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ORIGINAL RESEARCH

RNF213 R4810K Variant in Suspected Unilateral Moyamoya Disease Predicts Contralateral Progression

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BACKGROUND: Early-stage unilateral moyamoya disease (MMD) is difficult to discriminate from isolated intracranial atherosclerotic stenosis, and identification of contralateral progression may aid in the diagnosis of MMD. The *RNF213* (ring finger protein 213) R4810K variant is a strong genetic susceptibility factor for MMD; however, the role of contralateral progression in unilateral MMD is unknown.

METHODS AND RESULTS: Patients who had undergone *RNF213* R4810K genotyping with suspected unilateral MMD between January 2017 and August 2021 from 2 tertiary university hospitals were retrospectively reviewed. We compared the clinical features and radiographic outcomes of patients with and without this variant. The risk factors of contralateral progression in patients with suspected unilateral MMD were evaluated. The *RNF213* R4810K variant was observed in 72 of 123 patients with suspected unilateral MMD, all of which were heterozygous. The allele frequency of the R4810K variant was significantly higher in the suspected unilateral MMD group compared with the historical control group (29.3% versus 1.2%; $P<0.0001$). Family history of MMD was significantly more common in patients with the variant than in those without (17% versus 4%; $P=0.003$). Eleven of 72 patients with the variant developed contralateral progression, whereas only 1 of 51 patients without the variant developed contralateral progression during a median follow-up period of 28 months (log-rank test; $P=0.03$). The presence of the *RNF213* R4810K variant significantly correlated with contralateral progression (adjusted odds ratio, 6.39 [95% CI, 1.11–36.63]; $P=0.04$).

CONCLUSIONS: Contralateral progression is more likely to occur in patients with suspected unilateral MMD with the *RNF213* R4810K variant than in those without the variant. However, because our study used a small sample size, this finding should be carefully interpreted and requires further studies with more patients and longer follow-up periods.

Key Words: intracranial stenosis ■ moyamoya disease ■ polymorphism ■ progression ■ *RNF213*

RNF213 (ring finger protein 213) is a 591 kDa cytosolic E3 ubiquitin ligase with 2 functional domains, a RING finger domain and an AAA+ ATPase domain. Although the function of the *RNF213* protein remains poorly understood, a few studies have reported that this protein plays an important role in regulating vascular endothelial function and angiogenesis.[1,2] This large protein is encoded by the *RNF213* gene, which is located on chromosome 17q25. The R4810K variant, a polymorphism in c.14576G > A in *RNF213*, was identified as a strong genetic susceptibility factor for moyamoya disease (MMD) in East Asia.[1,3]

The current diagnostic criteria for MMD are based on the characteristic angiographic findings. However, depending on the stage of MMD, diagnostic angiographic findings may not be observed.[4] Intracranial atherosclerotic stenosis, a common cause of stroke in the Asian population, is difficult to distinguish from early stages of

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CLINICAL PERSPECTIVE

What Is New?

- Diagnosis of "true" moyamoya disease is often difficult in general clinical settings when presented unilaterally, because of the current angiography-based diagnostic criteria.
- Patients with suspected unilateral moyamoya disease with the *RNF213* (ring finger protein 213) R4810K variant are more likely to have contralateral progression than those without the variant.

What Are the Clinical Implications?

- The *RNF213* R4810K variant may be a useful biomarker for early diagnosis of moyamoya disease in patients with unilateral intracranial artery occlusive lesions.

Nonstandard Abbreviations and Acronyms

MMD	moyamoya disease
RNF213	ring finger protein 213

MMD, especially with unilateral involvement. In previous studies, 22% to 24% of patients with intracranial atherosclerotic stenosis (unilateral and bilateral involvement) had the *RNF213* R4810K variant; thus, the authors proposed that they may have had MMD in an earlier stage that was misdiagnosed as intracranial atherosclerotic stenosis based on the angiographic-based diagnostic criteria of MMD.[5–7] In contrast, it may also be possible to misdiagnose intracranial atherosclerotic occlusion with basal collaterals as MMD.[8]

Up to 18% of patients with MMD present with unilateral involvement, and 8% to 59% of patients experience contralateral progression.[9–15] Previous studies have shown genotype–phenotype correlation of the *RNF213* R4810K variant; homozygous variant showing an earlier age of onset, higher penetrance, higher proportion of cerebral infarction as an initial presentation, and significant systemic vascular involvement compared with the heterozygous form of the variant.[16,17] The association between the *RNF213* R4810K variant and contralateral progression in suspected unilateral MMD is unknown. Therefore, we hypothesized that patients with suspected unilateral MMD with the *RNF213* R4810K variant would be more susceptible to disease progression than those without the variant. Contralateral progression would be a strong supportive diagnostic factor of MMD in patients with unilateral intracranial artery occlusive lesions. In this study, we aimed to evaluate the association between the *RNF213* R4810K variant and contralateral progression in suspected unilateral MMD.

