

ORIGINAL ARTICLE



Genetic Susceptibility Loci for Cardiovascular Disease and Their Impact on Atherosclerotic Plaques

BACKGROUND: Atherosclerosis is a chronic inflammatory disease in part caused by lipid uptake in the vascular wall, but the exact underlying mechanisms leading to acute myocardial infarction and stroke remain poorly understood. Large consortia identified genetic susceptibility loci that associate with large artery ischemic stroke and coronary artery disease. However, deciphering their underlying mechanisms are challenging. Histological studies identified destabilizing characteristics in human atherosclerotic plaques that associate with clinical outcome. To what extent established susceptibility loci for large artery ischemic stroke and coronary artery disease relate to plaque characteristics is thus far unknown but may point to novel mechanisms.

METHODS: We studied the associations of 61 established cardiovascular risk loci with 7 histological plaque characteristics assessed in 1443 carotid plaque specimens from the Athero-Express Biobank Study. We also assessed if the genotyped cardiovascular risk loci impact the tissue-specific gene expression in 2 independent biobanks, Biobank of Karolinska Endarterectomy and Stockholm Atherosclerosis Gene Expression.

RESULTS: A total of 21 established risk variants (out of 61) nominally associated to a plaque characteristic. One variant (rs12539895, risk allele A) at 7q22 associated to a reduction of intraplaque fat, $P=5.09 \times 10^{-6}$ after correction for multiple testing. We further characterized this 7q22 Locus and show tissue-specific effects of rs12539895 on *HBP1* expression in plaques and *COG5* expression in whole blood and provide data from public resources showing an association with decreased LDL (low-density lipoprotein) and increase HDL (high-density lipoprotein) in the blood.

CONCLUSIONS: Our study supports the view that cardiovascular susceptibility loci may exert their effect by influencing the atherosclerotic plaque characteristics.

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The full author list is available on page 9.

Key Words: atherosclerosis
■ cardiovascular disease ■ coronary artery disease ■ genetic loci ■ plaque, atherosclerotic ■ quantitative trait loci ■ stroke

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Atherosclerosis refers to the lifelong process of lesion formation and progression in the inner linings of arteries¹ and underlies coronary artery disease (CAD) and large artery ischemic stroke (LAS).² CAD and LAS are complex diseases with a shared genetic architecture.^{3,4} To date, meta-analyses of genome-wide association studies (GWAS) identified multiple genetic loci for CAD⁵ and 4 for LAS.^{6–8} Despite this progress, it has proven challenging to interpret these findings in terms of the underlying biological mechanisms or to formulate testable therapeutic hypotheses.

Histological analyses of atherosclerotic plaques have provided insight into the process of atherosclerosis.¹ The high-risk lesion characterized by variability in lipid, inflammatory, calcific, and thrombotic components is prone to destabilization and rupture,^{1,9,10} resulting in acute cardiovascular disease.^{10–13} But the extent to which common sequence variation that associates to CAD and LAS risk also relates to advanced destabilizing atherosclerotic lesion characteristics remains unclear.

The Athero-Express (AE) Biobank Study,⁹ STAGE (Stockholm Atherosclerosis Gene Expression),¹⁴ and BiKE (Biobank of Karolinska Endarterectomy)^{15,16} are 3 independent, deeply phenotyped biobank studies comprising individuals undergoing surgical interventions. The AE assesses the genetic architecture of histologically analyzed plaque specimens and plaque-derived DNA methylation. STAGE and BiKE focus on the genetics of tissue-specific differential gene expression.

Here we specifically investigated the association of 61 established susceptibility loci for CAD and LAS to plaque characteristics in the AE study. We find that these risk loci are broadly albeit nominally associated with human atherosclerotic plaque characteristics. We report that the risk allele (A) of 1 variant (rs12539895) on chromosome 7q22 near *COG5* and *HBP1* was significantly associated with a reduction in intraplaque fat ($P=5.09\times 10^{-6}$ after correction for multiple testing). To further increase our understanding of this association, we investigated the effects of common variants at 7q22 on carotid plaque DNA methylation and tissue-specific gene expression, by combining data from the AE, STAGE, and BiKE studies.

METHODS AND MATERIAL

This study complies with the Declaration of Helsinki, and all participants provided informed consent. The medical ethical committees of the respective hospitals approved these studies.

The data, analytic methods, and study materials will be made available to other researchers for purposes of reproducing the results or replicating the procedure. The raw omics data are available through the European Genome-Phenome Archive. The main scripts used for the quality control and the (meta-)analysis of the data are available through GitHub (<https://github.com/swvanderlaan/publications> under doi: 10.5281/zenodo.1069531).

The Methods and Material can be found in the [Data Supplement](#).

RESULTS

Clinical Characteristics of the 3 Cohorts

For this study, we included individuals from 3 independent biobank studies, the AE,⁹ BiKE,^{15,16} and STAGE.¹⁴ Each study included patients with clinically significant arterial stenosis that are similar at baseline (Table 1).

Single-Variant Analysis of CAD and LAS Associated Loci With Plaque Characteristics

In the AE we correlated 61 established susceptibility loci for CAD and LAS^{5–8} to commonly assessed plaque characteristics; 5 Loci identified in a GWAS for bipolar disorder^{17,18} served as negative controls (these 66 variants are listed in Table I in the [Data Supplement](#)). There were 21 (out of 61) cardiovascular risk alleles nominally associated with plaque characteristics and 0 (out of 5) bipolar disorder associated variants (at a nominal $P<0.05$; Tables 2 and 3).

