independent loci achieved the genome-wide significance threshold ($P < 5 \times 10^{-8}$) in the combined meta-analysis. We observed the most significant signal for rs17398575, situated 96.5 kb from *PIK3CG* on 7q22. Per copy of the T allele (AF = 0.25), we observed an 18% increased odds of the presence of plaque ($P = 2.3 \times 10^{-12}$). The second signal was centered at rs1878406, located 8.5 kb from *EDNRA* on 4q31. Each copy of the T allele (AF = 0.13) was associated with a 22% increased odds of the presence of plaque ($P = 6.9 \times 10^{-12}$). Furthermore, two SNPs showed suggestive evidence for association in our combined meta-analysis. The first suggestive locus was rs17045031 on 3p13, where each copy

Table 1 Discovery, second stage and combined meta-analysis for common cIMT and plaque

					Discovery GWAS					Second stage meta-analysis					Combined meta-analysis		
	SNP	Chr.	Nearest gene	Alleles	AF	β	s.e.m.	*N*	*P*	AF	β	s.e.m.	*N*	*P*	β	s.e.m.	*P*
cIMT	rs11781551	8	*ZHX2*	A/G	0.48	−0.0081	0.0014	30,894	1.3×10^{-8}	0.47	−0.0072	0.0020	10,401	0.0004	−0.0078	0.0012	2.4×10^{-11}
	rs445925	19	*APOC1*	A/G	0.11	−0.0179	0.0033	12,395	5.2×10^{-8}	0.10	−0.0116	0.0047	4,790	0.01	−0.0156	0.0028	1.7×10^{-8}
	rs6601530	8	*PINX1*	G/A	0.45	0.0078	0.0015	28,124	2.5×10^{-7}	0.46	0.0073	0.0029	4,507	0.01	0.0078	0.0014	1.7×10^{-8}
	SNP	Chr.	Nearest gene	Alleles	AF	OR (95% CI)		*N*	*P*	AF	OR (95% CI)		*N*	*P*	OR (95% CI)		*P*
Plaque	rs17398575	7	*PIK3CG*	A/G	0.25	1.17 (1.12–1.23)		23,520	2.8×10^{-10}	0.25	1.20 (1.07–1.35)		5,735	0.002	1.18 (1.12–1.23)		2.3×10^{-12}
	rs1878406	4	*EDNRA*	T/C	0.13	1.21 (1.13–1.28)		24,089	3.1×10^{-9}	0.13	1.31 (1.13–1.52)		5,738	0.0003	1.22 (1.15–1.29)		6.9×10^{-12}

The alleles listed are the coded (named first) and non-coded allele. Chr., chromosome; AF, the allele frequency for the coded allele, which is an average weighted by study size; OR, odds ratio; CI, confidence interval; *N*, effective sample size calculated by taking the sum of each study's sample size multiplied by the SNP's imputation quality. Where more than one SNP at a locus surpassed our *P* value threshold, we present the SNP with the lowest *P* value.

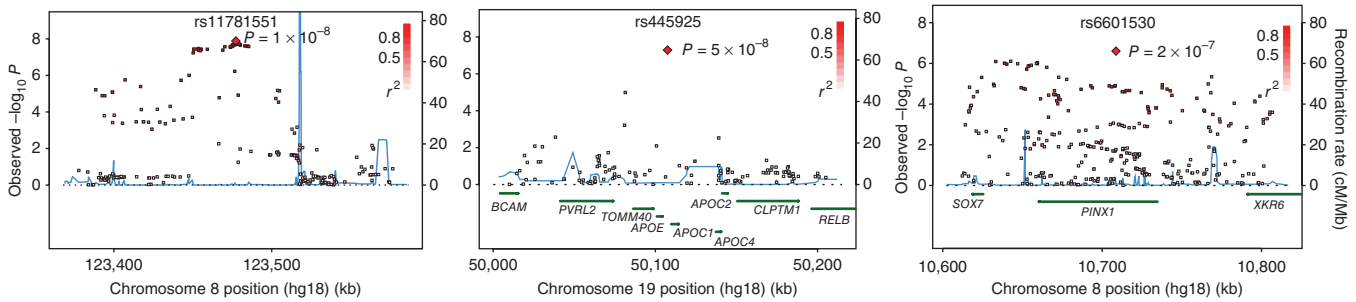

Figure 2 Regional plots for common cIMT SNPs. Plots are centered on the most significant SNP at a locus along with the meta-analysis results for SNPs in the 100-kb region surrounding it. All SNPs are plotted with their discovery meta-analysis *P* values against their genomic position, with the most significant SNP in the region indicated as a red diamond and the other SNPs shaded according to their pairwise correlation (r^2) with the signal SNP. The light blue line represents the estimated recombination rates. Gene annotations are shown as dark green lines.

of the A allele was associated with a decreased odds of the presence of plaque ($P = 1.0 \times 10^{-7}$). Our second suggestive locus was rs6511720, near *LDLR* on 19p13. Per copy of the T allele, we observed a decreased odds of the presence of plaque ($P = 3.8 \times 10^{-7}$).