METHODS

Data Availability

The data that support the findings of this study are available from the corresponding author on reasonable request.

Study Design and Patient Selection

We conducted a retrospective observational cohort study using the medical records from 2 tertiary university hospitals. We reviewed 531 patients who had undergone *RNF213* genotyping between January 2017 and August 2021. We initially selected 279 patients with brain vascular imaging data obtained at least 12 months apart via conventional cerebral angiography or magnetic resonance angiography. Of the 279 patients, 123 patients with bilateral MMD, 27 patients without significant intracranial artery stenosis, 3 patients with certain signs of intracranial artery dissection, and 3 patients who underwent endovascular intervention were excluded. A total of 123 patients with suspected unilateral MMD were analyzed in this study (Figure S1–S4). Suspected unilateral MMD was defined as severe stenosis (≥70%) or occlusion of the proximal middle cerebral artery and/or distal internal carotid artery, without contralateral arterial stenosis. Baseline characteristics including underlying vascular risk factors (hypertension, diabetes, hyperlipidemia, and current smoking status), and radiographic data were collected from all patients. A family history of MMD was obtained from patient medical records and interviews. The absence of family history reports was considered indicative of no family history of MMD. Age at diagnosis was defined as the age at the time of the initial angiographic image showing suspected unilateral MMD. The follow-up period was defined as the time to the latest brain vascular image or the time to the image showing contralateral progression. The *RNF213* genotyping was done by the judgment of treating physicians mostly for the following reasons: diagnostic support in patients showing angiographic findings of MMD, diagnostic support in patients with suspected MMD (especially those with a family history of MMD) but not fulfilling the diagnostic criteria of MMD, and genetic counseling of patients with a family member carrying a *RNF213* R4810K variant. Analysis of the R4810K variant of the *RNF213* gene (GenBank accession number, NM_001256071.1) was performed on the blood samples from the patients. The analysis was performed at a commercial laboratory (Seoul Clinical Laboratories, Gyeonggi-do, South Korea). This study was reviewed and approved by the Severance

Hospital Yonsei University Health System institutional review board (2021–0364-001). The requirement for written informed consent for participation was waived in this retrospective study.

Primary Outcome Measurement

The primary outcome measure was the occurrence of contralateral progression during the follow-up period after diagnosis of suspected unilateral MMD. Contralateral progression was defined as severe stenosis (≥70%) or occlusion of the proximal middle cerebral artery and/or the distal internal carotid artery on the initially unaffected side. The primary outcome was investigated by a neurology specialist blinded to the *RNF213* R4810K genotyping results. We then evaluated the risk factors associated with contralateral progression.

Statistical Analysis

We compared the baseline clinical characteristics of suspected unilateral MMD, with and without the *RNF213* R4810K variant. The genotype and allele frequencies of the *RNF213* R4810K variant in suspected unilateral MMD and historical controls were compared. Continuous variables are presented as means with SDs or as medians with interquartile ranges and were compared using the independent 2-sample t test or Mann–Whitney *U* test, respectively. Categorical variables are presented as counts (percentages) and were compared using chi-square test or Fisher exact test. Statistical significance was defined as a 2-sided *P* value<0.05.

Risk factors for contralateral progression were evaluated using logistic regression. For rare event, Firth method was used to reduce the bias. Univariable analyses were performed with variables, including the presence of the *RNF213* R4810K variant, age at diagnosis, follow-up period, sex, family history of MMD, vascular risk factors, and medication history. Multivariable analyses were performed, adjusted for variables with *P* values <0.1 in the univariable model. We conducted Kaplan–Meier survival analysis with a log-rank test to compare the cumulative incidences of contralateral progression during the follow-up period between patients with and without the *RNF213* R4810K variant. Statistical analyses were performed using SAS version 9.4 (SAS Institute Inc) and SPSS software version 25.0 (IBM).

RESULTS

Clinical and Radiographic Characteristics

The genotype and allele frequencies of the *RNF213* R4810K variant in suspected unilateral MMD and historical controls are shown in Table 1. The R4810K homozygous (A/A) genotype was not detected in the suspected unilateral MMD group and in the historical control group. The allele frequency of the R4810K variant was significantly higher in the suspected unilateral MMD group compared with the historical control group[18] (29.3% versus 1.2%; *P*<0.0001).

