Chapter 3
Molecular Epidemiology in East Asian Countries and in the World

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Abstract Although the etiology of moyamoya disease (MMD) remains unknown, recent genetic studies have identified *RNF213* p.R4810K in the 17q25-ter region as the most important founder susceptibility gene for MMD among East Asian populations, mainly including Japanese, Korean, and Chinese. Following the discovery studies, *RNF213* p.R4810K was replicated as an important founder susceptibility gene for MMD among many additional cohorts of East Asian populations. Moreover, many rare variants other than the founder one in *RNF213* also contributed to MMD across different populations in the world. Possibly because of the presence of the major founder effects of *RNF213* p.R4810K in East Asian patients, but not in Caucasian patients, the incidence and prevalence of MMD is relatively higher in East Asian countries than those in other countries. This chapter will discuss the molecular epidemiology of MMD in Asian and other populations in the world.

Keywords Moyamoya disease • Intracranial major artery stenosis/occlusion • *RNF213* • Molecular epidemiology • East Asian

Although moyamoya disease (MMD) etiology remains unknown, strong Asian ethnicity-related effect, a high disease concordance rate among monozygotic twins, and approximately 10–15% familial history of the patients with MMD strongly suggest the genetic components play an important role in the development of MMD. So far, five genetic loci have been identified to be linked to MMD in Japanese patients, including 3p24.2–p26, 6q25, 8q23, 12p12, and 17q25 [1–5]. Although the previously genetic results were conflicting and unrepeatable, a dozen of candidate genes or alleles have been reported to increase the susceptibility of MMD among the different ethnicities in the world [6–12]. Until recently, p.R4810K in *RNF213* located

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in 17q25.3-ter was identified as the first major founder susceptibility gene for MMD in East Asians, such as Japanese, Korean, and Chinese (Table 3.1) [13, 14]. Shortly after the discovery studies, p.R4810K, and other rare variants in *RNF213*, was collectively confirmed to be an important susceptibility gene among the different populations in the world by many research groups (Table 3.1) [15–32]. This chapter will mainly focus on the research findings of the genetic studies of *RNF213* of MMD among different populations in East Asia and in the world.

3.1 Discovery Stage: Identification of *RNF213*, the Most Important Founder Susceptibility Gene for MMD

In 2011, *RNF213* located in the previous linkage region of 17q25.3 was identified as the first important susceptibility gene for MMD by two Japanese research groups using the different approaches (Table 3.1) [13, 14]. Liu et al. performed a combined study of genome-wide linkage analysis and whole exome sequencing (WES) in eight Japanese familial MMD with at least three-generation affected members. Genome-wide linkage analysis revealed MMD linkage to 17q25.3 with a LOD score of 8.46 at D17S784. Fine mapping in the linkage region further narrowed the region to a 1.5-Mb disease locus bounded by D17S1806 and rs2280147, harboring 21 candidate genes. WES revealed p.R4810K in *RNF213* in the 1.5-Mb linkage region common to the eight index cases from eight families. Sequencing *RNF213* in 42 Japanese pedigrees with familial history for MMD confirmed p.R4810K to be segregated with disease perfectly. The SNPs genotyping around *RNF213* p.R4810K revealed a common haplotype transmitted in 42 families, which strongly suggested that *RNF213* p.R4810K was a founder susceptibility gene for MMD. A case-control association study across different ethnicities revealed that *RNF213* p.R4810K was significantly associated with sporadic MMD in East Asian populations (P=6.61×10^{-120}, OR=111.84, 95% CI=64.01–195.39), including Japanese (P=1.05×10^{-100}, OR=338.94, 95% CI=147.82–777.44), Korean (P=7.58×10^{-27}, OR=135.63, 95% CI=43.03–427.52), and Chinese (P=2.63×10^{-5}, OR=14.70, 95% CI=3.05–70.81). Five novel variants, p.D4863N, p.E4950D, p.A5021V, p.D5160E, and p.E5176G, were identified in non-p.R4810K East Asian patients. The p.R4810K and its founder haplotype were not identified among Caucasian cases; however, four rare variants p.N3962D, p.D4013N, p.R4062Q, and p.P4608S were identified (Table 3.1) [13]. This East Asian specific founder susceptibility gene of *RNF213* p.R4810K may be responsible for the high prevalence of MMD among East Asians and its low prevalence among Caucasians. The identification of MMD susceptibility gene in this study indicates that the combined WES with linkage analysis is useful to identify novel susceptibility loci to human diseases or traits.

