Effects of Coronary Artery Disease–Associated Variants on Vascular Smooth Muscle Cells

Charles U. Solomon, PhD*; David G. McVey, PhD*; Catherine Andreadi, PhD*; Peng Gong, PhD*; Lenka Turner, MSc*; Paulina J. Stanczyk, PhD*; Sonja Khemiri; Julie C. Chamberlain, BTec; Wei Yang, MSc; Tom R. Webb, PhD; Christopher P. Nelson, PhD; Nilesh J. Samani, MD; Shu Ye, MD, PhD

BACKGROUND: Genome-wide association studies have identified many genetic loci that are robustly associated with coronary artery disease (CAD). However, the underlying biological mechanisms are still unknown for most of these loci, hindering the progress to medical translation. Evidence suggests that the genetic influence on CAD susceptibility may act partly through vascular smooth muscle cells (VSMCs).

METHODS: We undertook genotyping, RNA sequencing, and cell behavior assays on a large bank of VSMCs (n>1499). Expression quantitative trait locus and splicing quantitative trait locus analyses were performed to identify genes with an expression that was influenced by CAD-associated variants. To identify candidate causal genes for CAD, we ascertained colocalizations of VSMC expression quantitative trait locus signals with CAD association signals by performing causal variants identification in associated regions analysis and the summary data–based mendelian randomization test. Druggability analysis was then performed on the candidate causal genes. CAD risk variants were tested for associations with VSMC proliferation, migration, and apoptosis. Collective effects of multiple CAD-associated variants on VSMC behavior were estimated by polygenic scores.

RESULTS: Approximately 60% of the known CAD-associated variants showed statistically significant expression quantitative trait locus or splicing quantitative trait locus effects in VSMCs. Colocalization analyses identified 84 genes with expression quantitative trait locus signals that significantly colocalized with CAD association signals, identifying them as candidate causal genes. Druggability analysis indicated that 38 of the candidate causal genes were druggable, and 13 had evidence of drug-gene interactions. Of the CAD-associated variants tested, 139 showed suggestive associations with VSMC proliferation, migration, or apoptosis. A polygenic score model explained up to 5.94% of variation in several VSMC behavior parameters, consistent with polygenic influences on VSMC behavior.

CONCLUSIONS: This comprehensive analysis shows that a large percentage of CAD loci can modulate gene expression in VSMCs and influence VSMC behavior. Several candidate causal genes identified are likely to be druggable and thus represent potential therapeutic targets.

Key Words: coronary artery disease ◼ genetics ◼ muscle, smooth, vascular ◼ transcriptomes

Editorial, see p 930

Genome-wide association studies (GWASs) have identified common genetic variants at >190 loci that are robustly associated with coronary artery disease (CAD). However, the underlying biological mechanisms are still unknown for most of these loci. This hinders translation from the genetic findings to a new
Solomon et al Effects of CAD Variants on VSMCs

understanding of disease mechanisms and the development of new treatments.

About two-thirds of the CAD-associated loci/variants identified by GWASs are not associated with the conventional risk factors (eg, elevated low-density lipoprotein cholesterol level and raised blood pressure), suggesting that the large majority of CAD susceptibility loci do not act through the traditional pathways and thus are not addressed by current treatment. Instead, many of the CAD loci identified by GWASs contain genes implicated in vascular cell biology, pointing to novel mechanisms directly involving cells of the blood vessel wall. Hence, identifying novel pathways and therapeutic targets in vascular cells can potentially aid the development of new treatments that complement current strategies that target conventional risk factors.

Vascular smooth muscle cells (VSMCs) are a major cell type in the blood vessel wall and play important roles in the development and progression of atherosclerosis, the pathological condition underlying CAD. Growing evidence suggests that the genetic influence on CAD risk may act, in part, through VSMCs. For example, genetic variants at a number of CAD loci have been shown to influence gene expression in VSMCs or affect VSMC behavior.

However, a systematic large-scale analysis that integrates genetic, transcriptomic, and phenotypic assays of a large biobank of VSMCs from different individuals has not been undertaken. Here, we report such an analysis and describe how the findings can be used to identify novel drug targets.