For both cIMT and plaque, secondary discovery genome-wide meta-analyses conditioned on the genome-wide significant and suggestive SNPs from the combined meta-analyses did not reveal any additional associations.

Internal cIMT

No SNP achieved our significance threshold for follow up in the discovery analyses of internal cIMT. Results for internal cIMT SNPs with $P < 1.0 \times 10^{-5}$ are shown in **Supplementary Table 4**.

Cross-phenotype comparisons

Supplementary Table 5 shows the results for the genome-wide significant and suggestive SNPs from our combined meta-analyses for common cIMT and plaque across the three carotid phenotypes. The directions of association were generally consistent, and three SNPs, rs445925 (*APOC1*) from the common cIMT analysis, and rs17398575 (*PIK3CG*) and rsrs1878406 (*EDNRA*) from the plaque analysis, were associated with all three phenotypes ($P < 0.05/8/2 = 0.003$) in cross-phenotype comparisons.

Associations with coronary artery disease

We investigated the genome-wide significant and suggestive SNPs from our combined meta-analyses for common cIMT and plaque for their potential associations with coronary artery disease (CAD) in the CARDIoGRAM consortium (**Table 2**). Two SNPs from our plaque analysis had a *P* value for association with CAD less than 0.006 (0.05/8 tests). The first SNP was rs6511720 near *LDLR*, where the G allele was associated with both higher plaque risk in our study and higher CAD risk ($P = 0.0002$), and the second SNP was rs1878406 near *EDNRA*, where the C allele was associated with lower risk of plaque and lower risk of CAD ($P = 2 \times 10^{-6}$). One SNP from the common cIMT analysis, rs445925 near *APOC1*, showed a suggestive association with CAD, with the same allele (A) being associated with higher common cIMT and higher CAD risk ($P = 0.02$). Another SNP identified in the plaque analysis, rs17045031 near *LRIG1*, showed a suggestive association with CAD, with the G allele being associated with both lower odds of plaque and lower risk of CAD ($P = 0.04$).

Conversely, none of SNPs reported to be associated with coronary artery disease in the CARDIoGRAM consortium[14] had a significant association ($P < 0.00072$, using a conservative Bonferroni correction for 23 tests across three phenotypes) in our discovery meta-analyses of common cIMT, internal cIMT or plaque (**Supplementary Table 6**).

DISCUSSION

In this meta-analysis of GWAS data from nine studies of common cIMT and seven studies of plaque, we identified genome-wide significant associations between three regions and common cIMT and between two regions and the presence of carotid plaque in over 40,000 participants of European ancestry. Notably, *EDNRA*, one of our genome-wide significant regions in the combined meta-analysis of plaque, was related to multiple carotid phenotypes and was also associated with coronary artery diseases in the recent large meta-analysis by the CARDIoGRAM consortium.

Three SNPs emerged as genome-wide significant from our combined meta-analysis of common cIMT. The strongest association, on chromosome 8 (rs11781551), is an intergenic SNP located 385 kb from *ZHX2*. Members of the zinc fingers and homeobox gene families encode nuclear homodimeric transcriptional repressors that interact with the A subunit of nuclear factor-Y (NF-YA) and contain two C2H2-type zinc fingers and five homeobox DNA-binding domains. Little information about these proteins exists regarding cardiovascular disease or population studies.

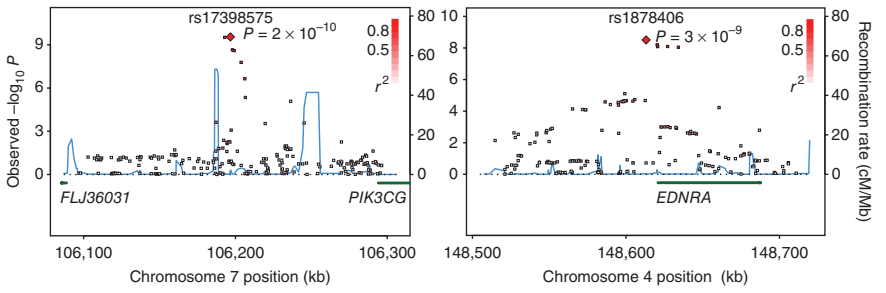

Figure 3 Regional plots for plaque SNPs. Plots are centered on the most significant SNP at each locus along with the meta-analysis results for SNPs in the 100-kb region surrounding it. All SNPs are plotted with their discovery meta-analysis *P* values against their genomic position, with the most significant SNP in the region indicated as a diamond and the other SNPs shaded according to their pairwise correlation (r^2) with the signal SNP. The light blue line represents the estimated recombination rates. Gene annotations are shown as dark green lines.