At the same time, Kamada et al. performed a genome-wide association study (GWAS) to identify MMD susceptibility gene in a Japanese cohort comparing 72 MMD patients and 45 controls. A genome-wide significant association locus was

Table 3.1 Genetic studies on MMD susceptibility genes *RNF213*

Author, year	Ethnicity (sample size: case/control)	Study approach	Results: identified loci/markers	Conclusions: significances/ implications
Liu et al. 2011 [13]	Japanese: (FMMD: 42, SMMD: 161/384), Korean (38/223), Chinese (52/150), Caucasian (50/384)	Genome-wide linkage analysis	Linkage to 17q25.3	*RNF213* was first major founder susceptibility gene for East Asian MMD, including Japanese, Korean, and Chinese
		WES to identify rare functional variants	Segregation with the *RNF213* p.R4810K and its founder haplotype	Rare variants in *RNF213* also confer the susceptibility for Caucasian and East Asian MMD without p.R4810K
		Association study for *RNF213* p.R4810K according to ethnicity	Strong association with the p.R4810K for East Asian sporadic MMD, but not for Caucasians	
			Rare *RNF213* variants identified in Caucasian and East Asian patients without p.R4810K	
Kamada et al. 2011 [14]	Japanese (72/45)	Linkage analysis focused on the previous candidate loci	Association of MMD with locus 17q25-ter	*RNF213* was identified as first major founder susceptibility gene for Japanese MMD
		GWAS and locus-specific association study	Strong association with the *RNF213* locus	Rare variants in *RNF213* also confer the susceptibility for Japanese MMD
		Association study for *RNF213* p.R4810K	Strong association with the p.R4810K in *RNF213*	
			Rare *RNF213* variants identified in Japanese patients without p.R4810K	

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Table 3.1 (continued)

Author, year	Ethnicity (sample size: case/control)	Study approach	Results: identified loci/markers	Conclusions: significances/ implications
Miyatake et al. 2012 [15]	Japanese (204/283)	Association study for *RNF213* p.R4810K	*RNF213* p.R4810K found in 95.1% of familial MMD cases, in 79.2% of sporadic cases, and in 1.8% of controls (OR = 259, 95% CI= 100–674, $P<0.001$)	*RNF213* was a major susceptibility gene for Japanese familial and sporadic MMD
		Analysis of genotype-phenotype correlation	Patients with homozygous p.R4810K present with an earlier onset, an increased risk of infarction at initial presentation, and an increased rate of PCA involvement	*RNF213* p.R4810K was a good biomarker for the progression and prognosis of disease
Miyatake et al. 2012 [16]	Japanese (Sibling cases)	Case-control study for *RNF213* p.R4810K	Earlier onset and more severe course in patients with homozygous p.R4810K	*RNF213* p.R4810K was a good biomarker on the progression and prognosis of disease
		Analysis of genotype-phenotype correlation		
Miyawaki et al. 2012 [17]	Japanese (48 MMD, 41 non-MMD ICASO)	Association study for the *RNF213* p.R4810K	*RNF213* p.R4810K found in 85.4% of MMD patients, in 21.9% of non-MMD ICASO patients, in 1.6% of cerebral aneurysm patients, in no cervical disease patients, and in no controls	*RNF213* p.R4810K was a major susceptibility gene not only for MMD but also for non-MMD ICASO with Japanese ethnicity
		Analysis of genotype-phenotype correlation	Significant associations of p.R4810K with MMD (OR= 292.8, 95% CI= 15.4–5153, $P<0.0001$) and non-MMD ICASO (OR= 14.9, 95% CI= 0.82–268.4, $P=0.01$); no association with either cerebral aneurysm or cervical disease	

