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Association of the *PHACTR1/EDN1* Genetic Locus With Spontaneous Coronary Artery Dissection

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ABSTRACT

BACKGROUND Spontaneous coronary artery dissection (SCAD) is an increasingly recognized cause of acute coronary syndromes (ACS) afflicting predominantly younger to middle-aged women. Observational studies have reported a high prevalence of extracoronary vascular anomalies, especially fibromuscular dysplasia (FMD) and a low prevalence of coincidental cases of atherosclerosis. *PHACTR1/EDN1* is a genetic risk locus for several vascular diseases, including FMD and coronary artery disease, with the putative causal noncoding variant at the rs9349379 locus acting as a potential enhancer for the endothelin-1 (*EDN1*) gene.

OBJECTIVES This study sought to test the association between the rs9349379 genotype and SCAD.

METHODS Results from case control studies from France, United Kingdom, United States, and Australia were analyzed to test the association with SCAD risk, including age at first event, pregnancy-associated SCAD (P-SCAD), and recurrent SCAD.

RESULTS The previously reported risk allele for FMD (rs9349379-A) was associated with a higher risk of SCAD in all studies. In a meta-analysis of 1,055 SCAD patients and 7,190 controls, the odds ratio (OR) was 1.67 (95% confidence interval [CI]: 1.50 to 1.86) per copy of rs9349379-A. In a subset of 491 SCAD patients, the OR estimate was found to be higher for the association with SCAD in patients without FMD (OR: 1.89; 95% CI: 1.53 to 2.33) than in SCAD cases with FMD (OR: 1.60; 95% CI: 1.28 to 1.99). There was no effect of genotype on age at first event, P-SCAD, or recurrence.

CONCLUSIONS The first genetic risk factor for SCAD was identified in the largest study conducted to date for this condition. This genetic link may contribute to the clinical overlap between SCAD and FMD.
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Spontaneous coronary artery dissection (SCAD) is an increasingly recognized cause of unheralded acute myocardial infarction (AMI) (1,2). SCAD occurs predominantly in young to middle-aged women, accounting for 23% to 36% of AMI cases in this population (3–6), and is a rare cause of sudden cardiac death (7). SCAD also is the most common cause of pregnancy-associated AMI, although AMI in pregnant women accounts for only 2% to 18% of all SCAD cases (8,9). SCAD is caused by the development of an intimal tear and flap or an intramural hematoma in the outer third of the tunica media of the vessel wall, which leads to external compression of the true lumen and coronary insufficiency, myocardial ischemia, and infarction (10).

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The causes of SCAD are poorly understood. Women with SCAD are typically not overweight and do not have many atherosclerotic risk factors. In observational studies, SCAD has been associated with a high prevalence of extracoronary arteriopathies, especially fibromuscular dysplasia (FMD) (1,2,11–16). FMD is a noninflammatory, nonatherosclerotic disease of medium size arteries, which may lead to complications arising from arterial stenosis, aneurysms, or dissections (17,18). FMD most commonly involves renal, carotid, and iliac arteries, but any arterial bed may be affected. The clinical overlap between SCAD and FMD includes a predilection for young to middle-aged women and a low prevalence of coexistent atherosclerotic disease (12,19–22).

Occasional familial cases of SCAD have been reported mainly in siblings or mother-daughter pairs (21). Hereditary connective tissue disorders appear rare, accounting for <5% of SCAD cases, and genetic screening for mutations in known connective tissue genes in SCAD survivors has a low yield (23,24), similar to genetic screening in FMD (25). The extent to which common genetic variants may affect susceptibility to SCAD is unknown.

