Journal of Clinical Neuroscience 22 (2015) 479–482

Contents lists available at ScienceDirect

Journal of Clinical Neuroscience

journal homepage: www.elsevier.com/locate/jocn

Clinical Study

Association between matrix metalloproteinase-3 gene polymorphism and moyamoya disease

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ARTICLE INFO

Article history:
Received 1 July 2014
Accepted 30 August 2014

Keywords:
MMP
Moyamoya disease
Polymorphisms

ABSTRACT

Genetic factors play an important role in the etiology and pathogenesis of moyamoya disease (MMD). Recently, several studies suggested the decreased expression of matrix metalloproteinase-3 (MMP3) was associated with an increased risk of MMD. This case-control study was performed to examine the association between MMP3 polymorphisms and the risk of MMD, comparing 86 Han Chinese MMD patients and 86 controls. We further conducted a meta-analysis, combining our results with all previous studies to provide a more precise estimate of this association. In our case-control study, MMP3 6A/6A (odds ratio [OR] = 1.93, 95% confidence interval [CI] 1.00–3.72; $p = 0.05$) and 6A allele frequencies (OR = 1.78, 95%CI 1.00–3.14; $p = 0.05$) in the MMD group were significantly higher than those in the control group. In the additional meta-analysis, only two other studies were identified. Meta-analysis with a total of 796 patients revealed 6A allele and 6A/6A genotype significantly increased the risk of MMD (OR = 1.64, 95% CI 1.26–2.13, $p = 0.0002$ and OR = 1.79, 95% CI 1.32–2.42, $p = 0.0002$, respectively). To confirm this finding, an additional analysis should be performed using a larger sample size. Moreover, larger and well-designed multicentric studies based on different races should be performed to evaluate the racial difference.

1. Introduction

Moyamoya disease (MMD) is an uncommon cerebrovascular disorder that is characterized by progressive stenosis of the intracranial internal carotid arteries and their proximal branches. Epidemiologically, MMD is most prevalent in East Asia with estimated rates of 0.94 per 100,000 in Japan and 0.43 per 100,000 in China [1–3]. Other factors, such as a family history of the disease and sex, are also associated with MMD. These phenomena imply that genetic factors may play an important role in the pathogenesis of MMD [4,5].

MMD is an important cause of cerebral stroke in children and adults. Generally, most children with MMD develop transient ischemic attack (TIA) or cerebral infarction, whereas about half of MMD adult patients develop intracranial bleeding, and half develop TIA or cerebral infarction or both. In either case, MMD might lead to irreversible and devastating neurological deficits and intellectual impairments [6–8]. Therefore prompt diagnosis and appropriate management are crucial to improve the long-term prognosis of patients, and identification of genetic variants associated with MMD may contribute to screening programs for individuals at risk of developing MMD.

Matrix metalloproteinases (MMP) are prime candidates for harboring genetic variants that contribute to the development or variable progression of MMD, as they actively degrade the extracellular matrix, playing a major role in the modulation of portal-based fibrosis and physiologic and pathologic remodeling of tissues [9,10]. One of the possible variations to contribute to MMD is MMP3. MMP3, also called stromelysin-1, is known to lyse basal membrane collagen and induce the synthesis of other MMP. MMP3 harbors a well-characterized insertion/deletion variant at the 1171 position of the MMP3 promoter region, characterized by the presence of either five or six adenine residues, commonly referred to as 5A or 6A. The 5A allele has been shown to result in higher MMP3 expression because of disrupted binding of a nuclear factor kappa B dimer [11–13]. Several studies have reported the decreased expression of MMP3 is associated with an increased risk of MMD, which can be caused by the MMP3 5A/6A variation [14,15]. However, the effect of MMP3 5A/6A polymorphism on susceptibility to MMD is still uncertain.

We performed a case-control study to investigate the possible impact of MMP3–1171 5A/6A polymorphisms on susceptibility to MMD. Moreover, we further conducted a meta-analysis, combining

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http://dx.doi.org/10.1016/j.jocn.2014.08.034
0967-5868/

our results with all previous studies to provide a more precise estimate of the associations between MMP3 and MMD.

2. Material and methods

2.1. Study population

The Ethics Committee of the West China Hospital of Sichuan University approved the study protocols, and all the participants gave written informed consent according to the Declaration of Helsinki. Peripheral blood specimens and demographic, medical, and family histories were obtained from 86 consecutive, unrelated patients at the West China Hospital of Sichuan University, China. The diagnostic criteria for MMD were based on the criteria from the Japanese Research Committee on Moyamoya disease of the Ministry of Health, Welfare and Labour [16]. Briefly, diagnosis was made by use of digital subtraction angiography and/or magnetic resonance angiography. The control group consisted of 86 unrelated, age-matched volunteers recruited from individuals admitted to the hospital for any reason other than neurological diseases. To minimize the genetic heterogeneity, subjects were ethnically limited to the Chinese Han population. Written informed consent was obtained from each eligible participant prior to enrollment.

