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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

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Keywords

genome-wide association study; genetic epidemiology; genetics; subclinical atherosclerosis; carotid intima media thickness; cardiovascular disease; cohort study; meta-analysis; risk

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

Measures of subclinical atherosclerosis, 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 cIMT and plaque, reflecting a thickening of the carotid artery wall or the presence of large irregular arterial wall deposits, respectively, are established measures of subclinical atherosclerotic

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disease. While 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.[7] While 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 provide evidence that carotid plaque is a better predictor of future cardiovascular disease risk than common cIMT. [8–10]

Numerous family studies 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 single nucleotide polymorphisms (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 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 to the approximately 2.5 million autosomal SNPs in the Phase II HapMap CEU reference panel. In addition, we followed-up our discovery findings in a second stage that included 11,273 participants from 7 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 to 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 between common cIMT and internal cIMT, from 0.36 to 0.67 (Supplementary Table 2).

The *a priori* threshold for genome-wide significance was 5×10^{-8}, and a p-value > 5×10^{-8} but $<4\times10^{-7}$, corresponding to not more than one expected false positive finding over 2.5 million tests, was considered suggestive evidence for association in our analyses.

Figure 1A provides a plot of $-\log_{10}$ (p-values) 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. 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 in Figure 1B and Supplementary Figure 1, respectively. Overall, from the discovery meta-analysis of common cIMT and plaque, we carried forward 3 genome-wide significant SNPs and 5 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.

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

Common cIMT

For common cIMT, 3 independent loci achieved our genome-wide significance threshold ($p<5\times10^{-8}$) 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=−0.0078, $p= 2.4\times10^{-11}$), i.e. 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 (β=-0.0156, $p= 1.7\times10^{-8}$). The third association was for rs6601530, located within the *PINX1* gene on 8q23.1. Each copy of the G allele (AF = 0.45) was associated with higher common cIMT (β=0.0078, $p= 1.7\times10^{-8}$). We also identified a suggestive locus, marked by rs4712972 near the *SLC17A4* gene on 6p22, where the A allele was associated with higher common cIMT (β=0.0099, $p= 7.8\times10^{-8}$).

While our genome-wide significant and suggestive SNPs from 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 Figure 6).

Plaque

In analysis of carotid artery plaque, 2 independent loci achieved the genome-wide significance threshold ($p<5\times10^{-8}$) in the combined meta-analysis.

The most significant signal was observed for rs17398575, situated 96.5 kb from the *PIK3CG* gene on 7q22. Per copy of the T allele (AF=0.25), we observed an 18% increased odds of presence of plaque ($p=2.3\times10^{-12}$). 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 ($p= 6.9\times10^{-12}$). Furthermore, two SNPs showed suggestive evidence for association in our combined meta-analysis. The first suggestive locus was rs17045031 on 3p13 where each copy of the A allele was associated with decreased odds of the presence of plaque ($p= 1.0\times10^{-7}$). Our second suggestive locus was

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rs6511720, near *LDLR* on 19p13. Per copy of the T allele we observed a decreased odds of the presence of plaque ($P=3.8\times10^{-7}$).

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, 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 rs1878406, near *EDNRA* where the C allele was associated with lower risk of plaque and lower risk of CAD ($p=2\times10^{-6}$). One SNP from 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 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 (i.e., a p-value less than 0.00072, 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 G WAS data from nine studies of common cIMT and seven studies of plaque, we identified genome-wide significant associations between 3 regions and common cIMT and between 2 regions and the presence of carotid plaque in over 40,000

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participants of European ancestry. Interestingly, *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 the ZHX2 gene. Members of this gene family are 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.