PHACTR1/EDN1 is a genetic locus on chromosome 6q24 reported to confer risk for coronary artery disease (CAD) and AMI (26,27). *PHACTR1/EDN1* is also associated with migraine (28) and cervicocerebral artery dissection (CeAD) (29). The putative causal genetic variant at the PHACTR1 locus has recently been reported to lie in a putative enhancer region for *EDN1*, the endothelin (ET)-1 gene (30). The present authors recently showed that this variant is also associated with the risk of FMD (31). Interestingly, the common allele, rs9349379-A, is associated with an increased risk for FMD, migraine, and CeAD, whereas the minor allele, rs9349379-G, associates with increased risk for atherosclerotic CAD and AMI (32). The present study aimed to investigate the association between rs9349379 and SCAD, to assess whether, at this locus, SCAD is genetically closer to FMD, given their clinical overlap, or to atherosclerotic CAD and AMI.

ABBREVIATIONS AND ACRONYMS

AMI = acute myocardial infarction

CAD = coronary artery disease

CeAD = cervicocerebral artery dissection

CI = confidence interval

EDN1 = endothelin gene

ET = endothelin

FMD = fibromuscular dysplasia

OR = odds ratio

PHACTR1 = phosphatase and actin regulatory 1 gene

P-SCAD = pregnancy-associated spontaneous coronary artery dissection

R-SCAD = recurrent spontaneous coronary artery dissection

SCAD = spontaneous coronary artery dissection

METHODS

STUDY POPULATIONS. Participants included in this study were predominantly from 4 different countries, all of European descent, which was determined by using ancestry markers and clinical records. The diagnosis of SCAD was confirmed by review of the index coronary angiogram by an experienced interventional cardiologist with expertise in the recognition of SCAD, along with contemporaneous medical records, following criteria described elsewhere (6,33). Individuals without a diagnostic angiogram were excluded from this analysis. Screening for FMD was undertaken in 491 SCAD patients from the Mayo Clinic study who underwent computed tomography angiography imaging of at least 2 arterial beds from brain to pelvis (14). Pregnancy-associated SCAD (P-SCAD) was defined as SCAD during pregnancy or occurring ≤12 weeks postpartum. Recurrent SCAD

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TABLE 1 Clinical Characteristics of Study Populations

Cohorts	N	Females	Median Age at Inclusion, yrs (Q1–Q3)	Median Age at 1st Event, yrs (Q1–Q3)	P-SCAD	Recurrent SCAD	Study Recruitment
French cases	189	170 (90)	51 (44–59)	NA	NA	NA	National register
French controls (PPS3)	3,964	1,012 (40)	58.73 ± 5.94	NR	NR	NR	Population-based
U.K. cases	202	194 (96)	46 (42–53)	44 (38–50)	18 (9)	23 (11)	Mainland U.K. nationwide
U.K. controls (B58)	606	582 (96)	44 (44–44)	NR	NR	NR	Mainland U.K. nationwide
AU/Mount Sinai cases	160	154 (96)	50 (45–57)	46 (41–54)	14 (9)	18 (11)	Social media platform
AU/Mount Sinai controls	1,127	672 (60)	>75	NR	NR	NR	Healthy volunteers
Mayo Clinic cases	504	482 (96)	48 (41–55)	45 (39–53)	53 (11)	81 (16)	Mayo Clinic patients, physician referrals, and social media
Mayo Clinic controls	1,493	1,423 (95)	48 (41–55)	NR	NR	NR	Healthy volunteers (Mayo Genome Consortia)

Values are n, n (%), or mean ± SD unless otherwise indicated. Note, for the birth cohort B85, median Q1 and Q3 are equal to 44 yrs.

AU = Australian study; NA = not available; NR = not relevant; PPS3 = Paris Prospective Study 3; P-SCAD = pregnancy spontaneous coronary artery dissection; Q1–Q3 = quartile 1–quartile 3; SCAD = spontaneous coronary artery dissection.

