ORIGINAL RESEARCH

RNF213 R4810K Variant in Suspected Unilateral Moyamoya Disease Predicts Contralateral Progression

Taedong Ok MD; Yo Han Jung MD, PhD; Jinkwon Kim MD, PhD; Sang Kyu Park MD, PhD; Goeun Park PhD; Sujee Lee MS; Kyung-Yul Lee MD, PhD

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
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. In contrast, it may also be possible to misdiagnose intracranial atherosclerotic occlusion with basal collaterals as MMD.

Up to 18% of patients with MMD present with unilateral involvement, and 8% to 59% of patients experience contralateral progression. 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. 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 (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 26 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...
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 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.

*χ² test was used.

Table 2. Baseline Characteristics of Patients With Suspected Unilateral MMD

| No. (%) | RNF213+ (n=72) | RNF213− (n=51) | P value |
|---------|----------------|----------------|---------|
| 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

|                | RNF213+ (n=72) | RNF213− (n=51) | P value |
|----------------|----------------|----------------|---------|
| 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

|               | RNF213+ (n=72) | RNF213− (n=51) | P value |
|---------------|----------------|----------------|---------|
| 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

|                | RNF213+ (n=72) | RNF213− (n=51) | P value |
|----------------|----------------|----------------|---------|
| 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.
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...
Ok et al RNF213 R4810K Variant in Suspected Unilateral MMD

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

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

| Variable                                | Univariable model           | P value | Multivariable model†                           | 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                              |                             |         |                                               |         |
| Antplatelet                             | 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.

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.\textsuperscript{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\textsuperscript{15} reported a large series of patients with unilateral MMD, including RNF213 gene analysis. However, \textasciitilde70\% 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\%.\textsuperscript{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).\textsuperscript{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.\textsuperscript{21,22}

Previous efforts to evaluate the factors predictive of contralateral progression have been performed in patients with unilateral MMD.\textsuperscript{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 antplatelet 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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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.

ARTICLE INFORMATION
Received February 7, 2022; accepted June 27, 2022.

Affiliations
Department of Neurology, Gangnam Severance Hospital (T.O., Y.H.J., K.L.); Department of Neurology, Yonsei Severance Hospital (J.K.); Department of Neurosurgery, Gangnam Severance Hospital (S.K.P) and Biostatistics Collaboration Unit (G.P., S.L.), Yonsei University College of Medicine, Seoul, South Korea.

Acknowledgments
We would like to thank Editage (www.editage.co.kr) for English language editing.

Sources of Funding
None.

Disclosures
None.