One variant (rs12539895) significantly associated with a reduction of intraplaque fat after correction for multiple testing, with a per A-allele odds ratio=0.63, 95% CI, 0.51–0.77; $P=5.09\times 10^{-6}$ (Table 3; Figure 1). This intronic single-nucleotide variant is located on chromosome 7q22 in the gene encoding for conserved oligomeric Golgi complex subunit 5 (*COG5*). Overall, intraplaque fat was the plaque characteristic that was the most commonly associated with CAD risk loci (6 out of 21 with a nominal $P<0.05$).

CAD loci also nominally associated with atherosclerotic plaque collagen content, smooth muscle cell (SMC) percentage, the percentage of macrophages, the extent of calcification, intraplaque hemorrhage, and intraplaque vessel density (Tables 2 and 3). Two LAS susceptibility loci (near *TSPAN2* on 1p13.2 and rs556621 on 6p21.1) nominally associated with intraplaque calcification and SMCs, respectively.

As expected, none of the negative control bipolar disorder risk-variants associated with any of the plaque characteristics.

Gene-Based Analysis of CAD and LAS Associated Loci With Plaque Characteristics

We mapped 787 genes to the 66 loci and applied gene-based association tests using VEGAS2 (Versatile Gene-based Association Study 2)^{21,22} on the 7 measured plaque characteristics (Material in the [Data Supplement](#)). One gene, HMG-box transcription factor 1 (*HBP1*), on chromosome 7q22 significantly associated with intraplaque fat ($P=9.0\times 10^{-7}$ corrected for 7 traits and 787 genes,

Table 1. Clinical Characteristics of the AE, BiKE, and STAGE

| Characteristic | AE | BiKE | STAGE |
|-----------------------------|---------------------|---------------------|---------------------|
| | n=1439 | n=127 | n=109 |
| Male, N (%) | 977 (67.89) | 100 (78.74) | 98 (90.00) |
| Age, y (SD) | 68.79 (9.31) | 70.56 (8.90) | 65.90 (8.00) |
| History, N (%) | | | |
| Cerebrovascular disease | 1184 (82.28) | 101 (79.53) | 9 (8.00) |
| Coronary artery disease | 429 (29.81) | 26 (20.47) | 100 (91.74) |
| Peripheral arterial disease | 251 (17.44) | n/r | n/r |
| Risk factors | | | |
| Type 2 diabetes mellitus | 332 (23.07) | 32 (25.20) | 24 (22.00) |
| Hypertension | 1231 (85.55) | 106 (83.46) | 70 (64.00) |
| Current smoker | 492 (34.19) | 61 (48.03) | 7 (6.00) |
| BMI | 25.95 (24.02–28.39) | 26.44 (23.70–28.40) | 25.93 (23.66–28.19) |
| eGFR | 72.26 (58.79–85.38) | n/r | 55.36 (48.02–62.70) |
| TC | 4.64 (3.84–5.50) | 4.30 (3.70–5.22) | 3.94 (3.24–4.63) |
| LDL | 2.70 (2.03–3.40) | 2.30 (1.90–3.00) | 2.00 (1.46–2.53) |
| HDL | 1.11 (0.90–1.38) | 1.10 (0.90–1.30) | 1.45 (1.28–1.62) |
| TG | 1.40 (1.00–2.00) | 1.45 (1.00–2.12) | 1.23 (0.85–1.60) |
| Medication, N (%) | | | |
| Antihypertensives | 1104 (76.72) | 110 (86.61) | 96 (88.00) |
| LLDs | 1112 (77.28) | 106 (83.46) | 13 (12.00) |
| Antithrombotics | 1272 (88.39) | 29 (22.83) | 93 (85.00) |
| Symptoms, N (%) | | | |
| Asymptomatic | 195 (13.55) | 40 (31.50) | n/r |
| Ocular | 221 (15.36) | 25 (19.68) | n/r |
| TIA | 635 (44.13) | 27(21.26) | n/r |
| Stroke | 383 (26.62) | 32 (36.78) | n/r |
| Surgery, N (%) | | | |
| De novo | 1363 (94.72) | n/r | n/r |
| Restenosis | 46 (3.20) | 2002 | n/r |
| Period, y | 2002–2013 | 2002 | 2009 |

For more details, refer to Material and Methods section. Cerebrovascular disease history includes ischemic stroke and TIA. Coronary artery disease history includes coronary artery disease, myocardial infarction, percutaneous coronary intervention, and coronary artery bypass graft. Peripheral disease history includes diagnosed peripheral arterial occlusive disease, femoral artery interventions, and ankle-brachial index <70. Type 2 diabetes mellitus and hypertension include all individuals with diagnosed type 2 diabetes mellitus or hypertension, respectively, and those on appropriate medication. Current smokers include all individuals smoking up to 6 mo until the surgery date. BMI, kg/m². eGFR rate was based on the Modification of Diet in Renal Disease formula, mL/min per 1.73 m². All lipids are in mmol/L. Antihypertensives include all antihypertension medication. Antithrombotics include clopidogrel, dipyridamole, acenocoumarin, ascal, and anti-platelet drugs. Carotid symptoms are the symptoms before surgery, which are the indication for surgery. Surgery includes de novo stenotic arteries, or re-stenotic arteries (restenosis), and the surgery period (in y) is indicated. Categorical risk factors are noted in N (%), continuous risk factors are in median (IQR) unless otherwise indicated. AE indicates Athero-Express; BiKE, Biobank of Karolinska Endarterectomy; BMI, body mass index; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; IQR, interquartile range; LDL, low-density lipoprotein; LLDs, lipid-lowering drugs including statins and other lipid-lowering drugs; STAGE, Stockholm Atherosclerosis Gene Expression; TC, total cholesterol; TG, triglycerides; and TIA, transient ischemic attack.