(R-SCAD) was considered when de novo SCAD was unrelated to the index dissection and affected different coronary artery segments. All participants provided written informed consent, and all individual studies were approved by national and institutional review boards. A detailed description of each study and the DISCO (Etude de la prévalence de la dysplasie fibromusculaire chez les patients présentant une DISsection COronaire) Consortium and CARDIoGRAMPlusC4D (Coronary ARtery DIsease Genome wide Replication and Meta-analysis (CARDIoGRAM) plus The Coronary Artery Disease [C4D] Genetics consortium) Study Group is provided in the Online Appendix. Genotypes were generated from different platforms in each of the 4 studies, and the genotype distributions did not significantly deviate from Hardy-Weinberg equilibrium (Online Table 1).

To compare the associations of rs9349379 with SCAD and with CAD/AMI, sex-specific associations of rs9349379 were evaluated in the meta-analysis of a genome-wide association study dataset of CAD assembled by the CARDIoGRAMPlusC4D consortium. This dataset included 43,171 AMI cases and 127,176 controls, 9,105 women with CAD and 30,428 female controls, and 30,428 men with CAD and 36,042 male controls (26).

STATISTICAL METHODS. To estimate the association between rs9349379 and SCAD, genotype distributions were compared between cases and controls in 4 independent studies. Analyses were performed using R software versions 3.3.1 and 3.4.3 (R Foundation for Statistical Computing, Vienna, Austria), PLINK version 1.07 or 1.9 software (Center for Human Genetic Research, Massachusetts General Hospital, Boston, Massachusetts), SAS version 9.4 software (Cary, North Carolina), or Stata version 15.1 software (StataCorp, College Station, Texas). Associations found using the additive genetic model were estimated using logistic regression adjusted for age and sex when relevant. In the U.K. study, controls were from a birth cohort and were all 44 years of age. Controls in the Mayo Clinic study were older and were matched for sex and ethnic group to cases.

To estimate the global effect on SCAD, a fixed effects inverse variance-weighted method was used, which combines the beta's (log-odds ratios) weighting by the inverse variance of the log-odds estimate, thereby accounting for study sample size.

The association with age at first SCAD occurrence was estimated by using linear (age continuous) or logistic (SCAD <40 years) regression. The genetic effect on P-SCAD under the additive model was only analyzed in women by using logistic regression analyses and time to R-SCAD using Cox proportional hazards regression. The meta-analyzed effect was estimated using the same method used for global association.

RESULTS

CLINICAL CHARACTERISTICS. Table 1 summarizes clinical characteristics of the 1,055 patients and 7,190 controls studied to estimate the association between rs9349379 and SCAD. Cases were recruited through diverse settings, including clinician referral to a national registry (French study), social media platforms, and a combination of both patient and physician referrals (United Kingdom, Mayo Clinic, and Australian/Mount Sinai studies), and showed overall similar clinical characteristics. SCAD patients were mostly women (87% to 96%) whose SCAD event occurred in middle age. P-SCAD or recurrence each occurred in approximately 10% of cases, as estimated from 3 of 4

cohorts where this information was available. In 491 SCAD patients in whom FMD screening was conducted, 45% (n = 206) showed at least 1 extracoronary artery was affected.

ASSOCIATION OF rs9349379 WITH SCAD. The rs9349379-A allele showed a higher prevalence among SCAD patients and its frequency was estimated to be 0.72 in the 1,100 patients studied compared to a frequency of 0.56 in the controls, and was significantly associated with increased risk for SCAD (Table 2). Under the additive model, the odds ratio (OR) per risk allele increment was estimated to be 1.67 (95% confidence interval [CI]: 1.50 to 1.86; $p = 1.10 \times 10^{-21}$) in the combined meta-analysis (Table 2, Figure 1). Overall, the Cochran Q statistic did not show evidence for heterogeneity in the combined meta-analyses (Table 2). The stratified analysis for the presence of FMD was conducted in the Mayo Clinic case control study (Table 3). The OR estimate was found to be higher for the association of SCAD in patients without FMD (OR: 1.89; 95% CI: 1.53 to 2.33; $p = 3.8 \times 10^{-9}$) than in SCAD cases with FMD (OR: 1.60; 95% CI: 1.28 to 1.99; $p = 3.5 \times 10^{-5}$) (Table 3).

