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Clinical Study

Association between the rs112735431 polymorphism of the RNF213 gene and moyamoya disease: A case-control study and meta-analysis

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ABSTRACT

Ring finger protein 213 (RNF213) gene polymorphisms are thought to be significant in the etiology and pathogenesis of moyamoya disease (MMD). Due to the rarity of MMD patients, their ethnic diversity, and the use of varying methodologies, studies of the association between these polymorphisms and MMD have not been repeatable. This lack of reproducibility affects the strength of the conclusions drawn from their results. We conducted the present case-control study and meta-analysis to provide more precise estimates of the association between the rs112735431 (c.14576G>A) polymorphism and the risk of MMD. A total of 81 MMD patients and 100 healthy controls were enrolled in our case-control study. The RNF213 rs112735431 (c.14576G>A) polymorphism was genotyped using Sanger sequencing after amplification with polymerase chain reaction (PCR). The genetic algorithm (GA) genotype and A allele frequencies of RNF213 rs112735431 (c.14576G>A) (odds ratio, OR = 7.10, 95% confidence interval, CI = 1.51–33.43, $p = 0.006$; OR = 9.37, 95% CI = 2.10–41.84, $p < 0.001$, respectively) were significantly higher in the MMD group than those in the control group. In our meta-analysis, we assessed a total of eight case-control studies, including 985 patients and 2335 controls. Pooled ORs indicated a significant association between the presence of the rs112735431 (c.14576G>A) polymorphism and MMD risk (dominant model: OR = 74.55, 95% CI = 35.86–154.98, $p < 0.00001$). Subgroup analysis based on country and sensitivity analysis verified these results. Our case-control study and meta-analysis both provide evidence of an association between the rs112735431(c.14576G>A) polymorphism in the RNF213 gene and MMD risk.

1. Introduction

Moyamoya disease (MMD), the etiology of which is unknown, is a chronic cerebral vascular disorder that is predominantly characterized by progressive stenosis of the intracranial internal carotid arteries and their proximal branches. This stenosis results in collateral circulation formation (including the typical "puff of smoke" vessels) [1,2]. The prevalence of MMD is low, and it has a worldwide distribution [3]. MMD is most prevalent in East Asian countries, including Japan [4], Korea [5] and China [6]. The global distribution of age at MMD onset shows two peaks: one at 5 to 9 years of age and another at approximately 40 years of age [2]. Generally, most children with MMD develop transient ischemic attack or cerebral infarction, while adults develop intracranial hemorrhage [4,6]. The majority of MMD cases manifest sporadically, although 10% to 15% of patients have family histories of the condition [7]. Growing evidence suggests that genetic factors may be important in the etiology and pathogenesis of MMD [8–10].

A previous genome-wide association study identified RNF213 as the first MMD-related gene [8]. Following this discovery, several epidemiological studies have evaluated the relationship between RNF213 gene polymorphisms and MMD in many countries. The results from these studies have indicated a strong association between RNF213 genotype, particularly with respect to the rs112735431 (c.14576G>A) polymorphism, and MMD. Interestingly, this mutation does not appear to occur in Caucasians with MMD [11]. The rs112735431 (c.14576G>A) RNF213 gene polymorphism has varying distributions in East and Southeast Asian populations. This variability suggests that MMD can possess regional characteristics [12]. Although the RNF213 gene has been widely researched in relation to MMD, the results from such studies cannot be extrapolated because of biases associated with different races and regions. To address these considerations and reinforce the results of previous research, we conducted a case-control study of a Chinese population and a meta-analysis of the association

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between the RNF213 rs112735431 (c.14576G>A) polymorphism and MMD susceptibility.

2. Materials and methods

2.1. Study population

A total of 81 consecutive, unrelated adult patients who were admitted to our hospital with a diagnosis of MMD were enrolled in this study from October 2013 to July 2015. The diagnostic criteria for MMD were based upon previously reported criteria from the Japanese Research Committee on Moyamoya Disease [13]. The control group consisted of 100 unrelated, age- and sex-matched healthy volunteers from our medical center. Details of all of the baseline characteristics that were collected are elucidated in the *Methods* section. These included age, sex, family history and other related clinical dates, all of which were collected via medical records and questionnaire surveys. This study was approved by the Ethics Committee of the First Affiliated Hospital of Guangxi Medical University, and all participants provided written informed consent according to the Declaration of Helsinki.

