Association of rare *RNF213* variants and intracranial aneurysm risk in a Chinese population

Yaqi Li[1#^], Junyu Liu[2,3#^], Chongyu Hu[4^], Chun Luo[1^], Jilin Zhou[3^], Bingyang Li[1,5^], Xin Liao[1,6^], Songlin Liu[3^], Dun Yuan[3^], Weixi Jiang[3^], Yifeng Li[3^], Junxia Yan[1,7^]

[1]Department of Epidemiology and Health Statistics, Xiangya School of Public Health, Central South University, Changsha, China; [2]Department of Pharmacology, Kyoto University Graduate School of Medicine, Kyoto, Japan; [3]Department of Neurosurgery, Xiangya Hospital, Central South University, Changsha, China; [4]Department of Neurology, Hunan People's Hospital, Changsha, China; [5]Changsha Hospital of Traditional Chinese Medicine (Changsha Eighth Hospital), Changsha, China; [6]The People's Hospital of Guangxi Zhuang Autonomous Region, Nanning, China; [7]Hunan Provincial Key Laboratory of Clinical Epidemiology, Xiangya School of Public Health, Central South University, Changsha, China

Contributions: (I) Conception and design: J Yan; (II) Administrative support: C Hu, S Liu, W Jiang, D Yuan, Yifeng Li; (III) Provision of study materials or patients: W Jiang, D Yuan, Yifeng Li, C Hu; (IV) Collection and assembly of data: J Liu, C Luo, J Zhou, B Li, X Liao; (V) Data analysis and interpretation: Yaqi Li, J Liu; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

[#]These authors contributed equally to this work.

Correspondence to: Junxia Yan, MD. Department of Epidemiology and Health Statistics and Hunan Provincial Key Laboratory of Clinical Epidemiology, Xiangya School of Public Health, Central South University, Shang Mayuanling, Kai Fu District, Changsha 410078, China. Email: 20457456@qq.com; Yifeng Li, MD. Department of Neurosurgery, Xiangya Hospital, Central South University, Changsha 410008, China. Email: liyifeng87@csu.edu.cn.

Background: Genetic factors play important roles in the development of intracranial aneurysm (IA). Rare *RNF213* variants have been identified as being susceptible to Moyamoya disease (MMD), non-MMD intracranial artery stenosis/occlusion disease, and other vascular disorders. This study aimed to investigate the association between rare *RNF213* variants and the risk of IA in a Chinese population.

Methods: We recruited 174 patients with IA for *RNF213* target exome sequencing. Information on the control subjects was obtained from the 1,000 Genome Project and GeneSky in-house database. After prioritizing rare *RNF213* variants, the filtered variants were confirmed by Sanger sequencing. Gene-based association analyses were performed to identify the association between variants and the disease using burden and variance component methods; that is, the weighted-sum statistic (WSS) and the sequence kernel association test (SKAT), respectively. The Student's *t*-test, Chi-squared test, and Fisher's exact test were used to compare the clinical characteristics between carriers and non-carriers of the *RNF213* variants.

Results: After filtering, there were 14 *RNF213* variants in 18 patients with IA, which were significantly associated with the disease after the gene-based association tests [minor allele frequency (MAF) <0.01, WSS P value 5.08×10^{-9}; SKAT P value 2.96×10^{-6}; SKAT-O P value 3.56×10^{-8}]. Significant difference was not obtained between the carriers and non-carriers of the *RNF213* variants in terms of the clinical characteristics.

Conclusions: Rare *RNF213* variants were associated with sporadic IA in a Chinese population. Our findings suggest that these rare *RNF213* variants might have potentially important roles in IA. However, more comprehensive studies need to be conducted to confirm this association and causality.

Keywords: Intracranial aneurysms (IAs); *RNF213* gene; rare variants; target exome sequencing

^ ORCID: Yaqi Li, 0000-0001-9661-3570; Junyu Liu, 0000-0002-3803-4068; Chongyu Hu, 0000-0002-4086-0916; Chun Luo, 0000-0001-9935-9467; Jilin Zhou, 0000-0003-2091-980X; Bingyang Li, 0000-0003-0138-773X; Xin Liao, 0000-0002-9640-8638; Songlin Liu, 0000-0002-2985-0266; Dun Yuan, 0000-0002-9665-4222; Weixi Jiang, 0000-0003-1570-0854; Yifeng Li, 0000-0002-7593-8453; Junxia Yan, 0000-0001-6067-1613.

Submitted Sep 28, 2022. Accepted for publication Dec 02, 2022.
doi: 10.21037/atm-22-5166
View this article at: https://dx.doi.org/10.21037/atm-22-5166

Introduction

Intracranial aneurysm (IA) is a complex disease characterized by the dilation or ballooning of intracranial arteries (1). The rupture of IAs has various clinical consequences, the most devastating of which is subarachnoid hemorrhage (SAH) (2). Overall, the incidence of crude aneurysmal SAH (aSAH) has been reported to be 6.9–9.0 per 100,000 people per year worldwide (3). Clinically, aSAH patients experience a sudden, severe headache, and half suffer a sudden loss of consciousness (4). The aSAH occurs in relatively young patients (aged 40–60 years on average) and leaves many with lasting disabilities that prevent them from returning to their previous jobs (5). However, the mechanisms of IA formation, growth, and rupture remain largely unknown.

