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Short communication

Mutation genotypes of *RNF213* gene from moyamoya patients in Taiwan

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ABSTRACT

Moyamoya disease (MMD) is a disorder characterized by stenosis of bilateral internal carotid arteries with compensatory angiogenesis of the perforating blood vessels. Familial transmission in MMD is common. Recently, mutations in human *RNF213* and *ACTA2* genes were identified to be responsible for MMD. The present study was to determine whether Taiwanese MMD patients carried mutations in these two genes. Of the 36 MMD patients, eleven was found to have *RNF213* mutations. Direct genetic sequencing identified four different *RNF213* mutations in the 11 patients from 8 families: five with a p.R4810K, one with p.A1622V, one with p.V3933M, and the other one with p.R4131C. The latter three represent novel missense mutations. No mutation in *ACTA2* gene was identified. Clinically, cerebral infarction was common in patients with an *RNF213* mutation (9/11). In addition, four mutant patients had developmental delay (4/11) and two had mental dysfunction (2/11). The magnetic resonance angiography of asymptomatic mutant carriers demonstrated high incidence of multiple stenosis of intracranial vessels (3/6, 50%). Since 30.6% (11/36) of Taiwanese moyamoya patients carry an *RNF213* mutation and intracranial arterial stenosis was found in half of the asymptomatic mutant carriers, it is suggested that the *RNF213* mutation should form part of the diagnostic workup for MMD in clinical practice.

1. Introduction

Moyamoya disease (MMD) is a rare cerebrovascular condition characterized by progressive bilateral stenosis or occlusion of the internal carotid artery (ICA) with frequent involvement of the proximal anterior cerebral arteries (ACAs) and middle cerebral arteries (MCAs) [1]. Two major categories of symptoms have been observed: 1) brain ischemia (such as stroke, reversible ischemic neurologic deficits and seizure); and 2) the deleterious consequences of compensatory mechanisms resulting from ischemia (e.g., hemorrhage from fragile collateral vessels and headaches from dilated transdural collaterals). Hemiparesis, dysarthria, aphasia and cognitive impairment are common in MMD patients who have had cerebral infarcts. Patients may also develop seizures, visual defects, syncope, or personality changes. Pediatric patients may develop choreiform movement which is thought to be implicated by the dilated moyamoya-associated collateral vessels in basal ganglia [2,3].

The majority of moyamoya patients are of Asian descent. An epidemiological study in Hokkaido, Japan showed an annual incidence of 0.94 per 100,000 population and a prevalence of 10.5 per 100,000 population [4]. In the same year, a nationwide epidemiology survey in Japan showed that the annual rate of newly diagnosed cases was 0.54 per 100,000 population [5]. In contrast, the incidence of MMD was 0.086 per 100,000 persons in two states of America [6]. A recent nationwide population-base study in Taiwan whose ethnicity is predominantly Chinese showed an annual incidence of 0.15 per 100,000 persons and a prevalence of 1.61 per 100,000 population [7]. Furthermore, approximately 70% of familial moyamoya cases occur in siblings and 24% occur between a parent and offspring [8]. In monozygotic twins, the concordance rate is close to 80% [9]. The racial difference in susceptibility to MMD as well as the familial segregation provides evidences for a genetic predisposition to the disease. Database OMIM reports five distinct loci for MMD associated specific genes (http://www.ncbi.nlm.nih.gov/omim/?term = moyamoya). Among these, mutations in *RNF213* (17q25, MYMY2 locus; Gene ID 57674) and *ACTA2* (10q23, MYMY5; Gene ID 59) were reported to be responsible for MMD in Japanese and European families, respectively [10–14].

In the present study, we recruited 36 Taiwanese moyamoya patients and screened them for mutations in both *RNF213* and *ACTA2* genes.

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Furthermore, the magnetic resonance angiography (MRA) of the asymptomatic mutant carriers was evaluated to see whether a mutation of *RNF213* or *ACTA2* gene would result in changes of the intracranial vessels.

2. Materials and methods

2.1. Subjects and sample preparation

Patients with MMD (n = 36) from the Departments of Neurosurgery and Neurology, National Taiwan University Hospital, Taipei, Taiwan were enrolled consecutively from 2004 to 2013. All patients were confirmed to have MMD by conventional cerebral angiographic studies. MRA study was undertaken to evaluate the intracranial vessels of the family members of the moyamoya patients with gene mutation. Peripheral blood (20 ml), collected in EDTA, was processed within 1 h after sampling.

To eliminate the sequence variation of our moyamoya patients that might represent a natural polymorphism within the Taiwanese population, a group of 500 normal individuals older than 60 years of age were recruited as control subjects to authenticate mutational changes detected in the patient group. The control subjects did not have the past history of cerebrovascular diseases, hypertension, or vascular dementia. Written informed consent was obtained from all participants and all procedures were approved by the Ethics Committee of the National Taiwan University Hospital.

