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Posterior circulation involvement and collateral flow pattern in moyamoya disease with the *RNF213* polymorphism

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Abstract

Purpose Moyamoya disease is a chronic cerebrovascular disorder characterized by progressive stenosis of the circle of Willis with a compensatory collateral vessel network. Recent studies have identified the ring finger protein 213 gene (*RNF213*) as the unique susceptibility gene for moyamoya disease. The purpose of this study was to compare clinical features of moyamoya disease, especially angiographic findings, between patients with and without the *RNF213* mutation.

Methods Blood samples from 35 patients with moyamoya disease were obtained between May 2016 and May 2017. Information on age at the time of diagnosis, sex, and initial symptom were obtained via retrospective chart review. Angiographic records were evaluated.

Results *RNF213* variants were detected in the 28 of 35 patients (80%), including all pediatric patients (100%) and 18 of 25 adult patients (72%) in our cohort. Leptomeningeal collateral flow from posterior to anterior circulation was more frequent in the *RNF213*-negative group than in the *RNF213*-positive group (100% versus 38.9%; $p = 0.020$). Posterior cerebral arterial territorial involvement was more frequently observed in *RNF213*-positive patients than in *RNF213*-negative patients (50% versus 0%; $p = 0.027$).

Conclusions *RNF213* may play a significant role in the development of collateral anastomoses.

Keywords RNF213 · Moyamoya disease · Cerebrovascular disorder · Collateral anastomoses

Introduction

Moyamoya disease (MMD) is a chronic cerebrovascular disorder characterized by progressive stenosis of the circle of Willis with subsequent development of a compensatory collateral vessel network [27]. The prevalence of MMD is highest in East Asian countries, including Korea. A recent study reported that the annual incidence in 2011 was estimated to be 16.1 per 100,000 person-years in Korea [1, 13]. In 2011, two Japanese groups identified the ring finger protein 213 gene (*RNF213*) on chromosome 17q25.3 as the unique susceptibility gene for MMD. In these studies, most Japanese patients carried p.R4810K (c.14429G>A) [10, 18]. In previous studies, *RNF213* p.R4810K variants were detected in approximately 80% of Korean MMD patients [14, 18, 19]. Although several risk factors for MMD have been identified, such as female sex, East Asian ethnicity, and family history of MMD, the etiology of MMD remains unclear. Furthermore, clinical features of MMD patients with *RNF213* polymorphisms have not been fully investigated. A few studies have revealed that *RNF213* polymorphisms are associated with high blood pressure, bilateral hemispheric involvement of MMD, early-onset MMD, and a poor prognosis [14, 16, 22]. Additionally, a recent animal study revealed a potential role of *RNF213* in the development of pathological angiogenesis in ischemia [3, 9]. Therefore, we sought to compare clinical features, particularly angiographic findings, between MMD patients with and without an *RNF213* mutation.

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Methods

Patients

Between May 2016 and May 2017, *RNF213* genetic variant testing was performed on patients who had been diagnosed with MMD and who had undergone follow-up for more than 1 year at our institute. We diagnosed 118 patients with MMD between January 2002 and May 2016 at our hospital on the basis of cerebral angiography and magnetic resonance angiography (MRA). Of 118 patients diagnosed with moyamoya disease, counseling for *RNF213* genetic variant testing was performed and 35 patients provided written informed consent to be involved in this study. This study was approved by the Korea University Ansan Hospital's Institutional Review Board (No. AS16032). Blood samples from 35 patients with MMD were obtained between May 2016 and May 2017. All patients were Koreans. A screening of *RNF213* for c.14429G>A (p.Arg4810Lys/p.R4810K), which was proven to have a strong association with Korean MMD patients, was done [14, 19]. Information on age at time of diagnosis, patient sex, and initial symptoms at diagnosis were obtained via retrospective chart review. Angiographic records were evaluated to determine ICA (internal cerebral artery) staging, posterior cerebral artery (PCA) involvement, aneurysms, and collateral flow patterns, such as leptomeningeal and transdural collateral flow.

