Myocardial Infarction–Associated SNP at 6p24 Interferes With MEF2 Binding and Associates With *PHACTR1* Expression Levels in Human Coronary Arteries

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Objective—Coronary artery disease (CAD), including myocardial infarction (MI), is the main cause of death in the world. Genome-wide association studies have identified dozens of single nucleotide polymorphisms (SNPs) associated with CAD/MI. One of the most robust CAD/MI genetic associations is with intronic SNPs in the gene *PHACTR1* on chromosome 6p24. How these *PHACTR1* SNPs influence CAD/MI risk, and whether *PHACTR1* itself is the causal gene at the locus, is currently unknown.

Approach and Results—Using genetic fine-mapping and DNA resequencing experiments, we prioritized an intronic SNP (rs9349379) in *PHACTR1* as causal variant. We showed that this variant is an expression quantitative trait locus for *PHACTR1* expression in human coronary arteries. Experiments in endothelial cell extracts confirmed that alleles at rs9349379 are differentially bound by the transcription factors myocyte enhancer factor-2. We engineered a deletion of this myocyte enhancer factor-2–binding site using CRISPR/Cas9 genome-editing methodology. Heterozygous endothelial cells carrying this deletion express 35% less *PHACTR1*. Finally, we found no evidence that *PHACTR1* expression levels are induced when stimulating human endothelial cells with vascular endothelial growth factor, tumor necrosis factor-α, or shear stress.

Conclusions—Our results establish a link between intronic SNPs in *PHACTR1*, myocyte enhancer factor-2 binding, and transcriptional functions at the locus, *PHACTR1* expression levels in coronary arteries and CAD/MI risk. Because *PHACTR1* SNPs are not associated with the traditional risk factors for CAD/MI (eg, blood lipids or pressure, diabetes mellitus), our results suggest that *PHACTR1* may influence CAD/MI risk through as yet unknown mechanisms in the vascular endothelium. **(*Arterioscler Thromb Vasc Biol*. 2015;35:1472-1479. DOI: 10.1161/ATVBAHA.115.305534.)**

Key Words: coronary artery disease ■ genetic association studies ■ myocardial infarction

Coronary artery disease (CAD), including myocardial infarction (MI), remains the main cause of death and disability worldwide, despite a large number of efficient drugs to manage the traditional risk factors (eg, blood lipids, blood pressure, and heart rate).[1] Recently, several new drugs have failed to show efficacy in large-scale clinical trials,[2–4] emphasizing the need for new therapeutic targets.

See accompanying editorial on page 1293

CAD/MI is heritable and the functional study of its genetic determinants could yield new biological pathways important for disease pathogenesis. Genome-wide association studies have already identified 45 single nucleotide polymorphisms (SNPs) robustly associated with CAD, including several markers that are not associated with the CAD epidemiological risk factors, such as hypertension, dyslipidemia, and type 2 diabetes mellitus.[5] One of these CAD/MI loci is located on chromosome 6p24 and is defined by a group of SNPs in

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Nonstandard Abbreviations and Acronyms

CAD	coronary artery disease
HUVEC	human umbilical vein endothelial cells
MEF2	myocyte enhancer factor-2
MHI	Montreal Heart Institute
MI	myocardial infarction
NFKB1	nuclear factor of kappa light polypeptide gene enhancer in B-cells 1
PHACTR1	phosphatase and actin regulator-1
SNP	single nucleotide polymorphism
TGF-β	transforming growth factor-β
VEGF	vascular endothelial growth factor

linkage disequilibrium within the third intron of the gene *PHACTR1*. In addition to the genetic association between *PHACTR1* SNPs and MI[6] or CAD,[5,7–9] these 6p24 SNPs have also been robustly associated with coronary artery calcification,[10] coronary artery stenosis,[11] migraine,[12] hemodynamic indexes,[13] and cervical artery dissection.[14]

It is currently unknown if *PHACTR1* is the causal gene at the locus, and if causal, what are the mechanisms underlying its effect on CAD/MI. The protein encoded by *PHACTR1* was initially identified in a yeast 2-hybrid screen as a protein phosphatase 1 interactor.[15] It is abundantly expressed in the nervous system, modulates protein phosphatase 1 activity in vitro and also interacts with actin.[15] In human umbilical vein endothelial cells (HUVEC), it has been reported that *PHACTR1* expression is induced on treatment with vascular endothelial growth factor (VEGF)[16] and that *PHACTR* depletion induces apoptosis and decreases tube formation.[17] In NIH3T3 fibroblasts, ectopically expressed *PHACTR1* translocates to the nucleus after serum stimulation and this translocation depends on the competition of protein phosphatase 1 and G-actin for the PHACTR1 RPEL motifs and C-terminal domain.[18] Disruption of these domains inhibits the cytoplasmic/nuclear localization of PHACTR1 and impairs actomyosin assembly.[18] Recently, it was shown that PHACTR1 acts downstream of TGF-β to mediate actin reorganization and migration of breast cancer cells.[19]