Table II in the [Data Supplement](#)). Consistent with this result, the top single-nucleotide variant in *HBP1* identified by VEGAS2, rs10953530 (odds ratio=1.63, 95% CI, 1.33–2.00 per A-allele, $P=1.84 \times 10^{-6}$; Table II in the [Data Supplement](#)), is in strong LD ($r^2=0.90$) with rs12539895, the top association signal in *COG5*.

Tissue-Specific Effects of Cardiovascular Risk Loci on Gene Expression in STAGE and BiKE

Given the single-variant and gene-based association presented above, we further explored the 7q22 locus

Table 2. CAD and LAS Susceptibility Loci and Their Association With Quantitative Plaque Phenotypes

| Phenotype | Locus | Variant | Chromosomal | BP | Alleles | CAF | β (SEM) | P Value | Disease | GWAS Dir. |
|----------------|------------------|------------|-------------|-------------|---------|-------|----------------|-----------------------|---------|-----------|
| Macrophages | <i>BCAS3</i> | rs7212798 | 17 | 59,013,488 | C/T | 0.154 | -0.137 (0.054) | 0.011 | CAD | - |
| SMCs | <i>LIPA</i> | rs1412444 | 10 | 91,002,927 | T/C | 0.363 | 0.083 (0.040) | 0.036 | CAD | - |
| | <i>COL4A1/A2</i> | rs11838776 | 13 | 111,040,681 | A/G | 0.261 | 0.103 (0.042) | 0.015 | CAD | - |
| Vessel density | <i>SWAP70</i> | rs10840293 | 11 | 9,751,196 | A/G | 0.569 | 0.079 (0.040) | 0.046 | CAD | + |
| | <i>KSR2</i> | rs11830157 | 12 | 118,265,441 | G/T | 0.395 | 0.109 (0.040) | 6.97×10^{-3} | CAD | - |
| | <i>UBE2Z</i> | rs46522 | 17 | 46,988,597 | T/C | 0.540 | -0.080 (0.038) | 0.034 | CAD | - |
| SMCs | <i>6p21.1</i> | rs556621 | 6 | 44,594,159 | G/T | 0.698 | 0.101 (0.041) | 0.015 | LAS | - |

Per variant the reported locus, the chromosomal BP, the effect and other allele (Alleles), as well as the CAF is given. For each variant the effect size (β) \pm SEM is relative to the coded allele with its *P* value of association with the quantitative plaque phenotype. Also indicated is the disease (CAD or LAS) to which the respective variant was originally associated in the GWAS. Additional per variant statistics are in Table I in the [Data Supplement](#). BP indicates base pair position; CAD, coronary artery disease; CAF, coded allele frequency; GWAS, genome-wide association study; GWAS Dir., the direction of effect in the CAD GWAS; LAS, large artery stroke; and SMCs, smooth muscle cells.

with respect to regional gene regulation and expression. Genetic variants could regulate gene expression by altering transcription factor binding or splice sites in plaques or other relevant tissues; such variants are known as expression quantitative trait loci. We associated variants in 7q22 with regional gene expression in diseased tissues from the STAGE and BiKE studies and tissues from the general population in the public Genotype-Tissue Expression project.²³

In STAGE, the rs12539895 associated with a reduction of *COG5* expression in atherosclerotic arterial wall tissue, internal mammary artery, and whole blood (Table III in the [Data Supplement](#)). Overall, 21 variants associated with expression of 10 genes across 6 tissues at a

false-discovery rate $Q \leq 0.05$; most expression quantitative trait loci were found in liver and whole blood tissue (Figure I in the [Data Supplement](#)).

In BiKE 3 correlated variants significantly associated with an increase of *HBP1* expression in carotid plaques, $P = 7.0 \times 10^{-6}$; these are proxies for rs12539895 ($LD r^2 > 0.90$; Figure 2; Table IV in the [Data Supplement](#)). The same alleles decrease CAD risk and intraplaque fat (Table IV in the [Data Supplement](#)). Given the association of rs12539895 with reduced intraplaque fat and the central role of the LDL (low-density lipoprotein) receptor (encoded by *LDLR*) in lipid uptake, we investigated if regional gene expression correlated to *LDLR* expression in carotid plaques from BIKE. Indeed, carotid plaque