Supplemental Material
Tables S1-S4
Figure S1

REFERENCES
1. Liu W, Monto D, Takashima S, Mineharu Y, Kobayashi H, Hitomi T, Hashikata H, Matsuura N, Yamazaki S, Toyoda A, et al. Identification of RNF213 as a susceptibility gene for moyamoya disease and its possible role in vascular development. PLoS One. 2011;6:e22542. doi: 10.1371/journal.pone.0022542
2. Roy V, Ross JP, Pepin R, Cortez Ghio S, Brodeur A, Touzel Deschenes L, Le Bel G, Phillips DE, Milot G, Dion PA, et al. Moyamoya disease susceptibility gene RNF213 regulates endothelial barrier function. Stroke. 2022;53:1263–1275. doi: 10.1161/STROKEAHA.120.032691
3. Kamada F, Aoki Y, Narisawa A, Abe Y, Komatsuzaki S, Kikuchi A, Kanno J, Niihori T, Ono M, Ishii N, et al. A genome-wide association study identifies RNF213 as the first Moyamoya disease gene. J Hum Genet. 2011;56:34–40. doi: 10.1038/jhg.2010.132
4. Suzuki J. Cerebrovascular “moyamoya” disease. Disease showing abnormal net-like vessels in base of brain. Arch Neurol. 1969;20:288–299. doi: 10.1001/archneur.1969.00480090076012
5. Bang OY, Ryoo S, Kim SJ, Yoon CH, Cha J, Yeon JY, Kim KH, Kim GM, Chung CS, Lee et al. Adult moyamoya disease: a burden of intracranial stenosis in east Asians? PLoS One. 2015;10:e0130663. doi: 10.1371/journal.pone.0130663
6. Miyawaki S, Imai H, Shimizu M, Yagi S, Ono H, Mukasa A, Nakatomi H, Shimizu T, Saito N. Genetic variant of RNF213 c.14576G>a in various phenotypes of intracranial major artery stenosis/occlusion. Stroke. 2015;46:2894–2897. doi: 10.1161/STROKEAHA.113.002477
7. Miyawaki S, Imai H, Takayanagi S, Mukasa A, Nakatomi H, Saito N. Identification of a genetic variant common to moyamoya disease and intracranial major artery stenosis/occlusion. Stroke. 2012;43:3371–3374. doi: 10.1161/STROKEAHA.112.663864
8. Yu LB, He H, Zhao JZ, Wang R, Zhang Q, Shi ZY, Shao JS, Zhang D. More precise imaging analysis and diagnosis of Moyamoya disease and Moyamoya syndrome using high-resolution magnetic resonance imaging. World Neurosurg. 2016;86:252–260. doi: 10.1016/j.wneu.2016.08.083
9. Church EW, Bell-Stephens TE, Bigder MG, Gummipudundi S, Han SS, Steinberg GK. Clinical course of unilateral moyamoya disease. Neurosurg. 2020;87:1262–1268. doi: 10.1093/neuros/iyaa284
10. Kelly ME, Bell-Stephens TE, Marks MP, Do HM, Steinberg GK. Progression of unilateral moyamoya disease: a clinical series. Cerebrovasc Dis. 2006;22:109–115. doi: 10.1159/000093328
11. Lee SC, Jeon JS, Kim JE, Chung YS, Ahn JH, Cho WS, Son YJ, Bang JS, Kang HS, Oh CW. Contralateral progression and its risk factor in surgically treated unilateral adult moyamoya disease with a review of pertinent literature. Acta Neurochirurgica. 2014;156:103–111. doi: 10.1007/s00706-013-1921-8
12. Park EK, Lee YH, Shim KW, Ooi JH, Kim DS. Natural history and process factors of unilateral moyamoya disease in pediatric patients. Childs Nerv Syst. 2011;27:1281–1287. doi: 10.1007/s00381-011-1469-y
13. Smith ER, Scott RM. Progression of disease in unilateral moyamoya syndrome. Neurosurg Focus. 2008;24:E17. doi: 10.3171/FOC/2008/24/2/E17
14. Yoon JY, Shin HJ, Kong DS, Seo HJ, Kim JS, Hong SC, Park K. The prediction of contralateral progression in children and adolescents with unilateral moyamoya disease. Stroke. 2011;42:2973–2976. doi: 10.1161/STROKEAHA.111.622522
15. Zhang Q, Wang R, Liu Y, Zhang Y, Wang S, Cao Y, Zhao Y, Liu X, Wang J, Deng X, et al. Clinical features and long-term outcomes of unilateral Moyamoya Disease. World Neurosurg. 2016;96:474–482. doi: 10.1016/j.wneu.2016.09.018
16. Kim EH, Yum MS, Ra YS, Park JB, Ahn JS, Kim GH, Goo HW, Ko TS, Yoo HW. Importance of RNF213 polymorphism on clinical features and long-term outcome in moyamoya disease. J Neurosurg. 2016;124:1221–1227. doi: 10.3171/2015.4.JNS142900
17. Miyatake S, Miyake N, Touho H, Nishimura-Tadaki A, Kondo Y, Okada I, Tsursuki Y, Doi H, Saka H, Saittsu H, et al. Homozygous c.14576G>a variant of RNF213 predicts early-onset and severe form of moyamoya disease. Neurology. 2012;78:803–810. doi: 10.1212/WNL.0b013e3182f497f1
18. Jang M-A, Min S, Yoon JH, Ki CS. Frequency of the moyamoya-related RNF213 p.Arg2410fs in South Korean families. Korean J Pediatr. 2013;56:168–173. doi: 10.3805/kjp.2013.56.2.168
19. Cecchi AC, Guo D, Ren Z, Flynn K, Santos-Cortez RL, Leal SM, Wang GT, Regalado ES, Steinberg GK, Shendure J, et al. RNF213 rare variants in an ethnically diverse population with Moyamoya disease. Stroke. 2016;47:3200–3207. doi: 10.1161/STROKEAHA.114.006244
20. Liu W, Hitomi T, Kobayashi H, Harada K, Koizumi A. Distribution of moyamoya disease susceptibility polymorphism p.R4810K in RNF213 in East and Southeast Asian populations. Neuro Med Chir (Tokyo). 2012;52:299–303. doi: 10.2176/nmc.32.299
21. Wang Y, Zhang Z, Wei L, Zhang Q, Zou Y, Yang L, Li D, Shang M, Han C, Mamiya M, et al. Predictive role of heterozygous p.R4810K of RNF213 in the phenotype of Chinese moyamoya disease. Neurology. 2020;94:e678–e686. doi: 10.1212/WNL.0000000000009891
22. Ko YJ, Choi SA, Kim SY, Yoo IH, Kim H, Lim BC, Lee JY, Phi JH, Kim SK, Wang KC, et al. The prognostic implication of RNF213 R4810K variant in pediatric moyamoya disease: early disease onset and poor prognosis. J Korean Child Neurol Soc. 2015;23:57–61.
23. Mineharu Y, Takagi Y, Takahashi JC, Hashikata H, Liu W, Hitomi T, Kobayashi H, Koizumi A, Miyamoto S. Rapid progression of unilateral moyamoya disease in a patient with a family history and an RNF213 risk variant. Cerebrovasc Dis. 2013;36:155–157. doi: 10.1159/000352065
24. Lee WJ, Jeong SK, Han KS, Lee SH, Ryu YJ, Sohn CH, Jung KH. Impact of endothelial shear stress on the bilateral progression of unilateral moyamoya disease. Stroke. 2003;34:757–763. doi: 10.1161/01.STR.0000082076.01192.1b
25. Okazaki S, Morimoto T, Kaminari Y, Kamamura T, Kobayashi H, Harada K, Tomita T, Higashiyama A, Takahashi JC, Nakagawara J, et al. Moyamoya disease susceptibility variant RNF213 p.R4810K increases the risk of ischemic stroke attributable to large-artery atherosclerosis. Circulation. 2019;139:295–298. doi: 10.1161/CIRCULATIONAHA.118.038439
| 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                     |