METHODS

The data, analytical methods, and study materials will be available to other researchers for purposes of reproducing the results or replicating the procedure. The RNA-sequencing (RNA-Seq) data from this study are available from the Gene Expression Omnibus with the accession number GSE189300. The study was approved by the East Midlands–Derby Research Ethics Committee, and the parents of umbilical cord donors gave written informed consent.

The methods used in this study are summarized here and detailed in Supplemental Methods.

VSMC Biobank

VSMCs were isolated from the artery of umbilical cords from 2114 different donors with a previously reported method. Immunocytochemical staining of cell-type markers was performed on randomly selected representative samples at passage 3 (n=231). The VSMC samples showed strong staining of the VSMC marker α-smooth muscle actin but not the endothelial cell marker von Willebrand factor or the fibroblast marker TE7 (Figure S1).

Genotyping and Imputation

A total of 1992 DNA samples were genotyped for 760,000 variants with Infinium Global Screening Array-24 2.0 BeadChips (Illumina), and imputation was performed to obtain genotypic information of 7,334,165 variants (Supplemental Methods).

Selection of CAD-Associated Variants

We compiled a list of single nucleotide polymorphisms (SNPs) reported by Erdmann et al, Aragam et al, and the GWAS catalog that had been previously associated with CAD at the
genome-wide significance level ($P<5\times10^{-8}$). Linkage disequilibrium between the SNPs was assessed with the LDlink SNPclip tool (Supplemental Methods). In total, 511 independent CAD-associated SNPs were identified (Table S1). Of these, 424 were available in this study, which were either typed by the Global Screening Array or imputed.

RNA Sequencing
RNA-Seq was performed on VSMC RNA samples from 1499 donors. Total RNA was extracted from an aliquot of passage 3 VSMCs stored in RNAlater solution. RNA concentration and integrity were assessed by RNA BR and RNA IQ assays. A strand-specific library with rRNA removal was prepared from total RNA of each of the 1499 VSMC samples, and 150-bp paired-end sequencing at a 30 million read depth was performed with the Illumina platform. RNA data were processed as described in the Supplemental Methods.

To determine whether RNA-Seq results were random or had repeatable differences between VSMCs from different individuals, VSMCs at passage 5 through 7 from 10 randomly selected samples of the VSMC bank were subjected to RNA-Seq using the strand-specific library preparation and paired-end sequencing methods mentioned previously. A hierarchical cluster analysis and a principal component analysis were performed on the RNA-Seq data (Supplemental Methods). The analysis showed that gene expression data from different passages (5–7) of VSMCs from each individual tended to cluster and to be more similar to each other than data from other individuals (Figure S2), indicating that RNA-Seq results had reproducible differences between VSMCs from different individuals.

To determine changes in gene expression in VSMCs with knockdown of YIPF6 or SLC25A36, RNA-Seq was performed on VSMCs transfected with YIPF6 siRNA, SLC25A36 siRNA, or negative control siRNA, as described in the Supplemental Methods.

Multidimensional Scaling Analysis
We used multidimensional scaling analysis to determine the relationship between the VSMC RNA-Seq data and other publicly available bulk RNA-Seq data and single-cell RNA-Seq data (Supplemental Methods). Details about the data sets included in the multidimensional scaling plot are available in Table S2.

Expression Quantitative Trait Locus and Splicing Quantitative Trait Locus Analyses
We performed expression quantitative trait locus (eQTL) and splicing quantitative trait locus (sQTL) analyses on 1486 VSMC samples that had both genotype and gene expression data (Supplemental Methods). The numbers of protein coding genes, long noncoding RNA (lncRNA) genes, pseudogenes, and other genes in the eQTL and sQTL analyses are given in Table S3. We used the eigenMT-BH method for multiple testing correction.