Miyawaki et al. 2013 [18]	Japanese (323/110)	Association study for the *RNF213* p.R4810K	*RNF213* p.R4810K found in 16/22 of the definite MMD patients, in 4/8 of the unilateral MMD patients, in 20/84 of non-MMD ICASO patients, in 1/34 of extracranial carotid atherosclerosis patients, in no patients with cerebral aneurysm and intracerebral hemorrhage, in 1.8% of controls	*RNF213* p.R4810K was a major susceptibility gene not only for MMD but also for non-MMD ICASO with Japanese ethnicity
		Analysis of genotype-phenotype correlation	Significant associations of p.R4810K with definite MMD (OR= 144.0, 95% CI= 26.7–775.9, $P<0.0001$), unilateral MMD (OR= 54.0, 95% CI= 7.5–386.8, $P=0.0001$), and non-MMD ICASO (OR= 16.8, 95% CI= 3.81–74.5, $P<0.0001$); no association with extracranial carotid atherosclerosis, cerebral aneurysm, and intracerebral hemorrhage	
Chung et al. 2016 [19]	Korean (146 cases only)	Association study for the *RNF213* p.R4810K with basal collaterals	*RNF213* p.R4810K observed in 65.8% of adult-onset MMD patients and not associated with the degree of basal collaterals ($P = 0.289$)	*RNF213* p.R4810K was a major susceptibility gene for Korean adult-onset MMD
Jang et al. 2015 [21]	Korean (1516 controls only)	Association study for the *RNF213* p.R4810K	*RNF213* p.R4810K found in 2.44% of controls	*RNF213* p.R4810K was a major susceptibility gene for Korean MMD
			Significant association of p.R4810K with MMD ($P < 0.001$) (OR=162.7, 95% CI=65.5–403.9; OR=137.8, 95% CI=55.8–339.9, based on the cord blood and adults samples, respectively)	

(continued)

Table 3.1 (continued)

Author, year	Ethnicity (sample size: case/control)	Study approach	Results: identified loci/markers	Conclusions: significances/ implications
Kim et al. 2016 [22]	Korean (165/294)	Association study for the *RNF213* p.R4810K	*RNF213* p.R4810K found in 75.8% of MMD patients and in 2.72% of controls. Significant association of p.R4810K with MMD ($P<0.001$)	*RNF213* p.R4810K was a major susceptibility gene for Korean MMD
		Analysis of genotype-phenotype correlation	Significant associations of homozygous p.R4810K with early-onset MMD, cerebral infarction at diagnosis, and cognitive impairment in long-term outcome	*RNF213* p.R4810K was a good biomarker for the progression and prognosis of disease
Bang et al. 2016 [23]	Korean (288 with MMD, 234 with ICASO)	Association study for the *RNF213* p.R4810K	*RNF213* p.R4810K observed in 69.1% MMD patients, 21.4% ICASO patients	*RNF213* is a susceptibility gene, not only for MMD but also for ICASO in Korean
		Analysis of genotype-phenotype correlation	Among ICASO patients, p.R4810K carriers were younger and more likely to have a family MMD history than noncarriers were	
			Independently association of the age of ICASO onset with the p.R4810K (OR =0.97, 95% CI =0.944–0.99)	

Wu et al. 2012 [24]	Chinese Han (170/507)	Association study for the *RNF213* p.R4810K	*RNF213* p.R4810K found in 13% of the patients, 0.4% of the controls	*RNF213* variants were the major susceptibility gene for Chinese MMD
		Analysis of genotype-phenotype correlation	Significant association of p.R4810K with MMD (P=6.1×10^{-15}). p.R4810K more frequently found in ischemic versus hemorrhagic MMD (P = 0.001)	*RNF213* p.R4810K was a good biomarker for the progression and prognosis of disease
		Mutational sequencing covering *RNF213* exon 40 to exon 68	Eight rare variants identified, p.A4399T found in 16.5% of cases and 8.9% of controls and associated with MMD (OR=2.0, 95% CI=1.2–3.3, P=0.004), especially with hemorrhage (OR =2.8, 95% CI=1.2–6.5, P=0.014)	
Wang et al. 2013 [25]	Chinese Han (96/96)	Association study for candidate polymorphisms in the *PDFRBR/MMP3/TIMP2/RNF213/Raptor* genes and searching for gene-gene interactions	Association of MMD with *RNF213* p.R4810K and rs148731719	*RNF213* p.R4810K and rs148731719 may independently exert a significant influence on MMD occurrence
			No significant gene-gene interaction detected	
Shang et al. 2015 [26]	Chinese Han (139 ICASO, 146 non-ICASO/300)	Association study for the *RNF213* p.R4810K	*RNF213* p.R4810K found in 0.35% of ischemic stroke patients, in 0.72% of ICASO patients, none in non-ICASO subgroup, and 0.33% of the controls	*RNF213* p.R4810K may be not associated with ischemic stroke and ICASO in Chinese Han population
		Analysis of genotype-phenotype correlation	No associations of p.R4810K with ischemic stroke and ICASO	

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Table 3.1 (continued)

