Significant Association of the *RNF213* p.R4810K Polymorphism with Quasi-Moyamoya Disease

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Background: Quasi-moyamoya disease is an angiographical moyamoya disease equivalent accompanied by known underlying diseases. *Mysterin/RNF213* is a major susceptibility gene for moyamoya disease, of which the p.R4810K variant is a founder polymorphism. The genetics of quasi-moyamoya disease is poorly understood, therefore, this study investigated a potential association between the p.R4810K polymorphism and quasi-moyamoya disease. *Methods:* Genotyping of the p.R4810K variant was performed on 18 quasi-moyamoya disease cases and 91 controls, who visited Kyoto University Hospital or Kobe City Medical Center, Japan, between 2006 and 2015. *Results:* The p.R4810K variant was found in 12 of 18 quasi-moyamoya disease patients. The frequency of p.R4810K carriers was significantly higher in quasi-moyamoya disease cases than in controls (66.7% versus 2.2%, odds ratio 89.0, 95% confidence interval: 19.2-669.4). *Conclusions:* Our data showed that the *RNF213* p.R4810K polymorphism was significantly associated with quasi-moyamoya disease. **Key Words:** Moyamoya disease—genetics—epidemiology—RNF213.

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Introduction

Moyamoya disease (MMD) is a steno-occlusive cerebrovascular disease accompanied by fine collaterals.[1] *Mysterin/RNF213* has been recognized as a major susceptibility gene for MMD, of which the p.R4810K variant (c.14429G>A: rs112735431, simply R4810K hereafter) was found to be a founder mutation common to East Asian patients with MMD.[2,3] The variant was also shown to be associated with other cerebrovascular disease such as middle cerebral artery occlusion.[4] Because all individuals with R4810K do not necessarily have MMD,[3] additional factors, either genetic, epigenetic, or environmental, are likely to be required for the manifestation of the disease phenotype. Quasi-MMD is angiographically equivalent to MMD and has underlying comorbidities such as neurofibromatosis type 1 (NF1), Down syndrome, thyroid disease, cranial irradiation, or sickle cell anemia,[5] suggesting that such comorbidities may have an influence on the progression of MMD. It is therefore important to determine whether R4810K is associated with quasi-MMD and its

comorbidities. Thus, we investigated the association between R4810K and quasi-MMD.

Methods

Study Population

This study was a case–control study based in 2 hospitals. A total of 311 MMD and 23 quasi-MMD cases visited Kyoto University Hospital or Kobe City Medical Center General Hospital (KCGH), Japan, between 2006 and 2015. Of these cases, 18 unrelated quasi-MMD patients agreed to join the study. One patient with Kawasaki disease was previously reported.[6] Diagnosis of quasi-MMD was based on the criteria of the Ministry of Health, Welfare and Labour, Japan.[5] Briefly, when cases were affected with autoimmune diseases, NF1, Noonan syndrome, meningitis, brain tumors, Down syndrome, hyperthyroidism, or other diseases, cases with angiographic characteristics of MMD (terminal portion of intracranial internal carotid arterial stenosis and moyamoya vessels) were diagnosed as quasi-MMD. Angiographic characteristics were diagnosed in 3 patients by magnetic resonance imaging and in 15 patients by magnetic resonance imaging and conventional angiography. Patients with atherosclerosis (i.e., irregular stenotic lesions other than the terminal portion of internal carotid artery with vascular risk factors such as diabetes mellitus or smoking) were not included in the study. Ninety-one Japanese subjects, confirmed as not having characteristics of MMD by angiography, were recruited as controls between 2007 and 2008. The study was approved by the Ethics Committees of Kyoto University and KCGH. Written informed consent was obtained from all participants.

DNA Extraction and Genotyping

Genomic DNA was obtained from peripheral blood samples using the DNA Blood Mini Kit (Qiagen, Hilden, Germany). Genotyping was performed using TaqMan SNP Assays (Applied Biosystems, Foster City, CA) as described previously.[3]

Statistical Analysis

Continuous variables were analyzed using the Mann–Whitney *U*-test and categorical variables using Fisher's exact tests. The association of R4810K with quasi-MMD was calculated using logistic regression based on an autosomal dominant model[3] with or without adjustment for sex. Statistical analyses were performed using JMP Pro Software version 11.2.0 (SAS, Cary, NC). A P value <.05 was considered significant.

Results

Table 1 shows the demographic characteristics of the study population. The female-to-male ratio was not statistically different between cases and controls. The R4810K genotype and clinical characteristics of quasi-MMD patients are shown in Table 2. Fifteen cases had bilateral lesions and 3 cases had a unilateral lesion. Angiographical images of representative cases are shown in Figure 1. The R4810K variant was found in all types of comorbidities, although the frequency was different for each comorbidity. The risk variant (A) was found in 25% (one fourth) of patients with NF1, 50% (two fourths) of patients with hyperthyroidism, 50% (one half) of patients with Down syndrome, and 100% of patients with other disorders. We then tested the association of the R4810K variant with quasi-MMD (Table 3). In the control population, the variant was within Hardy–Weinberg equilibrium (data not shown) and the minor allele frequency was similar to that in Japanese general population.[2,3] The frequency of R4810K carriers was significantly higher in cases than in elder controls without cerebrovascular stenosis (66.7% versus 2.2%, $P = 4.79 \times 10^{-10}$), with a high odds ratio (OR) of 89.0 (95% confidence interval: 19.2-669.4; $P = 2.68 \times 10^{-7}$). The association did not change after adjustment for sex.

Previous studies suggested that R4810K might be associated with a younger age at onset[7,8] and bilateral involvement of MMD.[8] However, there was no apparent association of R4810K with age at onset in our studied population. Among patients with NF1, age at onset was 11 years for those with R4810K, and it was 4, 9, and 35 years for those without the variant. The age at onset for patients with hyperthyroidism with R4810K was 26 and 28 years, and for those without the variant it was 24 and 38 years. However, the mean age at onset in patients with NF1 was lower than in the other patients ($P = .044$).

In terms of association of R4810K with bilateral lesions, 1 patient with NF1 who had R4810K was affected by bilateral quasi-MMD, whereas 2 of 3 patients with NF1 who had wild-type R4810K were affected by unilateral

Table 1. *Clinical characteristics of the study population*

	Quasi-MMD	Controls	*P* value
Number of individuals	18	91	
Age, mean ± SD	34.3 ± 21.7	58.6 ± 10.2	3.61×10^{-11}
Female, n (%)	14 (77.8%)	66 (72.5%)	.78

Abbreviations: MMD, moyamoya disease; SD, standard deviation.