2.2. DNA extraction and genotyping

Genomic DNA was extracted from peripheral blood using a DNA blood kit (Sangon Biotech, Shanghai, China) according to the manufacturer's instructions. The RNF213 rs112735431 (c.14576G>A) polymorphism was genotyped by Sanger sequencing after amplification via polymerase chain reaction (PCR). The following PCR primers were used: forward 5′-CTGCATCACAGGAAATGACACTG-3′ and reverse 5′- TGACGAGAAGAGCTTTCAGACGA-3′. Each PCR reaction was performed in a 40 μl volume containing 1.0 μl template, 20 μl 2× GoldStar Best Master Mix, 1.0 μl of each primer, and 17 μl ddH_2O. The following program was used in a thermal cycler: 5-min initial denaturation at 95 °C; 35 cycles consisting of 95 °C for 30 s, 58 °C for 30 s, and 72 °C for 30 s; and a 7-min final extension step at 72 °C. The PCR products were visualized on a 1.2% agarose gel (Agarose LE, Sangon Biotech) and then subjected to direct sequencing. All fragments were sequenced using an ABI 3130XL Avant Genetic Analyzer (Applied Biosystems Inc., Foster City, CA, USA). The sequencing results were analyzed with Chromas software (Technelysium Pty Ltd., South Brisbane, QLD, Australia) with signal/noise set to >98%. For quality control, genotyping analysis was performed blinded to knowledge of the subjects. Randomly selected PCR-amplified DNA fragments (approximately 5% of the samples) were also examined by DNA sequencing to validate the genotyping. The results were 100% concordant.

2.3. Study selection and inclusion criteria for meta-analysis

To further investigate the association between RNF213 and MMD, a meta-analysis of published studies was conducted. We searched the electronic databases of PubMed, EMBASE, Web of Science, Wanfang Date and the China National Knowledge Infrastructure (NCKI), which was last updated on July 11, 2015 at the time of this writing. We identified all publications on the association between the RNF213 gene rs112735431 (c.14576G>A) polymorphism and MMD susceptibility. We used various combinations of customized terms and the Medical Subject Headings (MeSH)-indexed terms "Moyamoya Disease" and "polymorphism". No restrictions on country or publication language were applied. For inclusion in the meta-analysis, studies had to (1) clearly describe the diagnosis of MMD, (2) apply a case-control design and clearly describe the sources of cases and controls, (3) analyze the relationship between the RNF213 gene rs112735431 (c.14576G>A) polymorphism and MMD, (4) present sufficient genotype data to calculate odds ratios (ORs) and 95% confidence intervals (CIs). If two or more studies had partly overlapping participants, the study with the larger sample size was selected. Major reasons for exclusion of studies were as follows: (1) duplicated studies, (2) review articles, (3) case reports, (4) animal models and (5) limited sample size.

2.4. Quality score assessment

We evaluated the quality of each eligible study using the Newcastle-Ottawa Scale (NOS), a widely used method in case-control studies [14]. To adapt the scale to study genetic associations, we made the following modifications: (1) all cases chosen had a clear and reliable method; (2) the cases in a study represented a specific population; (3) the control group source was clear; (4) the control group matched the case group; (5) there was sufficient sample quantity; (6) the same method was used to measure cases and controls; (7) the control group met Hardy-Weinberg equilibrium (HWE); (8) the age, race and family history of each case was considered when the study was designed or the data were analyzed. Scores ranged from 0 (worst) to 9 (best) based upon the above conditions. Studies with scores >6 were considered high quality. Discordance was resolved as mentioned above until a consensus was reached.

2.5. Statistical analysis

Statistical analyses were conducted using SPSS software (version 16.0) (SPSS, Chicago, IL, USA) and Review Manage 5.3 (The Nordic Cochrane Centre, The Cochrane Collaboration, 2014, Copenhagen N, Denmark). Distributions of genotype and allele frequencies between patients and controls were tested using the chi-squared (chi^2) test or Fisher's exact test, as appropriate. ORs and corresponding 95% CIs were calculated to characterize the association of the RNF213 polymorphism with MMD. We determined HWE results using a web-based program (http://www.oege.org./software/hwe-mr-calc.shtml). Two-sided p values less than 0.05 were considered statistically significant. To evaluate country-specific effects, subgroup analysis was performed according to the nationality of the study population. We used Cochran's Q statistic and I^2 statistics to assess and quantify statistical heterogeneity for each pooled summary. Substantial heterogeneity was considered to exist when $I^2 > 50\%$ and $p < 0.1$. Meta-analysis was performed via a fixed effects model if there was no evidence of statistical heterogeneity. Otherwise, a random effects model was chosen.

3. Results

3.1. Case-control study

A total of 81 patients and 100 healthy controls were enrolled in this study. Their demographic data are summarized in Table 1.

The genotype distribution for the controls was in accordance with HWE ($p = 0.919$). Genotype results are presented in Table 2. RNF213 rs112735431 (c.14576G>A) GA frequencies were significantly higher in the MMD group than in the control group (OR = 7.10, 95% CI: 1.51–33.43; $p = 0.006$). Considering the rarity of the AA genotype, we combined the AA and GA genotypes to form a dominant model in which rs112735431 (c.14576G>A) was associated with a significantly increased risk of MMD (OR = 8.52, 95% CI: 1.85–39.29; $p = 0.006$). When using a recessive model, no significant association was observed.