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

RNF213, Ring finger protein 213; FHx, family history; MMD, Moyamoya disease; m, month; M, male; F, female; TIA, transient ischemic attack.
Table S2. Results of Multivariable Logistic Regression Analysis for Contralateral Progression in Patients with Suspected Unilateral MMD

| Variable                        | Multivariable Model |   |
|--------------------------------|---------------------|---|
| 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

| Variable                              | Univariable Model | 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

| Variable                        | Univariable Model |        |        |        |        | Multivariable Model |        |        |
|---------------------------------|-------------------|--------|--------|--------|--------|---------------------|--------|--------|
|                                 | OR (95% CI)       | P      | 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   |        |        |        | 1.00 (0.98–1.02)  | 0.88   |        |
| Sex, female (ref: male)         | 1.39 (0.37–5.25)  | 0.63   |        |        |        | 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

531 Patients with \textit{RNF213} R4810K genotyping

\begin{itemize}
\item 252 Patients without brain vascular imaging data obtained at least 12 months apart via conventional cerebral angiography or magnetic resonance angiography
\end{itemize}

279 Patients with at least 12 months follow-up

\begin{itemize}
\item 156 Were excluded
\item 123 Bilateral MMD
\item 27 Without significant intracranial artery stenosis
\item 3 Intracranial artery dissection
\item 3 Endovascular intervention
\end{itemize}

123 Suspected Unilateral MMD underwent analysis

\textit{RNF213} indicates \textit{Ring finger protein 213}; and MMD, Moyamoya disease.