Patient characteristics are summarized in Table 2. We identified 123 patients with suspected unilateral MMD. The mean age at diagnosis was 43 years (SD, 14 years), and 6 patients were younger than 18 years. The median follow-up period was 28 months (interquartile range, 18–54 months). Seventy-two (59%) patients were women. Of the 123 patients, 14 had a family history of MMD. The initial events noted at the time of the first brain imaging were transient ischemic attack in 29 patients, cerebral infarction in 34 patients, cerebral hemorrhage in 8 patients, and incidental findings in 28 patients.

Among the 123 patients, 72 (59%) were heterozygous for the *RNF213* R4810K variant (*RNF213*+ group) and 51 (41%) lacked the *RNF213* R4810K variant (*RNF213*– group). The *RNF213*+ group had significantly greater family history of MMD than the *RNF213*– group. There were no significant differences in age, follow-up periods, proportion of women, vascular risk factors, and initial events between the *RNF213*+ and *RNF213*– groups.

Clinical events and radiographic progression during the follow-up period were compared (Table 3). Patients in the *RNF213*+ group presented with more stroke events caused by the initially unaffected arterial side during the follow-up period (7 of 72 versus 0 of 51; *P*=0.04). The incidence of contralateral progression was significantly higher in the *RNF213*+ group than in the *RNF213*– group (11 of 72 versus 1 of 51; *P*=0.01). There was no significant difference in events caused by the initially affected arterial side between the *RNF213*+ and *RNF213*– groups.

Further analysis of 12 patients with contralateral progression was performed (Table S1). Five patients had a family history of MMD, and 9 had stroke as an initial presenting symptom. Although the time of contralateral progression could not be calculated without regular imaging follow-up, 3 patients showed contralateral progression within 1 year. Figure 1 shows example images of unilateral MMD with normal contralateral arteries progressing to bilateral MMD within a relatively short period.

Risk Factors of Contralateral Progression in Patients With Suspected Unilateral MMD

Among the risk factors, the presence of the *RNF213* R4810K variant (unadjusted odds ratio [OR], 6.30 [95% CI, 1.09–36.52]; *P*=0.04) and family history of MMD (unadjusted OR, 8.10 [95% CI, 2.13–30.75]; *P*=0.002) was a statistically significant factor associated with

Table 1. Genotype and Allele Distribution of R4810K Variant of *RNF213* in Patients With Suspected Unilateral MMD and Historical Controls

	No. of patients	Genotype frequency				Allele frequency, No. (%)		
		G/G	G/A	A/A	*P* value*	Minor, A	Major, G	*P* value*
All cases in the study	123	51	72	0	<0.0001	72 (29.3)	174 (70.7)	<0.0001
Historical controls	1516	1479	37	0		37 (1.2)	2995 (98.8)	

MMD indicates moyamoya disease; and *RNF213*, ring finger protein 213.
*χ^2 test was used.

contralateral progression in patients with suspected unilateral MMD in the univariable analysis (Table 4). Multivariable analysis including the presence of the *RNF213* R4810K variant, age, and family history of MMD was performed (Table S2). The result showed that family history of MMD was a stronger risk factor of contralateral progression than the presence of the *RNF213* R4810K variant. However, since family history of MMD is significantly associated with the presence of the *RNF213* R4810K variant, problems considering multicollinear bias exist. Therefore, since the object of our study was to investigate the association between the presence of the *RNF213* R4810K variant and contralateral progression, multivariable analysis was performed without the family history of MMD. After adjusting for age, the presence of the *RNF213* R4810K variant (adjusted OR, 6.39 [95% CI, 1.11–36.63]; *P*=0.04) was independently associated with contralateral progression. Age at diagnosis was inversely correlated with contralateral progression; however, this did not reach statistical significance. None of the other variables, including follow-up period, sex, vascular risk factors, or medication history (antiplatelet and/or statin), were associated with contralateral progression.

Further investigation was performed among patients without family history of MMD. The patients with contralateral progression carried a significantly higher proportion of the *RNF213* R4810K variant compared with those without contralateral progression (100% versus 52%; *P*=0.02).