A second association, on 19q13 (rs445925), fell upstream of the *APOC1* gene. While this region has been of interest for its role in neurological genetics because of the *APOE* gene, it is also been 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 4 of our discovery studies that had independently measured the APOE epsilon variants, the correlation between rs445925 and the e4 allele was less than 0.05. Further, models that included both the *APOE* e4 and the *APOC1* variant indicated that the *APOE* gene was not associated with common cIMT in these studies (Supplementary Table 7), while the *APOC1* variant still showed a significant association with common cIMT. While *APOE* variants have been implicated in cases of familial dyslipidemia and premature atherosclerosis and in recent genome-wide association studies 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 the Pin2-interacting protein 1 (*PINX1*) gene. The 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 the *SLC17A4, SLC17A1,* and *SLC17A3* genes 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] the contribution to atherosclerotic vascular disease remains controversial.[23]

Plaque associations

For plaque, two regions were genome-wide significant in our combined meta-analysis. The first region was within 100kb of the *PIK3CG* gene, 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 an important role of endothelin in maintenance of the structural and functional integrity of epithelia. The

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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 the *EDNRA* gene. 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 the 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 the *LDLR* gene is a particularly interesting candidate for subclinical atherosclerosis because of its role in familial hypercholesterolemia and its appearance in recent genome-wide association studies for lipid traits [30–33] and myocardial infarction. [14,34] Notably, the *LDLR* SNP recently reported to be associated with MI (rs1122608) is located 38 kb away and is in modest LD (r^2=0.2 in HapMap CEU) with the signal SNP (rs6511720) in our analysis that also showed an association with CAD in the CARDIoGRAM consortium. The second was in the vicinity of *LRIG1*, which negatively regulates growth factor signaling and is involved in the regulation of epidermal stem cell quiescence.

Interestingly, 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 CHD suggests a common etiology for subclinical and clinically apparent disease that warrants further investigation.

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 due to population stratification since the discovery sample was restricted to whites of European origin 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 participating studies. For example, plaque definition included the presence of any plaque in most studies and stenosis greater than 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 utilizing a larger sample size or a higher resolution technique such as magnetic resonance imaging. Further, few studies had internal cIMT measures since

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these are more difficult to obtain than common cIMT measurements and thus 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 5 new loci for common cIMT and plaque. These loci implicate LDL metabolism (*APOC1*), endothelial dysfunction (*EDNRA*), platelet biology (*PIK3CG*), and telomere maintenance (*PINX1*). Two of our identified loci are 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 novel insights into the pathophysiology of clinical and subclinical cardiovascular disease.

Supplementary Material

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Genome-wide Association Studies of Carotid Intima Media Thickness and Plaque: Meta-analysis from the CHARGE Consortium

Carotid intima media thickness (cIMT) and plaque determined by ultrasonography are established measures of subclinical atherosclerosis that each predict 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 7 additional studies. In the combined meta-analysis, we identified three genomic regions associated with common cIMT and two different regions associated with the presence of carotid plaque ($p < 5\times10^{-8}$). 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 CARDIoGRAM consortium. Our findings may provide new insight into pathways leading to subclinical atherosclerosis and subsequent cardiovascular events.