ASSOCIATION OF rs9349379 WITH SCAD VERSUS CLASSICAL AMI AND CAD. Figure 2 summarizes the association between rs9349379 and SCAD and compares the effect size estimates of CAD/AMI globally and stratified by sex. The direction of effect of the association of rs9349379-A allele with SCAD (OR: 1.67; 95% CI: 1.50 to 1.86; $p = 6.76 \times 10^{-21}$) is identical to that of FMD (OR: 1.39; 95% CI: 1.25 to 1.54; $p = 7.40 \times 10^{-10}$) but opposite to the more common atherosclerotic forms of AMI/CAD (OR: 0.88; 95% CI: 0.86 to 0.89; $p = 1.81 \times 10^{-42}$), including when the analysis was restricted to women only (OR: 0.92; 95% CI: 0.88 to 0.96; $p = 6.09 \times 10^{-5}$) (Figure 2, Central Illustration). The specific association of the *PHACTR1* locus alleles with FMD and SCAD, which are predominantly affecting women and CAD/AMI, which are predominantly affecting men, is summarized in the Central Illustration.

PREVALENCE OF rs9349379 IN SCAD SUBGROUPS. Among SCAD patients in whom data were available, 87 of 835 patients presented with P-SCAD, and 118 of 872 had documented R-SCAD. Differences in the risk allele distribution were not identified in P-SCAD, R-SCAD. SCAD risk allele did not influence age at first event (Online Table 2).

ASSOCIATION WITH CIRCULATING ET-1. ET-1 expression was measured in a subsample of U.K. SCAD patients with clinical characteristics that were similar to those of patients studied in the U.K. case control study (98% women; mean 46.7 ± 8.01 years of age). This analysis showed that, as in healthy subjects (30), there was an association between lower levels of ET-1 in plasma of patients and the presence of the rs9349379-A allele ($p < 0.05$) (Online Figure 1, Online Methods).

TABLE 2 Association Between rs9349379 and SCAD in 4 Case Control Studies

Case Control Study	N	GG	GA	AA	EAF	OR*† (95% CI)	p Value*†
French cases	189	12	65	112	0.76		
French controls (PPS3)	3,964	574	1,795	1,595	0.63	1.81 (1.39–2.35)	1.03×10^{-5}
U.K. cases	202	16	99	87	0.68		
U.K. controls (B58)	606	105	275	226	0.60	1.38 (1.09–1.75)	7.00×10^{-3}
AU/Mount Sinai cases	160	12	70	78	0.71		
AU/Mount Sinai controls	1,127	187	536	404	0.60	1.66 (1.27–2.15)	1.56×10^{-4}
Mayo Clinic cases	504	40	199	265	0.72		
Mayo Clinic controls	1,493	255	703	535	0.59	1.77 (1.51–2.07)	1.00×10^{-12}
Total cases	1,055	80	433	542	0.72		
Total controls	7,190	1,121	3,309	2,760	0.61	1.67 (1.50–1.86)	6.76×10^{-21}

*OR and p values were computed using logistic regression under the additive genetic model. †Meta-analysis was performed using the inverse variance-weighted method. Heterogeneity among cohorts was tested using Cochran's Q statistic and was not significant (chi-square value: 3.38; *ddf* = 3; p = 0.337).

