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EDITORIAL

# Rare and Low-Frequency Variant of *ARHGEF17* Is Associated With Intracranial Aneurysms

See Article by Yang, Li, and Fang et al

**Li He, MD, PhD**
**Richard W. Pierce, MD, MS**
**Wang Min, PhD**

Intracranial aneurysms (IAs) are a group of cerebrovascular disorders characterized by irreversible dilatation or ballooning of the intracranial arterial wall. IAs are often classified both by size and shape with small aneurysms, <5 mm; medium-to-large aneurysms, 6 to 25 mm; and large aneurysms, >25 mm.[1–3] IAs affect ≈1% to 3% of general population, and among which ≈0.5% to 1% of people may experience rupture annually. The incidence rate of IAs is increasing along with the ease and availability of imaging studies, such as computed tomography angiography and magnetic resonance angiography.[4,5] IAs are usually asymptomatic before rupture unless they reach sufficient size to disrupt surrounding structures inducing neurological symptoms or leakage preceding rupture causing headache. Because of the lack of symptoms, detection of IAs pre-rupture is difficult opposed to postrupture IAs which usually present as hemorrhagic stroke or death. Endovascular treatment is now the first therapeutic choice, but the complications and high mortality resulted from operation need to be paid more attention.[6] Certainly, prophylactic embolization may significantly decrease morbidity and mortality of this life-threatening vascular disease, highlighting the need to better understand the development and rupture of IAs.[7] Risk factors, including aging, sex, smoking, hypertension, and hemodynamics and inflammation in the aneurysmal arterial wall, contribute to formation and rupture of IAs.[8–10] However, many studies support that there are underlying genetic risk factors for large fraction of IA cases. Therefore, the identification of more specific risk factors and stratifying individual risk are extremely important.

Epidemiological studies have shown that individuals with a positive family history are more susceptible to IAs compared with the general population,[11–13] suggesting genetic factors play an important role in IAs formation. Current investigations to uncover the genetic contribution have focused on genome-wide association studies of humans with positive family and sporadic IA cases.[14] Many of the variant genes found in these studies fail to replicate positive associations in meta-analyses.[15] One reason may be that most of the disease-associated single nucleotide polymorphisms reported by genome-wide association studies are common (denoted by minor allele frequency >5%) and located in intron and intergenic regions.[16] Recent studies observed common variants have weak and rare (minor allele frequency, <1%) and low-frequency (minor allele frequency, 1%–5%) variants have small to modest effect on complex diseases.[16] The appearance of next-generation sequencing technology raises the hope to discover novel variants of greater significance, particularly some rare and low-frequency variants located in the exome region. There is evidence that rare and low-frequency variants are related to many complex diseases,[17,18] as well as IAs,[19] unfortunately, further investigations have been scarce. Thus, more new rare and low-frequency variants should be discovered to better understand the mechanisms underlying IAs formation.



**Key Words:** Editorials ■ cerebrovascular disorders ■ genomics ■ intracranial aneurysm ■ risk factors



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In this issue of *Circulation: Genomic and Precision Medicine*, Yang et al[20] demonstrated that a rare and low-frequency variant (rs2298808) of *ARHGEF17* is significantly associated with IAs. The study design has used sophisticated and comprehensive genetic analyses and leveraged multiple types of existing databases combined with targeted sequencing, conducting variant discovery, variant replication, and risk gene prioritization (Figure A). Using next-generation sequencing technology, they obtained whole-genome sequencing and whole-exome sequencing of 20 Chinese individuals with a mix of familial and sporadic IA and compared them to 208 Han Chinese subjects in the 1000G[21] as controls, and keeping the variants with allele frequency ≤5%. Using this strategy, they verified 30 candidate variants. After replication analyses of the 30 candidate variants to 3 unsolved familial IA cases, which included Japanese cohort, European American cohort, and French-Canadian cohort, 6 of them were identified significantly associated with IA: rs115753757 of *ADAM15*, rs2298808 of *ARHGEF17*, rs35217482 of *AOX1*, rs114777682 of *AKAP13*, rs150645471 of *ACSM5*, and rs2397084 of *IL17F*. To identify the most distinct rare deleterious variants, the authors performed a gene-level variant enrichment test and found *ARHGEF17* was the only gene with higher variant burden compared with control cases. Association analysis in sporadic IAs was further ascertain rs2298808 ($P$=0.041) of *ARHGEF17* with an odds ratio of 1.51 was significantly related to IA. The authors went on to show that *ARHGEF17* variants segregated with disease in 5 families, and knockdown of *ARHGEF17* resulted in intracranial hemorrhage in zebrafish model. Through this thorough investigation, Yang et al[20] have provided an elegant example for rare variant association studies. Replicating rare variants in ethnically different cohorts would be problematic as allele frequencies are different. Nevertheless, this type of replication analysis helped the prioritization of potential risk genes. It would certainly have benefited from increased number of IA cases in the discovery stage. In the future, an unbiased whole-exome sequencing study with gene burden scores in large cohorts with and without disease could provide more stringent approach to identify pathogenic variants.