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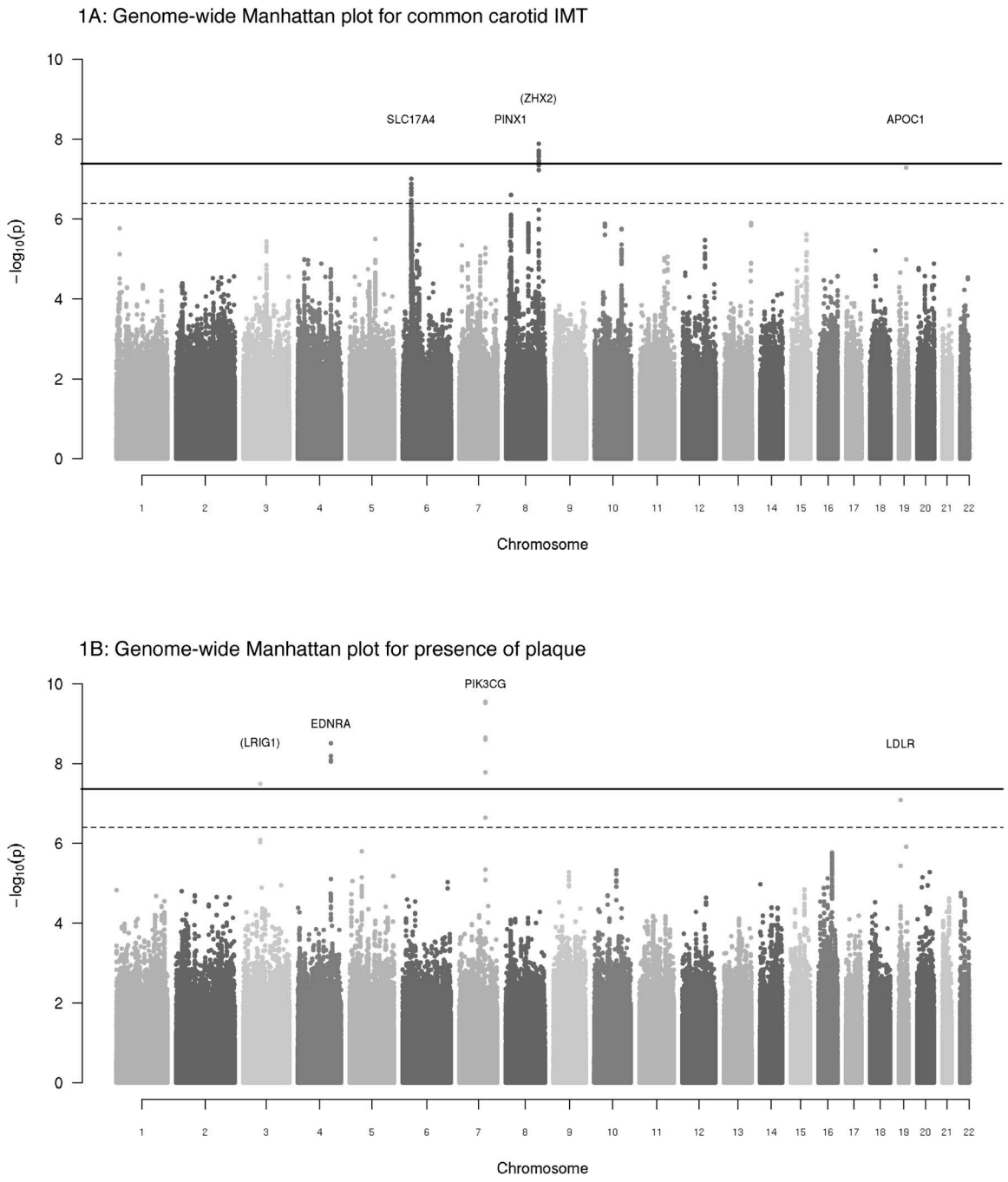

Figure 1. A–B: Genome-wide Manhattan plots for common cIMT and plaque

Plots show the individual p-values (based on discovery meta-analysis) against their genomic position for common carotid IMT (Figure 1A), the presence of plaque (Figure 1B). Within each chromosome, shown on the *x*-axis, the results are plotted left to right from the p-terminal end. The dashed line indicates the threshold for follow-up, $p<4 \times 10^{-7}$ and the solid line indicates the threshold for genome-wide significance, $p<4 \times 10^{-8}$. The nearest genes are indicated above points that surpassed our genome-wide significance threshold; genes that are greater than 100 kb from the signal SNP are indicated in parentheses.