Table 2. Baseline Characteristics of Patients With Suspected Unilateral MMD

	No. (%)			*P* value
	All (N=123)	*RNF213*+ (n=72)	*RNF213*− (n=51)	
Age, mean (SD), y	43 (14)	43 (15)	43 (14)	0.99
Age <18 y	6 (5)	4 (6)	2 (4)	>0.99
Follow-up period, median (IQR), mo	28 (18–54)	31 (19–55)	25 (18–49)	0.56
Female sex	72 (59)	41 (57)	31 (61)	0.67
Family history of MMD	14 (11)	12 (17)	2 (4)	0.003
Vascular risk factors				
Hypertension	50 (41)	33 (46)	17 (33)	0.16
Diabetes	17 (14)	10 (14)	7 (14)	0.98
Hyperlipidemia	44 (36)	31 (43)	13 (26)	0.06
Current smoking	16 (13)	12 (17)	4 (8)	0.15
Initial event				
Transient ischemic attack	29 (24)	17 (24)	12 (24)	0.99
Cerebral infarction	34 (28)	22 (31)	12 (24)	0.39
Cerebral hemorrhage	8 (7)	5 (7)	3 (6)	>0.99
Headache	15 (12)	6 (8)	9 (18)	0.12
Dizziness	6 (5)	3 (4)	3 (6)	0.69
Syncope	3 (2)	2 (3)	1 (2)	>0.99
Seizure	0 (0)	0 (0)	0 (0)	>0.99
Incidental	28 (23)	17 (24)	11 (22)	0.79
Medication				
Antiplatelet	100 (81)	57 (79)	43 (84)	0.47
Statin	93 (76)	54 (75)	39 (77)	0.85

IQR indicates interquartile range; MMD, moyamoya disease; and *RNF213*, ring finger protein 213.

Table 3. Clinical Events and Radiographical Progression During the Follow-Up Period

	All (N=123)	*RNF213*+ (n=72)	*RNF213*− (n=51)	*P* value
All stroke (affected side)	30 (24)	21 (29)	9 (18)	0.14
Transient ischemic attack	22 (18)	15 (21)	7 (14)	0.31
Cerebral infarction	5 (4)	3 (4)	2 (3)	>0.99
Cerebral hemorrhage	3 (2)	3 (4)	0 (0)	0.27
All stroke (unaffected side)	7 (6)	7 (10)	0 (0)	0.04
Transient ischemic attack	4 (3)	4 (6)	0 (0)	0.14
Cerebral infarction	3 (2)	3 (4)	0 (0)	0.27
Cerebral hemorrhage	0 (0)	0 (0)	0 (0)	>0.99
Radiographic characteristics				
Contralateral progression	12 (10)	11 (15)	1 (2)	0.01

RNF213 indicates ring finger protein 213.

In addition, the presence of the *RNF213* R4810K variant showed an OR of 13.87 for contralateral progression but with a large 95% CI (0.75–256.35; *P*=0.08) (Table S3).

Kaplan–Meier survival analysis with the log-rank test showed a significant difference in the cumulative incidence of contralateral progression during the follow-up period between the 2 groups (*P*=0.03) (Figure 2).

To identify the factors that may promote contralateral progression among patients with the *RNF213* R4810K variant, an additional logistic regression analysis was performed in the *RNF213*+ group. None of the factors included in the univariable and multivariable analysis showed significant associations with contralateral progression among patients with the *RNF213* R4810K variant (Table S4).

DISCUSSION

In this study, we showed that patients with suspected unilateral MMD who had the *RNF213* R4810K variant