Table 3. CAD and LAS Susceptibility Loci and Their Association With Semi-Quantitative Plaque Phenotypes

| Phenotype | Locus | Variant | Chromosomal | BP | Alleles | CAF | OR (95% CI) | P Value | Disease | GWAS Dir. |
|-----------------|----------------------------|------------------|-------------|-------------|---------|-------|------------------|-----------------------|---------|-----------|
| Calcification | <i>WDR12</i> | chr2:203828796:1 | 2 | 203,828,796 | CTA/C | 0.126 | 0.74 (0.58–0.94) | 0.012 | CAD | + |
| | <i>ZNF259-APOA5-APOA1</i> | rs964184 | 11 | 116,648,917 | C/G | 0.861 | 1.26 (1.01–1.58) | 0.043 | CAD | + |
| | <i>LDLR</i> | rs56289821 | 19 | 11,188,247 | A/G | 0.106 | 1.59 (1.21–2.07) | 6.29×10^{-4} | CAD | - |
| | <i>KCNE2 (gene desert)</i> | rs28451064 | 21 | 35,593,827 | A/G | 0.114 | 0.71 (0.55–0.91) | 7.92×10^{-3} | CAD | - |
| Collagen | <i>NOS3</i> | rs3918226 | 7 | 150,690,176 | T/C | 0.076 | 1.73 (1.03–2.90) | 0.031 | CAD | - |
| | <i>SMG6</i> | rs216172 | 17 | 2,126,504 | C/G | 0.330 | 0.78 (0.64–0.95) | 0.012 | CAD | - |
| Intraplaque fat | <i>MIA3</i> | rs67180937 | 1 | 222,823,743 | G/T | 0.715 | 1.27 (1.02–1.56) | 0.029 | CAD | + |
| | <i>ZEB2-ACO74093.1</i> | rs17678683 | 2 | 145,286,559 | G/T | 0.088 | 1.38 (1.00–1.92) | 0.049 | CAD | - |
| | 7q22 | rs12539895 | 7 | 107,091,849 | A/C | 0.234 | 0.63 (0.51–0.77) | 5.09×10^{-6} | CAD | - |
| | <i>NOS3</i> | rs3918226 | 7 | 150,690,176 | T/C | 0.076 | 1.73 (1.03–2.90) | 0.031 | CAD | + |
| | <i>TRIB1</i> | rs2954029 | 8 | 126,490,972 | T/A | 0.475 | 0.77 (0.65–0.92) | 3.54×10^{-3} | CAD | - |
| | <i>ABO</i> | rs2519093 | 9 | 136,141,870 | T/C | 0.199 | 1.27 (1.02–1.59) | 0.034 | CAD | - |
| IPH | <i>LIPA</i> | rs1412444 | 10 | 91,002,927 | T/C | 0.362 | 1.27 (1.07–1.52) | 6.33×10^{-3} | CAD | - |
| Calcification | <i>TSPAN2 (1p13.2)</i> | rs12122341 | 1 | 115,655,690 | G/C | 0.257 | 1.19 (1.00–1.43) | 0.048 | LAS | + |

Per variant the reported locus, the chromosomal BP, the effect and other allele (Alleles), as well as the CAF is given. The OR \pm 95% CI is relative to the risk allele with its associated *P* value. Also indicated is the disease (CAD or LAS) to which the respective variant was originally associated in the GWAS. Additional per variant statistics are in Table I in the [Data Supplement](#). Intraplaque fat, as <10% vs >10% fat per the total plaque area. BP indicates base pair position; CAD, coronary artery disease; CAF, coded allele frequency; GWAS, genome-wide association study; GWAS Dir., indicates the direction of effect in the CAD GWAS; IPH, intraplaque hemorrhage; LAS, large artery stroke; and OR, odds ratio.