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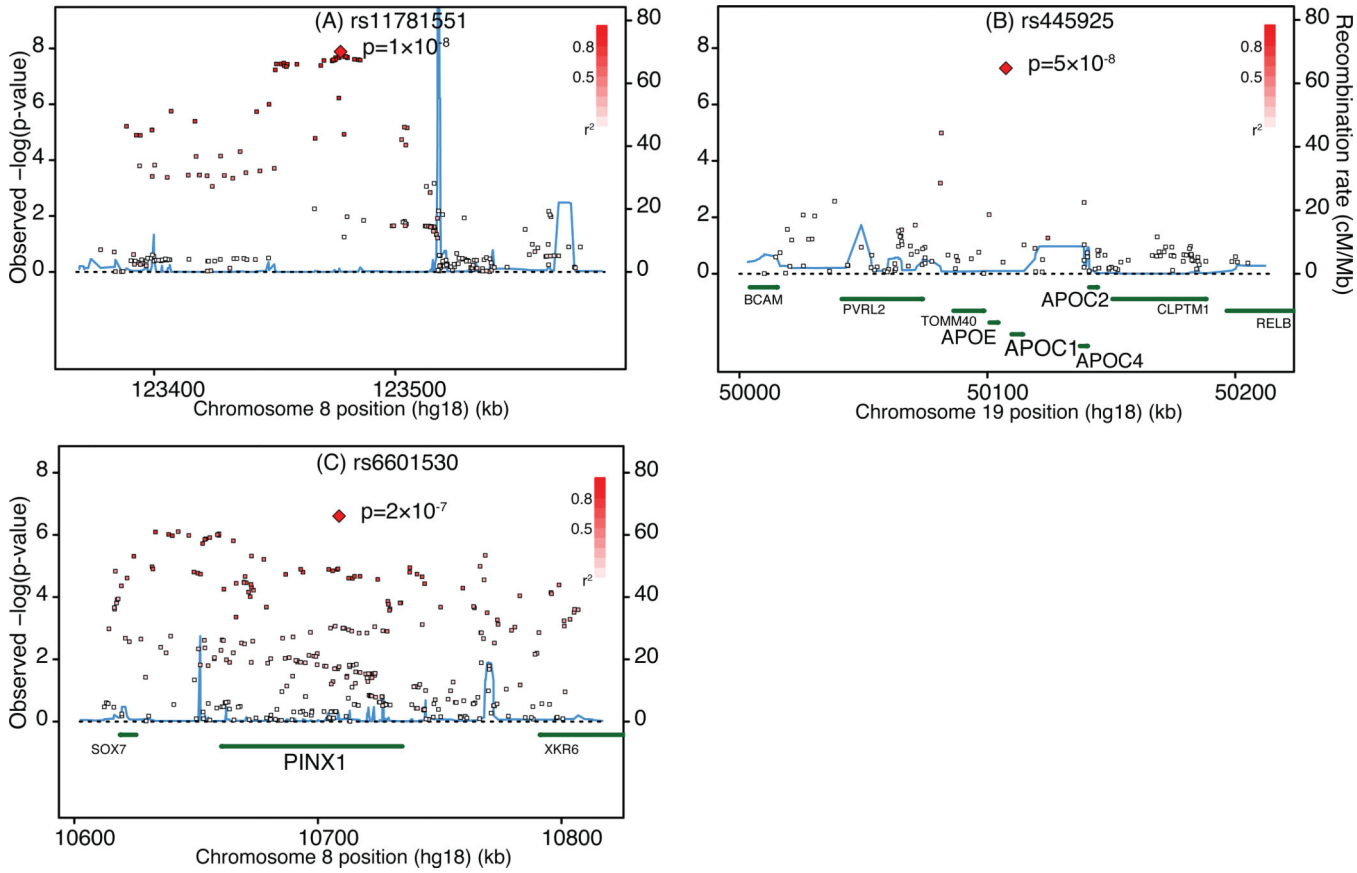

Figure 2. Regional plots for common carotid IMT SNPs

Plots are centered on the most significant SNP at locus along with the meta-analysis results for SNPs in the 100kb 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 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.