Colocalization With CAD GWAS
We used eQTL and GWAS causal variant identification in associated regions (eCAVIAR) to test for colocalization of CAD GWASs with VSMC cis-eQTL or cis-sQTL (Supplemental Methods). The eCAVIAR analysis used only SNPs that had been reported to associate with CAD at the genome-wide significance level ($P<5\times10^{-8}$) and had genome-wide significant quantitative trait loci in VSMCs in our study. The top quantitative trait locus SNPs (ie, SNPs with the lowest $P$ value) of genes or splice clusters were selected, and SNPs within 500 kb of the top SNP were overlapped with GWAS summary data. The eCAVIAR colocalization test was performed for genes/splice clusters that had >4 overlapping SNPs with GWAS summary data. The number of causal SNPs was set to 2, and colocalization events with $>0.05$ colocalization posterior probability were considered significant.

We also used summary data–based mendelian randomization (SMR)/heterogeneity in dependent instruments (HEIDI) to test for colocalization between CAD GWAS and VSMC cis-eQTL and cis-sQTL (Supplemental Methods). Although we ran transcriptome-wide SMR/HEIDI analysis, we focused only on SNPs that had been reported to associate with CAD at the genome-wide significance level ($P<5\times10^{-8}$) in GWASs. We applied a threshold of $P_{\text{SMR}}<0.05$ and $P_{\text{HEIDI}}>0.05$ to identify significant colocalizations of CAD GWAS signals with VSMC eQTL or sQTL signals.

Druggability and Drug–Gene Interaction Analyses
Genes with eQTL signals in VSMCs that showed significant colocalization with CAD GWAS signals in the eCAVIAR or SMR analyses were interrogated for druggability and drug-gene interactions in the Drug Gene Interaction Database.

Pathway Analyses
Genes with eQTL signals in VSMCs that showed significant colocalization with CAD GWAS signals in the eCAVIAR or SMR analyses were subjected to functional pathway analyses performed with DAVID version 6.8, Panther version 17.0, and the ToppFun function of ToppGene.

VSMC Behavior Assays
VSMCs at passage 3 were subjected to proliferation, migration, and apoptosis assays with the use of an Operetta CLS High-Content Analysis System (PerkinElmer; Supplemental Methods). In each of these assays, each VSMC preparation was analyzed in 4 replicate wells on a 96-well plate. All 3 assays have low coefficient of variation: 2.12%–3.87% for the proliferation assay, 1.81% (95% CI, 1.62%–4.53%) for the apoptosis assay. To verify that the assays have low coefficient of variation: 2.99% (95% CI, 2.12%–3.87%) for the proliferation assay, 1.81% (95% CI, 1.62%–4.53%) for the apoptosis assay. To verify that the cell behavior data generated from this study are reproducible, we repeated the assays on a random selection of the VSMC preparations (n=50). For each preparation, the 2 experiments were performed at 2 different times (1 at passage 3 and the other at passage 4). The results of the 2 independent assays were highly consistent (Figure S3), indicating high reproducibility of the data generated.

Weighted Gene Coexpression Network Analysis
We used weighted gene coexpression network analysis (WGCNA) to identify gene coexpression modules that
were correlated with VSMC behavior, as detailed in the Supplemental Methods.

**siRNA Transfection**

VSMCs were transfected with YIPF6 siRNA, SLC25A36 siRNA, or negative control siRNA (Supplemental Methods). Successful knockdown of YIPF6 and SLC25A36 was confirmed by reverse-transcription polymerase chain reaction and Western blot analyses (Figures S5 and S6).

**Statistical Analysis of CAD Risk Variants in Relation to VSMC Behavior Parameters**

We ascertained associations of the aforementioned 424 CAD SNPs with each VSMC behavior parameter in an additive model (Supplemental Methods).

**Polygenic Score Analysis**

A polygenic score analysis was carried out using variants that had been reported to be associated with CAD in GWASs \(^{1–3}\) and that were nominally associated (\(P<0.05\)) with VSMC behavior in our study (Supplemental Methods).