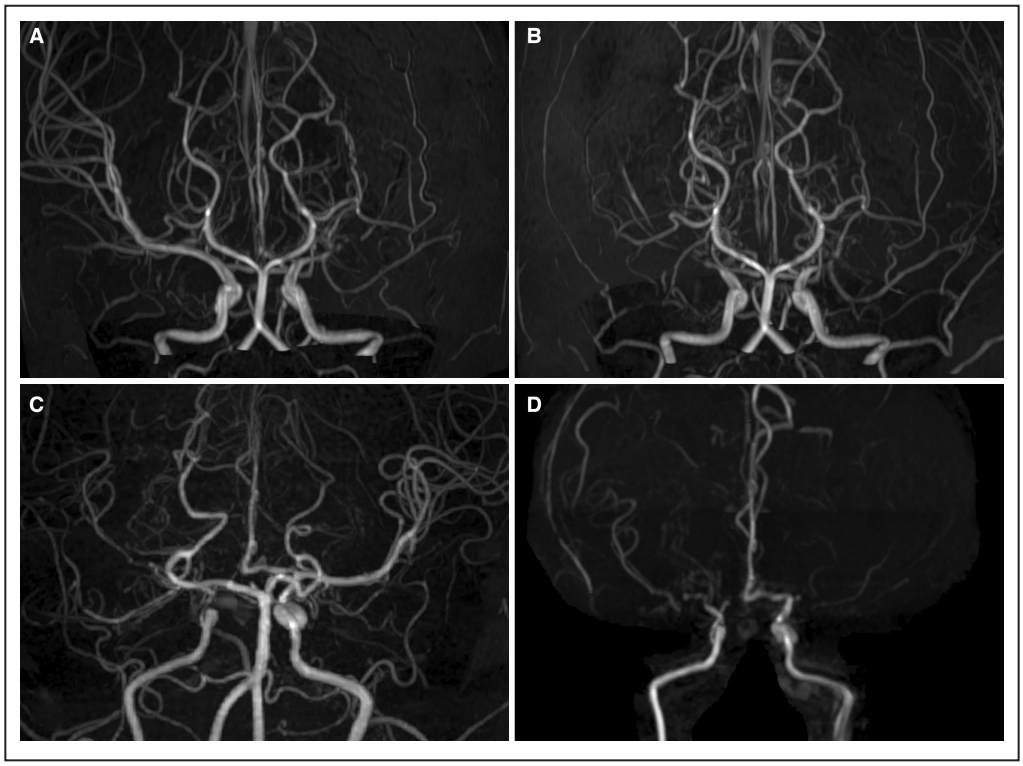

Figure 1. Magnetic resonance angiography imaging of patients with contralateral progression. (**A** and **B**) A 4-year-old girl presented with right-sided weakness. (**A**) Initial magnetic resonance angiography (MRA) shows occlusion of the left internal carotid artery (ICA). (**B**) Follow-up MRA taken 7 months later shows complete occlusion of the right ICA. (**C** and **D**) A 64-year-old woman presented with left-sided weakness. (**C**) Initial MRA shows occlusion of the right ICA. (**D**) Follow-up MRA taken 15 months later for right-sided weakness shows occlusion of the left middle cerebral artery.

Table 4. Results of Logistic Regression Analysis for Contralateral Progression in Patients With Suspected Unilateral MMD

Variable	Univariable model		Multivariable model†	
	OR (95% CI)	*P* value	OR (95% CI)	*P* value
RNF213 R4810K variant*	6.30 (1.09–36.52)	0.04	6.39 (1.11–36.63)	0.04
Age, per y	0.97 (0.93–1.00)	0.08	0.97 (0.93–1.00)	0.08
Follow-up period, per mo	1.00 (0.98–1.01)	0.88		
Female sex (reference: male)	0.99 (0.30–3.32)	0.99		
Family history of MMD	8.10 (2.13–30.75)	0.002		
Vascular risk factors				
Hypertension	1.05 (0.31–3.51)	0.94		
Diabetes	0.54 (0.07–4.47)	0.57		
Hyperlipidemia	0.89 (0.25–3.13)	0.85		
Current smoking	1.39 (0.27–6.99)	0.69		
Medication				
Antiplatelet	0.66 (0.16–2.66)	0.56		
Statin	0.41 (0.12–1.39)	0.15		

MMD indicates moyamoya disease; and OR, odds ratio.
*Firth method was used.
†Multivariable model included *RNF213* (ring finger protein 213) R4810K variant and age.

were more likely to have contralateral progression than those without the *RNF213* R4810K variant. Eleven of the 72 patients with the *RNF213* R4810K variant developed contralateral progression, while only 1 of the 51 patients without the *RNF213* R4810K variant developed contralateral progression within a relatively short follow-up period. The sole patient in the *RNF213*–group with contralateral progression had a son with

