Association between *RNF213* c.14576G>A Variant (rs112735431) and Peripheral Pulmonary Artery Stenosis in Moyamoya Disease

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Keywords

RNF213 · Polymorphism · Homozygote · Moyamoya disease · Peripheral pulmonary artery stenosis

Abstract

Background: Moyamoya disease (MMD) and peripheral pulmonary artery stenosis (PPAS) are relatively rare and demonstrate steno-occlusive vascular lesions in different organs. Genetic studies identified *RNF213* polymorphism c.14576G>A (rs112735431) as a susceptibility variant for East Asian MMD. *RNF213* polymorphism c.14576G>A is further associated with various vascular lesions of other organs. In this study, we aimed to clarify the incidence and clinical manifestations of PPAS in MMD patients and analyze the correlation between *RNF213* genotype and PPAS. **Methods:** This retrospective case-control study investigated the association between *RNF213* polymorphism and PPAS in 306 MMD/quasi-MMD patients, reviewing the medical charts and imaging records of consecutive patients with MMD admitted from January

2015 to December 2020. **Results:** PPAS was observed in 3 MMD/quasi-MMD patients (0.98%, 3/306). *RNF213* polymorphism c.14576G>A was determined for all 306 MMD/quasi-MMD patients. The incidence of PPAS in *RNF213*-wildtype, *RNF213*-heterozygote, and *RNF213*-homozygote MMD/quasi-MMD patients was 0% (0/101), 0.5% (1/200), and 40% (2/5), respectively. The association between PPAS and homozygote polymorphism of *RNF213* c.14576G>A was statistically significant in MMD/quasi-MMD patients ($p = 0.0018$). In all cases, pulmonary artery hypertension due to PPAS was evident during their childhood and young adolescent stages. Surgical indications for MMD were discouraged in 1 case due to her severe cardiopulmonary dysfunction. **Conclusions:** The homozygote variant of *RNF213* polymorphism c.14576G>A can be a potential predisposing factor for PPAS in MMD/quasi-MMD patients. Despite the relatively rare entity, PPAS should be noted to determine surgical indications for MMD/quasi-MMD patients.

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Introduction

Moyamoya disease (MMD) is a cerebrovascular disease characterized by chronic and progressive stenocclusive lesions at the terminal portion of internal carotid arteries and the development of “Moyamoya vessels” [1, 2]. Although intracranial carotid arteries are preferentially affected in MMD, extracranial vascular involvement, such as stenotic lesions in pulmonary, coronary, and renal arteries, has been reported [3–6].

Genetic studies identified a single-nucleotide polymorphism c.14576G>A (rs112735431) in the *RNF213* gene as a susceptibility variant for East Asian MMD [7, 8]. In particular, the homozygote variant of *RNF213* c.14576G>A is associated with early and severe onset of MMD [9]. Recent studies revealed that *RNF213* polymorphism c.14576G>A is associated with non-MMD stenocclusive lesions in intracranial [10, 11] and coronary arteries [12]. In addition, MMD cases with pulmonary artery stenosis (PPAS) had homozygote polymorphism of *RNF213* c.14576G>A [3–5]. This observation raised the hypothesis that *RNF213* polymorphism c.14576G>A plays essential roles in the development of PPAS in MMD patients. In this study, we aimed to clarify the incidence and clinical manifestations of PPAS in MMD patients and analyze the correlation between *RNF213* genotype and PPAS.

Materials and Methods

This is a retrospective case-control study. This study adhered to the Declaration of Helsinki and was approved by the Ethics Committee of Tohoku University (Approval No. 2016-1-212 and 2018-1-675) and Kohnan Hospital (Approval No. 2020-0520-03). Written informed consent was obtained from all the participants or their guardians.