**RESULTS**

The design of this study is summarized in Figure 1. For this study, we developed a large bank of VSMCs derived from the umbilical artery of 2114 individuals. We genotyped a total of 1992 samples of this VSMC biobank using Infinium Global Screening Array-24 2.0 BeadChips for 760,000 variants and performed imputation to obtain genotypic information for 7,334,165 variants. We also undertook RNA-Seq on 1499 VSMC samples (at culture passage 3) at a 30 million read depth (150 bp paired end) using a strand-specific library with ribosomal RNA removal prepared from each sample. Furthermore, assays of VSMC proliferation (\(n=2025\) samples), migration assay (\(n=2019\), and apoptosis (\(n=2075\)) were performed with passage 3 cells. The resulting data sets of genotypes, RNA-Seq, and VSMC behavior parameters were analyzed as outlined in Figure 1 and described here.

**Influences of CAD-Associated Variants on Gene Expression/Splicing in VSMCs**

Using data from the RNA-Seq mentioned previously, we performed multidimensional scaling analysis comparing the transcriptomes of VSMCs in this study with reported transcriptomic data from human coronary artery smooth muscle cells \(^{16}\) and transcriptomic data of other types of cell/tissue. The analysis showed that the gene expression profile of VSMCs in this study mapped closer to human coronary artery smooth muscle cells than any other cell/tissue types (Figure 2).

Because the vast majority of disease-associated genetic variants identified by GWASs are located in non-coding regions of the genome, \(^{8,27}\) a prevailing hypothesis is that these variants alter the individual’s disease risk through their effect on gene expression. \(^{8,27}\) Using the genotypic and RNA-Seq data sets from VSMCs in this study, we performed eQTL and sQTL analyses to systematically identify genetic variants that were associated with gene expression or splicing and to determine the genes with an expression or splicing that was associated with these variants.

These analyses generated a comprehensive compilation of eQTLs and sQTLs in VSMCs, with stringent statistical significance (summarized in Table S3). We then looked up genetic variants that had been reported to be associated with CAD in GWASs \(^{1–3}\) in this eQTL/sQTL catalog. Approximately 60% of the CAD-associated variants \(^{1–3}\) in this study showed statistically significant eQTL and/or sQTL effects (summarized in Figures S7 and S8 and detailed in Tables S4–S6). Two-thirds of these variants had eQTL effects on \(>1\) gene. The CAD risk allele of \(\geq 20\%\) of such variants had higher expression (positive \(\beta\) value) of all of the associated genes, whilst the CAD risk allele of some \(\geq 25\%\) variants had lower expression (negative \(\beta\) value) of all associated genes. However, the CAD risk allele of the majority \(\geq 55\%\) of such variants was associated with higher expression of some gene(s) but lower expression of other gene(s). Of the associated genes, \(\approx 80\%\) were protein coding and \(\approx 20\%\) were of lncRNAs (highlighted in green and yellow, respectively, in Tables S4–S6).

**Colocalization of CAD Association With eQTL Signals in VSMCs**

Colocalization between disease GWASs and eQTL signals has been used as a fine-mapping approach to successfully identify candidate causal variants and candidate causal genes at disease risk loci. \(^{16,24,28,29}\) Therefore, we carried out colocalization tests using publicly available CAD GWAS summary statistics \(^{7,30,31}\) and VSMC eQTL data from our study. We applied 2 colocalization tools: eCAVIAR \(^{23}\) and SMR/HEIDI \(^{24}\). The loci/variants/gene identified from these analyses are described in Tables S7 and S8. A recent colocalization study \(^{16}\) of human coronary artery smooth muscle cells reported 5 genes (\(SIPA1, TCF21, SMAD3, FES,\) and \(PDGFRA\)) with eQTL signals that showed significant colocalization with CAD GWAS signals in either eCAVIAR or SMR analysis. Three of these genes (\(TCF21, SMAD3,\) and \(FES\)) showed significant colocalization in both the eCAVIAR and SMR analyses in our study. Furthermore, our study detected significant colocalizations of eQTL signals of 81 other genes (thus, 84 genes in total, including \(TCF21, SMAD3,\) and \(FES\)) with CAD GWAS signals in eCAVIAR or SMR analyses, among which 18 showed significant colocalization in both analyses (Tables S7 and S8).
colocalizations between VSMC eQTL and CAD GWAS signals in several representative loci observed in this study are presented in Figure 3. The chromosomal locations of the 84 candidate causal genes are shown in Figure 4A. Functional pathway analyses revealed enrichment of several biological pathways, for example, the transforming growth factor-β (TGFβ) signaling pathway, which included TGFB1, BMPR2, BMP1, and SMAD3 (Figure 4B and Table S9).