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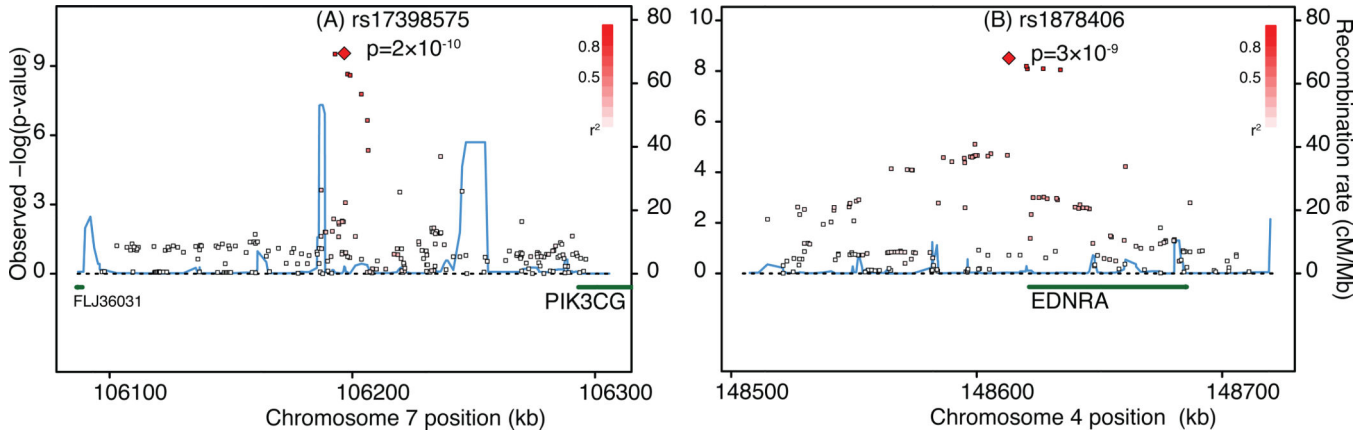

Figure 3. Regional plots for plaque SNPs

Plots are centered on the most significant SNP at each locus along with the meta-analysis results for SNPs in the 100kb 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 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.

