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RNF213 as the major susceptibility gene for Chinese patients with moyamoya disease and its clinical relevance

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OBJECTIVE Moyamoya disease (MMD) is a rare, genetically heterogeneous cerebrovascular disease. The authors conducted a genetic study of really interesting new gene (RING) finger protein 213 (*RNF213*); actin alpha 2 (*ACTA2*); BRCA1/BRCA2-containing complex subunit 3 (*BRCC3*); and guanylate cyclase 1, soluble, alpha 3 (*GUCY1A3*) as well as a clinical phenotype analysis in Chinese MMD patients to determine whether genetic differences are responsible for the different clinical features that appear in MMD in different ethnicities.

METHODS A panel was designed to identify disease-causing mutations in MMD genes and those involved in related disorders (*RNF213*, *ACTA2*, *BRCC3*, and *GUCY1A3*). The panel was used to detect disease-causing mutations in 255 Chinese MMD patients. Genotype and allele frequencies were compared between patients and 300 controls. A mutation segregation analysis was performed in 34 families, and genotype-phenotype correlations were made.

RESULTS Twenty-seven rare missense variants of *RNF213* were identified and were not found in controls. Among them, p.R4810K was identified in 31.4% of patients (80 of 255) with MMD. Significantly higher frequencies of the A allele and G/A genotype of p.R4810K were observed in MMD patients compared with controls ($\chi^2 = 104.166$, $p < 0.000$). Twenty-five rare variants were identified in 10.6% of patients (27 of 255) without p.R4810K variants. Segregation analysis supported an association between MMD and 3 variants. No possible disease-causing mutations were identified in *ACTA2*, *BRCC3*, or *GUCY1A3*. Compared with patients without the rare variants in *RNF213*, the p.R4810K heterozygous patients were younger at diagnosis (25 vs 29 years old, $p = 0.049$) and had more familial cases (24% vs 4.4%, $p = 0.000$), ischemic cases (81.3% vs 67.5%, $p = 0.037$), and involvement of the posterior cerebral artery (52% vs 32.5%, $p = 0.007$).

CONCLUSIONS *RNF213* is the major susceptibility gene in Chinese MMD patients. The spectrum of rare variants identified in Chinese MMD patients was diverse. Compared to patients without the rare variants in *RNF213*, the p.R4810K heterozygous patients exhibited different clinical features.

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KEY WORDS moyamoya disease; *RNF213*; variant; clinical features; vascular disorders

Moyamoya disease (MMD) is a vasculopathy characterized by progressive stenosis of the internal carotid arteries and its proximal branches accompanied by the development of a compensatory collateral vessel network.[27] Despite substantial investigation, the molecular etiology and pathogenesis of moyamoya angiopathy remain unclear. Several studies have explored genetic factors, and 4 loci associated with MMD have been reported: 3p24–p26,[10] 6q25,[11] 8q23,[25] and 17q25.[13,18] Mutations in really interesting new gene (RING) finger protein 213 (*RNF213*);[13,18] actin alpha 2 (*ACTA2*);[7] BRCA1/BRCA2-containing complex, subunit 3 (*BRCC3*);[22] and

ABBREVIATIONS MAF = minor allele frequency; MMD = moyamoya disease; MMS = moyamoya syndrome; mRS = modified Rankin Scale; NGS = next-generation sequencing; PCA = posterior cerebral artery; RING = really interesting new gene.

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guanylate cyclase 1, soluble, alpha 3 (*GUCY1A3*)[8] have been reported in patients with MMD or moyamoya syndrome (MMS).

RNF213 is located at 17q25 and encodes a RING finger protein, a single protein that possesses both ubiquitin ligase activity and ATPase activity.[18] As reported in previous studies, there are differences in the spectrum of mutations between East Asians and whites. For example, p.C3997Y, p.D4013N, and p.R4019C variants in *RNF213* have been identified only in white MMD patients,[3,18] who did not exhibit p.R4810K, a highly recurrent variant in Asian patients. In East Asia, the attributable risks of a p.R4810K mutation in Japanese (145 [90%] of 161 cases) and Korean (30 [79%] of 38 cases) populations were significantly larger than in the Chinese population (12 [23%] of 52 cases).[18] Moreover, patients with heterozygous *ACTA2* mutations have been reported to be predisposed to a variety of diffuse and diverse vascular diseases, including thoracic aortic aneurysms and dissections, ischemic stroke, and MMD.[7] Xq28 deletions removing mature T-cell proliferation 1/mature T-cell proliferation 1 neighbor and *BRCC3* may lead to moyamoya angiopathy, short stature, and a stereotyped facial dysmorphism.[22] An autosomal-recessive disease associated with moyamoya and achalasia was identified recently, and it was caused by loss-of-function mutations in *GUCY1A3*.[8] Whether *ACTA2*, *BRCC3*, and *GUCY1A3* are causative genes of MMD in Chinese patients remains unknown. To date, there are no large-scale genetic studies including Chinese MMD patients and families.