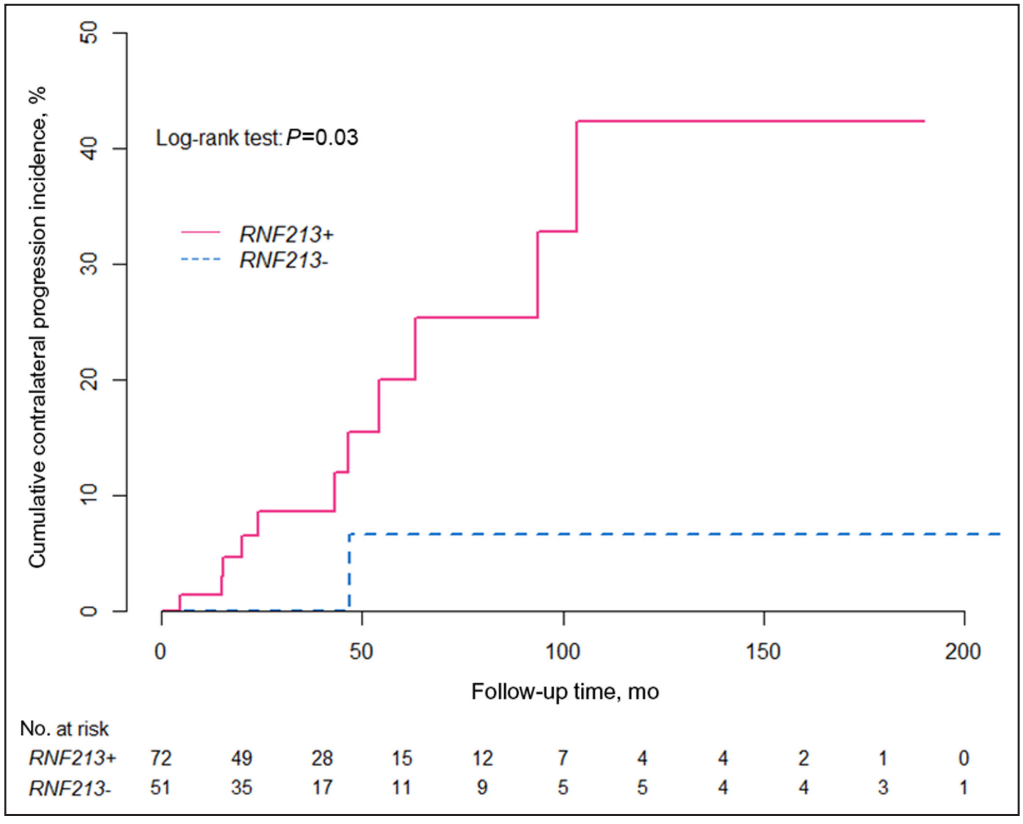

Figure 2. Kaplan–Meier estimates of contralateral progression during the follow-up period.
During the follow-up period, 11 of 72 (15%) patients with the *RNF213* (ring finger protein 213) R4810K variant had contralateral progression. Contralateral progression was observed in 1 of 51 (2%) patients without the *RNF213* R4810K variant (log-rank test; *P*=0.03).

a history of MMD, which implied that a genetic factor other than the *RNF213* R4810K variant may have been involved.[19]

To date, this is the largest study to address the clinical features and radiological outcomes of unilateral MMD via the analysis of the *RNF213* gene. Zhang et al[15] reported a large series of patients with unilateral MMD, including *RNF213* gene analysis. However, ≈70% of the study patients had their genes analyzed and only a few cases of the *RNF213* R4810K variant were identified. The prevalence of the *RNF213* R4810K variant in unilateral MMD is not well known and ranges from 12% to 67%.[3,15,17] In our study, 59% of the patients with suspected unilateral MMD had the *RNF213* R4810K variant. The difference in the prevalence of the *RNF213* R4810K variant may be explained by the small number of patients with unilateral MMD included in the studies and by differences in ethnicity.

Interestingly, all patients with the *RNF213* R4810K variant were heterozygous, including 4 pediatric patients. Previous studies of patients with bilateral MMD have shown that the number of *RNF213* R4810K variant alleles was associated with earlier disease onset, higher severity, and higher penetrance rates (79% in homozygous MMD and <1% in heterozygous MMD).[17,20] Additionally, patients with the *RNF213* R4810K variant present various phenotypes possibly because of the low penetration rate of the *RNF213* R4810K variant and the difference in susceptibility of the gene between individuals. As no homozygous *RNF213* R4810K variant was found in our study, we assume that the homozygous *RNF213* R4810K variant progresses early and quickly to a bilateral phenotype from a unilateral phenotype or presents initially as bilateral MMD.[21,22]

Previous efforts to evaluate the factors predictive of contralateral progression have been performed in patients with unilateral MMD.[9–15] Baseline stenosis of the contralateral arteries was the most frequently reported factor predictive of contralateral progression, along with hyperlipidemia, family history of MMD, congenital cardiac anomalies, and earlier age at diagnosis. In this study, the presence of the *RNF213* R4810K variant and the family history of MMD were significantly associated with contralateral progression in suspected unilateral MMD. Earlier age at diagnosis showed a nonsignificant but positive association with contralateral progression. Vascular risk factors including hyperlipidemia and intake of antiplatelet or statin were not associated with contralateral progression in our study.

The mean time to contralateral progression in unilateral MMD has been reported to vary from 1.2 to 5.8 years. However, whether the progression process was gradual or rapid is unknown. There are cases of rapid contralateral progression that occurred within 1 year.[23] In this study, 3 patients showed contralateral progression within 1 year. It can be assumed that there are factors that result in relatively rapid disease progression in patients with the *RNF213* R4810K variant. However, we could not identify any relevant factors. A more comprehensive study that includes factors such as inflammatory markers, immune-related markers, and changes in vascular flow is necessary.[24]

We assessed the clinical events in patients with suspected unilateral MMD with contralateral progression. Seven of the 11 (64%) patients with the *RNF213* R4810K variant had an ischemic stroke or a transient ischemic attack during the follow-up period caused by the initially unaffected side. The presence of the *RNF213* R4810K variant increases the risk of ischemic stroke attributable to large-artery atherosclerosis.[25] Regardless of whether intracranial atherosclerotic stenosis with the *RNF213* R4810K variant and unilateral MMD is the same disease entity, the presence of the *RNF213* R4810K variant may be a prognostic factor for contralateral progression and future ischemic stroke.