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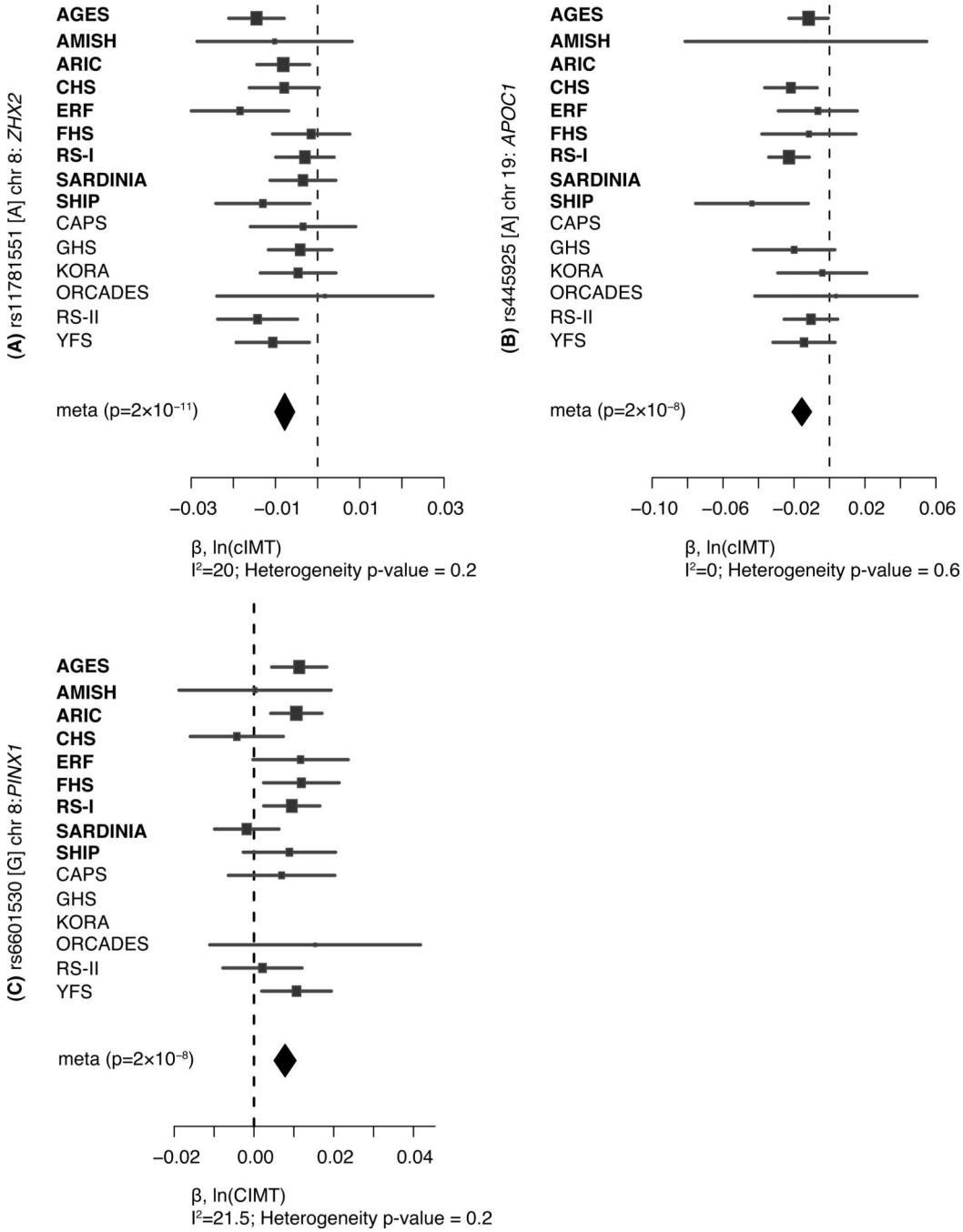