Moyamoya disease occurs worldwide, but its prevalence is highest in East Asian countries including Japan (6.03 cases/100,000 persons), Korea (6.3 cases/100,000 persons), and China (3.92 cases/100,000 persons).[1,5,9,17,21] Its incidence in Europe and America appears to be approximately one-tenth of that in Japan.[26] In China, a female predominance is less apparent than those in Japan and Western countries.[4,15,30] Moreover, symptoms at the onset of MMD in China are different from those in Japan and South Korea but similar to those in the United States and Europe.[4] The epidemiological and clinical characteristics of Chinese MMD are also different from those of other ethnicities of MMD.[4] Whether genetic differences are responsible for the different clinical features remains unclear. We conducted a comprehensive genetic study of *RNF213*, *ACTA2*, *BRCC3*, and *GUCY1A3* and a clinical phenotype analysis of Chinese MMD patients to address this question.

Methods

Study Population and Sample Collection

We recruited 255 MMD patients and 300 healthy controls at Beijing Tiantan Hospital, Capital Medical University, from June 2012 to October 2014. The MMD patients included 107 men and 148 women, with a mean age at participation of 26.7 ± 14.7 years (mean ± standard deviation). The 255 cases consisted of 220 sporadic cases with no family history and 35 familial MMD cases from 16 families (> 1 family member with MMD). The other members of these 16 families and the parents of 18 childhood-onset patients out of the 220 sporadic MMD cases were also included in the study. Our 300 controls, 128 men and 172 women, consisted of age- and sex-matched healthy individuals who were seen for routine laboratory tests (mean age at participation 28.0 ± 15.9 years). All subjects were Han Chinese.

Diagnosis of MMD was based on MR angiography and/or diagnostic angiography findings demonstrating stenosis or occlusion of the terminal portion of the internal carotid artery with the formation of collateral vessels compensating for the arterial occlusion.[24] Patients diagnosed with both unilateral and bilateral MMD were included in this study. Other causes of arterial occlusion, such as atherosclerosis, meningitis, Down syndrome, or systemic vasculitis, were excluded via medical record and imaging review. Information on sex, age at diagnosis, family history, onset of symptoms, and preoperative modified Rankin Scale (mRS) score were obtained through clinical chart review. Angiography profiles, including combined aneurysm, stenoocclusive lesions of the posterior cerebral artery (PCA), dilation of the anterior choroidal artery, and Suzuki stage, were classified by 2 neurosurgeons. Blood samples were collected from the samples mentioned above after written informed consent had been obtained. The Ethics Committee of Beijing Tiantan Hospital, Capital Medical University, approved the study.

DNA Sequencing and Data Analysis

Genomic DNA from the 255 patients was extracted from blood leukocytes using a QIAamp blood kit (Qiagen) and was sequenced with next-generation sequencing (NGS) using a custom-designed Ion AmpliSeq Panel (Life Technologies) and Ion Torrent personal genome machine (PGM).[28] As key words, "moyamoya disease" was searched in Online Mendelian Inheritance in Man (OMIN). *RNF213* (moyamoya disease 2), *ACTA2* (moyamoya disease 5), *BRCC3* (moyamoya disease 4), and *GUCY1A3* (moyamoya disease 6) had been reported as susceptibility or causative genes in MMD or MMS patients and were selected as target genes. A panel was designed to identify disease-causing mutations in the exons and exon-intron boundaries (± 50 bp) of these genes. The descriptions of these genes (*RNF213 ACTA2*, *BRCC3*, and *GUCY1A3*) in this paper are primarily based on the longest isoforms (NM_001256071, NM_001141945, NM_024332, and NM_000856, respectively). Exon sequencing was conducted according to the manufacturer's protocol. The details are shown in the Supplemental Materials and Methods.