Diagnosis of MMD and ICA staging

All patients older than 18 years underwent cerebral angiography, including external carotid arteriography. All patients younger than 18 years underwent MRA. All patients were diagnosed based on published guidelines [4]. ICA staging of steno-occlusive lesions was classified according to the staging system as defined by Mugikura et al. [24]. The classification of ICA stage is described below: stage 1, mild to moderate stenosis around carotid bifurcation; stage 2, severe stenosis around carotid bifurcation of occlusion of either of proximal ACA or MCA (middle cerebral artery); stage 3, near occlusion of both proximal ACA and MCA, only a few of either ACA or MCA branches or both are faintly opacified in antegrade fashion; and stage 4, complete occlusion of both proximal ACA and MCA with absent or small amount of ICA moyamoya.

Variant detection of *RNF213*

In this study, we referred to NM_001256071.1 as the reference transcriptional sequence of *RNF213*. Genomic DNA was isolated from peripheral blood leukocytes. *RNF213* on chromosome 17q25.3 was analyzed. Polymerase chain reaction and direct sequencing were performed for the selected mutation (c.14429G>A, p.R4810K in exon 60).

Statistical analysis

Differences in clinical features between patients with the *RNF213* variant and those patients without the *RNF213* variant were analyzed only in adult patients since all pediatric patients in our cohort carried the *RNF213* variant. Statistical analysis was performed using a chi-squared test or Fisher's exact probability test, as appropriate. Statistical analyses were performed using the Statistical Package for the Social Sciences version 18.0 for Windows (SPSS Inc.). Differences were considered significant at p values of less than or equal to 0.05.

Results

RNF213

RNF213 variants were detected in the 28 of 35 patients (80%). All genotypes of the minor allele frequency of the c.14429G>A variant were heterozygous. *RNF213* variants were detected in all pediatric patients (100%) and in 18 of 25 adult patients (72%).

Pediatric group

A total of ten pediatric patients were included in our study. Unfortunately, all pediatric patients in our cohort carried the p.R4810K variant in *RNF213*, and therefore we could not analyze the difference between *RNF213*-negative and *RNF213*-positive pediatric patients. The mean age was 7.4 ± 3.2 years at the time of diagnosis. The male to female ratio was 1.5:1 (6 versus 4). Cerebral ischemia was observed in 90% of all patients including transient ischemic symptoms. One patient expressed having a headache. Leptomeningeal collateral flow from the posterior circulation to anterior circulation was observed in one patient. Significant transdural collateral flow from the middle meningeal artery (MMA) was observed in three patients (30%). Finally, 57% of cases were ICA stage 3 or 4. The characteristics of patients are shown in Table 1.

Adult group

A total of 25 adult patients were included in our study, of which 18 carried the *RNF213* p.R4810K variant. The clinical and angiographic characteristics of the patients are shown in Table 1.

The mean age at diagnosis was 45 ± 10.4 years in the *RNF213*-positive group and 51.1 ± 7.7 years in the *RNF213*-negative group. No significant differences were observed in age ($p = 0.443$). Leptomeningeal collateral flow from posterior to anterior circulation was more frequently observed in the *RNF213*-negative group than in the *RNF213*-positive group

Table 1 Patient characteristics

Characteristics	All patients (n = 35)	Pediatric patients (n = 10)	Adult patients (RNF213+) (n = 18)	Adult patients (RNF213−) (n = 7)	p value (adult patients)
Age at diagnosis (years)	35.5 ± 20.0	7.4 ± 3.2	45.5 ± 10.4	51.1 ± 7.7	0.443
Sex					0.355
Male (%)	15 (43)	6 (60)	8 (44)	1 (14)	
Female (%)	20 (57)	4 (40)	10 (56)	6 (86)	
Initial presentation					0.316
Hemorrhage (%)	10 (29)	0 (0)	8 (44)	2 (29)	
Infarction (%)	21 (60)	9 (90)	7 (39)	5 (71)	
Headache (%)	4 (11)	1 (10)	3 (17)	0 (0)	
Collateral flow pattern					
Leptomeningeal (posterior to anterior) (%)	15 (43)	1 (10)	7 (39)	7 (100)	0.020
Bilateral (%)	10 (29)		4 (22)	6 (86)	
Unilateral (%)	5 (14)		3 (17)	1 (14)	
STA collateral (%)	5 (14)	1 (10)	1 (6)	4 (57)	0.012
MMA collateral (%)	12 (34)	3 (30)	8 (44)	1 (14)	0.355
Aneurysm (+) (%)	4 (11)	0 (0)	3 (17)	1 (14)	0.693
PCA involvement (+) (%)	15 (43)	6 (60)	9 (50)	0 (0)	0.027
Bilateral (%)	12 (34)	5 (50)	7 (39)	0 (0)	
Unilateral (%)	3 (9)	1 (10)	2 (11)	0 (0)	
ICA stage					0.564
1 (%)	5 (14)	1 (10)	4 (22)	0 (0)	
2 (%)	10 (29)	6 (60)	3 (17)	1 (14)	
3 (%)	13 (37)	3 (30)	7 (39)	4 (57)	
4 (%)	7 (20)	0 (0)	4 (22)	2 (29)	