Studies have revealed that hypertension, cigarette smoking and excessive alcohol consumption increase the risk of IA, while regular physical activity and healthy lifestyles can reduce the risk (6,7). Some unmodifiable factors (such as increasing age, and females) also increase the risk of IA (7). Besides, genetic factors also play a key role in the development of IA (8,9). The risk of IA is increased among family members with a history of IA or certain genetic disorders, such as autosomal dominant polycystic kidney disease or Ehlers-Danlos syndrome (8). Additionally, people living in specific geographical locations, such as Finland and Japan, have a higher risk of IA rupture (10). Genome-wide and candidate gene association studies have identified several common variants associated with IA, including *SOX17* (rs9298506 and rs10958409), *EDNRA* (rs6841581), *CDKN2B-AS1* (rs10757278), *COL1A2* (rs42524), *COL3A1* (rs1800255), *HSPG2* (rs3767137), *SERPINA3* (rs4934), and *VCAN* (rs251124 and rs173686) (8,11). However, these common variants only explain a small fraction of IA, and the genetic predispositions of IA are largely unknown.

Rare and low-frequency variants, which are not usually detected by genome-wide genotyping arrays, affect phenotypes (12,13). An early study on rare variants also suggested a large phenotypic effect (14). *RNF213* has been identified as a susceptibility gene for Moyamoya disease (MMD), a cerebrovascular disorder characterized by progressive occlusive lesions in the circle of Willis (15,16). The founder mutation p.R4810K (rs112735431) of *RNF213* was found to be associated with intracranial artery stenosis/occlusion diseases, coronary artery stenosis, and pulmonary artery stenosis in East Asians (17). Our previous study also identified the association between rare *RNF213* variants and cerebral arteriovenous malformation (cAVM) in a Chinese population (18). Considering the association of *RNF213* with various vascular diseases, the results of studies focusing on the *RNF213* and its role in vasculature implied this gene could be a key antimicrobial protein in response to the infectious or autoimmune signaling signals in the process of vascular disease (19). Till now, several *RNF213* variants were identified their association with IA in the French-Canadian population (1), whereas the study in the German and Japanese populations failed to obtain a significant result, revealing the existence of racial diversity (20,21). It is not yet known whether rare *RNF213* variants play a role in the pathogenesis of IA in the Chinese population. Thus, we performed exploratory *RNF213* target exome sequencing in a group of Chinese patients with IA and explored the association between rare *RNF213* variants and the risk of IA by conducting a single-variant association study and gene-based burden analysis. We present the following article in accordance with the MDAR reporting checklist (available at https://atm.amegroups.com/article/view/10.21037/atm-22-

Highlight box

Key findings

- A total of 14 *RNF213* variants were identified in 18 Chinese patients with IA. The gene-based association tests showed that rare *RNF213* variants were significantly associated with IAs.

What is known and what is new?

- Rare *RNF213* variants have been identified associated with MMD, non-MMD intracranial artery stenosis/occlusion disease, and other vascular disorders.
- We identified the association between rare *RNF213* variants and sporadic IAs in a Chinese population.

What is the implication, and what should change now?

- *RNF213* might play a role in IAs. A multi-center cohort with a lager sample size is needed to confirm this association and further exploration should be performed to identify the function of RNF213 in the pathological process of IA.

5166/rc) (22).

Methods

Study subjects

Patients diagnosed with IA at the Department of Neurology and Neurosurgery of Xiangya Hospital and Hunan People's Hospital from December 2018 to December 2019 were included in the study. All the patients with IA were enrolled and their diagnoses were confirmed by computed tomography angiography, magnetic resonance imaging, or digital subtraction angiography. The imaging results were interpreted by more than two physicians, including more than one radiologist and one neurological physician, and any disagreements were resolved by consensus or by a third neurological physician. Patients with traumatic or infectious IA or IA concomitant with other vascular diseases (e.g., MMD and cAVM) or hereditary disorders (e.g., autosomal dominant polycystic kidney disease, Ehlers-Danlos syndrome, and Marfan syndrome) were excluded from the study. Demographic and lifestyle information (e.g., age, gender, and behavioral factors, such as smoking and alcohol consumption), and clinical phenotype data (e.g., clinical symptoms, IA location and shape, and disease history) were collected through clinical interviews, health examinations, or imaging checkups. The minor allele frequency (MAF) of the control subjects was obtained from the following two online databases: the 1,000 Genome Project database of the Chinese Han Population (a public genome sequencing database of 208 normal Chinese Han individuals, http://www.internationalgenome.org/home), and the Shanghai GeneSky company in-house database (a private exome sequencing database of 1,007 Chinese individuals without known diseases, http://www.geneskybiotech.com/index.html). The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). This study was approved by the Medical Ethics Committee of Central South University (No. CTXY-150002-1), and written informed consent was obtained from all the participants.

DNA extraction and targeted exome sequencing

Peripheral blood samples were collected from all the enrolled patients using 5 mL EDTA-K2 vacuum tubes (LiuyangSanli Medical Technology Development Co., Ltd., Liuyang, China) and stored at –80 ℃. We used TIANamp Blood DNA Extraction Kit (Tiangen Biotech Co., Ltd., Beijing, China) for Genomic deoxyribonucleic acid (DNA) extraction from the leukocytes in peripheral blood. To identify the rare *RNF213* variants, protein coding sequences were selected, and custom polymerase chain reaction (PCR) primers were designed according to the primerXL pipeline. Ultimately, 100 oligonucleotide pairs were produced, encompassing all coding sequences and untranslated regions of the target *RNF213* gene (https://cdn.amegroups.cn/static/public/atm-22-5166-1.xlsx). The capture target regions contained 25-base flanking sequences at each exon's borders. The AB 2720 Thermal Cycler (Applied Biosystems, Waltham, MA, USA) was used for amplification reactions. The DNA-adapter-ligated and -indexed fragments were pooled and hybridized after PCR products were processed to build a library for further detection. After the sequencing primer was hybridized, targeted sequencing was conducted using an Illumina HiSeq high-throughput sequencing platform (Illumina, San Diego, CA, USA) in accordance with the manufacturer's instructions.