Author, year	Ethnicity (sample size: case/control)	Study approach	Results: identified loci/markers	Conclusions: significances/ implications
Huang et al. 2015 [27]	Chinese Guangxi Zhuang (52 MMD, 64 ICASO/80)	Association study for the *RNF213* rs138130613,p.R4810K and rs148731719 polymorphism	Significant associations of *RNF213* p.R4810K with both patients with MMD (P=0.006) and non-MMD ICASO (P=0.045), but no associations for rs138130613 and rs148731719 in *RNF213*	*RNF213* p.R4810K might be a genetic marker for MMD and might be related to the formation of intracranial major artery stenosis/occlusion in Guangxi Zhuang population
		Analysis of genotype-phenotype correlation		
Zhang et al. 2016 [28]	Chinese (255/300)	Association study for the *RNF213*, *ACTA2*, *BRCC3*, and *GUCY1A3* polymorphism	Twenty-seven rare variants of *RNF213* identified in MMD and not found in controls. Among them, p.R4810K identified in 31.4% of patients (P<0.000). Twenty-five rare variants identified in 10.6% of patients without p.R4810K variants. No possible disease variants identified in *ACTA2*, *BRCC3*, or *GUCY1A3*. Compared with patients without the *RNF213* rare variants, 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 PCA (52% vs 32.5%, P=0.007)	*RNF213* is the major susceptibility gene for Chinese MMD patients
		Analysis of genotype-phenotype correlation		The p.R4810K heterozygous patients exhibited different clinical features, compared to patients without the rare variants in *RNF213*
Lee et al. 2015 [29]	Taiwanese (36/500)	Mutational sequencing covering all exons in *RNF213*	Four different *RNF213* variants, p. R4810K, p.A1622V, p.V3933 M, and p. R4131C, identified in 11 of 36 patients	*RNF213* was a major susceptibility gene for Taiwanese MMD
		Association study for the *RNF213* p.R4810K		

Liu et al. 2013 [30]	Central European (38/41)	GWAS	No significant association with polymorphisms in *RNF213* and *ACTA2* genes	No major founder variant in Caucasian MMD was found, although several suggestive association regions were identified
		Sequencing of candidate genes in suggestive association regions	Suggestive associations with four loci	
Cecchi et al. 2014 [31]	Asian descent (16/−), non-Asian descent (94/−)	Mutational sequencing in 86 patients covering *RNF213* exon 43–45 and exon 60	*RNF213* p.R4810K found in 56% of MMD patients of Asian descent, in no of 94 patients of non-Asian descent	The variants in *RNF213* predispose patients of diverse ethnicities to MMD. The p.R4810K variant predisposes individuals of Asian descent in the United States to MMD
		WES in 24 additional patients covering the entire *RNF213* exons	Rare variants identified by targeted exon sequencing and WES	
Shoemaker et al. 2016 [32]	Asian descent and non-Asian descent (125/125)	WES to further develop variant landscape of MMD in multiethnic population	*RNF213* p.R4810K enriched among East Asians (P=6.01×10^{-5}) and none in Caucasian cases and controls. The most enriched variant in Caucasian (P=7.93×10^{-4}) and non-*RNF213* founder mutation (P=1.51×10^{-3}) cases was *ZXDC* p.P562L, a gene involved in MHC class II activation, and *OBSCN*, a gene involved in myofibrillogenesis	*RNF213* p.R4810K was the East Asian origins.
				Novel, alternative candidate variants and genes in addition to *RNF213* may be important in multiethnic MMD etiology and diagnosis

Abbreviations: *MMD* moyamoya disease, *FMMD* familial MMD, *SMMD* sporadic MMD, *WES* whole exome sequencing, *GWAS* genome-wide association study, *OR* odds ratio, *CI* confidence interval, *PCA* posterior cerebral artery, *ICASO* intracranial major artery stenosis/occlusion

found at 17q25-ter with Japanese MMD risk ($P<10^{-8}$). A locus-specific association study in the 17q25-ter further confirmed the genome-wide association locus and identified a haplotype at the *RNF213* locus significantly associated with MMD risk ($P=5.3\times10^{-10}$). Sequencing of *RNF213* revealed a founder variant p.R4810K, in 95% of MMD families, 73% of non-familial MMD cases, and 1.4% of controls among Japanese population. The p.R4810K dramatically increased the risk of MMD with an odds ratio of 190.8 ($P=1.2\times10^{-43}$, 95% CI=71.7–507.9). Three additional missense variants, p. M3891V, p.V4567M, and p.V4765M, were detected in three non-p.R4810K sporadic patients (Table 3.1) [14]. These two discovery studies successfully identified the same susceptibility genes of *RNF213* p.R4810K for MMD in various East Asian ethnicities using the different approaches, which suggest MMD with different ethnicities may share common major susceptibility genes and disease etiology. Further genetic studies are needed to validate this hypothesis in the different ethnicities with a large number of sample sizes.