Software, including Torrent Suite 4.0 (Life Technologies), SeattleSeq Annotation version 9.00 (University of Washington), and Integrative Genomics Viewer 2.1 (IGV, Broad Institute), was used to perform bioinformatics analyses such as optimizing signal processing, base calling, sequence alignment, and variant analysis. Gene variants identified via exome sequencing were filtered. Only variants with a minor allele frequency (MAF) < 5% in the Exome Variant Server database (http://evs.gs.washington.edu/EVS/) and the 1000 Genomes database (http://1000genomes.org) were retained for further investigation. Finally, identified rare variants (arbitrarily defined

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as MAF < 5% in this study) were confirmed by Sanger sequencing using an ABI 3730 DNA analyzer (Applied Biosystems). The details of the primers used for amplification are shown in Supplemental Table 1. Potential effects of amino acid substitutions were predicted by PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2/) and SIFT (http://sift.jcvi.org/).

Clinical Phenotype Analysis in Different Genotypes

We assessed the clinical and angiographic features according to genotypes, including sex ratio, age at diagnosis, family history, frequency of childhood onset, clinical manifestations, preoperative mRS score > 2, combined aneurysm, PCA involvement, dilation of anterior choroidal artery, and Suzuki stage.

Statistical Analysis

All statistical analyses were performed using SPSS Statistics version 19 software (IBM Corp.). Differences in genotype and allele frequencies between patients and controls were compared. Differences in allele frequency were quantified by odds ratios and 95% confidence intervals. A chi-square distribution was used to analyze associations between categorical clinical characteristics and genotypes. Nonnormally distributed continuous variables, such as age at diagnosis and Suzuki stage, were compared using the Mann-Whitney U-test between different genotypes. Statistical significance was defined as 2-tailed $p < 0.05$.

Results

DNA Sequencing Results

Two hundred fifty-five MMD patients were sequenced with NGS using a custom-designed Ion AmpliSeq Panel. The mean depth was more than 150 times (that is, each base was sequenced 150 times), and the on-target rate exceeded 95%. There were no possible disease-causing mutations identified in *ACTA2*, *BRCC3*, and *GUCY1A3*. Thirty-seven rare missense variants of *RNF213*, 16 of which were novel, were identified. No frame-shift or nonsense mutations were detected. These variants were p.P61L, p.A1041T, p.A1041V, p.R1228G, p.E1707K, p.T1727M, p.T1866I, p.P2107L, p.N2971Y, p.E3061K, p.G3470R, p.R3580W, p.M3666T, p.I3693L, p.D3899N, p.G3936E, p.D4013N, p.H4014T, p.A4050V, p.R4062Q, p.N4066S, p.K4160Q, p.P4250T, p.M4289I, p.S4389G, p.A4399T, p.P4576S, p.G4640R, p.R4810K, p.D4863N, p.V4884I, p.Q4938R, p.E4950D, p.S5012R, p.A5021V, p.T5037I, and p.R5153H and were confirmed by Sanger sequencing (Table 1 and Supplemental Table 2).

Comparison of the Genotype and Allele Frequencies Between Patients and Controls

Among the 37 rare variants of *RNF213*, 27 were not found in the 300 healthy controls (Fig. 1; Supplemental Table 3). The MAF values of these 27 mutations were less than 0.08% in the Exome Variant Server database and the 1000 Genomes database. Among these mutations, p.R4810K was identified in 31.4% of patients (80 of 255) with MMD (65.7% familial cases and 25.9% nonfamilial patients). The p.T1727M variant was identified only in patients with the p.R4810K mutation (29 [36.3%] of 80). Significantly higher frequencies of the A allele and G/A genotype of p.R4810K (rs112735431) and the T allele and T/C genotype of p.T1727M (rs371978343) were observed in MMD patients compared with controls ($\chi^2 = 104.166$, $p < 0.000$; $\chi^2 = 35.033$, $p < 0.000$, respectively). The other 25 rare variants (not found in controls) were identified in 10.6% of patients (27 of 255) without p.R4810K variants. No significant association between MMD and any of the 25 rare mutations in *RNF213* was detected. In total, 42% of patients (107 of 255) carried the p.R4810K variants or the possible susceptibility variants in *RNF213* (Table 1 and Fig. 1).

Mutation Segregation Analysis

Ten (62.5%) of the 16 MMD families (> 1 family member with MMD) carried the p.R4810K variant in *RNF213*. It co-segregated with MMD and was not fully penetrant. A novel variant, p.R4160Q, was confirmed to co-segregate with MMD in 1 family, but it was not fully penetrant. A de novo p.H4014T variant in the RING finger domain of *RNF213* was detected in 1 family (Fig. 2).