Read mapping, variant calling, and annotation

The sequencing read quality was evaluated using FastQC (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/). The reads were aligned to the human reference sequence [National Center for Biotechnology Information (NCBI) Genome build GRCh37, https://www.ncbi.nlm.nih.gov/genome/guide/human/#download] using the Burrows-Wheeler Aligner (http://bio-bwa.sourceforge.net/). The variants calling was performed using the HaplotypeCaller from the Genome Analysis Toolkit (GATK, https://software.broadinstitute.org/gatk/best-practices/) and VarScan (http://varscan.sourceforge.net/). The coverage analysis was performed using the Picard software CalculateHsMetrics tool. The reads matching the exonic regions, including the exon-intron boundaries, were analyzed. The potential effects on protein function by the variants were assessed using polymorphism phenotyping V2 (Polyphen2, genetics.bwh.harvard.edu/pph2) and Sorting Intolerant From Tolerant (SIFT, http://sift.bii.a-star.edu.sg/). The protein BLAST search engine (http://blast.ncbi.nlm.nih.gov/Blast.cgi) was used for determination of variant homology.

Variant filtration

Filtering criteria were used to prioritize the variants identified from targeted exome sequencing. The variants were regarded

as potentially functional variants low-frequency variants (MAF <0.05) or rare variants (MAF <0.01) if they were: (I) located in the exonic or splicing region; (II) predicted to affect protein coding sequences (including missense, nonsense, read-through, frameshift insertion or deletion, and splice-site variants); (III) less common in reference databases (MAF <0.05 in 1,000 Genome Project Chinese Han population database and Shanghai GeneSky company in-house Database); (IV) predicted to have a functional effect on proteins evaluated by SIFT or PolyPhen-2 (damaging, possibly damaging, or unknown); (V) sequenced with a read depth ≥10×.

Sanger sequencing

We performed the Sanger sequencing to re-confirm the existence of filtered *RNF213* variants. The primers for PCR amplification were designed based on human reference sequences (NCBI, http://www.ncbi.nlm.nih.gov/tools/Primer-blast/index.cgi?LINK_LOC=BlastHome). Sanger sequencing was performed using the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Buenos Aires, Argentina) on an ABI 3730XL DNA sequencer (Applied Biosystems, Buenos Aires, Argentina). The peak map was generated by comparing variant sequences with human reference sequences using Seqman Pro 11.

Statistical analysis

SPSS software (version 21.0; SPSS Inc., Chicago, IL, USA) was used for the statistical analysis. The normally distributed continuous variables are described as means and standard deviations (SDs), and the categorical variables are described as proportions. Odds ratio (ORs) and 95% confidence intervals (CIs) were used to evaluate the associations of IA with single variants by binary logistic regression analysis assumed in an autosomal dominant inheritance model. The aggregate effects of non-common variants (MAF <0.05) were assessed by the gene-based burden tests, including the weight-sum statistic (WSS), the sequence kernel association test (SKAT), and the SKAT-optimal test (SKAT-O) (23,24). The continuous weight function was used to analyze the non-common variants. The logistic weights of the *RNF213* variants were calculated and then applied to the analysis as follows (23):

$$w_j = 1/\left[\mathrm{MAF}_j\left(1-\mathrm{MAF}_j\right)\right]^{1/2} \quad [1]$$

which allows non-common variants to be considered in the overall statistic. For the comparison of the clinical characteristics between carriers and non-carriers of rare *RNF213* variants, the Student's *t*-test was used for the continuous variables of normal distribution, and the chi-squared test or Fisher's exact test was used for the categorical variables. P less than 0.05 showed a statistical significance.

Results

Characteristics of the population

In total, 174 IA patients were recruited for this study, of whom 105 (60.3%) were female. The patients had an average age of 56.8±10.9 years (range, 18.2–87.7 years). In total, 129 (74.2%) patients had a single aneurysm. The location of IAs were as follows: internal carotid artery (46.8%), middle cerebral artery (16.4%), anterior communicating artery (14.8%), and other locations (22.0%). Detailed demographic and clinical characteristics are summarized in *Table 1*.

Rare variant identification and gene-based association analysis

RNF213 exome sequencing was performed using the FastTarget technology. We obtained 3.68–53.44 Mb of targeted sequence suitable for mapping, with a mean sequencing depth between 92.8- and 1,292.9-fold. The average target sequencing depth was 520-fold. Of these, 99.87% of the reads had a Phred-like quality score (Q score) >20, and 87.07% had a Q score >30. The average proportions of target bases with read depths of 2-, 10-, 20- and 30-fold were 95.02%, 91.65%, 88.74%, and 85.91%, respectively.

After the variant annotation, we focused primarily on less common non-synonymous variants (missense and nonsense variants), splice-acceptor and donor-site variants, and coding indels based on the assumption that these variants were more likely to be pathogenic, compared to those synonymous ones which do not change amino acids of proteins. We filtered our data according to the MAF information in the 1,000 Genome Project Chinese Han Population and Shanghai GeneSky database (MAF <0.05). Overall, 14 *RNF213* variants were identified in 18 IA patients, including 10 missense variants, 3 splice-site variants, and 1 frameshift insertion variant (*Tables 2,3*). All the missense variants were predicted as deleterious variants.