3.2 Replication Stage: Replication of *RNF213* as a Susceptibility Gene for MMD Across Different Ethnicities

3.2.1 Japanese MMD

Shortly after the discovery studies, *RNF213* has been subsequently confirmed to be a major susceptibility gene in many additional studies across different ethnicities, including Japanese, Korean, Chinese, Europeans, and American (Table 3.1) [13–32]. Miyatake et al. sequenced the entire coding regions of the *RNF213* gene in 204 patients with MMD and evaluate the risk of corresponding variants detected in the parents of the patients and 283 controls. The p.R4810K variant was confirmed to be strongly associated with MMD in this additional Japanese cohort ($P<0.001$, OR=259, 95% CI=100–674). The p.R4810K was identified in 95.1% of familial MMD cases, 79.2% of sporadic cases, and 1.8% of normal controls. Moreover, the correlations of genotype-phenotype associated with gene dosage were also observed. Compared with heterozygotes or wild types, homozygotes of p.R4810K had a significantly earlier age at onset, significantly higher frequencies of infarctions at initial presentation, and involvement of posterior cerebral arteries (PCAs) than those in heterozygotes and wild types. Eighteen other rare variants were also identified in the patients without p. R4810K and were not associated with any clinical phenotypes of MMD (Table 3.1) [15]. Followed by their first study on the gene dosage effects of the *RNF213* variant, *RNF213* p.R4810K also showed the gene doses effects in Japanese sibling cases with MMD. In this study, *RNF213* p.R4810K showed a significant association with MMD, as well as different clinical course and disease severity. The patients with the homozygote of p.R4810K in the sibling pair showed an early-onset age and rapid disease progress, compared with those with the

heterozygote of the variant (Table 3.1) [16]. These two studies suggest an obvious correlation of genotype-phenotype of *RNF213* p.R4810K with MMD.

Additional two studies reported by the same research group replicated the previous association of the *RNF213* p.R4810K with Japanese MMD risk (Table 3.1) [17, 18]. The first study evaluated the frequencies of p.R4810K variant in 48 patients with MMD, as well as in 41 patients with intracranial major artery stenosis/occlusion (ICASO) without signs of MMD (non-MMD ICASO). The 85.4% patients with MMD and 21.9% patients with non-MMD ICASO had the risk allele of p.R4810K. The p.R4810K showed significant associations with MMD (P<0.0001, OR=292.8, 95% CI=15.4–5153) and with non-MMD ICASO (P=0.01, OR=14.9, 95% CI=0.82–268.4), but no association with either cerebral aneurysm or cervical disease. This study, for the first time, suggests the p.R4810K variant in *RNF213* common to both MMD and non-MMD ICASO (Table 3.1) [17]. To confirm the reality of the association of p.R4810K variant with non-MMD ICASO, Miyawaki et al. investigated the frequencies of p.R4810K variant in 323 patients with various phenotypes of intracranial major artery stenosis/occlusion and 110 control subjects in a different larger Japanese cohort from the previous one. Sixteen of 22 patients with definite MMD, 4/8 patients with unilateral MMD, 20/84 patients with non-MMD ICASO, 1/34 patients with extracranial carotid atherosclerosis, 0/44 patients with cerebral aneurysm, 0/21 patients with intracerebral hemorrhage, and 1.8% controls had the risk allele of p.R4810K. *RNF213* p.R4810K showed significant associations with definite MMD (P<0.0001, OR=144.0, 95% CI=26.7–775.9), unilateral MMD (P=0.0001, OR=54.0, 95% CI=7.5–386.8), and non-MMD ICASO (P<0.0001, OR=16.8, 95% CI=3.81–74.5), but no associations with extracranial carotid atherosclerosis, cerebral aneurysm, or intracerebral hemorrhage (Table 3.1) [18]. The authors replicated the previous findings and proposed the existence of a new entity of ICASO caused by the p.R4810K variant in *RNF213* at least for Japanese ethnicity.