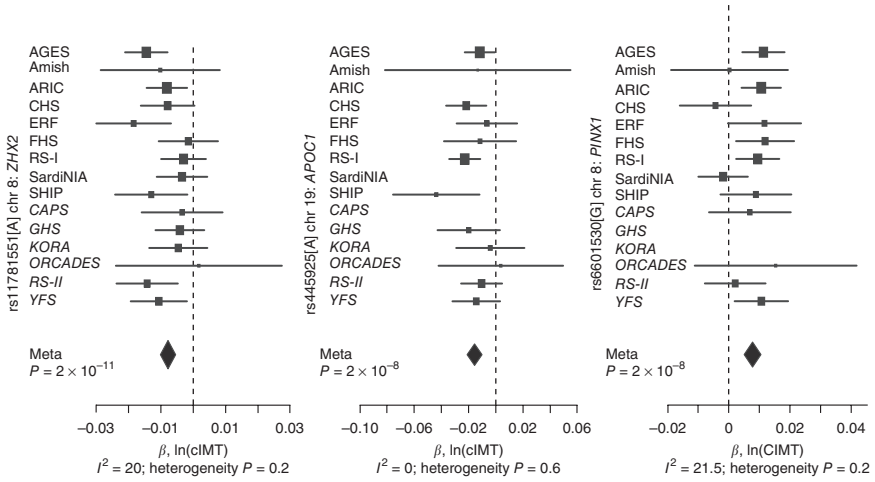

Figure 4 Forest plots for common cIMT SNP associations. Plots show the study-specific association estimates (β) and 95% confidence intervals for the nine discovery and six second-stage studies (listed in italics) presented as bars. The scale is in ln(cIMT). The association estimate and confidence interval for the meta-analysis combining the discovery and second-stage results are shown as a diamond. Blank spaces indicate occasions in which a particular study was not able to provide results for a given SNP.

A second association, on 19q13 (rs445925), fell upstream of *APOC1*. Although this region has been of interest for its role in neurological genetics because of *APOE, APOC1* has also been a frequent candidate gene for cardiovascular disease traits[15]. Although some previous studies have found associations of variation at the *APOE* locus and common cIMT[16], among four of our discovery studies that had independently measured the APOE ε variants, the correlation between rs445925 and the ε4 allele was less than 0.05. Further, models that included both the *APOE* ε4 and the *APOC1* variant indicated that *APOE* was not associated with common cIMT in these studies (**Supplementary Table 7**), whereas the *APOC1* variant still showed a significant association with common cIMT. Although *APOE* variants have been implicated in cases of familial dyslipidemia and premature atherosclerosis and, in recent GWAS, with variation in multiple lipoprotein measures[17], our results suggest that *APOC1* is the primary variant of interest for carotid traits.

The third association (rs6601530) was located in an intron of *PINX1*, encoding Pin2-interacting protein 1. This protein, a telomerase inhibitor[18] that plays a role in chromosomal segregation in mitosis[19], has been investigated in relation to cancers but was not considered a candidate gene for cardiovascular phenotypes.

The region on chromosome 6 marked by rs4712972, which includes *SLC17A4*, *SLC17A1* and *SLC17A3*, showed suggestive evidence for association with common cIMT in our combined meta-analysis. This region may merit further investigation, as recent genome-wide association studies have implicated this region with uric acid levels[20,21]. Although high uric acid levels have been associated with cardiovascular disease and all-cause mortality[22], their contribution to atherosclerotic vascular disease remains controversial[23].

For plaque, two regions were genome-wide significant in our combined meta-analysis. The first region was within 100 kb of *PIK3CG*, which encodes one of the pi3/pi4-kinase family of proteins. These proteins are important modulators of extracellular signals, including those elicited by E-cadherin–mediated cell-cell adhesion, which plays the important role of endothelin in maintenance of the structural and functional integrity of epithelia. The fact that this region was reported as a top hit in a recent GWAS of both platelet volume[24] and aggregation[25] suggests pleiotropy and highlights the interconnectedness of multiple cardiometabolic traits.

The second genome-wide significant region was near *EDNRA*. Because of the role of endothelin as a potent vasoconstrictor, the endothelin receptor, type A is a target for pharmacologic treatments to reduce blood pressure[26]. In addition, variation in this gene was associated with blood pressure[27], atherosclerosis[28] and cardiovascular disease endpoints[29] in candidate gene studies.

Two more regions showed suggestive evidence for association in our combined meta-analysis for plaque. The first region, near *LDLR*, is a particularly interesting candidate for subclinical atherosclerosis because of its role in familial hypercholesterolemia and its appearance in recent GWAS for lipid traits[30–33] and myocardial infarction[14,34]. Notably, the *LDLR* SNP recently reported to be associated with myocardial infarction (rs1122608) is located 38 kb away and is in modest linkage disequilibrium ($r^2 = 0.2$ in HapMap CEU) with the signal SNP (rs6511720) in our analysis, which also showed an association with CAD in the CARDIoGRAM consortium. The second region was in the vicinity of *LRIG1*, which negatively regulates growth factor signaling and is involved in the regulation of epidermal stem cell quiescence.