The aim of our study was to determine if *PHACTR1* is a good biological candidate for a role in CAD/MI. Here, we show that CAD/MI-associated SNPs at 6p24 correlate with *PHACTR1* expression levels in coronary arteries. This result suggests that changes in *PHACTR1* expression levels may modulate CAD/MI risk. We fine-mapped the association signal to an SNP in the third intron of *PHACTR1*, and showed that its alleles disrupt binding of the transcription factors myocyte enhancer factor (MEF) 2. Deleting this MEF2-binding site using CRISPR/Cas9 reduces *PHACTR1* expression in endothelial cells. Our findings support a possible role for PHACTR1 in the vascular endothelium and provide a framework to further understand how *PHACTR1* SNPs, which are not associated with the epidemiological risk factors of cardiovascular diseases, can influence CAD/MI risk.

Materials and Methods

Materials and methods are available in the online-only Data Supplement.

Results

SNPs at *PHACTR1* Associate With MI in French Canadians

To characterize the genetic risk factors of CAD/MI in French Canadians, we genotyped SNPs previously associated with MI or CAD in 1176 MI cases and 1996 controls selected from the Montreal Heart Institute (MHI) Biobank (Table I in the online-only Data Supplement).[5] Of the 45 SNPs that we genotyped successfully, 35 had an odds ratio consistent with the literature (binomial $P=1.2\times10^{-4}$) and 12 of these 35 SNPs were nominally significant (1-tailed $P<0.05$ and binomial $P=1.5\times10^{-6}$; Table II in the online-only Data Supplement). The strongest genetic association with MI risk that we observed was with rs12526453, an SNP located in the third intron of *PHACTR1* (G allele odds ratio=0.79; $P=8.4\times10^{-4}$).[6] To fine-map the genetic association between the *PHACTR1* locus and MI risk in our population, we selected and genotyped 13 additional SNPs that cover common genetic variation at the locus based on genetic variation patterns in European-ancestry individuals from the 1000 Genomes Project (Table III in the online-only Data Supplement).[20,21] We also imputed ungenotyped markers using reference haplotypes from the 1000 Genomes Project.[21,22] After filtering on imputation quality, we analyzed association between MI and genotypes for 387 DNA sequence variants (Table IV in the online-only Data Supplement). The strongest association with MI was with a genotyped SNP, rs9349379, located ≈24 kilobases downstream of rs12526453 but still in intron 3 of *PHACTR1* (G allele odds ratio=1.37; $P=8.4\times10^{-6}$; Figure 1; Table 1).[23] When we conditioned on genotypes at rs9349379, no additional DNA markers were significantly associated with MI at the *PHACTR1* locus ($P>0.10$).

We tested association between genotypes at *PHACTR1* rs9349379 and several risk factors for MI in the MHI Biobank

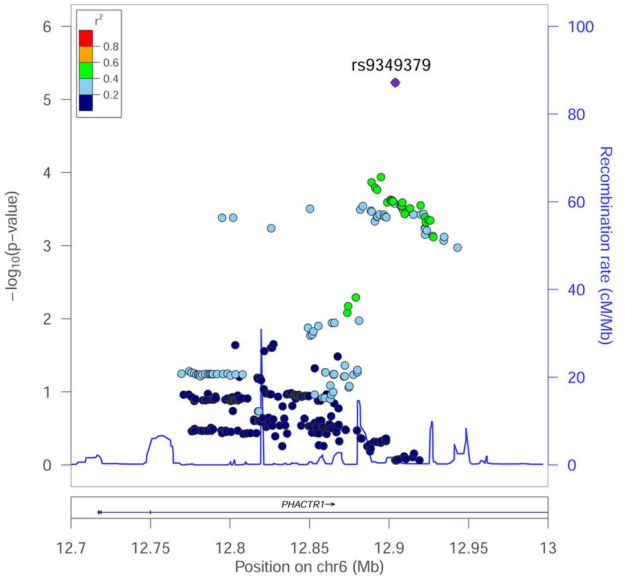

Figure 1. Association results between 387 genotyped or imputed DNA markers in *PHACTR1* and myocardial infarction status in 3172 French Canadians from the Montreal Heart Institute Biobank. rs9349379 has the strongest association signal ($P=8.4\times10^{-6}$). We used the LocusZoom tool to plot association results.