3.2.2 Korean MMD

Following on from the discovery studies [13, 14], several studies have replicated the association of *RNF213* p.R4810K with MMD risk in Korean population (Table 3.1) [19–23]. Chung et al. evaluated the association between clinical, genetic, and radiologic factors and basal collaterals in 146 Korean patients with MMD. The *RNF213* p.R4810K was observed in 50 (65.8%) adult-onset MMD patients and not associated with the degree of basal collaterals (P=0.289) (Table 3.1) [19]. Actually, this study did not evaluate the association between the p.R4810K and MMD risk, due to a lack of allele frequency information in the controls. However, the significant association should exist in this Korean cohort based on the frequency data from other studies (Table 3.1) [12, 13, 20, 21].

Another study investigated the *RNF213* p.R4810K genotype and genotype-phenotype correlations in 165 Korean MMD patients and 294 controls by direct

sequencing of the major *RNF213* SNPs. The p.R4810K was detected in 75.8% of MMD patients and in 2.72% of controls, respectively. The p.R4810K significantly associated with the risk of Korean MMD, with an OR of 52.11 ($P<0.001$) compared with controls. Moreover, p.R4810K risk genotypes occurred more frequently in familial MMD patients than in sporadic patients. The homozygous p.R4810K showed a significant association with early age at onset, cerebral infarction at initial diagnosis, and cognitive impairment in long-term outcome (Table 3.1) [22]. The findings indicate that the p.R4810K risk allele is strongly associated with Korean patients with MMD and homozygous p.R4810K may be a good biomarker for early-onset MMD or unstable MMD with cerebral infarction, which requires early diagnosis and revascularization treatment.

Due to the high prevalence of both intracranial atherosclerotic stenosis (ICAS) and MMD in Asians, Bang, et al. hypothesized that the *RNF213* p.R4810K is also a susceptibility gene for the Korean patients with ICAS. The participants included 234 patients with ICAS and 288 with MMD. The *RNF213* p.R4810K was observed in 21.4% patients with ICAS and in 69.1% patients with MMD. ICAS patients with *RNF213* p.R4810K were younger and more likely to have a family MMD history than the patients without the variant were. Multivariate analysis revealed that only the age of ICAS onset was independently associated with the *RNF213* p.R4810K (OR=0.97, 95% CI=0.944–0.99) (Table 3.1) [23]. This study further demonstrated that *RNF213* is a susceptibility gene not only for MMD but also for ICAS in East Asians. Further studies on the association of *RNF213* variants in ICAS patients outside East Asian populations are needed.

3.2.3 Chinese MMD

After the identification of the founder variant p.R4810K and other rare patient-specific variants in *RNF213* as the important susceptibility genes for Chinese MMD (Table 3.1) [13], several genetic studies have also perfectly replicated the association of *RNF213* variants with MMD in Chinese population (Table 3.1) [24–29]. Wu et al. performed the first replication study on molecular analysis of *RNF213* in 170 MMD patients and 507 controls from Chinese Han population. The p.R4810K was detected in 13% of cases with MMD and 0.4% of 507 normal controls, respectively. The association of p.R4810K was perfectly replicated to greatly increase the risk for Chinese Han MMD ($P=6.1\times10^{-15}$, OR=36.7, 95% CI=8.6–156.6). The allele frequency of R4810K was significantly higher in ischemic versus hemorrhagic MMD ($P=0.001$, OR=5.4, 95% CI=1.8–16.1). Genomic sequencing covering later part of *RNF213* also identified eight other non-p.R4810K variants: p.P4007R, p.Q4367L, p.A4399T, p.T4586P, p.L4631V, p.E4950D, p.A5021V, and p.M5136I. Among them, p.A4399T variant was found in 16.5% of cases and 8.9% of controls and was significantly associated with MMD ($P=0.004$, OR=2.0, 95% CI=1.2–3.3), especially with hemorrhage ($P=0.014$, OR=2.8, 95% CI=1.2–6.5) (Table 3.1) [24].

This study validated the association of p.R4810K and expanded the spectrum of *RNF213* mutations in Chinese Han MMD.

Wang et al. evaluated the contributions and interactions of the polymorphisms of *RNF213* and other previously associated genes in 96 Chinese Han cases and 96 controls. Again, p.R4810K in *RNF213* showed a significant association with Chinese Han MMD (8.33%) compared with controls (1.04%) (*P*=0.018, OR=8.74). The significant association of the polymorphism rs148731719 in *RNF213* was also identified, but no association between MMD and other three loci in the genes of *PDGFRB*, *MMP-3*, and *TIMP-2*. There was no any significant interaction among these five loci by multifactor dimensionality reduction analysis. This study indicated that *RNF213* p.R4810K and rs148731719 may exert a significant influence on MMD occurrence than other genes in Chinese Han population (Table 3.1) [25]. Further studies in a larger sample size across different ethnicities are necessary to validate these contributions and interactions.

