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 9 participating discovery studies and 7 participating second stage studies can be found in the Supplementary Note.

Carotid artery phenotypes

Each study evaluated the carotid arteries using 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 greater than 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 one to twelve 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 Amish, FHS, and ERF 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 to 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 adjusted for age and sex. Some studies made additional adjustments including study site, familial structure, or for whether the DNA had been whole genome amplified. 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. After verification of strand alignment across studies and QC, filtering, and imputation within each study, we restricted our meta-analysis to autosomal SNPs that were reported in at least 2 studies and had an average minor allele frequency of at least 1%. Prior to 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. Inflation was low, with λ_{gc} 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 express the association of each SNP and ln(IMT) as the regression slope (β), its standard error [SE(β)] and a corresponding p-value. For the presence of plaque, we calculated a meta-analysis log odds ratio (OR), 95% confidence interval, and p-value. In this case, the OR 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 $p < 4\times10^{-7}$, 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 (6 studies) or plaque (3 studies). We provided each collaborating second stage study a list of all SNPs that attained genome-wide significant p-values for common carotid IMT, internal carotid IMT, or plaque and combined the results from these studies using a fixed-effects meta-analysis as described previously.

Combined meta-analysis

Finally, we combined results from the discovery and second stage analyses using inverse variance weighting, as described above, and considered SNPs with a p-value$<5\times10^{-8}$ as genome-wide significant.