Table 1. Association Results Between rs9349379 and MI in the MHI Biobank*

		rs9349379 (chr6:12903957)	
Phenotype	n	OR (95% CI) or β (SE)	*P* Value
MI	1176 cases/1996 controls	1.37 (1.19–1.57)	8.4×10^{-6}
Hypertension	1543 cases/1534 controls	0.93 (0.83–1.05)	0.25
Type 2 diabetes mellitus	473 cases/2604 controls	0.95 (0.81–1.10)	0.49
Smoking	2018 cases/1075 controls	0.96 (0.86–1.07)	0.46
Systolic blood pressure	3060	–1.08 (0.51)	0.035
Diastolic blood pressure	3058	–0.37 (0.32)	0.25
LDL-cholesterol	1668	–0.013 (0.031)	0.67
HDL-cholesterol	1679	0.0002 (0.012)	0.99

CI indicates confidence interval; HDL, high-density lipoprotein; LDL, low-density lipoprotein; MHI, Montreal Heart Institute; MI, myocardial infarction; and OR, odds ratio.

*Genetic associations were also tested between rs9349379 and several MI risk factors: hypertension, type 2 diabetes mellitus, smoking, systolic and diastolic blood pressure, and LDL- and HDL-cholesterol levels. For dichotomous traits (MI, hypertension, and type 2 diabetes mellitus), OR and 95% CI are provided. For blood pressure measures and cholesterol levels, effect sizes (β) and SE are in mm Hg and mmol/L, respectively. The direction of effect is given for the G allele on the positive strand (NCBI [National Center for Biotechnology Information] build 37.1). The rs9349379 G allele frequency in the MHI Biobank is 37%.

samples: hypertension and blood pressure, type 2 diabetes mellitus, low- and high-density lipoprotein–cholesterol levels, and smoking.[24] After accounting for the number of phenotypes tested, none of the associations were significant (Table 1), consistent with results from large meta-analyses of genome-wide association results for these traits.[25–27] This suggests that genetic variation at the *PHACTR1* locus may influence MI risk through an unanticipated risk factor.

Coding Variants in *PHACTR1* Are Not Associated With MI

We tried to link genetic variation at the *PHACTR1* locus with a specific gene, focusing initially on the *PHACTR1* gene itself. Identification of rare and functional DNA sequence variants by exon resequencing can be used to establish gene causality.[28,29] As part of a parallel study, we resequenced all 14 exons of *PHACTR1* in 500 early onset MI cases and 500 matched controls from the MHI Biobank (Table V in the online-only Data Supplement).[30] We identified 4 novel rare exonic variants: 2 missense variants (ss836901033 [p.Ser190Pro] and ss836901061 [p.Glu196Lys]), as well as a 3′ splice site variant (exon 12, ss836901074) and a 3′ untranslated region variant (ss836901090; Table III in the online-only Data Supplement). We also queried data from the 1000 Genomes Project and found 3 additional rare nonsynonymous DNA sequence variants: 1 frameshift indel (rs36000655) and 2 missense variants (rs61746695 [p.Arg94Pro] and rs17602409 [p.Ile247Met]).

We genotyped 6 of these markers (the 3′ splice site variant ss836901074 failed assay design) in the MHI Biobank MI panel. The three 1000 Genomes Project variants were monomorphic in our DNA collection. After excluding the resequenced samples, the carrier frequencies for the remaining 3 *PHACTR1* variants (ss836901033, ss836901061, and ss836901090) were 1.0% in MI cases and 0.7% in controls (0.3% and 0.1% in cases and controls, respectively, when restricting to missense variants only). The gene-based MI association results for the 3 coding variants or only the 2 missense variants were, *P*=0.38 and *P*=0.089 (In the Materials and Methods section of this article). We also genotyped the same 3 rare *PHACTR1* coding variants in an additional subset of the MHI Biobank (870 MI cases and 1494 controls). The carrier frequencies were 1.4% in cases and 1.3% in controls for the 3 variants (*P*=0.95) and 0.2% in cases and 0.3% in controls for the 2 missense variants alone (*P*=0.51). Consistent with our results, rare coding variants in *PHACTR1* were not associated with MI risk in a recent large-scale whole-exome sequencing project performed in 4703 MI cases and in 5090 controls (gene-based *P*>0.3; Table VI in the online-only Data Supplement).[31] Thus, we cannot conclude that rare coding genetic variation implicates *PHACTR1* in MI.