RNF213 p.R4810K has been associated with non-MMD ICASO patients in Japanese population (Table 3.1) [17, 18], but a lack of data in other populations. To validate the previous findings, Shang et al. explored the association between *RNF213* p.R4810K and ischemic stroke in a Chinese Han population with 285 patients and 300 controls. The patients with ischemic stroke were divided into ICASO subgroup (139) and non-ICASO subgroup (146). One of 285 patients with ischemic stroke, one of 139 patients with ICASO, none in non-ICASO subgroup, and one of 300 controls had the p.R4810K risk variant. Compared with controls, p.R4810K variant had no associations with ischemic stroke (*P*=1, OR=1.053, 95%CI=0.066–16.912) and ICASO (*P*=0.533, OR=2.167, 95%CI=0.135–34.894), respectively. This study failed to replicate the associations previously reported in Japanese patients with ICASO, indicating that *RNF213* p.R4810K variant may be not associated with ICASO in Chinese Han population (Table 3.1) [26]. Further studies are needed to validate the association between p.R4810K and ICASO phenotype in different ethnicities. Huang et al. investigated the association between rs138130613, p.R4810K, and rs148731719 variants of *RNF213* and the genetic susceptibility of adult MMD in a Guangxi Zhuang population. The participants consisted of 52 consecutive adult patients with MMD, 64 non-MMD ICASO, and 80 gender- and age-matched healthy controls. Compared with the control group, *RNF213* p.R4810K was found to be significantly associated with both MMD group and non-MMD ICASO group (*P*=0.006, OR=12.29, 95% CI=1.47–103.10; *P*=0.045, OR=8.17, 95% CI=0.96–69.74, respectively), but no associations for rs138130613 and rs148731719 in *RNF213*. These findings suggest that *RNF213* p.R4810K might be a genetic marker for MMD and might be related to the formation of ICASO in Guangxi Zhuang population (Table 3.1) [27]. Interestingly, the frequency difference in *RNF213* p.R4810K between Han [26] and Guangxi Zhuang populations with non-MMD ICASO indicates that the genetic background may be different even in different ethnic groups among Chinese population. Further genetic studies are needed to clarify the genetic etiology among different Chinese nationalities.

Zhang et al. performed a genetic study to identify disease-causing mutations in MMD association genes, including *RNF213*, *ACTA2*, *BRCC3*, and *GUCY1A3* in 255 Chinese MMD patients and 300 controls. Twenty-seven rare missense variants of *RNF213* were identified. Among them, p.R4810K was identified in 31.4% (80 of 255) of patients with MMD. Significantly higher frequencies of the p.R4810K in MMD patients were observed compared with controls (χ^2=104.166, P<0.000). Twenty-five rare variants were identified in 10.6% (27 of 255) of non-p.R4810K patients. The frequencies of rare and founder variants identified in this study (42%) were relatively higher than those of the previous studies (10–20%). In contrast, no disease-causing variants were identified in *ACTA2*, *BRCC3*, or *GUCY1A3*. Compared with patients without the rare *RNF213* variants, the patients with p.R4810K 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 PCA (52% vs 32.5%, P=0.007) (Table 3.1) [28]. Although the total proportion of *RNF213* variants was relatively lower than that in Japanese and Korean, *RNF213* is the major susceptibility gene in Chinese MMD patients. Compared to patients without the rare *RNF213* variants, the patients with p.R4810K showed relatively severe clinical features of MMD. One study sequenced all coding exons of *RNF213* in 36 Taiwanese MMD patients. Four different *RNF213* variants, p.R4810K, p.A1622V, p.V3933M, and p.R4131C, were identified in 11 patients (30.6%) (Table 3.1) [29]. This study replicated the previous association data and was the first genetic epidemiological study for MMD in Taiwan.

3.2.4 Caucasian MMD

Although several *RNF213* rare variants have been identified in Caucasian MMD [13], the mutation spectrum of MMD in Caucasian population still remains largely unknown. Liu et al. performed a GWAS in 38 cases and 41 controls to identify whether there is a major founder susceptibility gene for Caucasian MMD. This study failed to identify any major founder variant in Caucasian MMD as it is in East Asian MMD. However, several suggestive association regions were identified for Caucasian MMD (Table 3.1) [30].