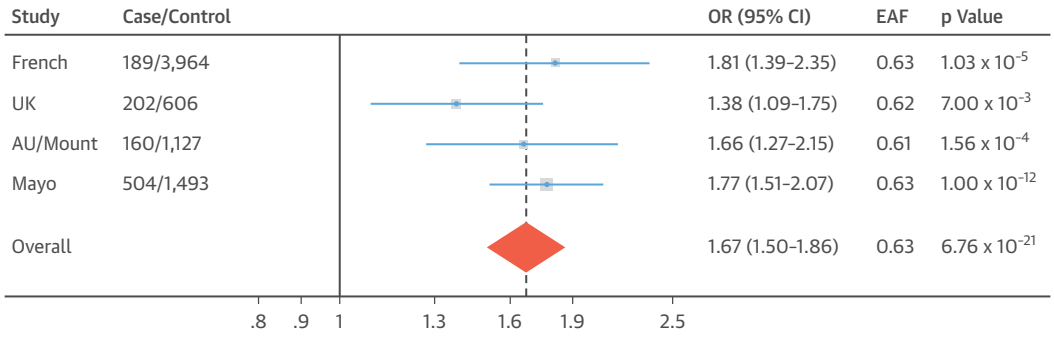
CI = confidence interval; EAF = effect allele frequency; OR = odds ratio; other abbreviations as in Table 1.

DISCUSSION

FIRST GENETIC RISK VARIANT FOR SCAD IS PROTECTIVE AGAINST ATHEROSCLEROTIC MYOCARDIAL INFARCTION. In this large genetic study conducted in >1,000 SCAD patients and ~7,200 controls, robust and replicated associations are reported between rs9349379, a common noncoding variant in the *PHACTR1/EDN1* locus, and the risk of SCAD. This is the first report showing that a genetic risk locus for SCAD is estimated to contribute to an increased risk of ~70% among carriers of the A allele, but the study did not partition age or the specific phenotypic subgroups, namely, R-SCAD and P-SCAD subjects.

A GENETIC LINK BETWEEN SCAD AND FMD. FMD has been reported to be highly prevalent in SCAD patients in multiple observational studies (1,2,11–16). A recent case report described evidence for histological FMD in the coronary event of a patient who died from SCAD (34). The finding of an association between rs9349379 and SCAD risk provides a molecular rationale for this clinical observation, given that rs9349379 has also recently been established as a risk variant for FMD (31). Compared to the prevalence of FMD, the prevalence of the risk allele seems to be higher among SCAD patients (frequency in FMD [FreqFMD]: 0.69 vs. 0.72 for frequency in SCAD [FreqSCAD]; p for trend = 0.06), with slightly overlapping estimation of risk for both diseases (OR for

FIGURE 1 Forest Plot Shows Associations in Individual Studies and the Global Genetic Association Between rs9349379 and SCAD



EAF was estimated from the total sample of cases and controls for each study using the additive model. Globally, rs9349379 increased the risk of SCAD by ~67% per A allele copy ($p = 6.76 \times 10^{-21}$). AU = Australian study; CI = confidence interval; EAF = effect allele frequency; OR = odds ratio.

FMD [ORFMD]: 95% CI: 1.25 to 1.54 vs. OR for SCAD [ORSCAD]: 95% CI: 1.50 to 1.86) (**Central Illustration, Figure 2**). This observation is supported by the bigger effect size estimated for the association of rs9349379 with SCAD without FMD, which also mitigates concerns for variable completeness of extracoronary arterial bed screening and the potential admixture of subtle or undetected FMD in the subgroup "SCAD without FMD." However, sample sizes in both FMD and SCAD analyses were relatively modest, and larger and clinically more diverse cohorts with systematic assessments of SCAD and FMD in the same patients will be required to confirm this trend for a higher prevalence of the rs9349379-A allele in SCAD than in FMD. These findings, however, do support the hypothesis that SCAD, like FMD, may be a complex genetic disease involving multiple genetic risk factors, each exerting a moderate effect in response to environmental triggers.

SCAD AND ATHEROSCLEROTIC CAD/AMI. Observational studies in SCAD have noted a low frequency of coincidental atherosclerotic CAD (3,6,20,22,33). Interestingly, rs9349379 is also a well-established risk locus for CAD and myocardial infarction (MI) (27). The association between the protective allele for CAD/AMI with SCAD provides a genetic explanation for this observation, given that the allele that increases the risk of SCAD is identical in FMD but opposite to the risk allele for CAD and MI, both in the overall SCAD cohort and in the group consisting of women only (**Figure 2, Central Illustration**) (27).