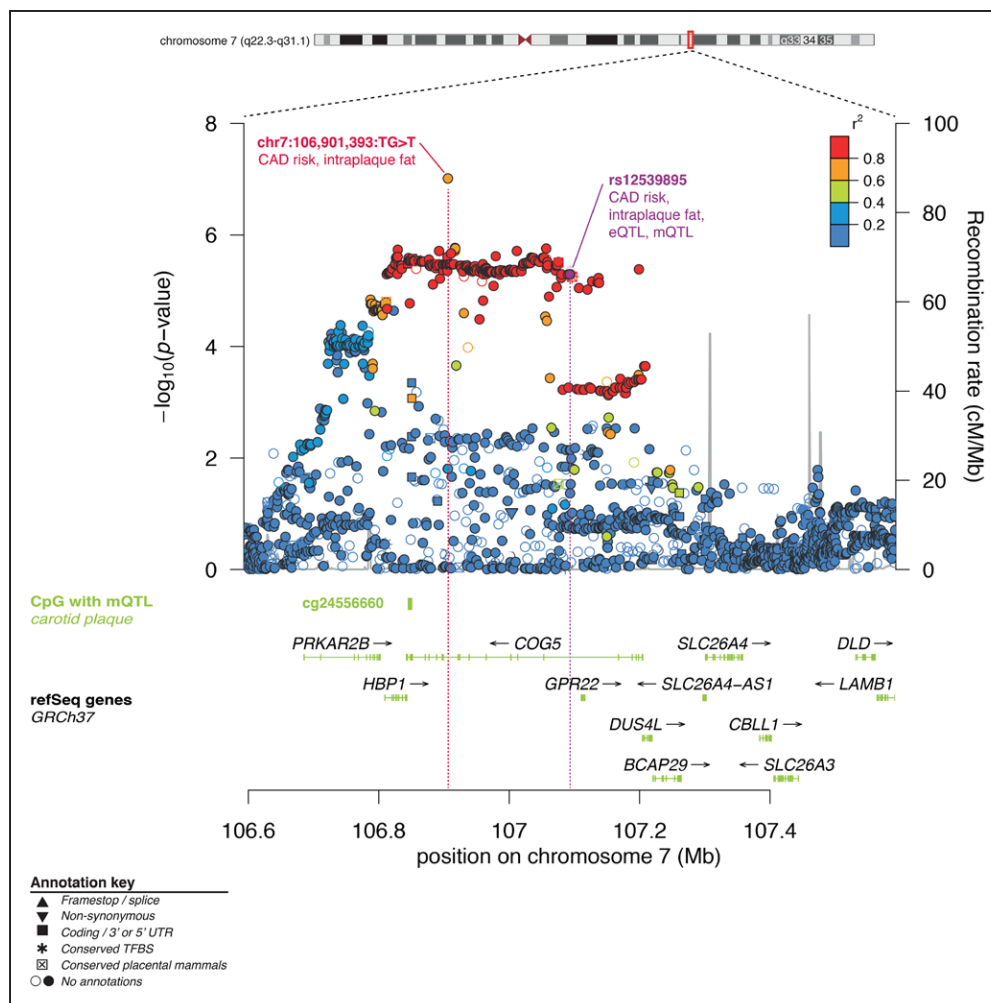


Figure 1. Associations of genetic variants in 7q22 with carotid intraplaque fat.

Rs12539895 was previously associated with coronary artery disease (CAD; purple). The strongest association was for a deletion chr7:106901393 (triglycerides [TG] >T; $P=2.14 \times 10^{-7}$; pink). The x axis shows the chromosomal position relative to 1000G (March 2012, Hg19). The **lower** shows refSeq canonical genes from UCSC (the black arrow indicates the direction of transcription). The **left** y axis shows the $-\log_{10}(P$ value) of the association with intraplaque fat. The **right** y axis shows the recombination rate (gray line in the **middle**). The **middle** shows each associated variant colored by the r^2 relative to rs12539895. The legend in the **upper right** corner shows the r^2 color scale. The per variant annotation key is depicted in the **bottom left** corner. Made using LocusZoom version 1.3 and SNIQA.^{19,20} COG5 indicates component of oligomeric Golgi complex 5; CpG, cytosine-guanine dinucleotide; eQTL, expression quantitative trait loci; HBP1, HMG-box transcription factor 1; and mQTL, methylation quantitative trait loci.

expression of *PRKAR2B*, *COG5*, *DUS4L*, and *CBL1* was significantly correlated to *LDLR* expression ($P < 4.6 \times 10^{-3}$ after correction for 11 genes tested; Table V in the [Data Supplement](#)).

Tissue-Specific Effects of Cardiovascular Risk Loci on Gene Expression in the General Population

In contrast, data from the Genotype-Tissue Expression project, comprising 48 pathology tissues of individuals from the general population, indicates that rs12539895 only associates to increased expression *HBP1* in testis, sun-exposed skin of the lower leg, and tibial artery (Table VI in the [Data Supplement](#)) and increased expression of *BCAP29* in lung tissues. In addition, proxies of rs12539895 ($LD r^2 > 0.80$) associate with

differential expression in tibial nerves, skeletal muscle cells, sigmoid colon, and basal ganglia (Table VI in the [Data Supplement](#)).

Tissue-Specific Sequence Variation Effects on Methylation

Sequence variation may regulate gene expression indirectly through changes in DNA methylation at sites rich of cytosine-guanine dinucleotides (CpGs) and in a tissue-specific manner.²⁴ To uncover cis-acting methylation quantitative trait loci we associated genetic variants in 7q22 with the methylation of CpGs mapped to the same region in plaques from the AE. A perfect proxy for rs12539895, rs80341862, was significantly associated with a decrease in methylation of a CpG at the 3' UTR of *COG5* and *HBP1* (false discovery rate