Comparison of Clinical Features Between Patients With the *RNF213* p.R4810K Heterozygous Variant and Those Without Rare Variants in *RNF213*

We analyzed the clinical features of patients with MMD according to the *RNF213* variants. Patients carrying the heterozygous p.R4810K variant were classified as Group 1, and those carrying no *RNF213* rare variants were classified as Group 2. Among the 255 MMD patients, 80 patients carried p.R4810K variants. Two homozygous patients and 3 patients without cerebral angiography were excluded; therefore, 75 cases were included in Group 1. Among the remaining 175 study entrants, 58 were excluded because they carried other *RNF213* rare variants mentioned above and 3 because they had no cerebral angiography; thus, 114 patients were enrolled in Group 2. Demographic data for the 189 patients are summarized in Table 2. The female/male ratio was 1.5:1. The mean age at diagnosis was 27.7 years (range 3–59 years). Of the 189 patients, 23 (12.2%) had a familial history, and 61 (32.3%) had a childhood onset (< 18 years old). Ischemic symptoms were identified in 138 patients (73.0%), and hemorrhage was observed in 51 (27.0%). Ten intracranial aneurysms (5.3%) were recorded. A preoperative mRS score > 2 was observed in 26 patients (13.8%). Angiographs from 76 patients (40.2%) showed stenoocclusive lesions of the PCA. The angiographic stages of anterior circulation were as follows: Stage 1 in 4 hemispheres (1.0%), Stage 2 in 111 (29.4%), Stage 3 in 60 (15.9%), Stage 4 in 135 (35.7%), Stage 5 in 45 (11.9%), and Stage 6 in 23 (6.1%). Dilation of the anterior choroidal artery was observed in 192 hemispheres (50.8%).

Compared with the patients without rare variants (MAF < 0.05) in *RNF213*, p.R4810K heterozygous patients were younger at diagnosis (25 vs 29 years old, $p = 0.049$) and included more familial cases (24% vs 4.4%, $p = 0.000$), more ischemic cases (81.3% vs 67.5%, $p = 0.037$), and more stenoocclusive lesions in the PCA (52% vs 32.5%,

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TABLE 1. Rare variants of *RNF213* (not found in controls) and the predicted effects of amino acid substitutions

Amino Acid Change	Position (chr17)	rs ID	MAF in NHLBI*	MAF in 1000 Genomes†	PolyPhen-2 HumDiv	SIFT
p.R1228G	78305970	—	0	0	Possibly D‡	D‡
p.E1707K	78313286	540300391	0.0002	0	Probably D‡	D‡
p.T1727M	78313347	371978343	0	0.000219	Benign	Tolerated
p.P2107L	78317793	—	0	0	Probably D‡	Tolerated
p.N2971Y	78321046	—	0	0	Probably D‡	Tolerated
p.E3061K	78321316	—	0	0	Benign	Tolerated
p.G3470R	78326844	—	0	0	Benign	Tolerated
p.R3580W	78328252	554959669	0.0002	0	Benign	D‡
p.I3693L	78333883	—	0	0	Benign	D‡
p.D3899N	78337535	—	0	0	Possibly D‡	D‡
p.G3936E	78338289	—	0	0	Benign	Tolerated
p.D4013N	78341825	397514563	0	0	Possibly D‡	Tolerated
p.H4014T	78341828	—	0	0	Probably D‡	D‡
p.A4050V	78341937	—	0	0	Benign	Tolerated
p.R4062Q	78343331	—	0	0	Probably D‡	D‡
p.N4066S	78343343	—	0	0	Probably D‡	D‡
p.K4160Q	78345726	—	0	0	Probably D‡	Tolerated
p.M4289I	78346890	374262786	0	0.00008	Benign	Tolerated
p.S4389G	78349650	187389872	0.0002	0	Benign	Tolerated
p.G4640R	78355467	138223459	0	0.00015	Benign	Tolerated
p.R4810K	78358945	112735431	0.0012	0	Possibly D‡	Tolerated
p.V4884I	78360160	146990608	0.0004	0.00008	Possibly D‡	Tolerated
p.Q4938R	78360582	—	0	0	Possibly D‡	D‡
p.E4950D	78360619	371441113	0.0008	0	Probably D‡	Tolerated
p.S5012R	78363006	—	0	0	Benign	Tolerated
p.T5037I	78363082	—	0	0	Possibly D‡	Tolerated
p.R5153H	78363984	528073196	0.0002	0	Probably D‡	Tolerated

— = novel variant; chr17 = chromosome 17; HumDiv = data set used by PolyPhen-2; NHLBI = National Heart, Lung, and Blood Institute; rs = reference single nucleotide polymorphism cluster.

* Minor allele frequencies in the National Heart, Lung, and Blood Institute Exome Sequencing Project.