Diagnosis of MMD/Quasi-MMD and PPAS

We retrospectively reviewed the medical charts and imaging records of consecutive patients with MMD who visited our institution from January 2015 to December 2020. Diagnosis of MMD and quasi-MMD was based on the diagnostic criteria of the Research Committee on Spontaneous Occlusion of the Circle of Wills of the Ministry of Health, Labor and Welfare of Japan [1, 2]. The patients were diagnosed as quasi-MMD, if the patients showed angiographic findings equivalent to MMD but also had comorbidities, such as autoimmune diseases (for instance, rheumatoid arthritis, systemic lupus erythematosus, anti-phospholipid antibody syndrome, and Sjogren syndrome), neurofibromatosis type 1, meningitis, intracranial tumors, Down syndrome, and sickle cell anemia. A total of 533 MMD/quasi-MMD (523 MMD and 10 quasi-MMD) patients were enrolled in this study. Examination of cardiopulmonary systems was conducted when patients manifested signs of

cardiopulmonary dysfunction. PPAS was diagnosed based on the clinical findings, including cardiopulmonary symptoms and the findings of imaging studies such as CT pulmonary angiography, ventilation-perfusion scan, and conventional pulmonary angiography. Peripheral pulmonary artery hypertension (PAH) was defined by a mean pulmonary artery pressure ≥ 25 mm Hg and a pulmonary arterial wedge pressure ≤ 15 mm Hg at rest at right heart catheterization. Indication of balloon pulmonary angioplasty for PPAS was to decrease pulmonary arterial pressure, to relieve of PAH symptoms, and to de-escalate PAH-specific drugs.

Identification of *RNF213* Polymorphism c.14576G>A (rs112735431)

We identified *RNF213* gene polymorphism c.14576G>A (rs112735431) (https://www.ncbi.nlm.nih.gov/projects/SNP/snp_ref.cgi?rs=112735431) for 306 MMD/quasi-MMD (300 MMD and 6 quasi-MMD) patients who agreed with genetic analysis, as described previously [13, 14]. In short, *RNF213* polymorphism rs112735431 was identified using the TaqMan SNP genotyping assay (Assay ID: C_153120198_10; Applied Biosystems, Foster City, CA, USA). Extracted DNA from peripheral blood or saliva was used for analysis. The real-time polymerase reaction was performed on a StepOnePlus real-time polymerase chain reaction system (Applied Biosystems) as follows: primary denaturation at 95°C for 10 min, followed by 40 cycles of denaturation at 95°C for 15 s and subsequent annealing and extension at 60°C for 1 min. The investigators who were blinded to patients' information determined genotypes using Applied Biosystems® TaqMan® Genotype™ software.

Statistical Analysis

All statistical analyses were performed using IBM SPSS (version 24; IBM Corp). The association between *RNF213* polymorphism c.14576G>A and the incidence of PPAS was evaluated by Fisher's exact test with Bonferroni correction. Significance was considered with 2-sided *p* values below 0.0167.

Results

The Association between *RNF213* Genotype and PPAS in MMD/Quasi-MMD Patients

A total of 306 MMD/quasi-MMD patients (300 MMD and 6 quasi-MMD) agreed for genetic testing, which revealed heterozygote and homozygote polymorphism in 1 and 2 MMD/quasi-MMD patients with PPAS, respectively (Table 1). The incidence of PPAS was higher in MMD/quasi-MMD patients with homozygote polymorphism of *RNF213* c.14576G>A (40%, 2/5) than in those without polymorphism or with heterozygote polymorphism of *RNF213* c.14576G>A (0% [0/101] and 0.5% [1/200], respectively) (Table 1). Statistical analysis demonstrated the significant association between homozygote polymorphism of *RNF213* c.14576G>A and PPAS in MMD/quasi-MMD patients ($p = 0.0018$), whereas there is no significant association between PPAS and heterozygote polymorphism of *RNF213* c.14576G>A ($p = 1.000$).

Table 1. Frequency of *RNF213* polymorphism c.14576G>A (rs112735431) in MMD/quasi-MMD patients associated with PPAS

	<i>RNF213</i> -wildtype (<i>n</i> = 101)	<i>RNF213</i> -mutant	
		<i>RNF213</i> -heterozygote (<i>n</i> = 200)	<i>RNF213</i> -homozygote (<i>n</i> = 5)
MMD/quasi-MMD w/PPAS	0%, 0/101	0.49%, 1/200	40.0%, 2/5

MMD, Moyamoya disease; PPAS, peripheral pulmonary artery stenosis.

