Journal of Clinical Neuroscience 21 (2014) 1740–1743

Contents lists available at ScienceDirect

Journal of Clinical Neuroscience

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

Clinical Study

Variant rs1906591 on chromosome 4q25 confers increased risk of cardioembolic stroke in Chinese patients

Man Luo [a,b,1], Jiaoxing Li [a,1], Rong Lai [a,1], Xunsha Sun [a], Yufang Wang [a], Xiaowei Xu [c], Wenli Sheng [a,*]

[a] Department of Neurology, First Affiliated Hospital, Sun Yat-Sen University, Guangzhou 510080, China
[b] Department of Neurology, First Affiliated Hospital, Guangxi Medical University, Nanning, China
[c] Department of Neurology, Weifang People's Hospital, Shandong, China

ARTICLE INFO

Article history:
Received 10 October 2013
Accepted 23 December 2013

Keywords:
Cardioembolic stroke
Chinese
Ischemic stroke
4q25

ABSTRACT

Ischemic stroke (IS) is a heterogeneous multifactorial disorder caused by both genetic and environmental factors. A genome-wide association study on stroke in Caucasians identified a variant on chromosome 4q25 that is significantly associated with IS, with the strongest risk for cardioembolic stroke (CES). The current study aims to investigate the association of the rs1906591 variant on 4q25 with IS through a case-control study in a Chinese Han population. A total of 712 IS patients and 774 control subjects were involved in the current research. Stroke subtyping was performed according to the Trial of Org 10172 in Acute Stroke Treatment criteria. The genotypes were determined using the SNaPshot technique. The association of the genotypes with the risk of IS was estimated using logistic regression analysis. The rs1906591 single nucleotide polymorphism variant was associated with the CES subtype in both recessive and additive models (recessive model: odds ratio [OR] = 2.58, 95% confidence interval [CI] 1.47–4.53, $p = 0.001$, adjusted OR = 2.71, 95% CI 1.48–4.96, $p = 0.001$; additive model: OR = 2.50, 95% CI 1.19–5.25, $p = 0.015$, adjusted OR = 2.83, 95% CI 1.24–6.50, $p = 0.013$). This result indicates that patients with the AA genotype have a higher rate of CES than other genotypes. However, the rs1906591 variant was not significantly associated with the overall incidence of stroke or other stroke subtypes. The rs1906591 variant is significantly associated with CES in the Chinese Han population, but not with other stroke subtypes.

1. Introduction

Stroke is one of the three most common causes of death, and a major cause of chronic disability in adults worldwide [1]. The situation is more serious in China, which has a population of 1.4 billion. The annual stroke mortality rate is approximately 157 per 100,000 which exceeds that of heart disease, making stroke the leading cause of death and adult disability [2]. China has 2.5 million new stroke cases each year and 7.5 million stroke survivors [2].

Ischemic stroke (IS), which accounts for 80% of all strokes, is a heterogeneous multifactorial disorder caused by both genetic and environmental factors and their interactions [3]. Studies in twins and families suggest that genetics significantly influence IS [4]. Stroke has several subtypes, including cardioembolic stroke (CES), large vessel disease, small vessel disease, and those resulting from undetermined or other causes [5]. Genetic predisposition may differ for these subtypes [6].

A genome-wide association study (GWAS) on stroke in Caucasians has identified that the rs2200733 variant on chromosome 4q25 is significantly associated with IS, with the strongest risk for CES [7]. The variant had been initially shown to associate with atrial fibrillation (AF) in a GWAS in patients with European ancestry [8]. Many replication studies have identified the association of this variant with AF in several populations with European ancestry, a Hong Kong Chinese population, and mainland Chinese Han [9–11]. Studies on the association of the rs2200733 variant and IS are few, and most of them are limited to Caucasian populations. The 4q25 haplotype block consists of approximately 1.5 million base pairs without any known genes [12]. The potential mechanism underlying the effects of this noncoding region remains unclear. It is interesting that the closest gene, located approximately 150 kilobases away from the variant, is the paired-like homeodomain transcription factor 2 (*PITX2*) which is involved in early cardiac development [8].

* Corresponding author. Tel.: +86 20 8733 1089; fax: +86 20 8775 5064.
E-mail address: shengwl@mail.sysu.edu.cn (W. Sheng).
[1] These authors have contributed equally to the manuscript.

http://dx.doi.org/10.1016/j.jocn.2013.12.034
0967-5868/

A replication study that did not consider stroke subtypes failed to show an association of rs2200733 with IS in the Chinese Han population [9]. Given that the rs2200733 variant is associated with AF [8], which in turn was related to most patients with CES, the 4q25 locus may be associated with AF and indirectly with CES [12]. Thus, classifying IS patients by subtype is important. In the present case-control study, we evaluated whether the rs1906591 variant, which is in full linkage with rs2200733 [12], confers a risk for IS and/or CES in the Chinese Han population.

