Association of *ACVRL1* Genetic Polymorphisms with Arteriovenous Malformations: A Case-Control Study and Meta-Analysis

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■ OBJECTIVE: **To investigate the association between polymorphisms in the gene encoding activin receptorlike kinase 1 (*ACVRL1*) with brain arteriovenous malformations (BAVMs) using a case-control study in a Chinese Han population, followed by a meta-analysis of the published literature.**

■ METHODS: **This study focused on the genotypic analysis of 4 single nucleotide polymorphisms (SNPs; rs2071219, rs706819, rs2293094, and rs11169953) in 50 patients with BAVM and 120 healthy volunteers attending Provincial Hospital in China. A meta-analysis was subsequently conducted involving an extensive literature search for relevant studies.**

■ RESULTS: **Our cohort study showed a significant association between *ACVRL1* rs706819 and increased risk for BAVM. Reduced BAVM risk was correlated with the G allele of rs2293094 and the C allele of rs11169953. However, neither the genotype nor allele frequencies of rs2071219 were found to be significantly different between the BAVM and control groups. Meta-analysis further confirmed that no significant evidence of association was found between rs2071219 and BAVM risk. Haplotype analysis of rs706819, rs2293094, and rs11169953 showed that the GGT haplotype could reduce the risk of BAVM, whereas the GAC haplotype may increase the risk of BAVM.**

■ CONCLUSIONS: **The present study indicates an association between 3 susceptibility SNPs, rs706819, rs2293094, and rs11169953, in the *ACVRL1* gene and BAVM. Follow-up functional studies on the *ACVRL1* gene are required to better understand its roles in BAVM development.**

INTRODUCTION

Sporadic brain arteriovenous malformations (BAVMs) are one of the major underlying causes of intracranial hemorrhages (ICHs) and comprise abnormal snarls of vessels that directly shunt blood from the arterial to venous circulation without capillary beds. These dilated vascular bundles, called a nidus, result in the formation of high-flow lesions that can easily rupture. ICHs caused by BAVMs have been linked to a high rate of disability and death, especially in children and young adults.[1,2] However, the cause of BAVMs is still largely unknown.

Activin receptorlike kinase 1 (ACVRL1/ALK1) is a type I transmembrane serine/threonine kinase receptor that binds proteins of the transforming growth factor (TGF) family during vascular remodeling, angiogenesis, and endothelial cell regulation.[3,4] In animal models, high levels of ACVRL1 induce abnormal endothelial differentiation and AVM development.[5] Multiple mutations in the ACVRL1 gene appear to be closely related to hereditary hemorrhagic telangiectasia 2, which is the most common syndrome associated with AVMs.[6] Although the AVMs in hereditary hemorrhagic telangiectasia 2 have some unique characteristics, there are numerous similarities between these inherited AVMs and sporadic lesions in terms of their clinical and morphologic characteristics,[7,8] suggesting common pathologic mechanisms between them. Various reports[4,8-11] have investigated the association of genetic polymorphisms in the

Key words
- ACVRL1
- Arteriovenous malformations
- Single nucleotide polymorphism

Abbreviations and Acronyms
ACVRL1: Activin receptorlike kinase 1
BAVM: Brain arteriovenous malformation
CI: Confidence interval
ICH: Intracranial hemorrhage
OR: Odds ratio
PCR: Polymerase chain reaction
SNP: Single nucleotide polymorphism
TGF: Transforming growth factor

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ACVRL1 gene with sporadic BAVMs. The most common *ACVRL1* polymorphism is IVS3-35A>G (rs2071219), which has been correlated with a greater risk of forming AVMs in some studies.[8,11] This association is inconsistent, and other researchers[4,9] have failed to identify any link between this genetic alteration in *ACVRL1* and AVM risk. Additional work is necessary to comprehensively understand the role of this particular genetic polymorphism in sporadic BAVMs.

Genetic polymorphisms, including those involved in the cause of BAVMs, can be influenced by population stratification.[10] In the present study, we sought to investigate the relationship between the *ACVRL1* IVS3-35A>G (rs2071219) polymorphism in Chinese Han patients with sporadic BAVMs. To do so, we genotyped and tagged 3 single nucleotide polymorphisms (SNPs) (rs706819, rs2293094, and rs11169953) to gain further insight into the association of *ACVRL1* genetic changes with sporadic lesion formation. Furthermore, a meta-analysis for rs2071219 was also performed to achieve a more comprehensive and reliable conclusion.