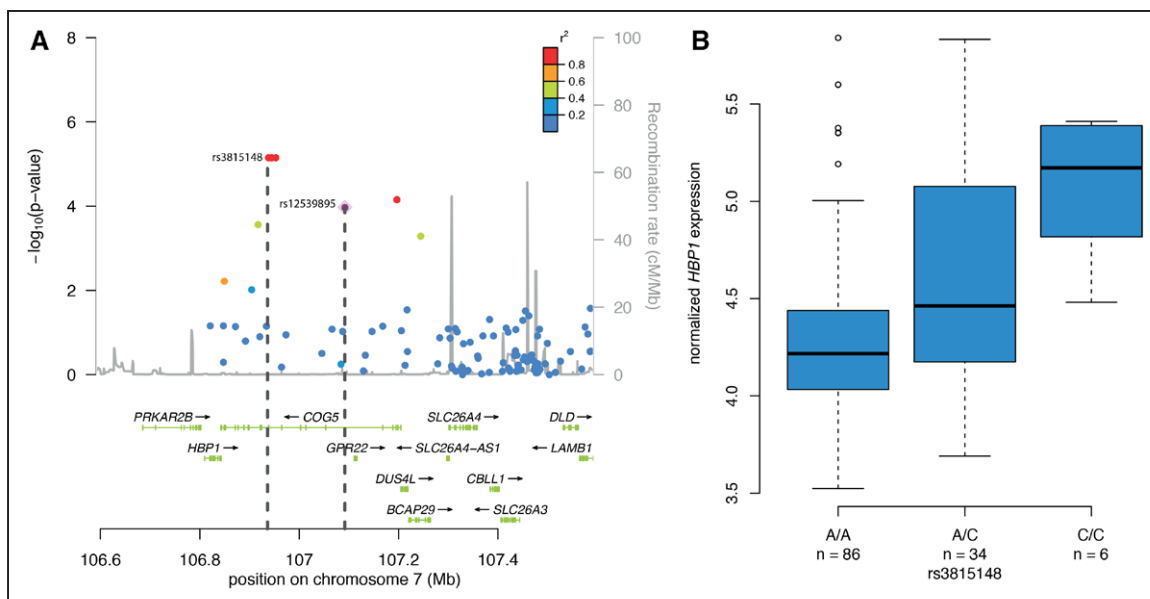


Figure 2. Association of rs3815148 with *HBP1* expression in carotid plaques.

Rs3815148 is a proxy (LD $r^2=0.91$) for rs12539895 and associated ($P=7.0\times 10^{-6}$) with *HBP1* expression in carotid plaques from BiKE. **A**, Regional association of variants with *HBP1* expression in carotid plaques. The x axis shows the chromosomal position relative to 1000G (March 2012, Hg19) and refSeq canonical genes (green) from UCSC (the black arrow indicates the direction of transcription). The left y axis shows the $-\log_{10} P$ value of the association with *HBP1* expression. The right y axis shows the recombination rate (gray line). The middle shows each associated variant colored by the r^2 relative to rs12539895. The legend in the upper right corner shows the r^2 color scale. **B**, Boxplot of the association of rs3815148 with *HBP1* expression. Proxy data based on 1000G phase 3, version 5 data from SNIPIA.²⁰ Made using LocusZoom version 1.3.¹⁹

Q value= 5.41×10^{-9} after 1000 permutations; Table VII in the [Data Supplement](#)). However, the decreased methylation of this CpG (cg24556660) did not correlate to intraplaque fat (odds ratio=0.98, 95% CI, 0.92–1.05; $P=0.62$).

COG5 and HBP1 Are Expressed in Carotid Plaques

Although it is clear that *COG5* and *HBP1* are expressed in various tissues (Tables IV and VI in the [Data Supplement](#)), no data exist on spatial distribution in atherosclerotic lesions. We investigated the spatial localization in carotid plaques from the AE using immunohistochemistry specific for *COG5* (component of oligomeric Golgi complex 5) and *HBP1* (HMG-box transcription factor 1; Figure II in the [Data Supplement](#)). Figure III in the [Data Supplement](#) shows that *COG5* is expressed in most cells in plaques, whereas *HBP1* is expressed primarily in cells with a foam-cell like morphology (Figure IV in the [Data Supplement](#)).

Cardiovascular Risk Factors and the 7q22 Locus

The dominant causal contributing factors for cardiovascular disease risk are circulating lipid proteins, presumably leading to changes in intraplaque fat. As the intraplaque fat and CAD associated alleles correlate with gene expression in relevant tissues for lipid metabolism (liver) and atherosclerosis (carotid plaques, arte-

rial wall tissues, and whole blood), we speculated that these variants may associate to circulating blood lipid levels too. Indeed, data from the Global Lipids Genetics Consortium²⁵ revealed that the risk-reducing allele of rs12539895 also associated with a decrease in circulating LDL and an increase in HDL (high-density lipoprotein) but not with other cardiovascular risk factors (Table VIII in the [Data Supplement](#)).

Polygenic Association of Clinically Relevant Variants With Plaque Phenotypes

Because of the central role of atherosclerosis in CAD and LAS and the presumed polygenic nature of these complex diseases, we further tested the contribution of modestly associated variants, ascertained through GWAS, on atherosclerotic characteristics. We summed genetic variation weighted by the effect on disease risk into polygenic risk scores.¹⁸ We made polygenic scores using imputed genotype data from AE and the allelic effects estimated through GWAS on CAD and LAS (Table IX in the [Data Supplement](#)); each score included variants selected by using increasingly liberal thresholds of significance (P_T ; Table X and Figure V in the [Data Supplement](#)).¹⁸ We then correlated each of these scores, which capture the effects of individual variants on clinical outcome, to the atherosclerotic plaque phenotypes. We found significant associations for the CAD score with intraplaque fat, while the LAS score was associated with intraplaque hemorrhage and SMCs (Figure 3; $P<0.05$).