Notably, we found three loci (*APOC1*, *PIK3CG* and *EDNRA*) that were associated with all three carotid phenotypes. Among these, the *EDNRA* locus was also significantly associated with coronary artery disease in the recent large meta-analysis by the CARDIoGRAM consortium. These associations may provide important insights into the pathophysiological mechanisms relating the genes to atherosclerosis and subsequent coronary artery disease. In particular, the concordance of association with SNPs in *EDNRA* with both carotid plaque and coronary heart disease suggests a common etiology for subclinical and clinically apparent disease that warrants further investigation.

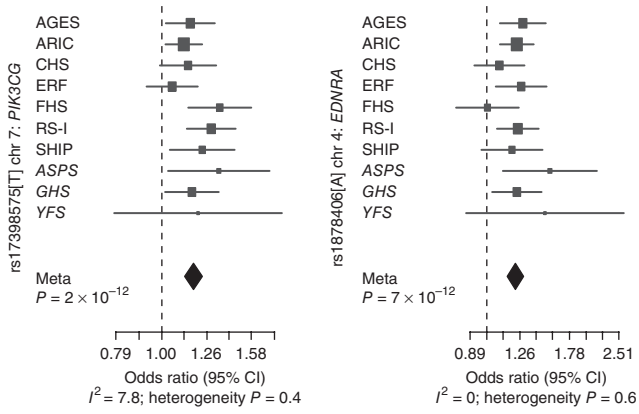

Figure 5 Forest plots for plaque SNP associations. Plots show the study-specific association estimates (odds ratios) and 95% confidence intervals for the nine discovery and three second-stage studies (listed in italics) presented as bars. The association estimate and confidence interval for the meta-analysis combining the discovery and second-stage results are shown as a diamond. Blank spaces indicate occasions in which a particular study was not able to provide results for a given SNP.

Table 2 Association of genome-wide significant and suggestive common cIMT and plaque SNPs with CAD in the CARDIoGRAM consortium

Source	SNP	Chr.	Nearest gene	Allele	AF	OR (95% CI)	*N*	*P*
cIMT	rs11781551	8	*ZHX2*	G	0.53	1.02 (0.99–1.05)	83,379	0.2
	rs445925	19	*APOC1*	G	0.91	1.11 (1.02–1.20)	34,216	0.02
	rs6601530	8	*PINX1*	G	0.40	1.02 (0.99–1.05)	79,512	0.1
	rs4712972	6	*SLC17A4*	G	0.86	1.02 (0.97–1.06)	84,001	0.5
Plaque	rs17398575	7	*PIK3CG*	G	0.73	0.98 (0.95–1.01)	83,028	0.2
	rs1878406	4	*EDNRA*	C	0.86	0.91 (0.87–0.95)	81,804	2×10^{-6}
	rs6511720	19	*LDLR*	G	0.90	1.13 (1.06–1.21)	56,420	0.0002
	rs17045031	3	*LRIG1*	G	0.94	1.09 (1.00–1.18)	80,655	0.04

The allele listed is the coded allele in the CARDIoGRAM consortium meta-analysis. AF, allele frequency for the coded allele; Chr., chromosome; OR, odds ratio; CI, confidence interval; *N*, sample size.

The strengths of the current study include the large sample size, the population-based designs, the collaboratively designed pre-specified analysis plan and the high quality of both genotyping and phenotyping. Further, our ability to relate our findings to the outcome of CAD in a large independent meta-analysis provides important additional context to our results. These associations are unlikely to be caused by population stratification because the discovery sample was restricted to individuals of European ancestry and was also investigated for global latent population substructure.

The study also has limitations. A single cross-sectional IMT assessment was used in all studies, and ultrasound protocols varied across the participating studies. For example, the plaque definition included the presence of any plaque in most studies but included stenosis >25% in others. The heterogeneity of measurement techniques may have compromised our ability to detect small associations. Despite this heterogeneity, the ability to detect consistent genetic associations for several carotid measures suggests that additional signals may be discovered in future studies using a larger sample size or a higher resolution technique, such as magnetic resonance imaging. Further, few studies had internal cIMT measures, as these measures are more difficult to obtain than common cIMT measurements, and this therefore limited our ability to discover associations with this phenotype. Although our sample size was reasonably large, we still had limited power to detect associations with small effect sizes. Genome-wide association studies are known for revealing associations with common variants and may miss rare variants not covered by the commercial genotyping arrays. For instance, the sparse coverage of the *APOC1* and *LDLR* gene regions resulted in varying imputation quality and a lower effective sample size for the analysis of these two regions.