2. Methods

2.1. Study population

This study was hospital-based in design. A total of 712 IS patients admitted to the Department of Neurology at the First Affiliated Hospital of Sun Yat-Sen University from September 2010 to December 2011, who were local residents of southern China, were involved in the current research. Confirmation of stroke was based on the results of strict neurological examination, CT scan, or MRI according to the International Classification of Diseases (10th Revision, code I63). Stroke subtyping was performed according to Trial of Org 10172 in Acute Stroke Treatment (TOAST) criteria [5] by two independent stroke neurologists blinded to genotype. The TOAST classification categories are: (1) large artery occlusive disease (large vessel disease), (2) CES, (3) lacunar stroke (small vessel disease), (4) other determined causative factor, and (5) undetermined (causative agent unknown despite diagnostic efforts, two possible causes, or incomplete evaluation). Control subjects were recruited randomly from the same geographical population and had no relevant neurovascular history. Both patients and controls were of Chinese Han descent. Exclusion criteria include other types of cerebrovascular diseases (transient ischemic attack, intracranial hemorrhage, subarachnoid hemorrhage, cerebral aneurysms, and cerebrovascular malformation) and severe systemic diseases such as tumors, severe inflammatory diseases and serious chronic diseases. This study was approved by the Ethics Committee of the First Affiliated Hospital of Sun Yat-sen University, and was conducted according to the Declaration of Helsinki principles. All participants signed the informed written consent form provided.

2.2. Clinical characteristics

At enrollment, age, sex and vascular risk factors were recorded according to a self-designed questionnaire. In addition to a history of ischemic heart disease (IHD), hypertension, diabetes mellitus (DM), or hyperlipidemia history or a family history of IS, the following vascular risk factors were also recorded: body mass index (BMI), systolic blood pressure, diastolic blood pressure, total plasma cholesterol, triglycerides, blood glucose, and cigarette smoking status. Cigarette smoking was categorized as never, former or current smoking (at least one cigarette per day).

2.3. Genotyping

Genomic DNA was isolated from 0.3 ml blood samples using TIANamp Blood DNA Kit (Tiangen Biotech, Beijing, China). The variant, rs1906591, was genotyped using the SNaPshot Multiplex Kit (Applied Biosystems, Foster City, CA, USA), as described by Lian [13]. The allelic reads from the two variants were then translated to rs1906591 genotypes (AA, AG, and GG). All genotyping personnel were blinded to clinical and neuroimaging data. The forward primer sequence used for polymerase chain reaction (PCR) was AAAATACCTTCATGCGGTGAAGAA, and the reverse PCR primer was TCAGAAATTCCATCAAAGGACCA. The primer sequence for minisequencing extension was TTTTTTTTTTTTTCTGCCTTTTCATTTCCCA. The fluorescently labeled fragments were resolved by capillary electrophoresis on an ABI Prism 3130XL genetic analyzer (Applied Biosystems). The resulting data were analyzed with GeneMapper 4.0 software (Applied Biosystems).

2.4. Statistical analysis

Student's *t*-test was used to compare age, blood pressure, cholesterol, triglycerides, and glucose levels between groups. The Mann–Whitney U test was used to compare BMI between groups. Comparisons between groups were conducted using the χ^2 test for the frequencies of sex, cigarette smoking, IHD, hypertension, DM, hyperlipidemia, rs1906591 genotype and A allele frequency. Hardy–Weinberg equilibrium was confirmed by χ^2 test. The association of the rs1906591 genotype or A allele with the risk of stroke was estimated using univariate logistic regression analysis. Multivariate logistic regression analysis was performed to adjust for potential confounding factors. Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS, Chicago, IL, USA) version 16.0. All tests were two-tailed, and a p value of <0.05 was considered significant.

3. Results

3.1. Study population

This study included 712 IS patients and 774 controls. There were 327 (45.9%) patients with large vessel disease, 221 (31.0%) patients with small vessel disease, and 54 (7.6%) patients with CES. The basic characteristics of the study subjects are shown in Table 1. The mean age ± standard deviation was 51.5 ± 16.9 years in controls and 65.2 ± 13.9 years in patients. Men accounted for 54.0% of controls and 65.3% of patients. As expected, the patient group had a higher prevalence of conventional risk factors for IS, including advanced age, higher blood pressure, higher glucose, cigarette smoking, family history of IS, IHD, DM, hypertension, and hyperlipidemia.

3.2. Distribution of rs1906591 genotype

The genotypes of single nucleotide polymorphism (SNP) rs1906591 among patients and controls are listed in Table 2. The distribution of genotypes in CES patients and controls was statistically significantly different ($p = 0.003$). However, no significant difference was found between controls and total IS patients or other stroke subtypes. There was no deviation from Hardy–Weinberg equilibrium regarding the SNP rs1906591 in the control group ($p = 0.691$).

3.3. Association of rs1906591 variant with total IS

We assessed whether the rs1906591 variant was associated with total IS by using logistic regression analysis. We found that the IS patient group had a higher rs1906591 A allele frequency (0.52 *versus* 0.48, $p = 0.039$) than the healthy control group, and the A allele was positively associated with the risk of IS (odds ratio [OR] = 1.16, 95% confidence interval [CI] 1.01–1.34, $p = 0.04$). Due to possible inconsistent results caused by different models of inheritance, the genotypic association analysis was performed under all inheritance models (dominant, recessive, and additive). Genotypic association between the rs1906591 variant and total IS was significant for dominant and additive models (dominant model: OR = 1.30, 95% CI 1.02–1.64, $p = 0.03$; additive model: OR = 1.36, 95% CI 1.02–1.81, $p = 0.04$) but not for the recessive