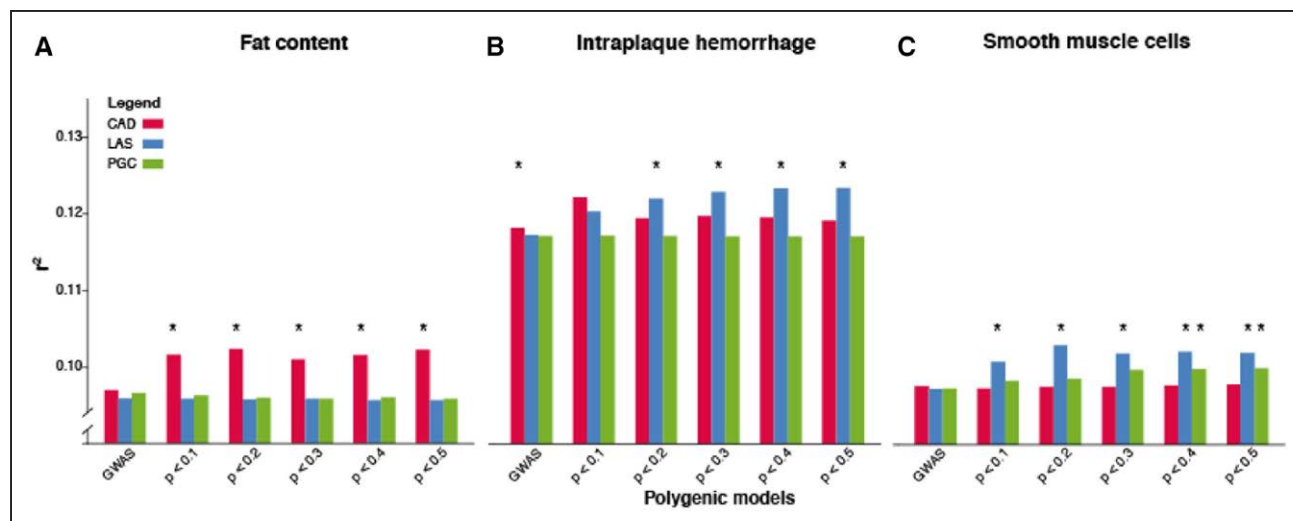


Figure 3. Polygenic scores of coronary artery disease (CAD; dark pink) and large artery stroke (LAS; blue) associate with different plaque characteristics. Polygenic scores for CAD associate with intraplaque fat (A), whereas polygenic scores for LAS associate with intraplaque hemorrhage (B) and smooth muscle cells (C). Scores were constructed based on GWAS (Genome-Wide Association Study) summary statistics using increasingly liberal P value thresholds (P_T , see also Information in the Data Supplement; Table II in the Data Supplement). Each P_T bin is depicted on the x axis. The y axis indicate the r^2 of the association; please note the different scales. * $P < 0.05$ for association of the score with the plaque characteristic. PGC indicates bipolar disorder from the Psychiatric Genetics Consortium (green).

DISCUSSION

In this study, we show that 21 out of 61 established cardiovascular genetic risk loci nominally associated to human plaque characteristics. One variant (rs12539895 at 7q22) significantly associated with a reduction of fat in carotid plaques. Tissue-specific expression analyses revealed effects of this variant on *COG5*, and *HBP1*, expression in arterial wall tissue, internal mammary artery, carotid plaques, and whole blood tissues, as well as differential methylation in carotid plaques. In human plaques, *COG5* is ubiquitously expressed (Figure III in the Data Supplement), while *HBP1* is concentrated near foam-like cells (Figure IV in the Data Supplement). Last, in line with the current understanding of the role of lipids in cardiovascular disease, rs12539895 also associates with a decrease in circulating LDL and an increase in HDL.

The polygenic score analyses showed the strongest correlation of a CAD polygenic score with intraplaque fat. In contrast, polygenic scores for LAS associated with intraplaque hemorrhage and SMCs.

The second strongest association was with calcification (rs56289821 near *LDLR*). Indeed, disruption of the *LDLR* pathway has been implicated in arterial calcification in mice²⁶ and aortic valve calcification in humans with a mutation in *LDLR*.²⁷ A further notable result involves a variant (rs2954029) near *TRIB1*, which was associated with a reduction of intraplaque fat. The T-allele of this variant was previously associated with a decrease in circulating triglycerides, LDL cholesterol, and ApoB (Apolipoprotein B), and an increase in HDL.²⁸ The same study also showed a decreased risk for ischemic heart disease and myocardial infarction.

We also note that some variants show directions of effects on plaque characteristics opposite to the direction observed in GWAS for primary disease risk. For instance, the risk allele A of rs11838776 near *COL4A1/A2* associates with more SMCs (Table 2) but is correlated to a reduction in CAD risk. This suggests that rs11838776 is reducing overall CAD risk, possibly through an overall increase in SMCs, in some individuals more (those carrying the risk allele) than others. Such paradoxical findings may point to potentially interesting biological mechanisms that need further scrutiny in future studies.

Given that rs12539895 at 7q22 was the only variant significantly associated to a plaque characteristic (intraplaque fat) after correction for multiple testing, we further explored this locus. Strong LD between variants extends between 106.7 and 107.2 Mb in this locus (Figure 1), complicating the identification of causal gene(s). However, our results converge around only a few genes at 7q22: in particular *HBP1* and *COG5*. The CAD risk associated variant rs12539895 associates with *COG5* and *HBP1* gene expression in relevant tissues with directionally consistent effects. Thus, although the current study is unable to point to the causal gene, it prioritizes the above genes.

HBP1 is a transcriptional repressor binding to promoter regions, it plays a role in *Wnt1* signaling²⁹ and cancer^{30,31}, and is mostly expressed in the heart. In a rat model of carotid injury therapeutic inhibition of *HBP1* (known as *Hmgb1* in rodents) was associated with a decrease in vascular SMC activation and neointimal formation.³² Another study showed that *HBP1* is targeted by miR-155.³³ Inhibition of miR-155 attenuated foam cell formation and reduced atherosclerotic plaques in ApoE^{-/-} mice, and *HBP1* knockdown

enhanced lipid uptake by macrophages.³³ Our expression quantitative trait loci analyses in BiKE provide confirmation of these results in humans, as the risk-reducing allele of rs12539895 proxies increase *HBP1* expression in carotid plaques (Figure 2; Table IV in the [Data Supplement](#)).