**Druggability of Candidate Causal Genes**

An interrogation of the Drug Gene Interaction Database of the 84 candidate causal genes with eQTL signals in VSMCs that showed significant colocalization with CAD GWAS signals in the eCAVIAR or SMR analyses identified 38 genes that were druggable (Figure 5 and Table S10), indicating that they are potential therapeutic targets. Furthermore, the interrogation of the Drug Gene Interaction Database showed that 13 of the 84 candidate causal genes had evidence of drug-gene interactions (Figure 5 and Table S11).

**Coexpressed Gene Modules Associated With VSMC Behavior**

Using the VSMC transcriptomic and cell behavior data sets, we investigated possible associations between gene expression levels and VSMC behavior. Because it is expected that VSMC behavior is influenced by many genes concurrently, we performed a WGCNA. The WGCNA algorithm clustered the VSMC transcriptome into 32 coexpression modules, each of which was given a name by the WGCNA program with a prefix ME (Module Eigengene) followed by a color such as MEred, MEyellow, and so on. WGCNA usually assigns genes (typically housekeeping genes) with an expression that has little variation throughout the data set to the MEgray module. In this study, the gray module contained 65% of all of the genes analyzed. The numbers of genes and the identity of the hub gene in each of the modules are described in Table S12.

The WGCNA analysis revealed highly significant associations of several of the coexpressed gene modules with the various VSMC behavior parameters (Figures 6 and 7).
Solomon et al: Effects of CAD Variants on VSMCs

and Figure S9); for example, the MEgreen module was inversely correlated with VSMC proliferation (Figure 6A and 6B), whereas the METurquoise module was inversely associated with apoptosis (Figure 7A and 7B). To test the effect of the MEgreen module hub gene YIPF6 on VSMC proliferation and the effect of the METurquoise module hub gene SLC25A36 on apoptosis, we performed a proliferation assay in VSMCs with siRNA-mediated knockdown of YIPF6 and an apoptosis assay in VSMCs with siRNA-mediated knockdown of SLC25A36. The experiments demonstrated that YIPF6 knockdown increased VSMC proliferation (Figure 6C), whereas SLC25A36 knockdown promoted VSMC apoptosis (Figure 7C). An RNA-Seq analysis showed that YIPF6 knockdown altered the expression of a panel of other genes (Figure S10 and Table S13), whereas SLC25A36 knockdown changed the expression of another panel of genes (Figure S11 and Table S14).

A gene ontology analysis showed that the various coexpressed gene modules had significant enrichment of genes in particular biological processes, cellular components, molecular functions, and functional pathways. For example, the MEgreen module had significant enrichment of genes in pathways related to phagosome, membrane trafficking, and vesicle-mediated transport (Figure 6D and Table S15), whereas the METurquoise module had significant enrichment of genes in pathways involved in cadherin signaling, Wnt signaling, and the ensemble of genes encoding extracellular matrix proteins (Figure 7D and Table S16).

Effects of CAD-Associated Variants on VSMC Behavior

Using genotype and VSMC behavior assay data, we ascertained whether there were relationships between VSMC behavior and genetic variants that had been reported to be associated with CAD in GWASs.1–3 At the Bonferroni-corrected $P$ value threshold of $8.42 \times 10^{-9}$ (0.05/14 behavior parameters$x$424 variants), none of the CAD variants showed significant association with the VSMC behavior parameters measured in this study. However, 139 CAD variants showed nominal associations with at least 1 of the VSMC behavior parameters ($P < 0.05$; Table S17). An interrogation of the VSMC eQTL and colocalization data sets described earlier showed that 63 of these 139 variants had eQTL effects on the expression of genes in VSMCs and that the eQTL signals of 38 of these genes significantly colocalized with CAD GWAS signals (Figure S12). Because genetic variants at multiple CAD loci showed suggestive association with VSMC behavior in our study, we calculated polygenic scores to ascertain and estimate possible additive effects of different CAD loci. The
polygenic score model was statistically significant for VSMC proliferation, migration straightness, and migration distance (Table S18). The polygenic score model explained up to 5.94%, 3.24%, and 5.02% of VSMC proliferation, migration straightness, and migration distance, respectively (Figure S13 and Table S18).