Table 1 Demographic characteristics of the 174 Chinese IA patients

Characteristics	IA (n=174)
Age (years)	
Mean ± SD	56.8±10.9
Range	18.2–87.7
Female, n (%)	105 (60.3)
Risk factors, n (%)	
Smoking	29 (16.7)
Drinking	15 (8.6)
Hypertension	92 (52.9)
Diabetes	13 (7.5)
Hyperlipemia	9 (5.2)
Coronary heart disease	10 (5.7)
Ruptured IA, n (%)	113 (64.9)
IA numbers	
1	129 (74.2)
2	30 (17.2)
3	12 (6.9)
4	3 (1.7)
Clinical symptoms	
Headache	108 (62.1)
Vomiting	74 (42.5)
Dizzy	45 (25.9)
Cognitive impairment	30 (17.2)
Blurred vision	8 (4.6)
Language barrier	8 (4.6)
Family history of IA	3 (1.7)
IA location	
Internal carotid artery	111 (46.8)
Middle cerebral artery	39 (16.4)
Anterior communicating artery	35 (14.8)
Anterior cerebral artery	13 (5.5)
Posterior communicating artery	8 (3.4)
Basilar artery	7 (3.0)
Other	24 (10.1)

Other includes the posterior cerebral artery, vertebral artery, posterior inferior cerebellar artery, and ophthalmic artery. IA, intracranial aneurysm; SD, standard deviation.

Twenty alternative alleles were identified in the IA patients (n=174) and 94 in the control subjects (n=1,215, cumulative allele OR, 1.73; 95% CI: 1.05–2.83; P=0.029).

A significant association was not identified among the variants (*Table 4*). The bene-based association analysis, which used both the burden and variance component tests, revealed that less common *RNF213* variants (MAF <0.05) were significantly associated with IA (WSS $P=2.27\times10^{-6}$; SKAT $P=4.97\times10^{-5}$; SKAT-O $P=1.13\times10^{-5}$). The allelic distribution for rare variants carried in IA patients was significantly different from controls (WSS $P=5.08\times10^{-9}$; SKAT $P=2.96\times10^{-6}$; SKAT-O $P=3.56\times10^{-8}$) (*Table 2*).

Clinical characteristics of patients with and without rare RNF213 variants

Among eighteen patients carrying *RNF213* variant, there was no significant difference between carriers and non-carriers in terms of any clinical characteristic, including age, gender, IA risk factors (including smoking, drinking, hypertension, diabetes, hyperlipidemia, and coronary heart disease), and IA rupture status, and number, and location (*Table 5*).

Discussion

In this study, we conducted *RNF213* exome sequencing in 174 IA patients and identified 14 variants in 18 IA patients, including 3 low-frequency variants and 11 rare variants. A gene-based association analysis revealed that the rare *RNF213* variants were significantly associated with IA in this Chinese population. However, there was no significant difference between the carriers and non-carriers of *RNF213* variants in terms of the clinical characteristics. Further comprehensive studies on the mechanisms how *RNF213* plays a role in the disease need to be conducted to confirm these results.

There is considerable evidence that genetic variants are involved in the pathogenesis of IA (25-27). IA-related genes might play different roles in the blood vessel wall; for example, these genes may be involved in endothelial formation and maintenance (i.e., *SOX17*), or endothelial cell integrity maintenance (i.e., the *ADAMTS* gene family) (28). *RNF213* is a ring-finger protein gene that encodes a 591-KDa protein that functions as both an AAA-type ATPase and an E3 ligase, which play a role

Table 2 Summary of the exome sequencing and gene-based association analysis of *RNF213* in 174 IA patients

Data	n=174 (mean ± SD or variants number)		
Sequencing and mapping data			
Raw data yield (M)	22.05±10.13		
No. of effective bases (Mb) mapped to genome	25.26±11.47		
Exons capture			
Effective bases on target region (Mb)	24.3±10.96		
Average sequencing depth in target region	497.01±224.10		
Fraction of target region covered with at least 10× (%)	91.65±0.07		
SNV and indel annotation			
No. of total SNVs	183		
No. of total indels	2		
Filtering steps			
SNVs located in exonic/splicing region	112		
MS/NS/SS/Indels[a]	64		
Less common variants (MAF <0.05)[b]	51		
Potential damaging variants[c]	17		
Read depth ≥10×	14		
Gene-based association analysis			
RNF213			
MAF <0.05	P_{WSS}: 2.27×10^{-6}	P_{SKAT}: 4.97×10^{-5}	P_{SKAT-O}: 1.13×10^{-5}
MAF <0.01	P_{WSS}: 5.08×10^{-9}	P_{SKAT}: 2.96×10^{-6}	P_{SKAT-O}: 3.56×10^{-8}

[a], MS/NS/SS/indels, missense, nonsense, splice-site, and insertion or deletion variants; [b], MAF, minor allele frequency in 1KGP (Chinese Han population) and GeneSky in-house Database; [c], variants judged as SIFT prediction = Damaging/unknown and PolyPhen-2 prediction = Possibly damaging/Probably damaging/Unknown. IA, intracranial aneurysm; SD, standard deviation; SNV, single nucleotide variant; MS, missense; NS, nonsense; SS, splice site; MAF, minor allele frequency; SIFT, sorting intolerant from tolerant; WSS, the weighted-sum statistic; SKAT, the sequence kernel association test.