Table 2. Clinical characteristics of MMD/quasi-MMD patients associated with PPAS

Case	Age	Sex	MMD/ quasi-MMD	<i>RNF213</i> c.14576G>A	Family history	Clinical manifestation	Treatment (MMD)	Treatment (PPAS)
1	19	F	MMD	Homozygote	None	11 Y: cardiac murmur 16 Y: TIA, shortness of breath	Antiplatelet therapy	BPA, prostacyclin agonist
2	56	M	MMD	Homozygote	None	25 Y: bloody sputum 31 Y: headache 43 Y: SAH	Routine follow-up	BPA, prostacyclin agonist
3	34	F	Quasi-MMD	Heterozygote	None	2 Y: TIA, headache Childhood: cardiac murmur 29 Y: shortness of breath	4, 9, 10, 13 Y: revascularization surgery	HOT, BPA, prostacyclin agonist

MMD, Moyamoya disease; PPAS, peripheral pulmonary artery stenosis; F, female; M, male; Y, year-old; TIA, transient ischemic attack; SAH, subarachnoid hemorrhage; BPA, balloon pulmonary angioplasty; HOT, home oxygen therapy.

Clinical Manifestations in MMD/Quasi-MMD Patients with PPAS

Clinical characteristics of 3 MMD/quasi-MMD patients with PPAS are summarized in Table 2. These 3 patients had a family history of MMD/quasi-MMD, of which 2 cases suffered from transient ischemic attacks (cases 1 and 3) and 1 patient had a history of subarachnoid hemorrhage (case 2). Only case 3 underwent revascularization for MMD/quasi-MMD before the manifestation of cardiopulmonary dysfunction. All 3 cases suffered from peripheral PAH due to PPAS when they were children or young adolescents. A prostacyclin agonist was administered, and balloon pulmonary angioplasty was performed in all 3 cases. We conservatively treated MMD in cases 1 and 2, considering the surgical risk due to severe cardiopulmonary dysfunction. Neither ischemic nor hemorrhagic events recurred so far. A representative case (case 1) is shown in Figure 1.

Discussion

This study revealed a significant association between homozygote variant of *RNF213* polymorphism c.14576G>A and PPAS in MMD/quasi-MMD patients. The homozygote variant of *RNF213* polymorphism

c.14576G>A can be a potential predisposing factor for PPAS in MMD/quasi-MMD patients.

All the MMD cases with PPAS in the previous literature studies [3–5] and the present study harbored homozygote polymorphism of *RNF213* c.14576G>A, except for 1 quasi-MMD case with heterozygote polymorphism in the present study. Likewise, a recent prospective study revealed the association among extracranial arteriopathies and *RNF213* polymorphism, especially the homozygote variant [6]. However, it did not include MMD patients associated with PPAS probably due to small number of enrolled cases. These data support the hypothesis that *RNF213* polymorphism c.14576G>A, especially the homozygote variant, can be the predisposing factor for various steno-occlusive vascular lesions [15].

RNF213 mediates various aspects of cellular homeostasis. Experimental studies have revealed the physiological roles of *RNF213* in nonmitochondrial oxygen consumption [16], lipid metabolism [17], and antigen uptake, processing, and presentation [18]. Given the observations that knock-in of the *RNF213* polymorphism c.14576G>A variant itself could not lead to the development of MMD [19], environmental stimuli could potentiate the endothelial dysfunction and proangiogenic activity. Intrinsic chronic inflammatory state can be one of the important environ-