DISCUSSION

For most of the CAD-associated loci identified by GWAS to date, the biological mechanisms through which the genetic variants influence CAD risk are unclear. The pathogenesis of atherosclerosis involves several different cell types, and it is plausible that different CAD loci may affect different cell types. To understand the biological mechanisms underlying the genetic associations with CAD and to translate the genetic findings into the development of new treatments, further studies to identify the cell types involved and the causal genes at the different CAD loci are required.

Previous studies have investigated some of the CAD loci individually in VSMCs, including LMOD1 at...
the 1q32.1 locus, GUCY1A3 at 4q32, PDGFRα at 4q12, TCF21 at 6q23, CDKN2A/CDKN2B/ANRIL at 9p21, SIPA1 at 11q13, COL4A1/COL4A2 at 13q34, SMAD3 at 15q22, ADAMTS7 at 15q25, and FES at 15q26. In addition, a recent study interrogated 95 CAD loci in relation to gene expression in 52 coronary artery smooth muscle cell lines, and another recent study examined 163 CAD loci in relation to...
VSMC phenotypes in cells derived from 151 heart transplant donors. The results from these previous and recent studies indicate that genetic influences on the transcriptome and behavior of VSMCs can be an important mechanism at many of the CAD loci.

Using a systematic approach including genetic, transcriptomic and cell behavior assays, and a larger VSMC bioresource, here we examined all CAD loci identified by GWASs to date. Our study shows that at the vast majority of the currently known CAD loci, the disease-associated variants have eQTL effects on gene expression in VSMCs. This provides a comprehensive catalog of genes with an expression that is influenced by CAD-associated variants, informing which of the CAD loci influence gene expression in VSMCs and which genes are modulated at those loci.

For loci for which the disease-associated variant influences the expression of >1 gene, evidence of colocalization of the disease-association signal with the eQTL signal can indicate which gene is causative. In a recent genetic study of coronary artery smooth muscle cells, Liu et al.16 identified 5 genes (SIPA1, TCF21, SMAD3, FES, and PDGFRA) with eQTL signals that significantly colocalized with CAD GWAS signals detected by either eCAVIAR or SMR analysis, suggesting that these genes are likely to be causal at their respective CAD loci. Our study confirmed the colocalization of 3 of these genes (TCF21, SMAD3, and FES) by both eCAVIAR and SMR analyses. In another recent genetic study of VSMCs, Aherrahrou et al.19 found an important role of MIA3 in CAD through modulation of VSMC behavior. In support, we observed colocalization of the MIA3 eQTL signal with
the CAD GWAS signal. Furthermore, our study identified 80 other genes with eQTL signals that significantly colocalized with CAD signals, which have not been reported in the literature and thus represent novel findings.

The novel finding of >80 candidate causal genes provides a broader understanding of the biological effects underlying the genetic associations with CAD. Currently known functions (if any) of these genes are summarized in Table S19. Pathway analyses of the 84 candidate causal genes showed enrichment of important functional pathways. Of particular interest is the TGFβ signaling pathway, which includes the candidate causal genes TGFB1, SMAD3, BMP1, and BMPR2. TGFβ signaling acts through the intracellular mediator SMAD3 and can interact with the BMPR2-mediated BMP signaling pathway.33 Previous investigations have indicated that TGFβ and BMP signaling can modulate VSMC behavior and is implicated in atherosclerosis and CAD.34,35 Another pathway of interest is related to type I collagen synthesis, which includes the candidate causal genes BMP1, MIA3, and SERPINH1. Studies have shown that type I collagen affects VSMC proliferation36 and plays a key role in atherogenesis.37 Our present study provides genetic data further indicating the importance of these pathways/genes in modulating VSMC behavior and in atherosclerosis.