in the construction of vascular walls (15,29). It was first identified as an important susceptible gene for MMD in East Asian populations (15,16). Subsequent studies showed that *RNF213* variants are not only susceptibility factors for MMD, but also for intracranial atherosclerosis (30) and systemic vascular diseases, such as coronary, pulmonary, and renal artery stenosis (17,31,32). Notably, patients with the *RNF213* rare variant (p.R4810K) may present successively with arterial dissection, SAH, intracranial artery stenosis, and MMD (31). From a functional perspective, Kobayashi *et al.* found that the p.R4810K mutation of *RNF213* resulted in the decreased angiogenic activity of vascular endothelial cells and the inhibition of angiogenesis in the presence of interferon or hypoxia in patients with MMD (33). A recent study showed that *RNF213* is critical in the ubiquitination of the bacterial lipopolysaccharide and that cells lacking *RNF213* suffer from cellular autoimmune deficiency (34). The *RNF213* variant causes reduced E3 ligase activity and ubiquitination, which enhances the stimulation of NFκB and inflammatory signaling, finally leading to vasculopathy (19). However, the mechanistic details of *RNF213* have not yet been elucidated.

RNF213 was first found to be associated with IA in a French-Canadian (FC) study in which the following 5 rare *RNF213* variants were identified in 4 affected families: p.E3806Rfs*27 (c.11413del), p.R673W (c.2017C>T), p.I4526T (c.13577T>C), p.N2327S (c.6980A>G), (NM_001256071.2), and p.S1045L (c.3134C>T)

Table 3 Variants of *RNF213* detected in IA patients

Position	Gene region	Function	Location	Variant[a]		SNP ID	Genotype[b]			SIFT[c]	POLYPhen V2[d]
				cDNA	Amino acid		Case	1000G_CHB	GENESKY		
Chr17:78261836	Exonic	Missense	Exon4	c.484G>A	p.G162S	rs376901868	173/1/0	208/0/0	1,007/0/0	D	P
Chr17:78280117	Exonic	Missense	Exon12	c.2276G>A	p.R759Q	rs755306581	173/1/0	208/0/0	1,007/0/0	D	D
Chr17:78311532	Splicing	–	Exon24	c.4668+6C>T	–	rs78795452	173/1/0	205/3/0	999/8/0	–	–
Chr17:78313764	Exonic	Missense	Exon26	c.5597C>T	p.T1866I	rs546687179	171/3/0	207/1/0	992/15/0	D	–
Chr17:78317642	Splicing	–	Exon28	c.6184-15C>T	–	rs140329743	173/1/0	208/0/0	1,007/0/0	–	–
Chr17:78319385	Exonic	Missense	Exon29	c.7250T>G	p.I2417S	rs181965032	172/2/0	202/6/0	992/15/0	D	P
Chr17:78320875	Exonic	Missense	Exon29	c.8740G>A	p.A2914T	rs187719193	173/1/0	207/1/0	1,007/0/0	D	D
Chr17:78321637	Exonic	Missense	Exon29	c.9502C>T	p.R3168W	rs199753442	173/1/0	208/0/0	1,007/0/0	D	D
Chr17:78321803	Exonic	Missense	Exon29	c.9668C>T	p.S3223L	rs567658257	173/1/0	208/0/0	1,007/0/0	D	P
Chr17:78332222	Exonic	Missense	Exon37	c.10997T>C	p.M3666T	rs375097553	171/2/0	206/2/0	1,003/4/0	D	D
Chr17:78350088	Splicing	–	Exon52	c.13186-13T>C	–	rs113236556	170/3/0	202/6/0	975/32/0	–	–
Chr17:78354716	Exonic	Missense	Exon56	c.13726C>T	p.P4576S	rs776390324	173/1/0	208/0/0	1,006/1/0	D	D
Chr17:78359421	Exonic	Missense	Exon61	c.14539G>A	p.G4847S	rs763289074	173/1/0	208/0/0	1,007/0/0	D	D
Chr17:78360058-78360058	Exonic	Frameshift Insertion	Exon62	c.14548_14549insACCTACCC	p.N4850fs	–	173/1/0	208/0/0	1,007/0/0	–	–

[a], Genbank accession number: ADAMTS15, NM_139055; THSD1: NM_018676; RNF213, NM_001256071; [b], Genotype presented as wild type/heterozygous/homozygous; [c], SIFT Score Prediction: D = damaging, T = tolerated; [d], POLYPHEN Score Prediction: P = possibly damaging, D = probably damaging. IA, intracranial aneurysm; SNP, single nucleotide polymorphism; 1000G_CHB, the database of Chinese Han Beijing population in the 1000 Genomes Project; SIFT, Sortig Intolerant From Tolerant; POLYPhen V2, Polymorphism Phenotyping V2.

Table 4 Single-variant association test of the identified variants of *RNF213*

Variant	Case	1000G_CHB	GENESKY	OR (95% CI)	P value
c.484G>A (p.G162S)	173/1/0	208/0/0	1,007/0/0	–	0.13
c.2276G>A (p.R759Q)	173/1/0	208/0/0	1,007/0/0	–	0.13
c.4668+6C>T	173/1/0	205/3/0	999/8/0	0.63 (0.08, 4.93)	0.99
c.5597C>T (p.T1866I)	171/3/0	207/1/0	992/15/0	1.32 (0.38, 4.56)	0.93
c.6184-15C>T	173/1/0	208/0/0	1,007/0/0	–	0.13
c.7250T>G (p.I2417S)	172/2/0	202/6/0	992/15/0	0.66 (0.15, 2.84)	0.81
c.8740G>A (p.A2914T)	173/1/0	207/1/0	1,007/0/0	7.02 (0.44, 112.70)	0.24
c.9502C>T (p.R3168W)	173/1/0	208/0/0	1,007/0/0	–	0.13
c.9668C>T (p.S3223)	173/1/0	208/0/0	1,007/0/0	–	0.13
c.10997T>C (p.M3666T)	171/2/0	206/2/0	1,003/4/0	3.54 (0.88, 14.27)	0.17
c.13186-13T>C	170/3/0	202/6/0	975/32/0	0.73 (0.26, 2.07)	0.55
c.13726C>T (p.P4576S)	173/1/0	208/0/0	1,006/1/0	7.02 (0.44, 112.70)	0.24
c.14539G>A (p.G4847S)	173/1/0	208/0/0	1,007/0/0	–	0.13
c.14548_14549insACCTACCC (p.N4850fs)	173/1/0	208/0/0	1,007/0/0	–	0.13

OR, odds ratio; CI, confidence interval; 1000G_CHB, the database of Chinese Han Beijing population in the 1000 Genomes Project.