† Minor allele frequencies in the 1000 Genomes project.

‡ Damaging.

p = 0.007). There was no significant difference in the sex ratio (1.4:1 vs 1.8:1, p = 0.707), frequency of childhood onset (38.7% vs 28.1%, p = 0.127), preoperative mRS score > 2 (9.3% vs 16.7%, p = 0.152), combined aneurysm (1.3% vs 7.9%, p = 0.123), dilation of anterior choroidal artery (56.0% vs 47.4%, p = 0.101), or Suzuki stage (p = 0.486) between the 2 groups.

Discussion

The results of this study indicate that *RNF213* is the major susceptibility gene for Chinese MMD patients. Moreover, no disease-causing mutations were identified in *ACTA2*, *BRCC3*, or *GUCY1A3*. Compared with the rate in previous studies of Chinese MMD patients, a much higher frequency of the p.R4810K variant was observed in our study (31.4% vs 9.4%–23%).[18,29,30] We also showed a higher prevalence of the p.R4810K mutation in familial MMD cases (65.7%) than in sporadic ones (25.9%), which was in line with Japanese MMD cases.[13,18] We noticed that 5 familial cases were enrolled in previous studies on Chinese patients with MMD. Thus, this difference may be due to the higher proportion of familial cases (35 [13.7%] of 255) in our study. In East Asia, the attributable risks for the p.R4810K mutation in the Japanese (145 [90%] of 161 cases) and Korean (30 [79%] of 38 cases) populations were significantly greater than that in the Chinese population (12 [23%] of 52 cases).[18] It has been suggested that other rare variants of *RNF213* may be causative mutations for those patients carrying them. By direct sequencing all exons and introns of *RNF213* in 40 Chinese MMD patients without a p.R4810K variant, 5 variants—p.D4863N, p.E4950D, p.A5021 V, p.D5160E, and p.E5176G—were identified in 7 cases. The 5 variants were not detected in 150 Chinese controls.[18] However, these variants were not verified in a large-scale case-control study. In our study,

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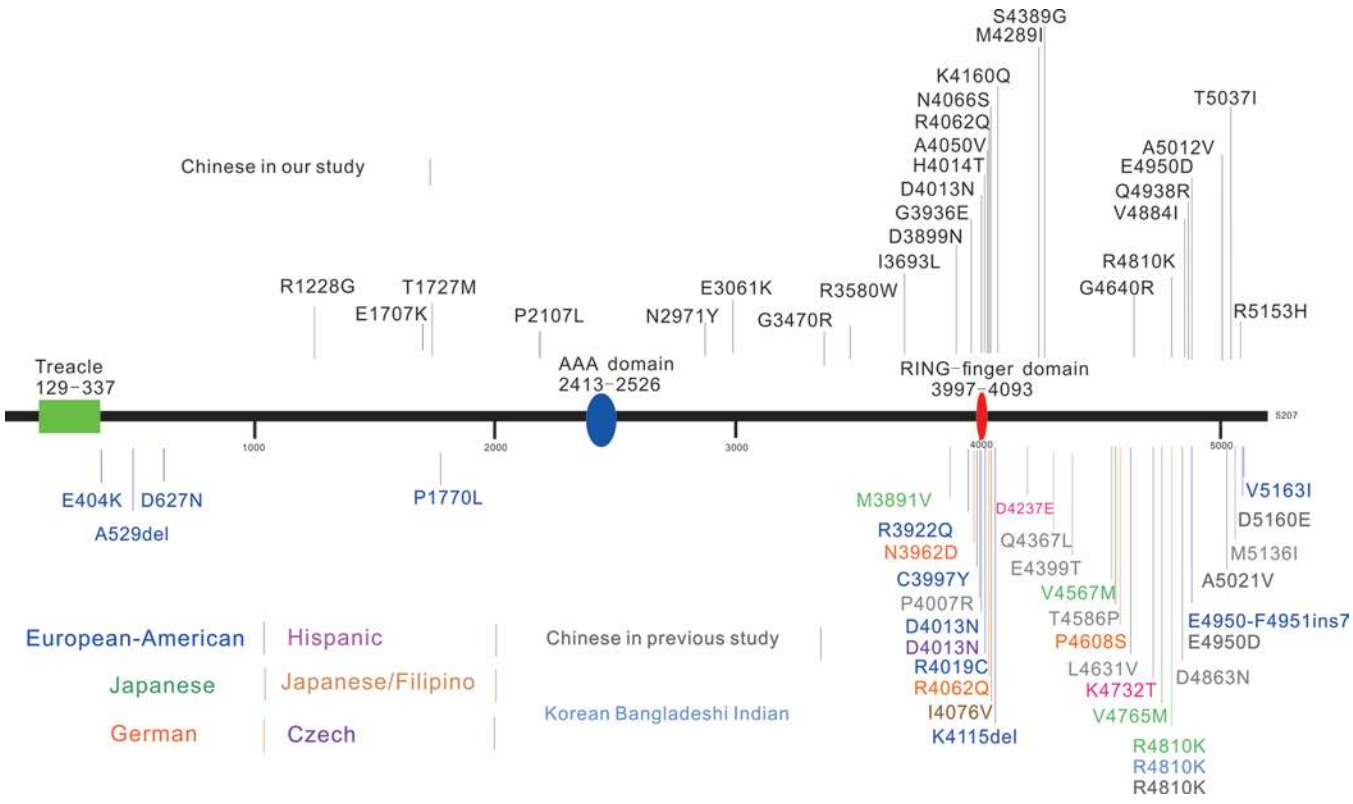