PHACTR1 rs9349379 is an eQTL in Human Coronary Arteries

Correlation between SNP genotypes and gene expression levels has also been used to identify causal genes involved in complex human diseases.[32] *PHACTR1* was originally identified as a gene highly expressed in the nervous system.[15] We screened several human tissues and cell lines and detected *PHACTR1* expression in the heart, aorta, and primary endothelial cells (Figure 2A).

On the basis of these expression results and the reported association between *PHACTR1* SNPs and CAD/MI, we obtained and extracted RNA from 25 human right coronary arteries to measure *PHACTR1* transcript levels. These samples were from patients undergoing heart transplant at the MHI (Table VII in the online-only Data Supplement). We also genotyped *PHACTR1* rs9349379 in the DNA of the same patients. We detected an association between genotypes at rs9349379 and *PHACTR1* expression levels in this tissue (r^2=0.21; *P*=0.018; Figure 2B). We also measured in the same coronary artery samples the expression of all coding genes located 1 megabase on either side of rs9349379 and found no association with genotypes at this marker (Figure I in the online-only Data Supplement). Thus, the association between genotypes and expression levels seems to be specific to *PHACTR1* within this locus in human coronary arteries.

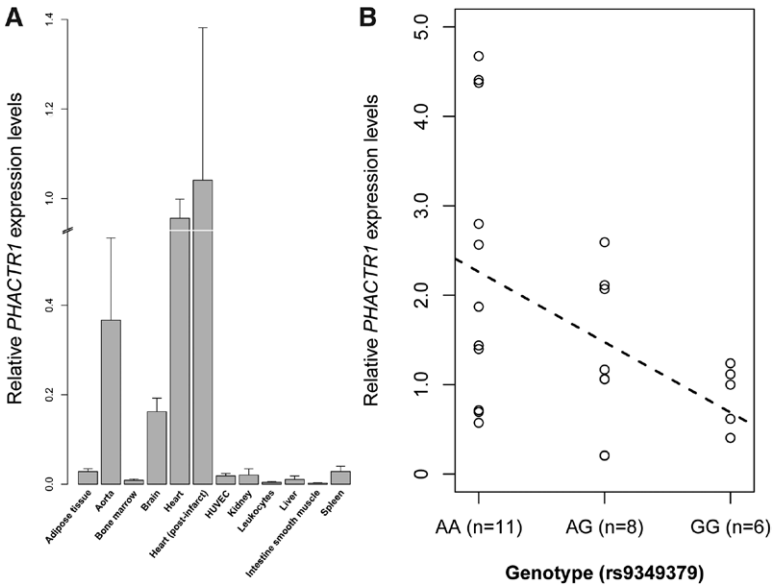

Figure 2. *PHACTR1* gene expression in human tissues. **A**, *PHACTR1* expression levels were measured by quantitative polymerase chain reaction (experiment done in quadruplicates). Results were normalized on the housekeeping gene *HPRT* and calibrated on the expression in the heart (postinfarct). Error bars represent standard deviations. **B**, rs9349379 is an expression quantitative trait locus for *PHACTR1* in human right coronary arteries (total n=25). The dashed line represents the best-fit regression line for *PHACTR1* expression levels. HUVEC indicates human umbilical vein endothelial cells.

We genotyped and imputed *PHACTR1* SNPs in the DNA extracted from the 25 right coronary artery donors, as described above (In the Materials and Methods section of this article; Table III in the online-only Data Supplement). When we conditioned on genotypes at rs9349379, no other markers were significantly associated with *PHACTR1* expression levels, consistent with the MI genetic association results (Table VIII in the online-only Data Supplement). This suggests that rs9349379 is an expression quantitative trait locus (eQTL) for *PHACTR1* in human right coronary arteries, providing an argument in favor of *PHACTR1* being at least 1 of the genes at the 6p24 locus implicated in CAD/MI development. Because we could not identify an independent collection of human coronary arteries to replicate this eQTL effect, we turned to functional characterization to understand how genotypes at rs9349379 may modulate *PHACTR1* expression levels.

rs9349379 Alleles Modulate MEF2 Binding at the *PHACTR1* Locus

There are no genetic variants in the 1000 Genomes Project European populations in strong linkage disequilibrium with rs9349379 (no variants with $r^2 \geq 0.8$). We queried data from the ENCODE and Roadmap Epigenomics Projects to identify how genotypes at rs9349379 can influence *PHACTR1* expression.[33] There is no functional annotation in ENCODE for rs9349379. In the Roadmap Epigenomics data, chromatin state predictions based on histone tail modifications suggest that rs9349379 is located in a transcriptional enhancer in skeletal and stomach smooth muscle. However, we could not measure *PHACTR1* expression in intestine smooth muscle, highlighting the importance to validate these bioinformatic predictions by direct biological experiments (Figure 2A). Although the Roadmap Epigenomics Project plans on testing several relevant human tissues for CAD/MI, notably the heart and aorta, these results are not yet publicly available.