Because we did not conduct follow-up fine mapping of the results and because some SNPs were distant from known genes, it is likely that the identified SNPs are not causal variants, but, instead, may be in linkage disequilibrium with variants that were not analyzed. Because some of our associations attained genome-wide significant *P* values only in the combined meta-analysis, confirmation of our findings in other populations and further exploration of these genomic regions with dense genotyping, expression and translational studies will be required to better understand the role of these genes in subclinical atherosclerotic disease.

In summary, our meta-analysis of GWAS data from nine community-based studies has revealed five new loci for common cIMT and plaque. These loci implicate low-density lipoprotein metabolism (*APOC1*), endothelial dysfunction (*EDNRA*), platelet biology (*PIK3CG*) and telomere maintenance (*PINX1*) as biological traits associated with subclinical atherosclerosis. Two of our identified loci were also associated with coronary artery disease in the recent large meta-analysis by the CARDIoGRAM consortium. Exploring the molecular, cellular and clinical consequences of genetic variation at these loci may yield new insights into the pathophysiology of clinical and subclinical cardiovascular disease.

URLs. METAL, http://www.sph.umich.edu/csg/abecasis/Metal/.

METHODS

Methods and any associated references are available in the online version of the paper at http://www.nature.com/naturegenetics/.

Note: Supplementary information is available on the Nature Genetics website.

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AUTHOR CONTRIBUTIONS

Study concept and design: J.C.B., M. Kavousi, N.F., G.R.A., L.A.C., T.L., S.R.H., K.N., C.H., O.R., C.S.F., V.N., R.B.S., T.A., M.S., M. Kähönen, P.S.W., A.R.S., J.I.R., J.S., D.H.O., E.G.L., B.M.P., M.U., E.B., J.V., W.K., S. Blankenberg, A.B.N., J.W., C.v.D., A. Scuteri, V.G., C.J.O.

Acquisition of the data: J.C.B., A.I., G.R.A., U.S., W.S.P., H.S.M., R.S., T.L., B.O., S. Bevan, E.-M.S., O.R., C.M., H.V., B.T., F.R., K.E.P., H.E.W., R.B.S., M.D., A.P., T.A., S.K., M.P.R., K.T., A.U., M.S., M. Kähönen, T.I., P.S.W., M.O., J.L., A.R.S., G.E., J.I.R., A.H., J.S., T.Z., G.U., F.E., L.J.L., R.B.D., D.H.O., J.T., T.B.H., P.A.W., B.M.P., J.F.P., W.R., E.B., N.K., H.S., J.F.W., J.V., W.K., S. Blankenberg, A.B.N., G. Heiss, C.v.D., A. Scuteri, G. Homuth, B.D.M., V.G., C.J.O.

Statistical analysis and interpretation of the data: J.C.B., M. Kavousi, N.F., A.I., G.R.A., A.V.S., L.A.C., J.E.H., T.L., J.B., S.R.H., A.D., K.N., C.H., O.R., A. Schillert, S.S., Y.-C.C., K.R., V.N., E.H., K.E.P., T.A., S.D., S.K., P.S.W., C.C.W., R.B.D., A.Z., R.K.C., H.S., C.J.O.

Drafting of the manuscript: J.C.B., M. Kavousi, N.F., A.I., K.N., O.R., M. Kähönen, J.F.P., J.V., C.J.O.

Critical revision of the manuscript: J.C.B., M. Kavousi, N.F., A.I., G.R.A., U.S., W.S.P., L.A.C., H.S.M., R.S., T.L., J.B., T.M., S.R.H., A.D., K.N., O.R., C.M., H.V., B.T., K.R., F.R., V.N., H.E.W., R.B.S., M.D., A.P., S.D., M.P.R., K.T., A.U., D.J.C., M.S., M. Kähönen, T.I., J.L., G.E., J.I.R., A.H., J.S., F.E., L.J.L., R.B.D., D.H.O., C.B., A.Z., E.G.L., R.K.C., T.B.H., B.M.P., J.F.P., X.L., W.R., E.B., J.V., W.K., S. Blankenberg, J.W., C.v.D., A. Scuteri, G. Homuth, B.D.M., V.G., C.J.O.