FIG. 1. Domains of *RNF213* and variants identified in MMD. The 27 missense variants identified in Chinese patients with MMD in this study are shown in black typeface above the *black line*. The *RNF213* rare variants identified in ethnically diverse populations in previous reports are shown below the *black line*. Different colors represent different ethnicities. Figure is available in color online only.

p.D4863N, p.E4950D, and p.A5021V were identified. Moreover, 16 novel variants were observed, and 27 rare variants (MAF < 0.08%) were not detected in controls. Segregation analysis supported an association between MMD and 2 novel variants (p.H4014T and p.R4160Q). We expanded the spectrum of rare variants in MMD patients. To date, most variants identified in *RNF213* of MMD patients were missense variants.[3,18,23] However, as the most widely reported variants, p.R4810K or p.D4013N did not affect ubiquitin ligase activity or cause other hallmark changes such as mRNA or protein instability in vitro.[18] Further studies on the protein function of *RNF213* may help to define the roles of these variants.

The spectrum of rare variants identified here supports the idea of diversity among ethnicities. The p.R4810K variant, a highly recurrent variant in Asian cases, was not found in white cases.[3,18] Unlike p.R4810K, the variants of p.M3891V, p.V4567M, and p.V4765M were only identified in Japanese MMD cases.[13] Other variants, such as A529del, p.R3922Q, p.C3997Y, R4019C, I4076V, K4115del, D4237E, K4732T, E4950_F4951ins7, and V5163I, have been identified only in white MMD patients.[3] To date, several studies have reported different rare variants in different populations.[3,6,13,18] The extensive allelic heterogeneity and ethnicity-specific variant spectrum of MMD were observed here (Fig. 1). It has been widely reported that the epidemiological and clinical characteristics of MMD vary in different ethnicities.[1,4,5,9,21] However, the effect of genotype on clinical features is poorly understood. The homozygous c.14576G > A (rs112735431) variant carriers showed a significantly earlier age at onset and greater PCA involvement.[23] However, no significant difference was observed between heterozygous and wild-type GG variants. Compared with a previous report,[23] more adult (67.7% vs 29.9%) and GG patients (60.3% vs 17.6%) were included in our study. Moreover, in our Group 2 (GG carriers), patients with rare variants of *RNF213* were excluded because of their potential susceptibility to MMD. Thus, to some extent, patients in Group 2 could represent a series of MMD patients without any disease-causing mutations in *RNF213*, *ACTA2*, *BRCC3*, or *GUCY1A3*. We observed that fewer familial cases were found in Group 2 ($p < 0.05$). Because of the complex nature of the disease's pathogenicity, it was reasonable to postulate that environmental factors, recessive inheritance, dominant inheritance with lower penetrance, or other polygenic inheritances were the possible pathogenic mechanisms in those patients. Recently, a genetic study of 170 Chinese patients with MMD showed the p.A4399T variation to be a potential risk factor for MMD, especially the hemorrhage subtype.[30] In our study, approximately 20% of adult MMD patients presented with intracranial hemorrhage. The variant p.A4399T,