*ARHGEF17* encodes the protein RhoGEF17 (also known as p164RhoGEF or Tumor endothelial marker 4) that is a guanine nucleotide exchange factor. Guanine nucleotide exchange factors, along with GTPase accelerating factors (guanine nucleotide exchange factors), regulate the GTP bound state, and therefor activity, of RhoGTPases. With GTP bound, RhoGTPase function as master switches controlling core cell functions, including tension and barrier function in endothelial cells.[22,23] RhoGEF17 localizes to cytoskeletal elements within the cytosol of endothelial cells and is known to interact with RhoA but not Rac1 or Cdc42.[24,25] The authors established the *ARHGEF17* genetic knockout zebrafish to detect its function in vivo and determined that *ARHGEF17* deficiency damaged endothelial cell integrity and induced intracranial hemorrhage in the zebrafish head region (Figure B). This new observation, combined with the population genetic studies outlined, indicates that *ARHGEF17* is a risk gene of IAs. A limitation of the current study is that the cellular mechanisms by which the identified *ARHGEF17* variations induces IA formation is not clear. Computation scores to predict how identified variation would impact protein function were not provided. In a rescue experiment in zebrafish model, rs22298808 variant mRNA can only partially rescued the hemorrhage phenotype in RhoGEF17-deficient embryos, suggesting that rs22298808 mutation can disrupt the RhoGEF17 function but not completely in zebrafish. RhoA is known to generally destabilize endothelial barriers as opposed to Rac1 or Cdc42 which promote barrier stability. Therefore, increased RhoA has been implicated in other vascular

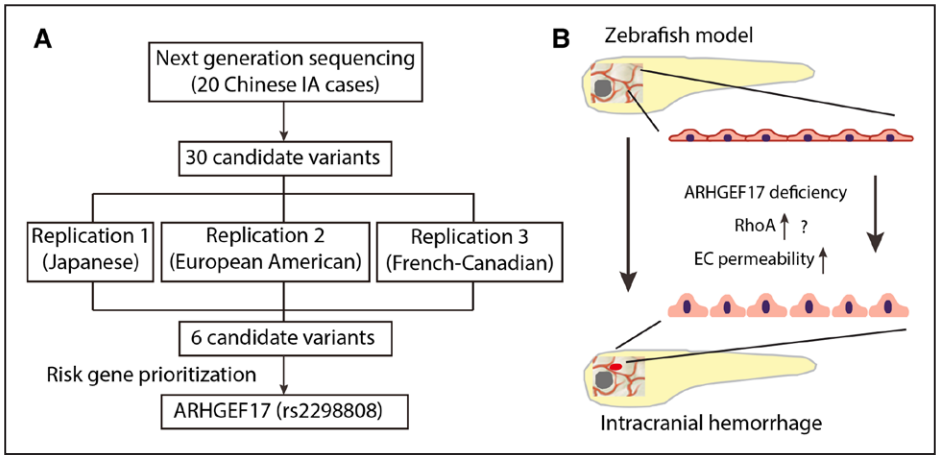


**Figure. Identification of ARHGEF17 rare variants and their functional analyses in zebrafish.**
**A**, Schematic illustration of the discovery and function of rare and low-frequency variant of ARHGEF17 in intracranial aneurysms (IAs). Next-generation sequencing technology was used to detect the rare and low-frequency variants in 20 Chinese familial and sporadic IA patients, after replication and risk gene prioritization analysis, *ARHGEF17* was considered as a risk gene of IAs. **B**, ARHGEF17 deficiency induced intracranial bleeding and vascular leakage in zebrafish. The mechanism by which ARHGEF17 mediates this process needs to be further defined. EC indicates endothelial cell.

diseases, such as cerebral cavernous malformation, and inhibition of RhoA effector rho-associated, coiled-coil-containing protein kinase kinase could rescue vascular defects in mouse models.[26] A defective guanine nucleotide exchange factor(or a broken on switch) for RhoA would be expected to promote vascular barriers through decreased active RhoA. However, active RhoA was not assessed in the IAs in their zebrafish model. Moreover, there are 20 RhoGTPases that have been identified with which RhoGEF17 may interact.[22] Most RhoGTPases have unassigned function and may act to promote or diminish endothelial barriers. Identification of novel binding partners for RhoGEF17 may lead to the discovery of new pathways of vascular stabilization. There are many established mouse models to induce IA formation where these mechanisms may be further investigated.[22] Even so, this is the first study to investigate the role of ARHGEF17 in vivo, and it sheds light on the potential risk role of *ARHGEF17* in animal model of IAs.

Overall, this is a fascinating and thought-provoking study that leveraged genomic databases, targeted sequencing, and in vivo investigations to establish the importance of *ARHGEF17* in IAs. The application of next-generation sequencing technology provides a new way to identify more rare and low-frequency variants in IA cases, as well as other diseases. Moreover, this study has identified a new risk factor *ARHGEF17* for IA development and vascular permeability and may stratify individual patient risk and facilitate precision medicine for IAs and endothelial permeability-related vascular diseases.

## ARTICLE INFORMATION

### Correspondence

Wang Min, PhD, Department of Pathology, Vascular Biology and Therapeutics Program, Yale University School of Medicine, 10 Amistad St, 401B New Haven, CT 06510. E-mail wang.min@yale.edu

### Affiliations

Center for Translational Medicine, The First Affiliated Hospital, Sun Yat-sen University, Guangzhou, China (L.H., W.M.). The Vascular Biology and Therapeutics Program, Yale University School of Medicine, New Haven, CT (R.W.P., W.M.).

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### Disclosures

None.

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