Recent genome-wide association studies (GWAS) for coronary artery disease (CAD) have identified >45 novel loci, the majority of which are not associated with traditional CAD risk factors. The lead single-nucleotide polymorphisms (SNPs) for each of these loci are generally common, displaying allele frequencies from 0.13 to 0.91 and allele-specific effect sizes (odds ratios) for CAD of between 1.06 and 1.3. Notably, the majority of CAD signals identified by the GWAS approach are in noncoding regions of the genome, not unexpected given that only 1% of the genome is protein coding. An important part of the noncoding genome is under purifying selection, implying important regulatory functions supported by the fact that an excess of GWAS signals are close to genic regions. However, for most of these variants including the PHACTR1 gene region on chromosome 6p24, the causal biological mechanisms have remained unclear.

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In this issue of ATVB, Beaudoin et al have sought to confirm previous reports that rs9349379A>G is the lead SNP in this region, to determine by expression quantitative trait loci (eQTL) analysis if PHACTR1 is likely to be the causal gene and using a combination of bioinformatic and laboratory analysis, and to explore possible causal mechanisms relating the risk allele (G; minor allele frequency, 0.41) to decreased PHACTR1 expression.

By resequencing and imputation from the 1000-genome data set, they identify rs9349379 as the top SNP associated with CAD in Montreal Heart Institute Biobank samples consisting of 1176 myocardial infarction cases and 1996 French Canadian controls (G allele odds ratio, 1.37; \( P=8.4 \times 10^{-6} \)). No other SNP in this region remained significant after conditional analysis on rs9349379 (Figure).

eQTL are genetic variants that associate with the RNA transcript level of a given gene. The majority of those identified by the GWAS approach seem to have cis-effects, thus acting on the expression of nearby genes. Evidence that a given SNP associates with expression of a candidate gene (eQTL) further supports a potential causal association. Given that these effects are often cell specific, eQTL analysis in a relevant cell or tissue is important. Because available eQTL data sets for this SNP did not include coronary artery cell types, analysis was performed in a sample of 25 genotyped coronary artery specimens. Despite the small sample size, the risk allele was found to associate with reduced expression of PHACTR1 but not with expression of all other coding genes in a 1-Mb region on either side of rs9349379.

As a complementary approach to eQTL analysis, public databases, generated by the Encyclopedia of DNA Elements and Roadmap Epigenomics projects, can be used to identify predicted functional regulatory elements. An important caveat is that these analyses require genome-wide chromatin data from a relevant cell type. Here, bioinformatic analysis using Roadmap data and in silico searches predicted that rs9349379(G) interrupts an myocyte enhancer factor (MEF)2–binding site. This was confirmed in vitro by electrophoretic mobility shift assay with human umbilical vein endothelial cell nuclear extracts, and by supershift assay, MEF2A and MEF2C were shown to be among the nuclear proteins interacting with this region. Finally, to support the premise that the MEF2-binding site is functionally important, they used CRISPR/Cas9 genome editing in human embryonic stem cells. They identified a clone carrying a heterozygous deletion of a 34-bp segment encompassing the MEF2-binding site that in differentiated endothelial cells attenuated PHACTR1 expression.

In summary, this important functional analysis of PHACTR1 illustrates the multifaceted approach required to unravel mechanisms of a GWAS locus in common diseases. In addition, rs9349379 does not associate with other traditional CAD risk factors, highlighting that altered binding of MEF2 or other factors at this region could represent an interesting novel CAD mechanism.

Nonetheless, important questions remain. Although the risk allele (G) associates with decreased PHACTR1 expression in human coronary arteries, it is not entirely clear which arterial wall cell type is susceptible to altered PHACTR1 expression. Because functional studies were carried out in human umbilical vein endothelial cell, the authors suggest that arterial endothelial cells are the key. However, Figure 2A highlights the high relative PHACTR1 expression in the aorta and heart and suggests that PHACTR1 is important in other vascular cell types. According to the Roadmap Epigenomics data, chromatin state predictions indicate that rs9349379 is located in a transcriptional enhancer in other smooth muscle types. The authors rationalized not using vascular smooth muscle...
Because of their expression in Figure 2A, it was suggested that PHACTR1 levels in intestinal smooth muscle were low. However, aortic smooth muscle cells may have vastly different PHACTR1 expression compared with intestinal smooth muscle. The MEF2 family of transcription factors has widely characterized roles in vascular smooth muscle.

The authors have also not firmly established that disruption of MEF2 binding per se is causal because other transcription factors may potentially bind to this site, and partial siRNA knockdown of 2 members of the MEF2 family of proteins, MEF2A and MEF2C, did not alter PHACTR1 expression. Of note, a putative association of a structural variant in MEF2A with CAD risk was refuted by later studies. It is curious that MEF2B and MEF2D were not examined because previous studies have implicated the important roles for these latter MEF2 members in vascular cell lines. It would also have been useful to include a reporter gene transactivation study to demonstrate that rs9349379 lies in an enhancer and alters expression in target cells. The inclusion of luciferase results would lend more support to finding that the 34-bp deletion via CRISPR/Cas9 has functional effect on enhancer function.

Perhaps more importantly, little information is yet available on how the PHACTR1 gene product may relate to atherosclerosis. It is known to modulate protein phosphatase 1 activity in vitro and to interact with actin. The locus associates with chronic CAD and coronary artery calcium rather than acute myocardial infarction supportive of effects on atherosclerosis per se. However, the rs9349379(G) allele for CAD has opposite effects on risk for cervical artery dissection, suggesting complex effects on arterial wall integrity. The MEF2-binding sequence and rs9349379 are conserved between humans and mice; so, future studies incorporating a murine model of atherosclerosis may shed further light on this fascinating but unfinished story.

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None.

References


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