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Joshua C Bis[1,80], Maryam Kavousi[2,3,80], Nora Franceschini[4,80], Aaron Isaacs[5,6,80], Gonçalo R Abecasis[7,80], Ulf Schminke[8,80], Wendy S Post[9,80], Albert V Smith[10,80], L Adrienne Cupples[11,12,80], Hugh S Markus[13], Reinhold Schmidt[14], Jennifer E Huffman[15], Terho Lehtimäki[16,17], Jens Baumert[18], Thomas Münzel[19], Susan R Heckbert[20,21], Abbas Dehghan[2,3], Kari North[22], Ben Oostra[6,23], Steve Bevan[13], Eva-Maria Stoegerer[14], Caroline Hayward[15], Olli Raitakari[24,25], Christa Meisinger[18], Arne Schillert[26], Serena Sanna[27], Henry Völzke[28], Yu-Ching Cheng[29], Bolli Thorsson[10], Caroline S Fox[12,30], Kenneth Rice[31], Fernando Rivadeneira[3,32], Vijay Nambi[33–35], Eran Halperin[36,37], Katja E Petrovic[38], Leena Peltonen[39,40], H Erich Wichmann[41], Renate B Schnabel[19], Marcus Dörr[42], Afshin Parsa[43], Thor Aspelund[10,44], Serkalem Demissie[11], Sekar Kathiresan[45–47], Muredach P Reilly[48], the CARDIoGRAM Consortium, Kent Taylor[49], Andre Uitterlinden[2,3,32], David J Couper[50], Matthias Sitzer[51], Mika Kähönen[17,52], Thomas Illig[53,54], Philipp S Wild[19], Marco Orru[27,55], Jan Lüdemann[56], Alan R Shuldiner[43,57], Gudny Eiriksdottir[10], Charles C White[11], Jerome I Rotter[49], Albert Hofman[2,3], Jochen Seissler[58], Tanja Zeller[19], Gianluca Usala[27], Florian Ernst[59], Lenore J Launer[60], Ralph B D'Agostino Sr[61], Daniel H O'Leary[62], Christie Ballantyne[33], Joachim Thiery[63,64], Andreas Ziegler[26], Edward G Lakatta[65], Ravi Kumar Chilukoti[59], Tamara B Harris[60], Philip A Wolf[12,66], Bruce M Psaty[1,21,67,68], Joseph F Polak[69], Xia Li[4], Wolfgang Rathmann[70], Manuela Uda[27], Eric Boerwinkle[71], Norman Klopp[53], Helena Schmidt[72], James F Wilson[73], Jorma Viikari[74,75], Wolfgang Koenig[76], Stefan Blankenberg[19], Anne B Newman[77,80], Jacqueline Witteman[2,3,80], Gerardo Heiss[4,80], Cornelia van Duijn[3,5,6,80], Angelo Scuteri[65,80], Georg Homuth[59,80], Braxton D Mitchell[43,78,80], Vilmundur Gudnason[10,44,80] & Christopher J O'Donnell[12,79,80]

[1]Cardiovascular Health Research Unit and Department of Medicine, University of Washington, Seattle, Washington, USA. [2]Department of Epidemiology, Erasmus Medical Center, Rotterdam, The Netherlands. [3]Netherlands Genomics Initiative (NGI)-Sponsored Netherlands Consortium for Healthy Aging (NCHA), Rotterdam, The Netherlands. [4]Department of Epidemiology, University of North Carolina Chapel Hill, Chapel Hill, North Carolina, USA. [5]Genetic Epidemiology Unit, Department of Epidemiology, Erasmus University Medical Center, Rotterdam, The Netherlands. [6]Centre for Medical Systems Biology, Leiden, The Netherlands. [7]Center for Statistical Genetics, Department of Biostatistics, University of Michigan School of Public Health, Ann Arbor, Michigan, USA. [8]Department of Neurology, Ernst Moritz Arndt University Greifswald, Greifswald, Germany. [9]Division of Cardiology, Department of Medicine, Johns Hopkins University, Baltimore, Maryland, USA. [10]Icelandic Heart Association, Kopavogur, Iceland. [11]Department of Biostatistics, Boston University School of Public Health, Boston, Massachusetts, USA. [12]National Heart, Lung, and Blood Institute's Framingham Heart Study, Framingham, Massachusetts, USA. [13]Centre for Clinical Neuroscience, St. George's University of London, London, UK. [14]Department of Neurology, Medical University Graz, Graz, Austria. [15]Medical Research Council (MRC) Human Genetics Unit, Institute of Genetics and