COG5 has not been associated with atherosclerotic disease previously. *COG5* is part of an evolutionary conserved protein complex in the Golgi apparatus that is involved in protein and lipid trafficking and sorting.³⁴ It plays a major role in glycosylation of proteins. One report discusses a mutation near the 3' UTR of *COG5* leading to impaired glycosylation of proteins, one of which was ApoC-III.³⁵ Another study reported that *COG5* depletion in HeLa cells did not significantly affect glycosylation or membrane expression of *LDLR*.³⁶ Strikingly, *COG5* knockdown in these cells leads to a significant dilation of Golgi-apparatus cisternae, as well as an impaired intracellular trafficking leading to an accumulation of intracellular *LDLR*.³⁶ In this study, we show that *COG5* expression negatively correlates with *LDLR* expression in human carotid plaques. The CAD risk-reducing allele of rs80341862 (a rs12539895 proxy) associated with decreased *COG5* expression in atherosclerotic arterial wall, internal mammary artery tissue, and whole blood tissues (Table III in the [Data Supplement](#)), and reduced intraplaque fat (Table 3). Although this variant associated with a decreased methylation at the 3' UTR of *COG5* in plaques (Table V in the [Data Supplement](#)), the methylation itself did not associate with the prevalence of fat in plaques. This indicates a direct effect on transcription through sequence variation rather than through gene regulation by methylation.

Our tissue-specific transcriptional analyses are consistently showing effects of rs12539895 and its proxies on *HBP1* and *COG5* in (atherosclerotic) arterial wall tissues. Interestingly, only *HBP1* was expressed in arterial wall tissue from Genotype-Tissue Expression, potentially indicating a tissue-specific effect on *HBP1*, and a disease-specific effect on *COG5*.

Furthermore, 2 studies tied variants (rs3815148³⁷ and rs4730250³⁸) at 7q22 to susceptibility of osteoarthritis and pointed to *COG5* and *DUS4L* as potential causal candidates; these variants are in high LD with rs12539895 ($r^2 > 0.80$). In turn, osteoarthritis is associated with carotid intima-media thickness,³⁹ a proxy of atherosclerosis severity, and circulating cholesterol.⁴⁰ In point of fact, some have speculated that a transition of chondrocytes to a foam-cell like morphology may be responsible to osteoarthritis.⁴⁰ Other GWAS also pointed to variants at 7q22 and mapped *PIK3CG* as potentially causal for ulcerative colitis,⁴¹ systolic blood pressure,⁴² mean platelet volume,⁴³ carotid plaque presence, and carotid intima-media thickness.⁴⁴ While none of the variants near *PIK3CG* are in LD with rs12539895,

of note is the fact that rs17398575, associated to plaque presence and carotid intima-media thickness, is an expression quantitative trait loci in aorta for *PRKAR2B* near *HBP1*.²³ Last, a large-scale genetic analysis of serum metabolite levels associated rs12539895 to lower levels of 10-undecenoate (11:1n1)/X-11438. However, these are speculations and strong evidence linking carotid intima-media thickness, osteoarthritis, and our findings have yet to be provided.

The association of 7q22 with circulating lipids, rather than other cardiovascular risk factors, implicates a role for lipid metabolism that may lead to reduced intraplaque fat and a reduction in CAD risk. Last, our histological analyses indicate a role for *HBP1* with a foam-like appearance in carotid plaques (Figure IV in the [Data Supplement](#)), but more specific staining, for example, for oxidized LDL scavenger receptors, are needed to determine the type of cells.

The lack of association for the 40 other cardiovascular risk variants may indicate mechanisms in play that are not captured by our histological measurements. However, some variants may simply not exert their effect through atherosclerosis directly but rather through changes in for example cardiac tissue and thereby modulate risk. Finally, we have studied the association of these variants with plaques at the end stage of atherosclerotic disease, thus we cannot exclude that a causal role in early plaque development.

Admittedly, these observations require confirmation, and reducing the noise in histological phenotype measurements through alternative methods might be a feasible way to increase power.^{45,46} Also, our measure of intraplaque fat is an overall measure of intraplaque fat as described by Verhoeven et al⁹; thus we cannot ascertain which fraction of lipids and which cells might be involved. Power may have impeded our polygenic score analyses, as well as the identification of CpGs associating with intraplaque fat. We were also unable to directly test tissue-specific gene expression to intraplaque fat; thus, further studies are needed to explore this. Still, although sample size and some phenotypic heterogeneity limit our power, the size and scope of the AE are unique (in its kind) and allow for the most in-depth study of genetic variation with human plaques characteristics to date.

In conclusion, our study supports the view that cardiovascular susceptibility loci may exert their effect by influencing the atherosclerotic plaque characteristics. Further functional studies will be needed to test the hypothesis that *HBP1* and *COG5* are indeed linked to atherosclerosis and specific plaque characteristics.

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Disclosures

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APPENDIX

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