TABLE 3 Association Analysis Stratified by the Presence of FMD in Mayo Clinic SCAD Patients

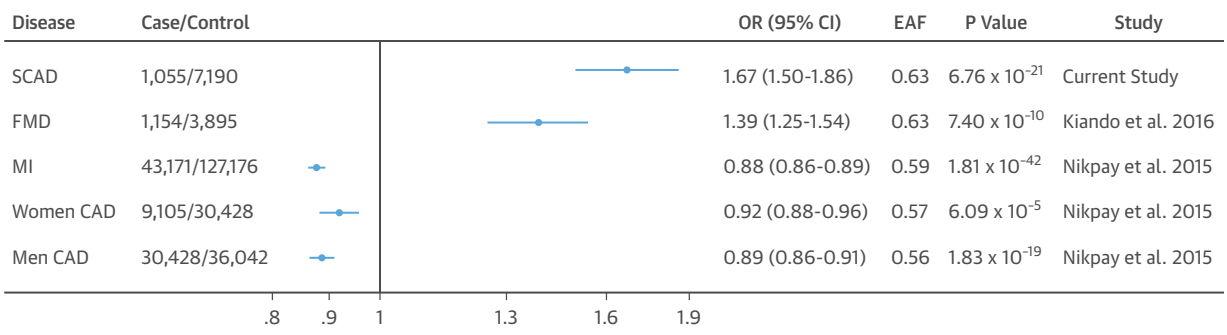
	N	GG	GA	AA	EAF	OR* (95% CI)	p Value*
All SCAD cases	504	40	199	265	0.72	1.77 (1.51–2.07)	1.00×10^{-12}
SCAD with FMD	206	25	72	109	0.70	1.60 (1.28–1.99)	3.18×10^{-5}
SCAD without FMD	253	13	108	132	0.73	1.89 (1.53–2.33)	3.83×10^{-9}
Mayo Clinic controls	1,493	255	703	535	0.59		

*Odds ratios and p values were computed by using logistic regression under the additive genetic model.
FMD = fibromuscular dysplasia; other abbreviations as in **Tables 1 and 2**.

A GENETIC LINK BETWEEN SCAD AND SEVERAL NEUROVASCULAR DISEASES. In addition to providing an explanation for the clinical association between SCAD and FMD, rs9349379 also links SCAD with both CeAD, a rare condition defined as an intimal flap or intramural hematoma in a carotid or vertebral artery that can cause stroke (29) and migraine (28). Importantly, rs9349379-A is a reported risk allele for both of these disorders. A higher prevalence of migraine has been consistently described in observational studies of SCAD patients, ranging from 33% to 43%, than in a population prevalence of ~15% (35–37). Although the population incidence of CeAD is rare, there are multiple series describing CeAD in SCAD patients either preceding the SCAD event or discovered during follow-up imaging, usually in association with cervical FMD (11,16,38). However, further global genetic investigation is required, using full genome-wide association studies in both SCAD and FMD patients to assess the extent to which CeAD shares genetic susceptibility with these diseases.