Additional in silico searches revealed that rs9349379 lies within a predicted binding site for the transcription factors MEF2. MEF2 is a family of transcription factors encoded by 4 genes in humans (*MEF2A-D*) and is important for cellular differentiation and stress response.[34] A previous report suggested that genetic variation in *MEF2A* might predispose to CAD risk,[35] although this result is controversial.[36,37] In the presence of the A allele at rs9349379, the DNA sequence matches perfectly the canonical MEF2-binding site except for a C-to-A change at the 5′ end of the motif (Figure 3). The G allele at rs9349379, associated with lower *PHACTR1* expression in right coronary arteries, disrupts the MEF2-binding motif (Figure 3). To determine if rs9349379 is bound by MEF2, we performed electrophoretic mobility shift assays with HUVEC nuclear extracts. These experiments showed that the probe with the A allele at rs9349379, but not the G allele, is shifted by proteins in the nuclear extract (Figure 3). When we added an antibody that recognizes either MEF2A or MEF2C, we observed a supershift of probe A, indicating that MEF2 is one of the binding proteins (Figure 3). Furthermore, the interaction is specific as addition of excess unlabeled probe A, but not probe G, could efficiently disrupt the shift (Figure 3). Although we cannot completely rule out a role for MEF2C in the observed supershift, short interfering RNA–mediated knockdowns indicate that MEF2A is the most abundant MEF2 transcription factor in HUVEC that is recognized by the anti-MEF2 antibody (Figure II in the online-only Data Supplement). When we reduced the expression of MEF2A or MEF2C using short interfering RNA in HUVEC, we did not detect a reduction in *PHACTR1* expression levels. However, the incomplete knockdowns of *MEF2A* and *MEF2C* (Figure II in the online-only Data Supplement), or other compensatory mechanisms, might explain the lack of effect on *PHACTR1* expression.

MEF2-Binding Site at rs9349379 Controls *PHACTR1* Expression In Vivo

To provide further in vivo evidence that the MEF2-binding site that overlaps with rs9349379 is important to modulate the expression of *PHACTR1* in endothelial cells, we engineered its deletion using the CRISPR/Cas9 system (Figure 4A). Starting with human embryonic stem cells, we introduced a guide RNA

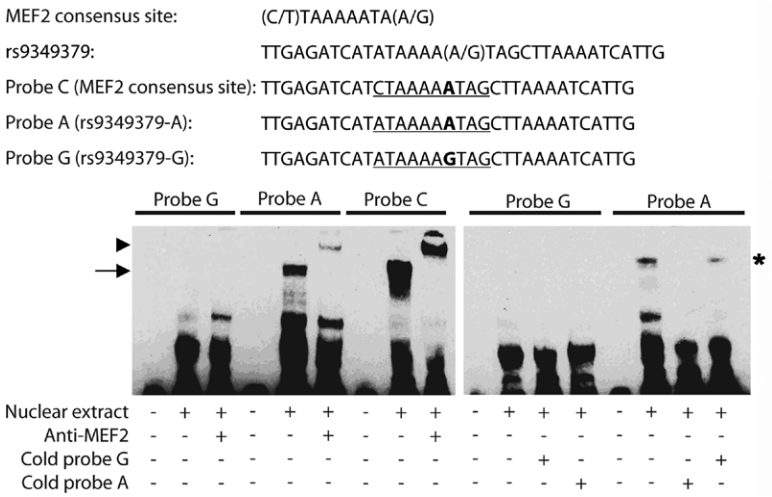

Figure 3. *PHACTR1*-rs9349379 is differentially bound by myocyte enhancer factor (MEF) 2. Electrophoretic mobility shift assays with human umbilical vein endothelial cell nuclear extract. Probe C contains the canonical MEF2-binding site and acts as positive control. Probes A and G differ by their respective allele at rs9349379: the G allele disrupts the MEF2 consensus binding site. Only probe A shifts in the presence of nuclear extract (arrow, **left**), and the complex supershifts when an antibody against MEF2 is added (arrowhead, **left**). The binding between probe A and MEF2 is specific: excess unlabeled (cold) probe A, but not unlabeled probe G, competes and disrupts the interaction between labeled probe A and MEF2 (star, **right**).