2.2. DNA extraction and genotyping

Peripheral venous blood from each participant was collected and used for the isolation of genomic DNA using a commercial kit (Wizard DNA Purification Kit; Promega, Madison, WI, USA). The MMP3 genotype was determined by the polymerase chain reaction (PCR)–restriction fragment length polymorphism assay. The PCR primers were as follows: forward 5′-GGAATTCACATCA CTGCCACCAC-3′ and reverse 5′-CGGCACCTGGCCTAAAGACATT-3′. The PCR reaction began with an initial denaturation step for 5 minutes at 95°C, followed by amplification for 35 cycles at 94°C for 1 minute, 55°C for 1 minute, 72°C for 1 minute, and a final extension at 72°C for 5 minutes. Digestion was performed at 37°C in 15 mL using 10 mL of the PCR product and 3 U of restriction endonuclease for 2 hours. The size of the digestion products was determined by agarose gel electrophoresis.

2.3. Study selection and inclusion criteria for meta-analysis

To further investigate the association between MMP3 and MMD, a meta-analysis based on published studies was carried out. A systematic literature search was performed using the PubMed, MEDLINE, EMBASE, China National Knowledge Infrastructure and Chinese Biomedicine databases from 1980 to April 2014, using a search strategy based on combinations of the keywords "MMP" or "polymorphism" or "genotype" and "Moyamoya Disease" or "MMD." Studies were selected when they met the following inclusion criteria: (1) it was a case-control study conducted to evaluate the association between MM3 polymorphism and the risk of MMD; (2) sufficient genotype data were presented to calculate the odds ratios (OR) and 95% confidence intervals (CI); and (3) the paper clearly described the diagnosis of MMD and the sources of cases and controls.

2.4. Statistical analysis

Statistical analysis in our case-control study was carried out using the Statistical Package for the Social Sciences version 15.0 (SPSS, Chicago, IL, USA). Deviations from Hardy–Weinberg equilibrium were tested using a simple chi-squared goodness-of-fit test. Differences between continuous variables were assessed by the Student's *t*-test, whereas those between categorical variables were evaluated using Pearson's chi-squared test. The existence of differences in allelic and genotypic frequencies between different groups was assessed by calculating the OR with 95% CI. Two-sided *p* values were used, with *p* values of less than 0.05 considered statistically significant. The meta-analyses were performed using STATA software (version 11.0; STATA, College Station, TX, USA) for each pooled summary estimate using Cochran's Q statistic and the I^2 statistic, respectively. Meta-analysis was performed via effect model if there was no evidence of statistical heterogeneity.

3. Results

The clinical and demographic data of patients with MMD and controls are listed in Table 1.

Sex distribution and age was similar between cases and controls. The distribution of all genotypes was within Hardy–Weinberg equilibrium. Most MMD patients had an initial presentation of cerebral ischemia or cerebral hemorrhage. Three patients presented with seizures, one with headache, and one was diagnosed incidentally.

The genotype results are presented in Table 2. MMP3 6A/6A (OR = 1.93, 95% CI 1.00–3.72; $p = 0.05$) and 6A allele frequencies (OR = 1.78, 95% CI 1.00–3.14; $p = 0.05$) were significantly higher in the MMD group than in the control group.

4. Meta-analysis

After detailed full article evaluation, two studies met the entry criteria. Both of them were conducted in China. Thus, two studies and our data were included for analysis with a total of 796 patients. The meta-analysis revealed one copy of the 6A allele significantly increased the risk of MMD (OR = 1.64, 95%CI 1.26–2.13, $p = 0.0002$) (Fig. 1). The risk of MMD was higher in the 6A/6A genotype *versus* the genotypes of 6A/5A and 5A/5A (OR = 1.79, 95%CI 1.32–2.42, $p = 0.0002$). The dominant model showed a greater risk of developing MMD (OR = 1.63, 95%CI 0.70–3.76, $p = 0.25$ for 6A/6A+ 6A/5A *versus* 5A/5A). No heterogeneity was found among the studies.

5. Discussion

The concept of genetic factors being involved in the development of MMD has led to many studies on possible genetic determinants for MMD in the last decade. A genetic meta-analysis showed that there was a likely association between ring finger protein (RNF)-213 gene polymorphism and MMD [17]. Other possible genetic variants included those of vascular endothelial growth factor, basic fibroblast growth factor, hepatocyte growth factor, transforming growth factor beta 1, granulocyte colony-stimulating factor, platelet-derived growth factor receptor beta, MMP and

Table 1
Characteristics of patients with moyamoya disease and controls

	MMD	Controls
Subjects, n	86	86
Males, n (%)	45 (52.3%)	40 (46.5%)
Mean age, years (±SD)	42.9 ± 6.8	41.0 ± 9.2
Symptoms at onset, n (%)		
Ischemia	44 (51.2%)	
Hemorrhage	37 (43.0%)	
Other	5 (5.8%)	

MMD = moyamoya disease, SD = standard deviation.