2.2. Mutation screening

The coding exons (with the flanking intronic regions) of *RNF213* and *ACTA2* genes were sequenced. DNA was extracted from the blood using the Genomic DNA Extraction Kit (Geneaid, Taiwan). Polymerase chain reaction (PCR) and sequencing primers for the 68 exons of *RNF213* (Gene ID: 57674) and nine exons of *ACTA2* (Gene ID: 59) were designed using Primer 3 software (sequences available on request). PCR amplification was performed on the GeneAmp® PCR System 2700 thermal cycler (Applied Biosystems, CA, USA) using a reaction mixture (50 μl) containing 2 mM of dNTP (5 μl), 10× PCR buffer (5 μl) (Thermo, MA, USA), 10 μM of each forward and reverse primers, 1.25 U Taq DNA polymerase (Thermo, MA, USA), and DNA template 100 ng. The amplification program involved an initial denaturation at 95 °C for 15 min followed by 25 cycles of 94 °C for 30 s, 60 °C (decreasing by 0.4 °C/cycle) for 30 s and 72 °C for 45 s followed by 12 cycles of 94 °C for 30 s, 50 °C for 30 s and 72 °C for 45 s, and a final extension step at 72 °C for 7 min. The PCR products were purified using the PCR DNA Fragment Extraction Kit (Geneaid, Taiwan) and sequenced using BigDye® sequencing chemistry (version III, Applied Biosystems, CA, USA) on an automated ABI3730™ DNA sequencer (Applied Biosystems, CA, USA).

The DNA samples of the 500 control subjects were analyzed by using the high-resolution melting curve method for each exon and comparing the curve characteristics with the mutant sequence. The amplicons were saturated with LCGreen® Plus dye (BioFire Diagnostics, Salt Lake City, UT, USA) and their melting curve characteristics were analyzed on the Idaho Technology LightScanner® Instrument (Hi-Res Melting®, Idaho, USA).

2.3. Statistical analysis

Comparison among the groups was performed using the nonparametric Student *t* test method. Data were considered to be statistically significant with $p < 0.05$.

3. Results

3.1. Clinical characteristics

Thirty six moyamoya patients were enrolled in this study. Twenty-nine of the 36 patients (80.6%) were younger than 18 years of age and, while not statically significant, there were more females than males (M:F = 14:22, Table 1). The clinical features included hemiparesis (77.8%), migraine or headache (27.8%), speech disturbance (16.7%), and sensory deficits (13.9%). In addition to ICA stenosis, the steno-occlusive features of the great vessels were also found at bilateral ACAs and MCAs in 88.9% and 83.3% of the patients, respectively (Table 1). Eight of 36 patients (22.2%) had a positive family history of MMD.

3.2. Mutation screening

Eleven of the 36 moyamoya patients carried a missense mutation in *RNF213* (Table 2). There are familial relationships among patient-MY3 and -MY3.3 (sisters), patient-MY6 and -MY6.1 (mother and son), and patient-MY34 and -MY34.5 (brothers). Six of the eleven patients had the same missense mutation (c.14429G>A) that is predicated to cause a p.R4810K amino acid change and has previously been reported in other Asian moyamoya patients [10]. We also identified three novel mutations, which are p.A1622V (c.4865C>T), p.V3933M (c.11797G>A), and p.R4131C (c.12391C>T) (Figs. 1 and 2, and Table 2). The p.V3933M (family MY34) and p.R4131C (family MY3) mutations were subsequently shown to be segregated among members established a genetic basis for the disease associated with these changes (Figs. 1 and 2). In family MY34, the intracranial vessels were affected in three members (II.2, III.1, and III.2, Fig. 1C). Typical moyamoya vessels were seen in patient-III.1 and -III.2. Marked stenotic change of unilateral MCA was noted in patient II.2. However, the MRA was normal in carrier I.2 (Fig. 1C). Both Valine at amino acids 3933 and Arginine at 4131 are conserved throughout evolution (Fig. 2). This p.V3933M mutation further strengthens the link between the genetic change and vascular pathology. Familial segregation could not be established for the p.A1622V mutation in patient MY17 owing to a lack of parental DNA and neither is Alanine conserved across vertebrate species. Thus the relationship between this sequence change and the disease in MY17 could not be established beyond doubt. However, neither p.A1622V nor the other two variants (p.V3933M and p.R4131C) were detected in 1000 genome database (http://www.1000genomes.org/) as well as our control group of 500 healthy individuals using high-resolution melting curve analytical methodologies represents an allele frequency of less than 0.1%. We did not find sequence variation of *ACTA2* gene in our moyamoya patients.

Clinically, all patients with an *RNF213* mutation suffered from transient or repeated motor paresis and most of them had cerebral

Table 1
Demographic data for the 36 moyamoya patients.

Patient	No. (%)
Age	36 (100)
≧18 y/o	7 (19.4)
<18 y/o	29 (80.6)
Sex (M:F)	14:22 (38.9:61.1)
Clinical features	
Hemiparesis	28 (77.8)
Headache or migraine	10 (27.8)
Speech disturbance	6 (16.7)
Sensory deficits	5 (13.9)
Seizure	4 (11.1)
Consciousness disturbance	3 (8.3)
Movement disorder	2 (5.6)
Mental abnormalities	1 (2.8)
Gait disturbance	1 (2.8)
Involved vessels	
Bilateral ACAs	32 (88.9)
Bilateral MCAs	30 (83.7)
One ACA	4 (11.1)
One MCA	6 (16.7)
Family history	8 (22.2)

No. = number, M = male, F = female, ACA = anterior cerebral artery, MCA = middle cerebral artery.