that specifically targets rs9349379. After screening, we identified a clone that carries a heterozygous 34-base pairs deletion that removes rs9349379, as well as all but 1 base pairs of the MEF2-binding site (Figure 4A). We then differentiated wild-type and heterozygous human embryonic stem cells into endothelial cells using an established protocol (In the Materials and Methods section of this article).[38,39] Endothelial cells that carry the 34-base pairs deletion at rs9349379 express 35% less *PHACTR1* than cells homozygous for the high-expressing A allele at rs9349379 (Figure 4B). This represents a promising in vivo validation of the potential regulatory role of rs9349379 in the control of *PHACTR1* expression in the vascular endothelium. This result is also consistent with our eQTL result.

No Ectopic Induction of *PHACTR1* Expression in HUVEC

In a previous report, VEGF treatment was shown to induce *PHACTR1* expression in HUVEC.[17] We attempted to reproduce this result using a similar protocol. First, we confirmed that our HUVEC can respond to VEGF by demonstrating the phosphorylation of the mitogen-activated protein kinase ERK (extracellular signal-regulated kinase) on VEGF treatment (Figure III in the online-only Data Supplement). However, we could not measure by quantitative polymerase chain reaction a significant difference in *PHACTR1* expression levels using different VEGF concentrations and induction times (Figure 5A). We also tested the effect of tumor necrosis factor-α, an inflammatory molecule that can trigger endothelial dysfunction, and shear stress, a stimulus known to protect blood vessels from atherosclerosis, on *PHACTR1* expression levels in HUVEC. In both the cases, we showed induction of the positive controls, *NFKB1* for tumor necrosis factor-α and *KLF2* for shear stress. However, we again did not measure a significant change in *PHACTR1* expression levels (Figure 5B and 5C).

Discussion

The genetic association between intronic SNPs in *PHACTR1* and CAD/MI is robust, relatively strong for a genome-wide association studies finding (odds ratio for the risk allele at rs9349379 is 1.37 in the MHI Biobank) and pleiotropic (the same SNPs are also associated with coronary artery calcification and stenosis, migraine, hemodynamic indexes, and cervical artery dissection). It is different from many of the known CAD/MI-associated SNPs because it is not associated with the

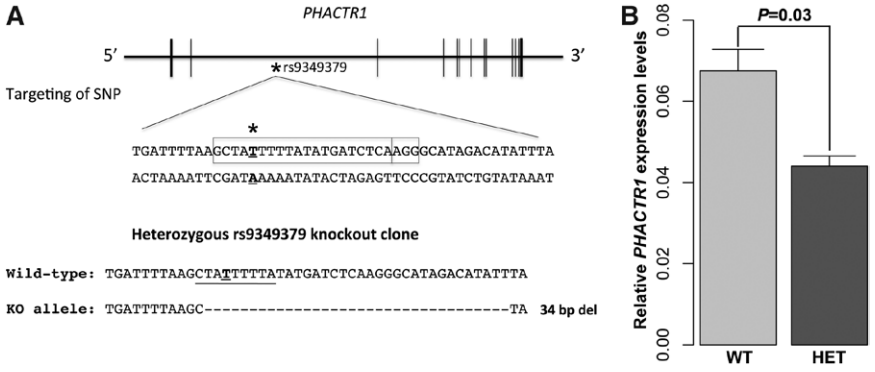

Figure 4. A CRISPR/Cas9-induced deletion that encompasses rs9349379 and the myocyte enhancer factor (MEF) 2–binding site reduces *PHACTR1* expression in endothelial cells. **A**, Schematic representation of the *PHACTR1* locus. The star (bold, underline) corresponds to rs9349379 in intron 3 of *PHACTR1* and the box highlights the DNA sequence targeted by the CRISPR guide RNA. We isolated a heterozygous clone that carries a 34-base pairs (bp) deletion (knockout [KO] allele) that removes rs9349379 (bold) and most of the MEF2-binding site (underline). **B**, Human endothelial cells that are heterozygous for the *PHACTR1* 34-bp deletion (HET) express 35% less *PHACTR1* than wild-type (WT) cells (*t* test *P*=0.03). Data shown is mean±SD. The experiment was done in triplicate. SNP indicates single nucleotide polymorphism.