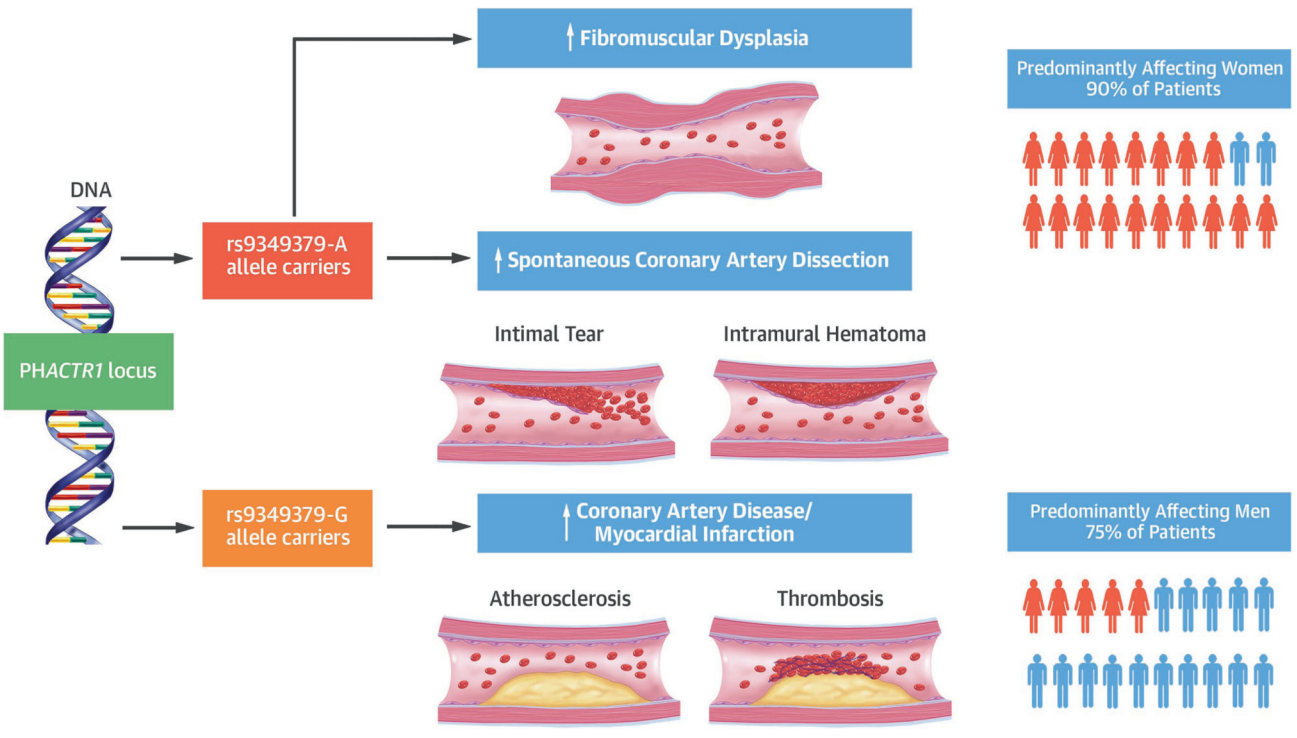
POTENTIAL REGULATORY MECHANISMS OF rs9349379. Involvement of the same genetic variant in a diverse panel of cardiovascular and neurovascular diseases is intriguing, and the underlying mechanisms remain to be fully elucidated. Initial molecular investigation at this locus was focused on

FIGURE 2 **Associations Between rs9349379 and SCAD Were Compared With the Associations Previously Reported Between FMD and CAD/AMI Globally and Those Stratified by Sex**



The effecting allele is the A allele for all diseases analyzed, which is the risk allele for SCAD and FMD, but which is protective for CAD/AMI. The association is the opposite between SCAD and CAD/MI, including in the analysis of female control cases. CAD/AMI = coronary artery disease/acute myocardial infarction; CI = confidence interval; EAF = effect allele frequency; FMD = fibromuscular dysplasia; OR = odds ratio; SCAD = spontaneous coronary artery dissection.

CENTRAL ILLUSTRATION **Genetic Variant rs9349379 Associates With Spontaneous Coronary Artery Dissection**



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Summary of the genetic associations between the *PHACTR1* locus represented by the common variant rs9349379 that associates with the genetic risk of FMD, SCAD, CAD, and MI. Our study provides evidence supporting an increased risk for FMD and SCAD among patients carrying the rs9349379-A allele. This association is the first step toward establishing a molecular mechanism for the clinical associations observed between SCAD and FMD. FMD and SCAD are predominantly affecting women who represent up to 90% of patients. The rs9349379-G allele on the other hand, has been reported to increase the risk of CAD and more classical MI, where most of patients are men. CAD/MI = coronary artery disease/myocardial infarction; EAF = effect allele frequency; FMD = fibromuscular dysplasia; SCAD = spontaneous coronary artery dissection.