Molecular Medicine, Western General Hospital, Edinburgh, Scotland. [16]Department of Clinical Chemistry, University of Tampere, Tampere, Finland. [17]Tampere University Hospital, Tampere, Finland. [18]Institute of Epidemiology II, Helmholtz Zentrum München, German Research Center for Environmental Health, Neuherberg, Germany. [19]Department of Medicine 2, University Medical Center Mainz, Mainz, Germany. [20]Cardiovascular Health Research Unit and Department of Epidemiology, University of Washington, Seattle, Washington, USA. [21]Group Health Research Institute, Group Health, Seattle, Washington, USA. [22]Carolina Center for Genome Sciences, University of North Carolina, Chapel Hill, North Carolina, USA. [23]Department of Clinical Genetics, Erasmus University Medical Center, Rotterdam, The Netherlands. [24]Research Centre of Applied and Preventive Cardiovascular Medicine, University of Turku, Turku, Finland. [25]Department of Clinical Physiology, Turku University Hospital, Turku, Finland. [26]Institute for Medical Biometry and Statistics, University of Lübeck, Universitätsklinikum Schleswig-Holstein, Campus Lübeck, Lübeck, Germany. [27]Institute of Genetic and Biomedical Research (IRGB), National Research Council (CNR), Cittadella Universitaria di Monserrato, Monserrato, Cagliari, Italy. [28]Institute for Community Medicine, Ernst Moritz Arndt University Greifswald, Greifswald, Germany. [29]Division of Endocrinology, Diabetes, and Nutrition, University of Maryland School of Medicine, Baltimore, Maryland, USA. [30]Division of Endocrinology, Metabolism, and Diabetes, Brigham and Women's Hospital and Harvard Medical School, Boston, Massachusetts, USA. [31]Department of Biostatistics, University of Washington, Seattle, Washington, USA. [32]Department of Internal Medicine, Erasmus Medical Center, Rotterdam, The Netherlands. [33]Baylor College of Medicine, Houston, Texas, USA. [34]Center for Cardiovascular Prevention, The Methodist DeBakey Heart and Vascular Center, Houston, Texas, USA. [35]Ben Taub General Hospital, Houston, Texas, USA. [36]The Blavatnik School of Computer Science, Tel-Aviv University, Tel Aviv, Israel. [37]The International Computer Science Institute, Berkeley, California, USA. [38]Department of Neurology, General Hospital and Medical University Graz, Graz, Austria. [39]Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Hinxton, Cambridge, UK. [40]Institute for Molecular Medicine Finland, Biomedicum, University of Helsinki and National Institute for Health and Welfare, Helsinki, Finland. [41]Institute of Epidemiology I, Helmholtz Zentrum München, German Research Center for Environmental Health, Neuherberg, Germany. [42]Department of Internal Medicine B, Ernst Moritz Arndt University Greifswald, Greifswald, Germany. [43]Department of Medicine, University of Maryland School of Medicine, Baltimore, Maryland, USA. [44]University of Iceland, Reykjavik, Iceland. [45]Cardiovascular Research Center and Cardiology Division, Massachusetts General Hospital, Boston, Massachusetts, USA. [46]Center for Human Genetic Research, Massachusetts General Hospital, Boston, Massachusetts, USA. [47]Program in Medical and Population Genetics, Broad Institute of MIT and Harvard, Cambridge, Massachusetts, USA. [48]The Cardiovascular Institute, University of Pennsylvania, Philadelphia, Pennsylvania, USA. [49]Medical Genetics Institute, Cedars-Sinai Medical Center, Los Angeles, California, USA. [50]Department of Biostatistics, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA. [51]Department of Neurology, Klinikum Herford, Herford, Germany. [52]Department of Clinical Physiology, University of Tampere, Tampere, Finland. [53]Research Unit of Molecular Epidemiology, Helmholtz Zentrum Muenchen, German Research Center for Environmental Health, Neuherberg, Germany. [54]Biobank of the Hannover Medical School, Hannover Medical School, Hannover, Germany. [55]Cardiology Operating Unit, Division of Medicine, Santa Barbara Hospital, Iglesias, Italy. [56]Institute of Clinical Chemistry and Laboratory Medicine, Ernst Moritz Arndt University Greifswald, Greifswald, Germany. [57]Geriatric Research and Education Clinical Center, Veterans Administration Medical Center, Baltimore, Maryland, USA. [58]Ludwig-Maximilians University of Munich, Medical Clinic Innenstadt, Diabetes Center, Munich, Germany. [59]Interfaculty Institute for Genetics and Functional Genomics, Ernst Moritz Arndt University Greifswald, Greifswald, Germany. [60]Intramural Research Program, Laboratory of Epidemiology, Demography and Biometry, National Institute on Aging, National Institutes of Health, Bethesda, Maryland, USA. [61]Department Mathematics and Statistics, Boston University, Boston, Massachusetts, USA. [62]St. Elizabeth's Medical Center, Tufts University School of Medicine, Boston, Massachusetts, USA. [63]Institute of Laboratory Medicine, Clinical Chemistry and Molecular Diagnostics, University Hospital Leipzig, Leipzig, Germany. [64]Leipzig Research Center of Civilization Diseases, Medical Faculty, University of Leipzig, Leipzig, Germany. [65]Gerontology Research Center, National Institute on Aging, Baltimore, Maryland, USA. [66]Department of Neurology, Boston University School of Medicine, Boston, Massachusetts, USA. [67]Department of Epidemiology, University of Washington, Seattle, Washington, USA. [68]Department of Health Services, University of Washington, Seattle, Washington, USA. [69]Department of Radiology, Tufts University School of Medicine, Boston, Massachusetts, USA. [70]Institute of Biometrics and Epidemiology, German Diabetes Center, Leibniz Center for Diabetes Research at Heinrich Heine University Düsseldorf, Düsseldorf, Germany. [71]University of Texas, School of Public Health, Human Genetics Center, Houston, Texas, USA. [72]Institute of Molecular Biology and Biochemistry, Medical University Graz, Graz, Austria. [73]Centre for Population Health Sciences, University of Edinburgh, Teviot Place, Edinburgh, Scotland. [74]Department of Medicine, University of Turku, Turku, Finland. [75]Turku University Hospital, Turku, Finland. [76]Department of Internal Medicine II–Cardiology, University of Ulm Medical Center, Ulm, Germany. [77]Department of Epidemiology, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, Pennsylvania, USA. [78]Department of Epidemiology & Public Health, University of Maryland School of Medicine, Baltimore, Maryland, USA. [79]Cardiology Division, Department of Medicine, Massachusetts General Hospital, Harvard Medical School, Boston Massachusetts, USA. [80]These authors contributed equally to this work. Correspondence should be addressed to J.C.B. (joshbis@uw.edu) or C.J.O. (odonnellc@nhlbi.nih.gov).