Statistical analysis was performed in only adult patients using chi-square test and Fisher's exact probability test

STA, superficial temporal artery; *MMA*, middle meningeal artery; *PCA*, posterior cerebral artery; *ICA*, internal cerebral artery

(100% versus 39%; $p = 0.020$) (Fig. 1). Transdural collateral flow via the superficial temporal artery (STA) was more frequently observed in the *RNF213*-negative group than in the *RNF213*-positive group (57% versus 6%; $p = 0.012$), but the frequency of transdural collateral flow via the middle meningeal artery (MMA) was not significantly different between the two groups. PCA involvement was more frequently observed in the *RNF213*-positive than in the *RNF213*-negative group (50% versus 0%; $p = 0.027$) (Fig. 2). Otherwise, no significant differences were found in ICA stage, presence of aneurysm, sex, or symptoms during initial presentation.

Discussion

The results of our study indicate that *RNF213* is a major susceptibility gene for Korean MMD patients, which has been reported in previous studies [14, 19]. In this study, compared with patients without the variants in *RNF213*, fewer leptomeningeal collateral flow patterns from the PCA to anterior circulation and greater PCA involvement were observed

in patients with *RNF213* variants. Previous studies suggested that more PCA involvement and transdural collateral flow patterns might be found in more advanced stages of moyamoya disease [6, 12, 17, 28]. In our study, there was no statistically significant difference in the ICA stage between *RNF213*-positive and *RNF213*-negative patients, despite having differences in PCA involvement and collateral flow patterns in the two groups. We classified angiographic grade mainly according to their state of steno-occlusive changes of ICA, MCA, and ACA as defined by Mugikura et al. [24]. Suzuki's staging system has been widely used to classify the severity of moyamoya disease [26, 27]. But the classification system was based mainly on serial angiographic changes in intensification and decrease in moyamoya vessels, and sometimes it cannot classify the angiographic findings accurately when ICA and PCA stages are far different [5, 24]. For example, in our study, there are some cases that complete occlusion of both proximal ACA and MCA without PCA involvement of moyamoya vessels. The staging system as defined by Mugikura et al. is more useful in the precise staging in such cases.