the closest gene that encodes PHACTR1, a phosphatase and actin regulator protein suggested to be involved in angiogenesis and cell migration (39). The rs9349379 allele is an expression quantitative locus for *PHACTR1* in skin fibroblasts (31), macrophages (40), and many arterial tissues, available in the GTeX catalog (Genotype-Tissue Expression [GTEx] Program, National Institutes of Health, Bethesda, Maryland) (41). Another study showed that the rs9349379-G allele disrupts a binding site for the myocyte enhancer factor 2 (MEF2) protein, but that study did not find the previously claimed regulation by VEGF (42). Gupta et al. (30) recently used genome editing of pluripotent stem cells to show that the rs9349379-G allele correlates with increased expression of ET-1, but not PHACTR1, during the differentiation to endothelial and smooth muscle lineages. This study suggests the ET-1 gene (*EDN1*), which maps ~600 kbp upstream of artery-specific enhancers, may mediate several important biological mechanisms for vascular diseases genetically linked to rs9349379 (e.g., vasoconstriction, proliferation, and vasodilation) (30). In addition, in the study by Gupta et al. (30), the rs9349379-A allele was reported to associate with lower levels of circulating ET-1 in healthy volunteers, which the present authors also found to be the case in SCAD patients. The well-known hemodynamic and vascular effects of ET-1 provide an attractive potential contributing mechanism for many of the vascular diseases where rs9349379 is genetically involved. However, whether ET-1 expression is decreased in SCAD, FMD, or CeAD compared to that in healthy individuals is not known. The present lack of evidence for significant hemodynamic differences in SCAD populations suggests that the effect on ET-1 would be insufficient to explain the large spectrum of clinical manifestations associated with this locus. ET-1 biological actions are diverse and compensatory through its receptors ET_A and ET_B, which mediate opposing vasoconstrictor and vasodilator effects, although human coronary arteries only express the ET_A subtype (43). Further investigation is needed to confirm whether reduced ET-1 levels may result in increased risks for SCAD, FMD, and CeAD. In addition, the possible contributions and roles of other coding and noncoding genes at this locus, including *PHACTR1*, cannot be ruled out at this stage, especially in the complex genetic context contributing to these vascular disorders, which includes multiple genetic and environmental triggering factors.

STUDY LIMITATIONS. This study is a cross-sectional case control design of a single genetic locus. Thus, the finding reported only represents one of the many of the genetic risk determinants for SCAD where we observe a positively shared genetic association between SCAD and FMD, which is opposite between SCAD and atherosclerotic CAD in women. Also, we have combined data from different national studies using different methodologies, and we still have applied unified diagnostic criteria for selection of patients and controls. Another limitation of our study is the relatively modest samples included in the stratified analyses for P-SCAD, R-SCAD and SCAD without FMD, which may have biased the current findings.

CONCLUSIONS

This study reports that rs9349379 is the first genetic risk locus for SCAD. The previously reported association between this common variant and other vascular disorders, especially FMD, provides a genetic explanation for the established clinical associations among these disorders. Further studies will be required to confirm the relative importance of the endothelin mechanistic pathway and its relevance to SCAD and FMD risks.

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PERSPECTIVES

COMPETENCY IN MEDICAL KNOWLEDGE: Identification of a risk locus suggests that SCAD may be genetically determined with a complex pattern of inheritance. Genetic susceptibility to SCAD through the *PHACTR1/EDN1* locus is shared with FMD.

TRANSLATIONAL OUTLOOK: Further research is necessary to establish the molecular mechanisms responsible for the clinically observed associations between SCAD and FMD.

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KEY WORDS cardiovascular disease in women, fibromuscular dysplasia, genetic association, myocardial infarction

APPENDIX For expanded methods, supplemental figures and tables, and a complete list of the DISCO Consortium and CARDIoGRAMPlusC4D Study Group members, please see the online version of this paper.