ONLINE METHODS

Participating studies. Our analyses were performed within the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) Consortium[35]. Details on the nine participating discovery studies and seven participating second stage studies can be found in the **Supplementary Note**.

Carotid artery phenotypes. Each study evaluated the carotid arteries with high-resolution B-mode ultrasonography using previously described reading protocols. For these analyses, we used data from the baseline examination or the first examination in which carotid ultrasonography was obtained. Our primary analysis concerned the common carotid artery using the intima-media thickness, typically summarized as the mean of the maximum of several measurements. For most studies, this was an average of multiple measurements from both the left and right arteries. All studies measured the far wall and several also included the near wall. We also examined the atherosclerotic thickening of the carotid artery wall, defined in seven of the nine studies by either the presence of plaque or the proxy measure of stenosis >25%. Secondary analyses considered the internal cIMT, which was characterized in three of the nine studies. As with the common carotid analyses, we used the mean of the maximal measurements from the near and far walls of the internal carotid arteries on both the left and right sides, which summarized the 1–12 measurements taken per participant. Specific details for each study's ultrasound, reading and plaque definition protocols are provided in the **Supplementary Note**.

Genotyping and imputation. The nine studies in these analyses used commercial genotyping platforms available from Illumina or Affymetrix. Each study performed genotyping quality control checks and imputed the approximately 2.5 million polymorphic autosomal SNPs described in the HapMap CEU population for each participant using available imputation methods. Details of per-study genotyping, imputation and quality control procedures are available in the **Supplementary Note**.

Statistical analysis within studies. Each study independently implemented a predefined GWAS analysis plan. For the continuous measures of common and internal cIMT, we evaluated cross-sectional associations of ln(IMT) and genetic variation using linear regression models (or linear mixed effects models in the Amish, FHS and ERF data to account for family relatedness). For each of the 2.5 million SNPs, each study fit additive genetic models regressing trait on genotype dosage (0–2 copies of the variant allele). For the dichotomous outcome of plaque, each study used logistic regression models (or general estimating equations clustering on family to account for familial correlations). In our primary analyses, all studies were adjusted for age and sex. Some studies made additional adjustments, including adjusting for study site, familial structure or for whether the DNA had been whole-genome amplified or not. Additional details of the statistical analyses are available in the **Supplementary Note**.

Discovery meta-analysis. We conducted a meta-analysis of regression estimates and standard errors using an inverse-variance weighting approach as implemented in METAL (see URLs). After verification of strand alignment across studies and after quality control, filtering and imputation within each study, we restricted our meta-analysis to autosomal SNPs that were reported in at least two studies and had an average minor allele frequency of at least 1%. Prior to the meta-analysis, we calculated a genomic inflation factor (λ_{GC}) for each study to screen for cryptic population substructure or undiagnosed irregularities that might have inflated the test statistics. The inflations were low, with λ_{GC} being below 1.09 in all studies. We applied 'genomic control' to each study whose genomic inflation factor was greater than 1.00 by multiplying all of the standard errors by the square root of the study-specific λ_{GC}. For cIMT, we expressed the association of each SNP and ln(IMT) as the regression slope (β), its standard error (s.e.m. (β)) and a corresponding P value. For the presence of plaque, we calculated a meta-analysis log odds ratio, 95% confidence interval and P value. In this case, the odds ratio represents the increase or decrease in the odds of plaque for each additional copy of the SNP's coded allele. Standardized gene and SNP annotations were created using a PERL program[36].

For follow up, we decided *a priori* on a significance threshold of $P < 4 \times 10^{-7}$, which corresponds to not more than one expected false positive finding over 2.5 million tests.

Second-stage meta-analysis. Second-stage samples were drawn from several external studies with available genetic data and measures of cIMT (six studies) or plaque (three studies). We provided each collaborating second-stage study with a list of all SNPs that attained genome-wide significant P values for common cIMT, internal cIMT or plaque and combined the results from these studies using a fixed-effects meta-analysis as described above.

Combined meta-analysis. Finally, we combined the results from the discovery and second-stage analyses using inverse-variance weighting, as described above, and considered SNPs with $P < 5 \times 10^{-8}$ as genome-wide significant.

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