(NM_020954.3). IA-affected individuals carried the *RNF213* variants with significantly higher rates compared to the controls (1). In a subsequent validation study with an increased sample size, the authors confirmed significant differences in *RNF213* functional variation between the patient group and control group in the FC population (1). An exome sequencing study in Germany identified another four variants in three patients with IA/SAH; that is, p.L133M (c.397C>A), p.I209N (c.626T>A), p.A2695V (c.8084C>T), and p.T4677L (c.14030G>T) (NM_001256071) (20). In the present study, we did not identify p.R4810K in the IA patients, consisted with a previous study in the Japanese population (35). Murai *et al.* found that patients with internal carotid artery cystic aneurysms had a similar risk of p.R4810K mutation compared to patients with internal carotid artery stenosis (21). Additionally, 14 *RNF213* variants were found in 18 patients in our study. Among them, all the variants except p.M3666T were not identified in cerebrovascular diseases (31), and none of the variants had been previously identified in patients with IA. However, the variant p.M3666T (c.10997T>C) was previously identified in a patient with MMD (36), and we identified the same variant in two patients with cAVM in our previous study (18).

A total of seven novel variants [four missense variants (p.R759Q, p.R3168W, p.S3223L, and p.G4847S), a splice-site variant (c.6184-15C>T), and two frameshift variants (p.N4850fs and p.V5193fs)] were identified in our study, which had not previously been reported neither in the online databases of the 1,000 human genomes nor 1,007 in-house controls (*Table 3*). In our study, four variants (i.e., p.I2417S, p.A2914T, p.R3168W, and p.S3223L) were located in the AAA-ATPase domain, and four variants [i.e., p.M3666T, splice-site variant (c.13186-13T>C), p.P4576S, p.G4847S, and p.N4850fs] were located in the E3 ligase domain; the remaining five were located in the N-arm region. Zhou *et al.* also identified two deleterious rare variants [i.e., p.R2438C (c.7312C>T), and p.A2826T (c.8476G>A)] in the AAA-ATPase domain and suggested that the domain might be a risk region for IA (1). Cecchi *et al.* conjectured that rare mutations in the C-terminus have a significant effect on MMD (37). Thus, the relationship between the pathogenesis of cerebrovascular disorders and the functional domain of *RNF213* needs to be explored.

Additionally, our gene-based association analysis showed that rare *RNF213* variants were significantly associated with IA, suggesting that *RNF213* may be a susceptible factor for IA, which was consistent with the results of the previous study (1). Among the 18 *RNF213* carriers, two patients carried two different variants, both of whom were elderly

Table 5 Characteristics of the carriers and non-carriers of the *RNF213* variants

Characteristics	Carriers (n=18)	Non-carriers (n=156)	P value
Age (years), mean ± SD	60.4±8.9	55.9±11.6	0.066
Female, n (%)	12 (66.7)	92 (59.0)	0.529
Risk factors, n (%)			
Smoking	4 (22.2)	27 (17.3)	0.606
Drinking	1 (5.5)	26 (16.7)	0.218
Hypertension	9 (50.0)	81 (51.9)	0.877
Diabetes	2 (11.1)	11 (7.1)	0.535
Hyperlipemia	1 (5.5)	8 (5.1)	0.938
Coronary heart disease	1 (5.5)	8 (5.1)	0.938
Ruptured IA, n (%)	8 (44.4)	88 (56.4)	0.334
IA numbers			
1	14 (77.8)	114 (73.1)	0.668
≥2	4 (22.2)	42 (26.9)	
IA location			0.121
Anterior circulation	19 (82.6)	177 (83.9)	
Internal cerebral artery	9 (39.1)	104 (49.3)	
Middle cerebral artery	8 (34.8)	28 (13.3)	
Anterior communicating artery	1 (4.3)	35 (16.6)	
Anterior cerebral artery	1 (4.3)	10 (4.7)	
Posterior circulation	4 (17.4)	34 (16.1)	
Posterior communicating artery	1 (4.3)	7 (3.3)	
Basilar artery	1 (4.3)	5 (2.4)	
Other	2 (8.7)	22 (10.4)	

Other includes the posterior cerebral artery, vertebral artery, posterior inferior cerebellar artery, and ophthalmic artery. IA, intracranial aneurysm; SD, standard deviation.

women with multiple unruptured IAs. Sauvigny *et al.* found that an IA patient with two rare *RNF213* variants had a giant aneurysm (20). However, the relationship between the number of *RNF213* variants and IA severity has yet to be investigated. Among MMD patients, homozygotes of c.14576G>A (p.R4810K) variant present at an earlier onset age, with more severe clinical symptoms, and result in a poorer cognitive prognosis than heterozygotes and wild types c.14576G>A variant (p.R4810K) (38,39). In addition, patients with intracranial artery stenosis/occlusion disease who carried a rare *RNF213* variant showed significant age differences compared to non-carriers (40). Based on these studies, we explored the association between genotype and phenotype in patients with IA. However, we did not find any significant differences between the carriers and non-carriers of *RNF213* variants in terms of their clinical characteristics, including age, gender, risk factors for IA (smoking, drinking, hypertension, diabetes, hyperlipemia, and coronary heart disease), or IA rupture status, number and location. An assumption arose that IA, as a multi-factor-caused disorder, could be influenced by environmental factors or other genetic factors beyond the *RNF213* variations. Thus, the effect of other risk factors on IA should also be explored in future studies.