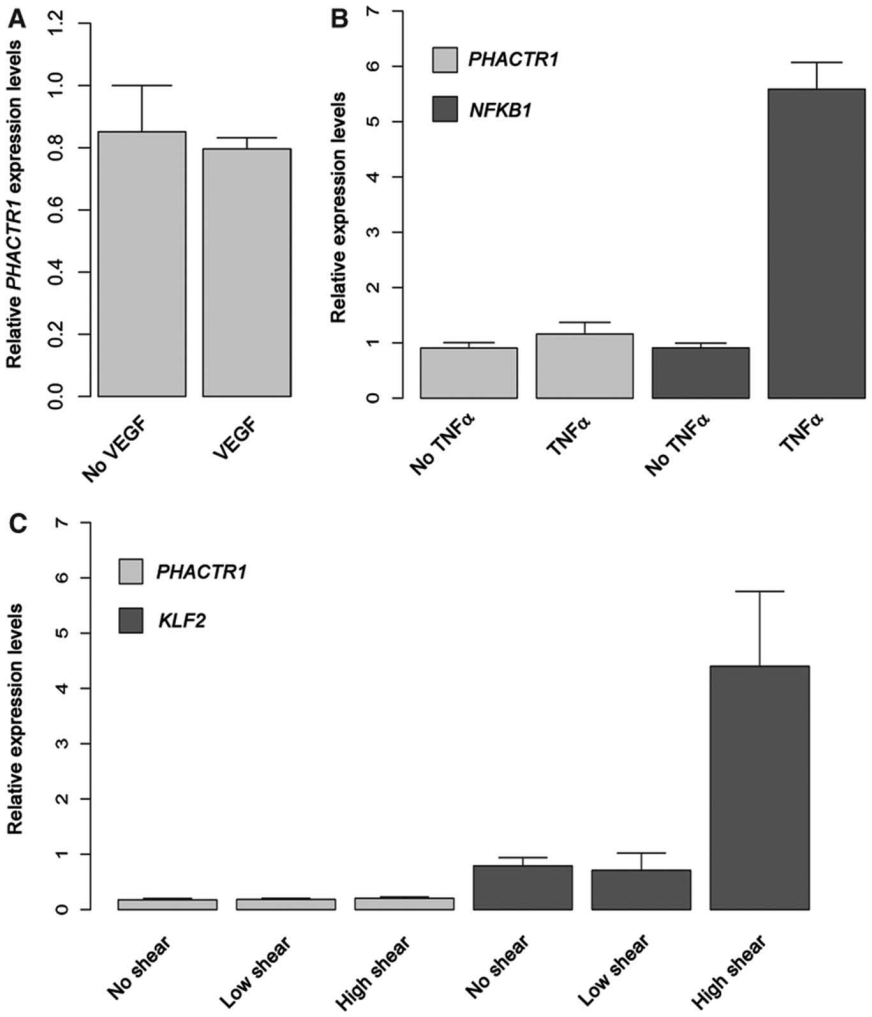

Figure 5. Different endothelial cell stimuli do not induce *PHACTR1* expression levels as measured by quantitative polymerase chain reaction (PCR). **A**, Serum-starved human umbilical vein endothelial cells (HUVECs) were treated with vascular endothelial growth factor (VEGF; 20 ng/mL) for 5 minutes before RNA was extracted and *PHACTR1* transcript levels measured. See Figure III in the online-only Data Supplement for the positive control of this experiment. **B**, HUVEC were treated with tumor necrosis factor (TNF)-α (10 ng/mL) for 16 hours. RNA was extracted and the levels of *PHACTR1* and *NFKB1* (positive control) were quantified by quantitative PCR. **C**, HUVEC were treated with no, low, or high shear stress. RNA was extracted 6 hours after the beginning of the experiments, and *PHACTR1* and *KLF2* (positive control) expression levels were measured by quantitative PCR. The same results were observed after 24 hours of shear stress. For all panels, data shown are mean±SDs. The VEGF and TNF-α experiments were done in triplicates; we had 4 replicates for the shear stress experiment. All comparisons are nonsignificant (*t* test $P>0.05$), except for *NFKB1* without and with TNF-α treatment ($P=7.8\times10^{-5}$) and for *KLF2* without and with high shear stress ($P=0.0012$).

traditional risk factors, such as lipid levels, blood pressure, or diabetes mellitus. This observation is promising, in as much as understanding how this genetic variation influences CAD/MI risk may yield new insights into the biology of atherosclerosis and potentially, in the long-term, new therapeutic strategies.