METHODS

Patients

A total of 50 patients with BAVM were recruited from the Department of Neurosurgery at Provincial Hospital in affiliation with Shandong University in Jinan from July 2014 to June 2016 and from Liaocheng People's Hospital in Liaocheng from March 2015 to June 2016. The study was approved by the local institutional ethical committee. AVM was diagnosed based on digital subtraction angiography results. As controls, we recruited 120 healthy volunteers (mean age, 36.15 years; standard deviation, 17.82 years; 46.7% male). All the individuals were carefully evaluated to avoid selection bias. Patients with AVM and healthy patients with a definite clinical diagnosis of hereditary hemorrhagic telangiectasia, AVMs in other organs/tissues, cardiovascular disease, or a family history positive for cerebrovascular dysplasia were excluded from the study.

SNP Selection

SNPs in the *ACVRL1* gene were selected from the HapMap CHB population data (dbSNP build 126 on NCBI human genome build 36) using a Tagger algorithm[12] available in Haploview.[13] We used pairwise tagging to select a minimal set of tagged SNPs with a minor allele frequency $\geq$5% such that all captured alleles are correlated at an $r^2 > 0.8$ with a marker in that set. Thus, each tagged SNP acts as a direct proxy to all other correlated untyped SNPs, and, by definition, is not highly correlated to other tagged SNPs selected for genotyping. In this study, 3 tagged SNPs were selected: rs706819, rs2293094, and rs11169953.

DNA Extraction and Genotyping

Peripheral blood samples (5 mL) were collected from the patients in a fasting state. EDTA was added to the blood samples as an anticoagulant, which were then stored at −80°C. DNA was extracted from the thawed samples using a Blood Genomic DNA Mini Kit (CWBIO, Beijing, China) following the manufacturer's protocol. The following polymerase chain reaction (PCR) primers were used: rs2071219, 5′-TCCCTTCCCTCCTTTCCTCT-3′ (sense) and 5′-TCCAGAAAGTGATGAGACAGTGAT-3′ (antisense); rs706819, 5′-AGAACTGGAGCTTGCAGACT-3′ (sense) and 5′-GGAAGATTTGGTCCTGATGTCC-3′ (antisense); rs2293094, 5′-ATTCCAGTGACCAGAGGACG-3′ (sense) and 5′-AGGGCAGT-GAAGAAAGCTCT-3′ (antisense); and rs11169953, 5′-AGGTACG-GACAGAGGATCCT-3' (sense) and 5′-GTGGGATGGGATGGAGAGAG-3′ (antisense). The reaction was carried out under the following conditions: initial denaturation at 95°C for 2 minutes followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing for 1 minute 30 seconds at 53°C, extension at 65°C for 30 seconds, and then 65°C for 10 minutes. The PCR products were first analyzed using 1% agarose gel electrophoresis and then genotyping of *ACVRL1* rs2071219, rs706819, rs2293094, and rs11169953 polymorphisms were carried out using the PCR-ligase detection reactions method.[14] Technical support for genotyping was provided by Shanghai Biowing Applied Biotechnology Co. Ltd. To validate the SNP genotyping assays, 5% of the genotyped samples were randomly selected and tested reciprocally, with 100% reproducibility.

Statistical Analysis

The independent segregation of alleles was tested using the Hardy-Weinberg equilibrium.[15] The differences among patients with BAVM and controls in clinical variables were evaluated by independent sample t tests. Categorical data are expressed as frequencies and percentages. Statistical analysis of the allele and genotype frequencies between patients with BAVM and controls was performed using χ^2 tests with the SPSS software, version 19.0 (IBM Corp., Armonk, New York, USA). Two-tailed statistical significance was set at 95% ($P < 0.05$).

The association between the SNPs and the frequency of BAVMs was tested using χ^2 tests performed using SHEsis (http://analysis.bio-x.cn).[16] The haplotype structure for the rs706819, rs2293094, and rs11169953 SNPs was constructed and analyzed with SHEsis.[16]

Meta-Analysis

A computed literature search for studies investigating the association of *ACVRL1* polymorphisms with AVM risk was performed. The search was conducted on PubMed, Embase, and the Cochrane Library from inception up to June 5, 2016. We used the following keywords and MeSH (Medical Subject Heading) terms: (AVM OR brain arteriovenous malformations) AND (ALK1 OR ACVRL1). There were no language restrictions. Duplicate publications were considered only once. Any clearly irrelevant studies, review articles, and editorials were excluded. The remaining articles were carefully checked in their entirety to identify whether they contained information on the topic of interest. Moreover, the reference sections were scanned manually for other relevant studies and review articles. The article search was performed independently by 3 investigators. All studies that reported the associations of *ACVRL1* polymorphisms with AVM risk were considered in this meta-analysis. The following selection criteria were used: 1) case-control studies; 2) investigations involving patients with sporadic BAVMs who did not have any hereditary disease-related cerebrovascular dysplasia; 3) studies evaluating *ACVRL1* polymorphisms and BAVM risk; 4) studies reporting genotype counts of *ACVRL1* polymorphisms between BAVM cases and controls; and 5) control genotype distributions consistent with