This study had several limitations. Firstly, it was an exploratory study in which only 174 patients were enrolled.

The relatively small sample size may have limited the statistical power in exploring the associations between rare *RNF213* variants and the risk of IA. Thus, further studies with larger sample sizes need to be conducted to validate our findings. Secondly, this was a regional study, and there might be a selection bias. Thus, multiple-center studies are needed in the future to further verify the association between *RNF213* and IA. Thirdly, due to the invasive examination and relatively expensive cost of cerebrovascular angiography, recruiting control subjects who have been clinically proven to be aneurysm-free is difficult. The rare variant information of the control group was obtained from the 1,000 Genome Project and Shanghai GeneSky in-house database rather than matched controls from the same area. This may have affected the results. However, these two databases consist of >1,000 exome sequencing data of Chinese individuals without known diseases, so the results may not have been significantly affected. Fourthly, we did not perform any functional studies, and the exact mechanism of action of these variants in IA remains unclear. Fifthly, IA is a multifactorial disease that is usually caused by multiple environmental and genetic factors. However, information on environmental factors, such as hypertension and smoking, were not available from the control groups; thus, we could not adjust for the effect of these environmental factors in the gene-based association study.

Conclusions

In our study, rare *RNF213* variants were associated with IA in a Chinese population. Our findings suggest that these rare *RNF213* variants might have potentially important roles in IA. In the future, more comprehensive studies on the etiology of IA need to be conducted.

Acknowledgments

We would like to thank all the participants who provided samples and those involved in the sample collection.
Funding: This work was supported by grant to JY from the Natural Science Foundation of Hunan Province, China (No. 2021JJ30911) and Central South University Case Database Construction Project for Graduate Students, China (No. 2020ALK24).

Footnote

Reporting Checklist: The authors have completed the MDAR reporting checklist. Available at https://atm.amegroups.com/article/view/10.21037/atm-22-5166/rc

Data Sharing Statement: Available at https://atm.amegroups.com/article/view/10.21037/atm-22-5166/dss

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://atm.amegroups.com/article/view/10.21037/atm-22-5166/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). This study was approved by the Medical Ethics Committee of Central South University (No. CTXY-150002-1) and written informed consent was obtained from all the participants.

References

1. Zhou S, Ambalavanan A, Rochefort D, et al. RNF213 Is Associated with Intracranial Aneurysms in the French-Canadian Population. Am J Hum Genet 2016;99:1072-85.
2. van Gijn J, Kerr RS, Rinkel GJ. Subarachnoid haemorrhage. Lancet 2007;369:306-18.
3. Etminan N, Chang HS, Hackenberg K, et al. Worldwide Incidence of Aneurysmal Subarachnoid Hemorrhage According to Region, Time Period, Blood Pressure, and Smoking Prevalence in the Population: A Systematic Review and Meta-analysis. JAMA Neurol 2019;76:588-97.
4. Huckhagel T, Klinger R, Schmidt NO, et al. The burden of headache following aneurysmal subarachnoid hemorrhage: a prospective single-center cross-sectional analysis. Acta Neurochir (Wien) 2020;162:893-903.
5. Neifert SN, Chapman EK, Martini ML, et al. Aneurysmal