Fig. 1 Vertebral angiography of a 49-year-old *RNF213*(−) female patient. Note the leptomeningeal collateral flow to the anterior circulation from posterior circulation without moyamoya involvement of the proximal posterior cerebral artery (PCA). Anteroposterior (**a**) and lateral view (**b**) of vertebral angiography
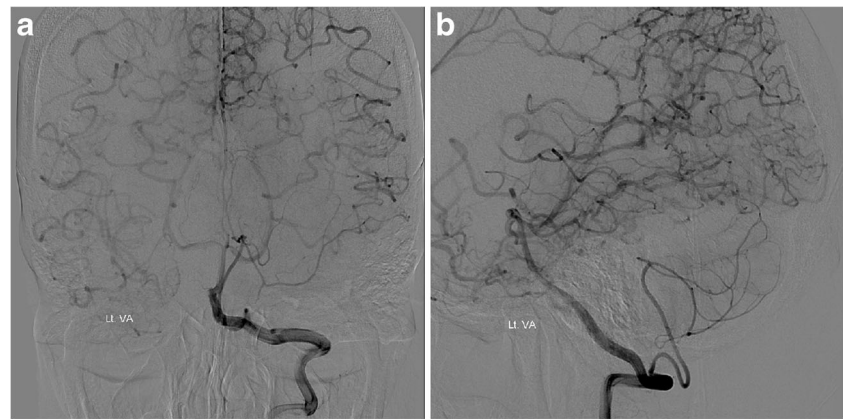
It appears that, in addition to the advanced stage of moyamoya disease, *RNF213* may have a significant role in vascular pathology, such as PCA involvement and angiogenesis in the development of collateral vessels. Tomohito et al. [5, 7] reported that pediatric patients with posterior circulation involvement had significantly fewer advanced ICA lesions than did adult patients. Furthermore, they found that posterior circulation involvement at the initial onset was significantly correlated with poorer outcomes. Moreover, recent animal studies have shown enhanced post-ischemic angiogenesis in mice lacking *RNF213* [9]. The study showed that angiogenesis under chronic ischemic conditions was significantly better in *RNF213* knockout mice. For these reasons, we speculated that greater PCA involvement of moyamoya vessels and more aggressive neoangeogenesis, such as transdural collateral anastomoses rather than leptomeningeal collateral flow, would be found in patients with *RNF213* variants. Yet, we observed that transdural collateral anastomoses via MMA occurred at similar frequencies between the two groups in our study. Our cohort of patients was small, so more studies will be needed in the future.

Previous studies have reported the clinical significance of posterior circulation involvement in MMD. The prevalence of posterior circulation involvement in MMD was reported to be 27–38% [5, 7, 14]. Additionally, some studies have demonstrated that clinical presentation, particularly cerebral

ischemia, is significantly correlated with the severity of posterior circulation involvement in MMD [21, 27].

It appears that not only the severity of steno-occlusive lesions in MMD but also collateral status may influence clinical outcomes. Collateral status, such as leptomeningeal collateral flow from the PCA to anterior circulation and transdural collateral flow, could influence clinical outcomes [5, 15, 23]. Moreover, Armide et al. [25] revealed that the presence of preoperative transdural collaterals significantly correlated with better postoperative angiographic results.

There are several surgical revascularization methods available to treat MMD, including direct revascularization of the STA and middle cerebral artery (MCA) anastomoses [11] and indirect revascularization via encephaloduroarteriosynangiosis (EDAS) [20]. Recent studies have emphasized that combining direct and indirect bypass surgeries produce good outcomes in pediatric and adult patients [2, 8]. We believe that different outcomes after indirect bypass or direct bypass surgery are expected in accordance with the presence of the *RNF213* mutation since different developmental collateral flow patterns were observed in this study. Although statistical analysis was not performed on surgical outcomes in our study due to the lack of postoperative angiographic evaluations, two patients with *RNF213* polymorphisms revealed good angiographic outcomes after indirect bypass surgery (Figs. 3 and 4). We believe