Figure 4. Forest plots for common carotid IMT SNP associations

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

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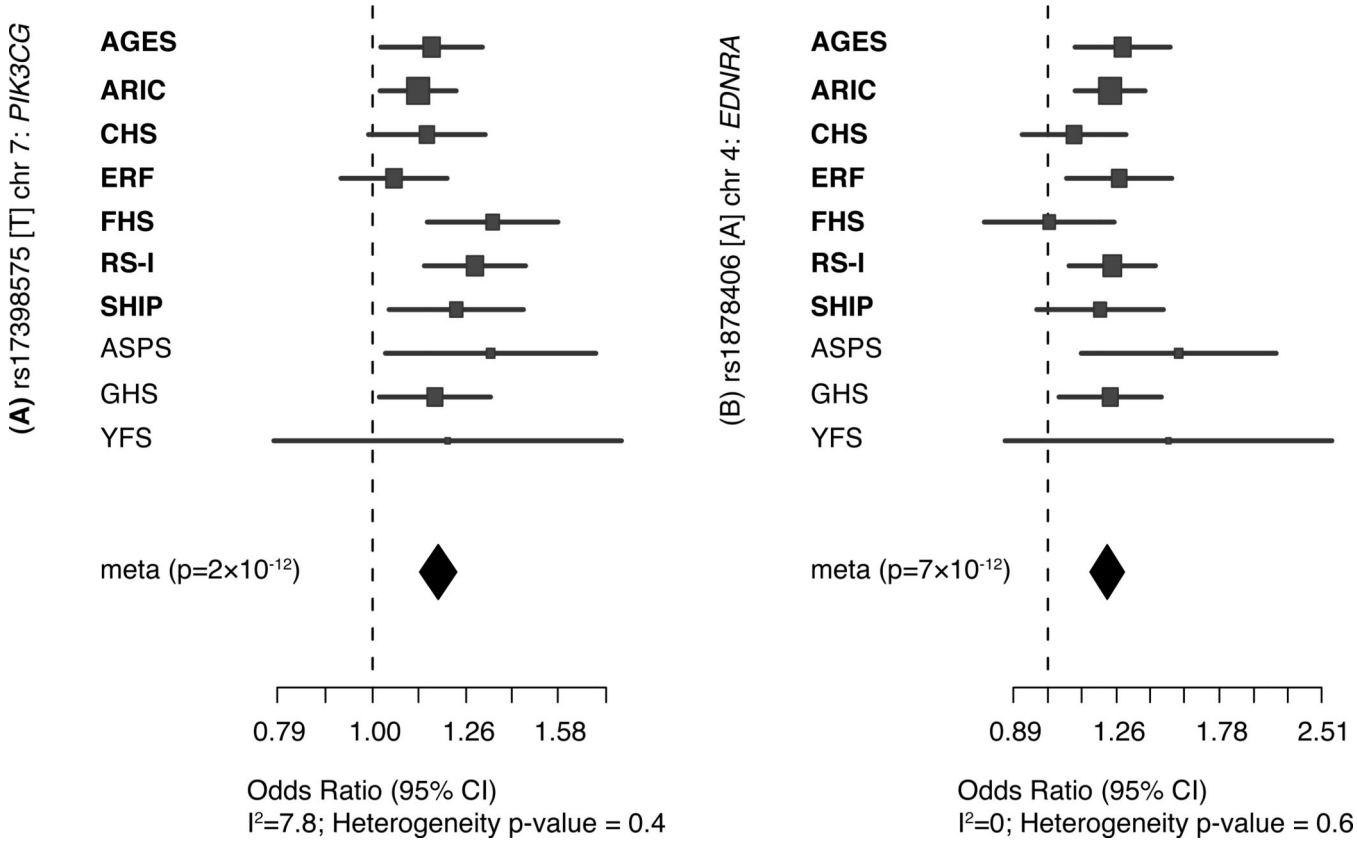

Figure 5. Forest plots for plaque SNP associations

Plots show the study-specific association estimates (OR) and 95% confidence intervals for the nine discovery and second stage studies, presented as bars. The association estimate and confidence interval for the meta-analysis combining discovery and second stage results is presented as a diamond. Blank spaces indicate occasions in which a particular study was not able to provide results for a given SNP.

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Table 1

A: Discovery, Second Stage, and Combined meta-analysis for common cIMT and plaque

				Discovery GWAS (cIMT)					**Second Stage Meta-analysis (cIMT)**					**Combined Meta-analysis (cIMT)**		
SNP	**Chr**	**Nearest gene**	**Alleles**	**AF**	**β**	**SE**	**N**	**p-value**	**AF**	**β**	**SE**	**N**	**p-value**	**β**	**SE**	**p-value**
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}

				Discovery GWAS (plaque)				Second Stage Meta-analysis (plaque)				Combined Meta-analysis (plaque)	
SNP	Chr	Nearest gene	Alleles	AF	OR (95% CI)	N	p-value	AF	OR (95% CI)	N	p-value	OR (95% CI)	p-value
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}

Alleles indicates the coded (named first) & non-coded allele; AF indicates allele frequency for the coded allele, an average weighted by study size; OR indicates odds ratio, CI, confidence interval; N indicates effective sample size, calculated by taking the sum of each study's sample size multiplied by the SNP's imputation quality. When more than one SNP at a locus surpassed our p-value threshold, we presented the SNP with the lowest p-value.

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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	N	p-value
Comm. 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

Allele indicates the coded allele in the CARDIoGRAM Consortium meta-analysis; AF indicates allele frequency for the coded allele; OR indicates odds ratio, CI, confidence interval; N indicates sample size.

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