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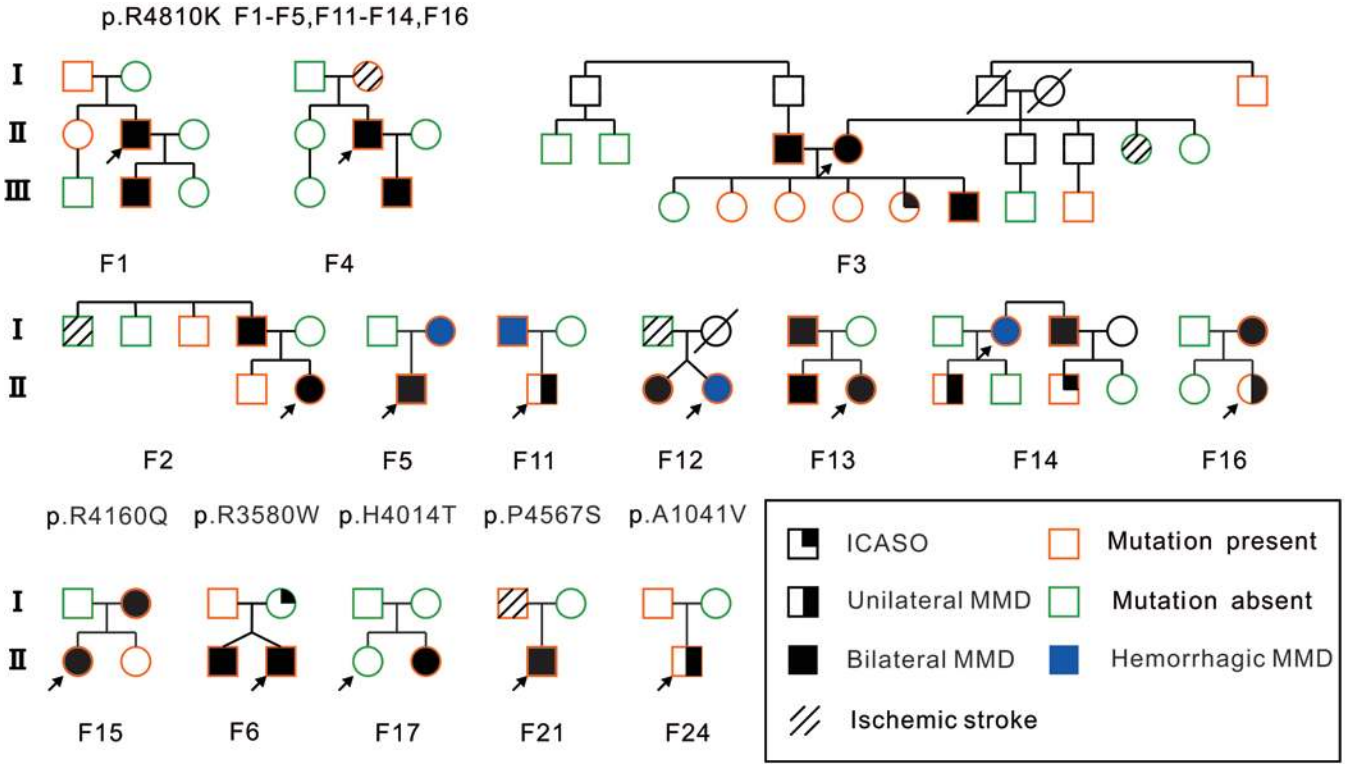

FIG. 2. Co-segregation of rare variants in *RNF213* with MMD in families. Pedigrees of 10 families (> 1 family member with MMD, **upper** and **center rows**) with the *RNF213* p.R4810K variant (F1–F5, F11–F14, and F16). The p.R4810K variant co-segregates with intracranial major artery stenosis/occlusion (ICASO), unilateral MMD, bilateral MMD, hemorrhagic MMD, and ischemic stroke. Pedigrees of 5 families (**lower row**) with other *RNF213* rare variants. The p.R4160Q alteration co-segregates with MMD in F15. The p.R3580W variant does not co-segregate with MMD in F6. A de novo variant, p.H4014T, was identified in F17. Disease and mutation status are indicated in the figure key. *Circles* represent women, *squares* represent men, and *arrowheads* indicate the proband in the family. A *diagonal line through a circle or square* indicates that the individual is deceased. Figure is available in color online only.

which is a common one, is not a risk factor for MMD and hemorrhage. However, patients without rare variants detected in *RNF213* were prone to exhibit hemorrhage. Although cerebral hemorrhage is mainly due to rupture of the fragile, deep, collateral vessels and aneurysms,[14] the presence of a combining aneurysm or the dilation of an anterior choroidal artery was not significantly different between the 2 groups. There was also no significant difference in the Suzuki stages between the 2 groups. However, Suzuki Stage 3, which is the classic angiographic finding of MMD, is more often observed in p.R4810K heterozygotes ($p < 0.05$). Mice in which *RNF213* was knocked out show enhanced postischemic angiogenesis.[12] This finding suggests a potential role for the *RNF213* malfunction in the development of pathological vascular networks. Our data suggest that MMD patients with the p.R4810K variant may have a distinct cerebrovascular disease entity and may represent a separate subgroup.