More recently, two research groups independently investigated disease variants in multiethnic populations of MMD patients based in the United States by target exons sequencing and WES. These two genetic studies consistently replicated previous results and identified many novel rare variants in *RNF213* and other genes (Table 3.1) [31, 32]. Cecchi et al. investigated the contribution of *RNF213* variants to MMD among an ethnicities diverse population in the United States using targeted exon sequencing of *RNF213* or WES. The East Asian founder variant *RNF213* p.R4810K was found in 56% (9/16) of MMD patients of Asian descent, but not in 94 patients of the European, Hispanic, or African descent. Among nine patients of Asian descent carrying *RNF213* p.R4810K, seven were East Asian origin as previously reported; other were in novel groups not previously reported, including

Bangladeshi, Indian, and Filipino origin. Four rare *RNF213* variants were identified by Sanger sequencing: p.C3997Y, p.D4013N, p.R4019C, and p.I4076V. Seven additional rare variants were also identified in 29% (7/24) via WES: p.A529del, p.R3922Q, p.K4115del, p.D4237E, p.K4732T, p.E4950_F4951ins7, and p.V5163I. This study further confirms that rare genetic variants in *RNF213* predispose patients of diverse ethnicities to MMD and that the p.R4810K founder variant predisposes patients of Asian descent in the United States to MMD (Table 3.1) [31]. To further search the mutations for MMD, Shoemaker et al. conducted WES of 125 unrelated ethnically diverse MMD patients and 125 matched controls. According to ethnicity of the studied subjects, they established three subpopulations: Asian, Caucasian, and non-p.R4810K case. This study replicated the previously identified *RNF213* p.R4810K founder variant in Asian cases (P=6.01×10^{-5}) that was enriched among East Asians compared to Southeast Asian and Pacific Islander cases (P=9.52×10^{-4}) and was completely absent in all Caucasian cases and controls. Among Caucasian and non-p.R4810K cases, two most enriched variants were identified: *ZXDC* (p.P562L), a gene involved in MHC class II activation, and *OBSCN*, a gene involved in myofibrillogenesis. This study provides additional evidence on the East Asian origins of the *RNF213* p.R4810K variant and revealed novel, alternative candidate variants and genes that may be important in the etiology of MMD with multiethnicities (Table 3.1) [32].

3.3 Significance and Future Perspectives of *RNF213* on MMD

To date, many loci and genes were identified to be associated with the risk of MMD; however, the results were completely unrepeatable in different studies. In contrast, *RNF213* was confirmed to be the most important founder susceptibility gene for MMD with various ethnicities by many research groups in the world. The carrier frequencies of p.R4810K in East Asia were about 1–3% for controls and 20–80% for cases [13–29], but none in Caucasian cases and controls (Table 3.1) [30–32]. The previous data strongly suggest that the genetic background of MMD in East Asian is distinct from that in Westerners and the high incidence of MMD in East Asian countries may be attributable to the major founder effect of *RNF213*.

The homozygotes of *RNF213* p.R4810K had a significantly earlier-onset age, higher frequency of cerebral infarctions at diagnosis, and involvement of PCAs compared with heterozygotes or wild types, which strongly suggest that the homozygous *RNF213* p.R4810K could be a good biomarker for predicting the severe type, progression, and prognosis of MMD, for which early clinical intervention is recommended (Table 3.1) [15, 16, 22, 24, 28]. In addition, *RNF213* p.R4810K was significantly associated with not only MMD but also non-MMD ICASO in East Asian (Table 3.1) [17, 18, 23, 27], suggesting *RNF213* may be also important in the occurrence of ICASO phenotypes.

However, several questions remain and need to be elucidated. First, the large gap between the prevalence of carriers of the p.R4810K (1–3%) and the prevalence of MMD (1 in 10,000) strongly suggests that p.R4810K is a susceptibility factor to MMD, with an involvement of other unknown factors in the moyamoya phenotype. Further studies will need to figure out the mysterious other factors involved in MMD etiology in addition to *RNF213*. Secondly, the frequency of *RNF213* variants is relatively lower in Chinese MMD patients, compared to that in Japanese and Korean patients. The p.R4810K and other rare variants in *RNF213* could only explain about 20% cases in Chinese population. The major novel susceptibility genes for Chinese, as well as Caucasian MMD, remain to be discovered by next-generation sequencing tools in a large number of study populations.

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