Limitations

Our study had some limitations. First, patients who underwent *RNF213* R4810K genotyping were retrospectively reviewed to identify those with suspected unilateral MMD. Thus, selection bias to enroll patients enriched for genetic disease may exist. Second, the number of patients with contralateral progression was small, presumably because of the small sample size and the relatively short follow-up period. Further studies with larger sample size and longer follow-up periods are required to confirm these results. Third, the patients did not undergo regular imaging follow-ups. Therefore, contralateral progression times were estimated based on the time intervals between the images with progression and those with stable status. Fourth, proximal anterior cerebral artery stenosis or occlusion was not included as an indication of suspected unilateral MMD or contralateral artery stenosis. Although the definition of MMD includes anterior cerebral artery steno-occlusive lesions, this was excluded from our analysis because of the presence of hypoplasia or aplasia, which may be easily mistaken for a pathological lesion. Fifth, family histories of MMD may have been underestimated because they were determined by interviewing patients. Therefore, the actual incidence of a family history of MMD may be increased by acquiring intensive family histories and by performing image screening on family members. Last, only the R4810K variant rather than the whole gene was analyzed. Since 1 patient showing contralateral progression without the R4810K variant had a family member with MMD, it is conceivable that a distinct genetic locus may have been involved. Therefore, comprehensive genetic analysis of *RNF213* is necessary to determine the association of other *RNF213* variants and contralateral progression.

CONCLUSIONS

In this study, patients with suspected unilateral MMD with the *RNF213* R4810K variant had a significantly higher risk of contralateral progression. The *RNF213* R4810K variant may be a useful biomarker for early diagnosis of MMD in patients with unilateral intracranial artery occlusive lesions. Moreover, we recommend more frequent imaging screening to detect disease progression in patients with the *RNF213* R4810K variant. However, because of the small sample size, this finding should be carefully interpreted and requires further studies with more patients and longer follow-up periods.

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Disclosures

None.

Supplemental Material

Tables S1–S4
Figure S1

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SUPPLEMENTAL MATERIAL

Table S1. Clinical Characteristics of Patients with Contralateral Progression and Suspected Unilateral Moyamoya disease

Patient	Age	Sex	*RNF213*	FHx of MMD	Initial symptom	Affected Side	Follow-up symptom	Progression period, m*
1	65	M	+	-	Dizziness	Left	Infarction	24
2	44	M	-	+	Infarction	Left	None	34
3	64	F	+	-	Infarction	Right	Infarction	15
4	51	F	+	-	Hemorrhage	Left	None	30
5	20	M	+	+	Infarction	Left	TIA	93
6	54	F	+	+	Headache	Left	TIA	63
7	35	M	+	+	TIA	Right	None	46
8	27	F	+	-	TIA	Right	TIA	54
9	50	F	+	-	Incidental	Right	None	44
10	8	F	+	+	TIA	Right	Infarction	12
11	4	F	+	-	Infarction	Left	TIA	7
12	6	M	+	-	TIA	Right	None	3

RNF213, *Ring finger protein 213*; *FHx*, family history; *MMD*, Moyamoya disease; *m*, month; *M*, male; *F*, female; *TIA*, transient ischemic attack

*Interval time from the date of the latest image of unilateral disease to the date of contralateral progression; estimate of actual progression period.

Table S2. Results of Multivariable Logistic Regression Analysis for Contralateral Progression in Patients with Suspected Unilateral MMD

	Multivariable Model	
Variable	**OR (95% CI)**	***P***
RNF213* R4810K variant	4.80 (0.82–28.11)	0.08
Age, per year	0.97 (0.93–1.01)	0.10
Family history of MMD	5.35 (1.36–21.10)	0.02

RNF213, *Ring finger protein 213*; *MMD*, Moyamoya disease; *OR*, odds ratio; *CI*, confidence interval.
*Firth's method was used
†Multivariable model included *RNF213* R4810K variant, age, and family history of MMD

Table S3. Results of Logistic Regression Analysis for Contralateral Progression among Patients without Family History of MMD

	Univariable Model	
Variable	**OR (95% CI)**	***P***
Age, per year	0.98 (0.93–1.03)	0.36
Follow-up period, month	1.01 (1.00–1.02)	0.24
Sex, female (ref: male)	1.68 (0.31–9.08)	0.55
RNF213* R4810K variant	13.87 (0.75–256.35)	0.08
Vascular risk factors		
Hypertension	1.91 (0.41–8.96)	0.42
Diabetes mellitus	0.97 (0.11–8.61)	0.98
Hyperlipidemia	2.55 (0.54–12.05)	0.24
Current smoking	0.53 (0.03–11.20)	0.68
Medication		
Anti-platelet	0.57 (0.10–3.18)	0.52
Statin	0.73 (0.13–4.00)	0.72