Fig. 2 Angiography of a 46-year-old *RNF213*(+) male patient. Note the prominent transdural collateral anastomoses via left external cerebral artery (**a**). Also note the moyamoya involvement of posterior circulation bilaterally and no significant leptomeningeal collateral flow to the anterior circulation from the posterior circulation was observed (**b**)
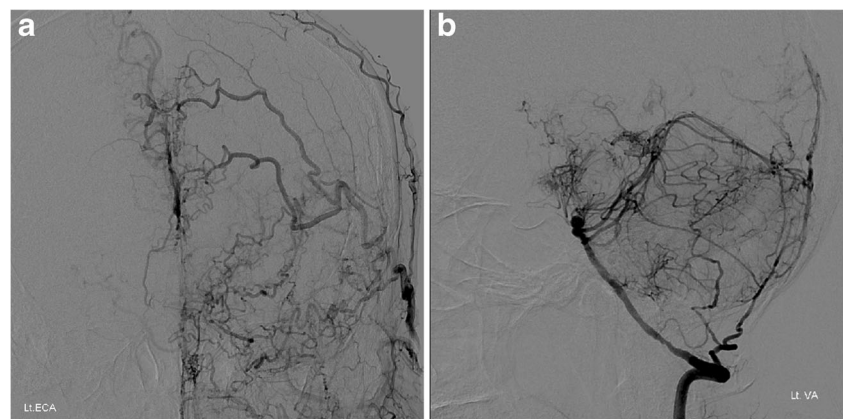
Fig. 3 Angiography of a 36-year-old *RNF213*(+) female patient who had a history of external ventricular drainage surgery via right Kocher's point for intraventricular hemorrhage 10 years ago. Note well-developed transdural anastomoses via burr hole site from the external carotid artery to the anterior cerebral artery (ACA) and middle cerebral artery (MCA) territories. Anteroposterior (**a**) and lateral views (**b**) of external carotid angiography
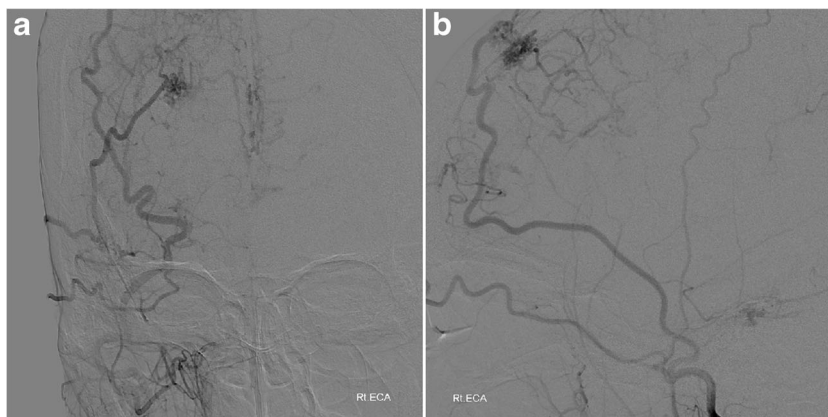
that indirect bypass surgery may be helpful not only in pediatric patients but also in adult patients with the *RNF213* polymorphism. Combined direct and indirect bypass surgeries may produce better outcomes than direct bypass surgery alone in those cases. Future studies will be needed to clarify this point.

There were several limitations in our study. First, the number of patients was small, and the study was retrospective, not randomized, and incorporated data from only one institute. Furthermore, only angiographic findings at initial diagnosis were analyzed, whereas data on perfusion were not analyzed in this study due to a lack of documented clinical results. Second, every pediatric patients involved in this study carried the *RNF213* polymorphism, so we could not analyze the differences. More number of cases will be needed to analyze the differences between *RNF213*-postive and *RNF213*-negative groups in pediatric population. Third, all patients who had undergone surgical revascularization already had operations before checking *RNF213* polymorphism. This means that we did not know whether the patients had *RNF213* polymorphism or not at the time of surgery. Only EDASs were

performed on all patients who had undergone surgical revascularization in this study. Among them, we thought that *RNF213*-positive cases had better postoperative revascularization than *RNF213*-negative cases. But there is no sufficient postoperative angiographic evaluation so we just introduced two cases of *RNF213*-positive cases with good postoperative revascularization. However, we currently take the postoperative angiographic evaluation on the patients and hope to compare the surgical outcomes between *RNF213*-positive and *RNF213*-negative cases in the near future. Nevertheless, we provide key findings that will assist in future efforts to identify the best surgical treatment for MMD patients according to their *RNF213* mutation. Thus, a prospective study is needed.

Conclusions

RNF213 is the major susceptibility gene in MMD patients. Our study indicates that collateral flow patterns are different according to the presence of the *RNF213* mutation. Leptomeningeal collateral flow patterns from posterior circulation to anterior circulation were observed more frequently in the *RNF213*-negative group. Meanwhile, PCA involvement is more common in the *RNF213*-positive group and may have a significant role in the development of collateral anastomoses. We suggest that different outcomes after indirect bypass or direct bypass surgery may be observed in accordance with *RNF213* mutations because different collateral flow patterns were observed in our study. Future research is needed to further elucidate the relationship between *RNF213* variants and collateral flow patterns.

Compliance with ethical standards

Fig. 4 Postoperative angiography of a 55-year-old *RNF213*(+) female patient who underwent encephaloduroarteriosynangiosis in the right hemisphere 5 years ago. Note the prominent, well-developed transdural anastomoses from the middle meningeal artery (MMA) and superficial temporal artery (STA)

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