Furthermore, several other candidate causal genes are also known to influence VSMC behavior and are involved in atherosclerosis. For example, a recent study revealed a role for TCF21 in VSMC phenotypic modulation, demonstrated that TCF21 plays a protective role against atherosclerosis, and showed that increased TCF21 expression is associated with decreased CAD risk.38 In support, our study observed an eQTL effect of

Figure 7. Coexpressed gene modules in relation to VSMC apoptosis and functional pathways.
A. Heat map representation of correlations of coexpression gene modules with parameters of vascular smooth muscle cell (VSMC) apoptosis. Values shown are correlation coefficients and P values (in brackets). The prefix ME in each module name stands for module eigengene. B. Scatterplot of MEturgeonquoise module membership (x axis) vs coefficient of correction between individual gene expression level and %D@4h (y axis). Each gene is indicated by an open dot in turquoise. C. Effect of the MEturgeonquoise module hub gene SLC25A36 on VSMC apoptosis. Data shown are mean (±SEM) values of the percentage of dead cells (propidium iodide positive) at 4 hours after staurosporine treatment (%D@4h) of VSMCs transfected with either SLC25A36 siRNA or negative control siRNA relative to nontransfected VSMCs (n=5; VSMCs from 5 different individuals; P value is from a 2-tailed Mann-Whitney test). SLC25A36 knockdown in VSMCs transfected cells is shown in Figure S6. D. Biological pathways enriched in the MEturgeonquoise module. ECM indicates extracellular matrix; EGF, epidermal growth factor; FDR, false discovery rate; FSGS, focal segmental glomerulosclerosis; NA@30m and NA@60m, change in nuclear area at 30 and 60 minutes after staurosporine treatment; NF@30m and NF@60m, change in nuclear fragmentation index at 30 and 60 minutes after treatment with the apoptosis inducer staurosporine; %D@8h, percentage of dead cells (propidium iodide positive) at 8 hours after staurosporine treatment; and TT50D, time in minutes for 50% of cells to become propidium iodide positive after staurosporine treatment.
the reported CAD-associated variant rs2327429 on TCF21 expression in VSMCs, with the nonrisk allele (C) having higher TCF21 expression than the CAD risk (T).

Growing evidence indicates that lncRNAs play important roles in many biological processes and diseases, including atherosclerosis, and a number of lncRNAs have been identified as potential therapeutic targets. In this study, we observed eQTL effects of CAD-associated variants on lincRNA expression at many loci, for example, CDKN2B-AS1 (Table S4) on chromosome 9p21, the locus that is more significantly associated with CAD in GWASs than any other locus. Furthermore, analyses of colocalizations of CAD association with eQTL signals in VSMCs indicated that several lncRNAs (TDRKH-AS1, MIA3-AS, ATP2B1-AS, EIF2B2-AS, MAP1S-AS, HCG27-AS, ENSG00000226453, and ENSG00000256879) are candidate causal genes. In addition, our study suggests a relationship of some of these lncRNA genes with VSMC behavior. For example, we observed that the CAD risk allele of rs11810571 was associated with decreased TDRKH-AS1 expression in VSMCs and that TDRKH-AS1, which was one of the genes in the MEgreen module in the WGCNA analysis, was inversely associated with VSMC proliferation.

Among the 84 candidate causal genes, 38 are classified as druggable in the Drug Gene Interaction Database, indicating that these genes are potential therapeutic targets. The candidate causal genes in the TGFβ/BMP signaling pathway (TGFβ1, SMAD3, BMP1, and BMPR2) and TCF21 highlighted previously are among the list of druggable genes. Furthermore, 13 of the 84 candidate causal genes had existing evidence of drug-gene interactions. These findings are likely to have utility in therapeutics development; recent studies have indicated that using genetic data to select the best targets can significantly increase the likelihood of success in the development of new drugs.