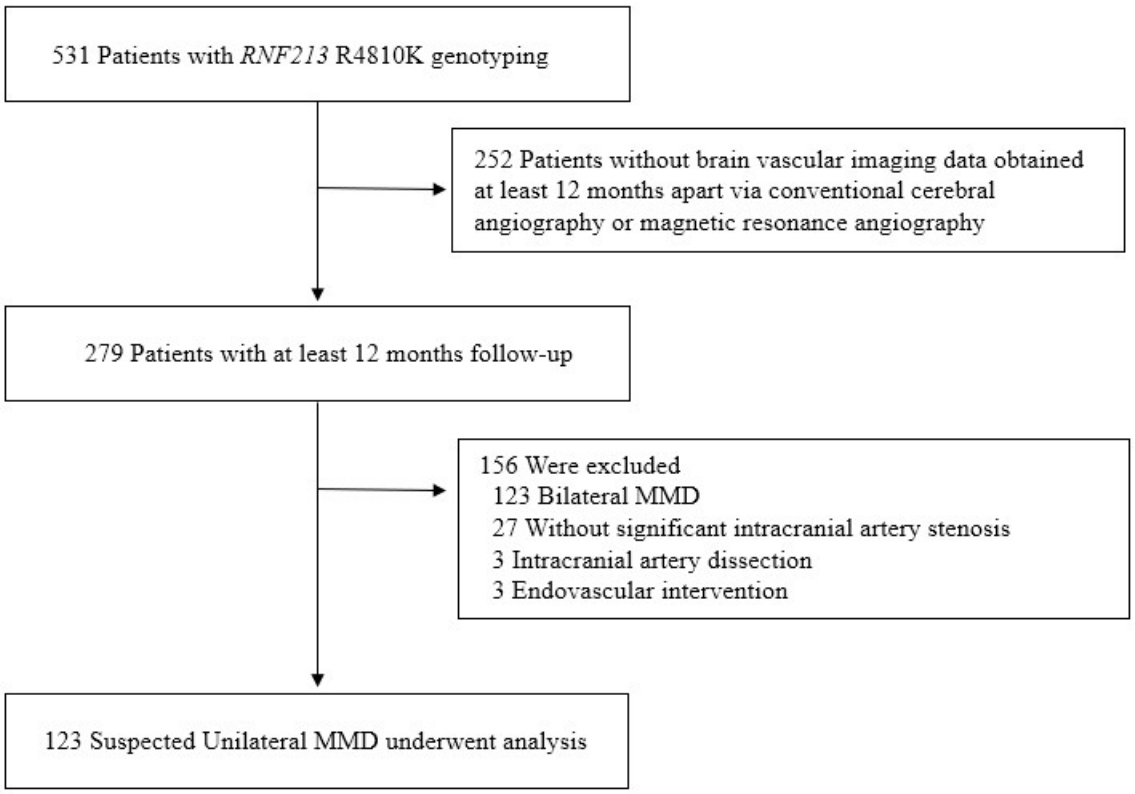
RNF213, *Ring finger protein 213*; *MMD*, Moyamoya disease; *OR*, odds ratio; *CI*, confidence interval.
*Firth's method was used

Table S4. Results of Logistic Regression Analysis for Contralateral Progression among *RNF213+* patients

	Univariable Model		Multivariable Model	
Variable	**OR (95% CI)**	***P***	**OR (95% CI)**	***P***
Age, per year	0.96 (0.92–1.00)	0.07	0.96 (0.92–1.00)	0.08
Follow-up period, month	1.00 (0.98–1.02)	0.88		
Sex, female (ref: male)	1.39 (0.37–5.25)	0.63		
Family history of MMD	3.79 (0.90–15.91)	0.07	3.63 (0.82–15.96)	0.09
Vascular risk factors				
Hypertension	0.98 (0.27–3.57)	0.98		
Diabetes mellitus	0.58 (0.07–5.08)	0.62		
Hyperlipidemia	0.72 (0.19–2.72)	0.63		
Current smoking	0.45 (0.05–3.93)	0.47		
Medication				
Anti-platelet	0.65 (0.15–2.84)	0.57		
Statin	0.52 (0.13–2.04)	0.35		

RNF213, *Ring finger protein 213*; *OR*, odds ratio; *CI*, confidence interval.

Figure S1. Flow chart for the selection of suspicious unilateral moyamoya disease with *RNF213* R4810K genotyping



RNF213 indicates *Ring finger protein 213*; and MMD, Moyamoya disease.