Fig. 1. Representative case: MMD which exhibited pulmonary hypertension due to peripheral pulmonary artery stenosis (case 1). **a** A 16-year-old female patient with frequent transient ischemic attack in her right extremities and exertional dyspnea was diagnosed as MMD and PAH due to peripheral pulmonary artery stenosis. Genetic analysis identified the homozygote polymorphism of *RNF213* c.14576G>A. Decreased cerebral blood flow suggested the need of revascularization surgery, but severe cardiopulmonary dysfunction discouraged us to perform surgery for MMD. We continued antiplatelet therapy for MMD and started prostacyclin agonist. Then, we performed multiple balloon pulmonary angioplasty to

improve PAH. The patient manifested neither further ischemic attacks nor the worsening of cardiopulmonary dysfunction. **a** Right common carotid artery angiography showing the occlusion of the right internal carotid artery and the development of Moyamoya vessels with transdural anastomosis. **b** Left common carotid artery angiography showing slight stenotic lesion at the terminal portion of the left internal carotid artery. **c** Pulmonary angiography showing multiple segmental stenotic lesions in peripheral pulmonary arteries. MMD, Moyamoya disease; PAH, pulmonary artery hypertension.

mental stimuli, as evidenced by high prevalence of co-existing autoimmune diseases [13, 20] and increased levels of circulating inflammatory factors [21–23]. Experimental studies showing the impaired T-cell response and enhanced angiogenic activities in *Rnf213*-mutant mice also support this idea [18, 24, 25]. Despite the need of the further investigations, *RNF213* appears to modulate metabolism and immune systems, which may affect vascular pathology.

Neurosurgical indications should be carefully determined if PAH is evident in MMD patients. Tokunaga et al. [3] reported 1 MMD case who passed away postoperatively due to the severe pneumonia associated with PPAS. This case suggests neurosurgical interventions are challenging once PAH is evident. In the present study, surgical treatment for an ischemic-onset MMD patient (case 1) was not performed due to severe cardiopulmonary

dysfunction. Fortunately, this case remained free from further ischemic attacks after antiplatelet therapy. A thorough follow-up of both MMD and PAH is warranted because the progression of PPAS can affect cerebral ischemia. These observations suggest the necessity of careful management in MMD/quasi-MMD patients with PPAS.

This retrospective study holds several limitations. First, the incidence of PPAS might be underestimated because cardiopulmonary system examination was performed only when the patients developed any symptoms, including cardiac murmur. Second, sampling bias may exist due to the availability of genetic testing and the lack of asymptomatic MMD/quasi-MMD patients in the present study. A future prospective study which screens PPAS in all the MMD/quasi-MMD cases is required to validate the findings of the current study.

Conclusion

There was a significant association between homozygote variant of *RNF213* polymorphism c.14576G>A and PPAS in MMD/quasi-MMD patients. The homozygote variant of *RNF213* polymorphism c.14576G>A can be a potential predisposing factor for PPAS in MMD/quasi-MMD patients. Despite the relatively rare entity, PPAS should be noted to determine surgical indications for MMD/quasi-MMD patients.

Statement of Ethics

This study adhered to the Declaration of Helsinki and was approved by the Ethics Committee of Tohoku University (Approval No. 2016-1-212 and 2018-1-675) and Kohnan Hospital (Approval No. 2020-0520-03). Written informed consent was obtained from all the participants or their guardians.

Conflict of Interest Statement

The authors have no conflicts to declare.

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Author Contributions

H.E., D.O., and R.T. contributed to conception and design. D.O., R.T., H.E., K.T., and M.F. contributed to genetic testing. H.E., K.S., S.T., T.E., K.N., and M.F. contributed to diagnosis and treatment of MMD patients with PPAS. D.O., H.E., R.T., and K.T. contributed to interpretation of the data. R.T. and Y.T. contributed to statistical analysis. D.O. and R.T. contributed to drafting. K.N., M.F., and T.T. contributed to funding acquisition. H.E., S.K., S.T., S.Y., Y.T., T.E., M.F., K.N., and T.T. contributed to critical revision of the manuscript. S.Y. and T.T. contributed to supervision. All authors read and approved the final version of the manuscript.

Data Availability Statement

All data generated or analyzed during this study are included in this article. Further enquiries can be directed to the corresponding author.

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