In a recent study, Aherrahrou et al observed nominal associations of 79 CAD loci with VSMC phenotypes. Our study also detected suggestive associations of variants at 139 CAD loci with parameters of VSMC behavior. However, the experimental conditions and measurements of VSMC behavior of these 2 studies were different. Aherrahrou et al measured VSMC proliferation in response to platelet-derived growth factor-BB, TGFβ1, or interleukin-1β; VSMC migration induced by platelet-derived growth factor-BB; and VSMC calcification. The VSMC behavior assays of our study were proliferation of nonstimulated cells, migration of nontreated cells, and apoptosis. Therefore, the results of these 2 studies are not directly comparable. Nevertheless, both studies have provided new information indicating that a considerable number of CAD loci likely act, in part, through genetic influences on VSMC behavior.

Studies have indicated that a large proportion of CAD heritability in humans is attributable to the collective polygenic effect of variants at many genetic loci, each having a moderate contribution. It is plausible that the polygenic influence of CAD susceptibility is a result of polygenic effects on the function/behavior of the various types of cells that are involved in the pathogenesis of CAD. The presence of a polygenic influence on VSMC behavior is evident from previous and our present studies, which have collectively shown the influences of genetic variants at many different CAD loci on VSMC behavior. The polygenic score analysis of the CAD-associated variants that showed nominal associations with VSMC behavior in the present study indicated that these variants collectively explained up to 5.94% of the between-sample variation of VSMC behavior. This is likely to be an underestimate because of the in vitro nature of the VSMC behavior assays. Nonetheless, these results support the notion of a polygenic influence on VSMC behavior.

The WGCNA of this study likely represents the most comprehensive analysis of coexpressed gene modules in VSMCs to date. The results of this analysis indicate that VSMC proliferation, migration, and apoptosis are associated with various coexpressed gene modules; for example, proliferation is inversely associated with the MEgreen module, whereas apoptosis is inversely correlated with the Meturquoise module. We experimentally validated the effects of the hub gene (YIPF6) of the MEgreen module and the hub gene (SLC25A36) of the Meturquoise module, demonstrating that knockdown of YIPF6 increased VSMC proliferation and knockdown of SLC25A36 promoted VSMC apoptosis. Therefore, the results of our study suggest that YIPF6 inhibits cell proliferation, which is in line with the finding of a previous study that overexpressing YIPF6 in a prostate carcinoma epithelial cell line reduced cell proliferation. It has been shown that YIPF6 interferes with the reassembly of the Golgi apparatus, which could be a reason for the inhibitory effect of YIPF6 on cell proliferation. With regard to SLC25A36, the results of our study indicate that it has an antiapoptotic effect. SLC25A36 is an intracellular transporter that imports/export pyrimidine nucleotides into and from mitochondria, thereby playing an important role in mitochondrial DNA and RNA synthesis. A recent study has shown that SLC25A36 deficiency causes a reduction in mitochondrial DNA and a decrease of mitochondrial membrane potential. It is well established that the loss of mitochondrial membrane potential is a key trigger of apoptosis.

Conclusions

This study, with integrative analysis of genetic, transcriptomic, and cell behavior data from a large bank of VSMCs, identified 84 genes that may modulate CAD risk through VSMCs, a number of which (eg, TGFβ1, SMAD3, BMP1, and BMPR2) are likely connected in functional pathways that influence VSMC behavior.
Among the candidate causal genes identified, 38 are potentially druggable and therefore represent promising therapeutic targets. These findings could be used to inform the development of new treatments that locally target VSMCs and complement current interventions that address conventional CAD risk factors.

ARTICLE INFORMATION

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Affiliations

Department of Cardiovascular Sciences, University of Leicester, and National Institute for Health Research Leicester Biomedical Research Centre, UK (C.U.S., D.G.M., C.A., P.G., L.T., P.J.S., S.K., J.C.C., T.R.W., C.P.N., J.N.S., S.Y.). Shantou University Medical College, China (W.Y., S.Y.). Cardiovascular Disease Translational Research Programme, Department of Medical, National University of Singapore (S.Y.).

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Disclosures

None.

Supplemental Material

Expanded Methods

Figures S1–S13

Tables S1–S19

References 49–52

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Solomon et al

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