Subarachnoid Hemorrhage: the Last Decade. Transl Stroke Res 2021;12:428-46.
6. Vlak MH, Rinkel GJ, Greebe P, et al. Independent risk factors for intracranial aneurysms and their joint effect: a case-control study. Stroke 2013;44:984-7.
7. Backes D, Rinkel GJ, Laban KG, et al. Patient- and Aneurysm-Specific Risk Factors for Intracranial Aneurysm Growth: A Systematic Review and Meta-Analysis. Stroke 2016;47:951-7.
8. Tromp G, Weinsheimer S, Ronkainen A, et al. Molecular basis and genetic predisposition to intracranial aneurysm. Ann Med 2014;46:597-606.
9. Zhou S, Dion PA, Rouleau GA. Genetics of Intracranial Aneurysms. Stroke 2018;49:780-7.
10. Greving JP, Wermer MJ, Brown RD Jr, et al. Development of the PHASES score for prediction of risk of rupture of intracranial aneurysms: a pooled analysis of six prospective cohort studies. Lancet Neurol 2014;13:59-66.
11. Alg VS, Sofat R, Houlden H, et al. Genetic risk factors for intracranial aneurysms: a meta-analysis in more than 116,000 individuals. Neurology 2013;80:2154-65.
12. Lettre G. Rare and low-frequency variants in human common diseases and other complex traits. J Med Genet 2014;51:705-14.
13. Cirulli ET, Goldstein DB. Uncovering the roles of rare variants in common disease through whole-genome sequencing. Nat Rev Genet 2010;11:415-25.
14. Kosmicki JA, Churchhouse CL, Rivas MA, et al. Discovery of rare variants for complex phenotypes. Hum Genet 2016;135:625-34.
15. Liu W, Morito D, Takashima S, et al. Identification of RNF213 as a susceptibility gene for moyamoya disease and its possible role in vascular development. PLoS One 2011;6:e22542.
16. Kamada F, Aoki Y, Narisawa A, et al. A genome-wide association study identifies RNF213 as the first Moyamoya disease gene. J Hum Genet 2011;56:34-40.
17. Chang SA, Song JS, Park TK, et al. Nonsyndromic Peripheral Pulmonary Artery Stenosis Is Associated With Homozygosity of RNF213 p.Arg4810Lys Regardless of Co-occurrence of Moyamoya Disease. Chest 2018;153:404-13.
18. Liu J, Hu C, Zhou J, et al. RNF213 rare variants and cerebral arteriovenous malformation in a Chinese population. Clin Neurol Neurosurg 2021;203:106582.
19. Asselman C, Hemelsoet D, Eggermont D, et al. Moyamoya disease emerging as an immune-related angiopathy. Trends Mol Med 2022;28:939-50.
20. Sauvigny T, Alawi M, Krause L, et al. Exome sequencing in 38 patients with intracranial aneurysms and subarachnoid hemorrhage. J Neurol 2020;267:2533-45.
21. Murai Y, Ishisaka E, Watanabe A, et al. RNF213 c.14576G>A Is Associated with Intracranial Internal Carotid Artery Saccular Aneurysms. Genes (Basel) 2021;12:1468.
22. Macleod M, Collings AM, Graf C, et al. The MDAR (Materials Design Analysis Reporting) Framework for transparent reporting in the life sciences. Proc Natl Acad Sci U S A 2021;118:e2103238118.
23. Madsen BE, Browning SR. A groupwise association test for rare mutations using a weighted sum statistic. PLoS Genet 2009;5:e1000384.
24. Wu MC, Lee S, Cai T, et al. Rare-variant association testing for sequencing data with the sequence kernel association test. Am J Hum Genet 2011;89:82-93.
25. Ruigrok YM, Elias R, Wijmenga C, et al. A comparison of genetic chromosomal loci for intracranial, thoracic aortic, and abdominal aortic aneurysms in search of common genetic risk factors. Cardiovasc Pathol 2008;17:40-7.
26. Broderick JP, Brown RD Jr, Sauerbeck L, et al. Greater rupture risk for familial as compared to sporadic unruptured intracranial aneurysms. Stroke 2009;40:1952-7.
27. Karschnia P, Nishimura S, Louvi A. Cerebrovascular disorders associated with genetic lesions. Cell Mol Life Sci 2019;76:283-300.
28. Samuel N, Radovanovic I. Genetic basis of intracranial aneurysm formation and rupture: clinical implications in the postgenomic era. Neurosurg Focus 2019;47:E10.
29. Morito D, Nishikawa K, Hoseki J, et al. Moyamoya disease-associated protein mysterin/RNF213 is a novel AAA+ ATPase, which dynamically changes its oligomeric state. Sci Rep 2014;4:4442.
30. Bang OY, Chung JW, Cha J, et al. A Polymorphism in RNF213 Is a Susceptibility Gene for Intracranial Atherosclerosis. PLoS One 2016;11:e0156607.
31. Lin J, Sheng W. RNF213 Variant Diversity Predisposes Distinct Populations to Dissimilar Cerebrovascular Diseases. Biomed Res Int 2018;2018:6359174.
32. Fukushima H, Takenouchi T, Kosaki K. Homozygosity for moyamoya disease risk allele leads to moyamoya disease with extracranial systemic and pulmonary vasculopathy. Am J Med Genet A 2016;170:2453-6.
33. Kobayashi H, Matsuda Y, Hitomi T, et al. Biochemical and Functional Characterization of RNF213 (Mysterin) R4810K, a Susceptibility Mutation of Moyamoya Disease,

in Angiogenesis In Vitro and In Vivo. J Am Heart Assoc 2015;4:e002146.
34. Otten EG, Werner E, Crespillo-Casado A, et al. Ubiquitylation of lipopolysaccharide by RNF213 during bacterial infection. Nature 2021;594:111-6.
35. Miyawaki S, Imai H, Takayanagi S, et al. Identification of a genetic variant common to moyamoya disease and intracranial major artery stenosis/occlusion. Stroke 2012;43:3371-4.
36. Shoemaker LD, Clark MJ, Patwardhan A, et al. Disease Variant Landscape of a Large Multiethnic Population of Moyamoya Patients by Exome Sequencing. G3 (Bethesda) 2015;6:41-9.
37. Cecchi AC, Guo D, Ren Z, et al. RNF213 rare variants in an ethnically diverse population with Moyamoya disease. Stroke 2014;45:3200-7.
38. Kim EH, Yum MS, Ra YS, et al. Importance of RNF213 polymorphism on clinical features and long-term outcome in moyamoya disease. J Neurosurg 2016;124:1221-7.
39. Miyatake S, Touho H, Miyake N, et al. Sibling cases of moyamoya disease having homozygous and heterozygous c.14576G>A variant in RNF213 showed varying clinical course and severity. J Hum Genet 2012;57:804-6.
40. Liao X, Zhang T, Li B, et al. Rare RNF213 variants and the risk of intracranial artery stenosis/occlusion disease in Chinese population: a case-control study. BMC Med Genet 2019;20:55.

Cite this article as: Li Y, Liu J, Hu C, Luo C, Zhou J, Li B, Liao X, Liu S, Yuan D, Jiang W, Li Y, Yan J. Association of rare *RNF213* variants and intracranial aneurysm risk in a Chinese population. Ann Transl Med 2022;10(24):1336. doi: 10.21037/atm-22-5166