To translate this genetic discovery, we first need to connect genetic variants with genes. Our own DNA resequencing project and a large whole-exome sequencing effort failed to identify coding variants in *PHACTR1* that might directly implicate this gene in MI.[30,31] Previous eQTL experiments to link CAD/MI-associated *PHACTR1* SNPs with its expression levels have equally been unsuccessful, despite large sample sizes and a wide variety of tissues tested (leukocytes [including monocytes], liver, fat, skin, omentum, aortic media and adventitia, mammary artery, and lymphoblastoid cell lines).[5,40] Reasoning that the transcriptional effect might be tissue-specific, we measured an association between genotypes at rs9349379 and *PHACTR1* expression levels in human coronary arteries. This is an important result because it supports *PHACTR1* as a potential causal gene at the locus and suggests that low *PHACTR1* expression levels in coronary arteries increase CAD/MI risk. Although coronary artery is not a homogenous tissue, the lack of *PHACTR1* expression in smooth muscle and of eQTL effect in leukocytes support the idea that the vascular endothelium may be the most relevant tissue to study how PHACTR1 functions influence CAD/MI.

A recent report showed that the G allele at *PHACTR1*-rs9349379 is associated with lower risk of cervical artery dissection.[14] The same G allele is also associated with reduced migraine risk,[12] a known risk factor for cervical artery dissection.[41] These results are in sharp contrast with the reported association between the G allele at rs9349379 and increased risk of CAD or MI. This is not the first report of a genome-wide genetic association of opposite effects of

the same SNP on 2 diseases.[42] We will need to understand PHACTR1 biological activities to explain its opposite roles in cervical artery dissection and CAD/MI. Maybe PHACTR1 modulates a downstream pathway differently in carotid or cervical arteries than in coronary arteries? Or maybe it affects different biological pathways in these different artery tissues? Although to our knowledge it has not been tested, we would predict that rs9349379 would be an eQTL for *PHACTR1* expression levels in human carotid or cervical arteries.

High-density genotyping and bioinformatic analyses pinpointed rs9349379 as a potential causal variant at the locus. Using in vitro assays, we showed that alleles at this intronic *PHACTR1* SNP bind differentially members of the MEF2 transcription factor family. Furthermore, deletion of the MEF2-binding site that overlaps with rs9349379 reduced *PHACTR1* expression in endothelial cells. A small exonic 21-base pairs deletion in *MEF2A* was originally described to cause familial CAD, although this result has not been widely replicated.[35–37] It is important to emphasize that our results are not invalidated by the lack of consistent association between genetic variation at *MEF2A* and CAD. First, we do not know if MEF2A or MEF2C (or both) recognize in vivo the binding motif that we characterized at rs9349379. Second, we do not need variation in *MEF2* expression levels or activities to explain the variation in *PHACTR1* expression levels. Changes in *PHACTR1* expression levels are controlled, at least in part, by genotypes at rs9349379. MEF2 transcription factors remain good candidates for a role in atherosclerosis as they are present in the endothelium and are involved in maintaining vascular integrity.[34]

A previous report suggested that *PHACTR1* expression levels in HUVEC can be induced on VEGF treatment, but we were not able to reproduce this result, despite trying different VEGF concentrations and induction times.[17] More generally, we explored if classic endothelial stimuli (VEGF, tumor necrosis factor-α, and shear stress) can induce *PHACTR1* expression. Under our stimulation protocols, we failed to ectopically change *PHACTR1* expression levels in HUVEC. These negative observations are informative in guiding future experiments to determine if other endothelial stresses, potentially through MEF2 activation, may act synergistically with genotypes at rs9349379 to influence CAD/MI risk. In conclusion, our results provide an initial model (CAD/MI-associated SNPs at 6p24 affects MEF2 binding and consequently *PHACTR1* expression levels) and a candidate tissue (vascular endothelium) to further characterize how PHACTR1 influences atherosclerosis independently of the traditional CAD/MI risk factors.

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Disclosures

None.

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Significance

Despite having efficient therapies and prevention strategies, coronary artery diseases remain one of the main causes of death in the World. Recent failed or futile clinical trials have highlighted the need to identify new therapeutic targets. Unbiased human genetic studies can provide such new entry points into disease pathophysiology. Genome-wide association studies for coronary artery disease risk have identified a robust signal for DNA polymorphisms within the *PHACTR1* gene on chromosome 6. *PHACTR1* single nucleotides polymorphisms are not associated with traditional atherosclerosis risk factors, such as blood lipids, hypertension, or diabetes mellitus. Our work establishes a link between these genetic variants and *PHACTR1* expression levels in human coronary arteries through a potential effect on the binding of the transcription factors MEF2 at the locus. These results set the stage to explore how PHACTR1 functions in the vascular endothelium influence coronary artery disease risk.