Moyamoya disease is a progressive disorder. Its natural history varies from slow progression to rapid neurological decline.[16,19,26] Preventing strokes and the resulting comorbidities depends on the early identification of individuals who are predisposed to MMD and MMS. Early diagnosis allows for timely surgical intervention to reduce the risk of stroke and possibly decrease the resulting cognitive deficits.[19] The NGS platform used in this study has great potential for clinical application.[2,20,28] Screening for rare variants of *RNF213* should be considered for patients with MMD. In addition, categorization of the clinical phenotypes and advances in NGS tools will undoubtedly accelerate the identification of novel familial MMD and MMS variants and patients.[20]

Our study is limited by its relatively small sample size and the absence of both protein functional tests and more detailed clinical data on the MMD patients.

Conclusions

RNF213 is the major susceptibility gene in Chinese MMD patients. The spectrum of rare variants identified in Chinese MMD patients underscores the ethnic diversity in the variants found in this disease. Finally, compared to patients without the rare variants in *RNF213*, p.R4810K heterozygous patients exhibited different clinical features.

Acknowledgments

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TABLE 2. Clinical features of patients with the p.R4810K heterozygous variant and those without rare and novel variants in *RNF213*

Feature	Total	Group 1, w/ p.R4810K Heterozygous Variant (%)	Group 2, w/o *RNF213* Rare & Novel Variants (%)	χ^2 or Z	p Value
No. of patients*	189	75	114		
Sex (F/M)	1.5:1	1.4:1	1.8:1	0.142	0.707
No. of females (%)	114 (60.3)	44 (58.7)	70 (61.4)	—	—
Mean age at diagnosis in yrs†	27.7 ± 14.6	25.2 ± 14.6	29.3 ± 14.4	−1.971‡	0.049
w/ familial history (%)	23 (12.2)	18 (24)	5 (4.4)	16.283	0.000
Frequency of childhood onset (%)§	61 (32.3)	29 (38.7)	32 (28.1)	2.324	0.127
Symptoms (%)					
Ischemia	138 (73.0)	61 (81.3)	77 (67.5)	4.366	0.037
Hemorrhage	51 (27.0)	14 (18.7)	37 (32.5)	4.366	0.037
Combined aneurysm	10 (5.3)	1 (1.3)	9 (7.9)	2.378	0.123
Preop mRS score >2	26 (13.8)	7 (9.3)	19 (16.7)	2.051	0.152
PCA involvement	76 (40.2)	39 (52)	37 (32.5)	7.187	0.007
Suzuki stage, 378 hemispheres (%)	378	150	228	−0.696‡	0.486
1	4 (1.0)	1 (0.67)	3 (1.3)	0.008	0.929
2	111 (29.4)	37 (24.7)	74 (32.5)	2.647	0.104
3	60 (15.9)	33 (22.0)	27 (11.8)	6.991	0.008
4	135 (35.7)	51 (34.0)	84 (36.8)	0.318	0.573
5	45 (11.9)	19 (12.67)	26 (11.4)	0.138	0.711
6	23 (6.1)	9 (6.0)	14 (6.1)	0.003	0.955
Dilation of AChA, 378 hemispheres (%)	192 (50.8)	84 (56.0)	108 (47.4)	2.697	0.101

— = not applicable; AChA = anterior choroidal artery.

* Five (2 homozygous patients and 3 patients without cerebral angiography) of 80 patients in Group 1 and 58 (58 patients with other *RNF213* rare variants and 3 patients without cerebral angiography) of 175 patients in Group 2 were excluded.

† Mean ± standard deviation.

‡ Z value.

§ Onset at age < 18 years.

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Disclosures

The authors report no conflict of interest concerning the materials or methods used in this study or the findings specified in this paper.

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Conception and design: Zhao, X Zhang. Acquisition of data: Q Zhang, Y Liu, D Zhang, R Wang, F Liu, Zhou. Analysis and interpretation of data: Q Zhang, Y Liu, D Zhang, R Wang, Y Zhang, S Wang, Yu, Lu, F Liu. Drafting the article: Q Zhang, Y Liu. Critically revising the article: Q Zhang, Y Liu. Reviewed submitted version of manuscript: Q Zhang, Y Liu. Statistical analysis: Q Zhang, D Zhang, R Wang.

Supplemental Information

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