The Single Nucleotide Polymorphism rs2208454 Confers an Increased Risk for Ischemic Stroke: A Case–Control Study

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Keywords

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SUMMARY

Aim: A recent genome-wide association study identified a strong association of covert magnetic resonance imaging infarcts with the single nucleotide polymorphism (SNP) rs2208454. The aim of this study was to determine whether the rs2208454 polymorphism is associated with an increased risk for ischemic stroke (IS). **Methods:** Ischemic stroke patients (n = 712) and control subjects (n = 774) from a southern Chinese Han population were included. The snapshot technique was used for genotype analysis. **Results:** Compared with the GT+GG or GG genotype, the frequency of the TT genotype was significantly higher in IS than in controls. After adjusting for age, gender, family history of IS, hypertension history, and history of diabetes mellitus, a significant correlation between the TT genotype and IS persisted (TT vs. GT+GG: adjusted OR = 1.79, 95% CI: 1.16–2.77; TT vs. GG: adjusted OR = 1.88, 95% CI: 1.20–2.94). In subgroup analyses, SNP rs2208454 was significantly associated with large artery atherosclerosis (LAA) (TT vs. GG: adjusted OR = 2.16, 95% CI: 1.19–3.93), but failed to show significant association with small-artery occlusion or cardioembolism IS subtypes. **Conclusions:** Single nucleotide polymorphism rs2208454 confers an increased risk for IS in a southern Chinese Han population. When the IS subtype was examined, the effect of the SNP was restricted to LAA.

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Introduction

Ischemic stroke (IS) is a disease that affects people across the world and endangers human health. Despite significant progress in its prevention, diagnosis, and treatment, the incidence of IS is still rising. IS now represents the second leading cause of death globally and the most common cause of disability in adults [1,2].

Although the pathogenesis of IS is not fully understood, a large number of studies have examined its etiology. Research has established that IS is a heterogeneous multifactorial disorder resulting from the interaction between and the combined effects of environmental and genetic factors [3,4]. There are several IS subtypes, including large artery atherosclerosis (LAA), small-artery occlusion (SAO), cardioembolism (CE), stroke of other determined etiology (SOE) and stroke of undetermined etiology (SUE) [5]. It is important to note that genetic predisposition to the different IS subtypes may vary [6].

In many cases, brain infarcts in the elderly fail to cause visible symptoms. Patients with this type of brain infarct usually present with no symptoms (or very mild symptoms) of acute IS and are

instead diagnosed by magnetic resonance imaging (MRI). Brain infarcts without outward symptoms are known as MRI-defined silent brain infarcts or covert MRI infarcts [7,8]. Epidemiological data have shown that silent brain infarcts are found in 20% of the healthy elderly.

Recently, a genome-wide association study (GWAS) on a population of MRI-defined silent brain infarcts found that the rs2208454 single nucleotide polymorphism (SNP) located on chromosome 20p12 was the most significant SNP associated with covert MRI infarcts. Currently, there are no studies reporting a correlation between the rs2208454 SNP and IS. To address this, we performed a case–control study to determine the association between rs2208454 and IS in a southern Chinese Han population.

Methods

Study Population

The design of this study was hospital-based. A total of 712 IS patients and 774 unrelated, healthy controls from a Han

compare age, blood pressure, cholesterol, triglycerides, and glucose levels between groups. A Mann–Whitney *U*-test was used to compare the body mass index (BMI) between the two groups. The frequencies of sex, cigarette smoking, IHD history, hypertension history, DM history, hyperlipidemia history, and rs2208454 genotype distribution were compared between patients and controls using a chi-square test. The genetic association was estimated using a univariate logistic regression analysis. A multivariate logistic regression analysis was performed to adjust for potential confounding factors, such as age, sex, family history of IS, hypertension history, and a history of DM. Genetic association was estimated under three different models (dominant, recessive, and additive). Statistical analysis was performed using the SPSS 16.0 statistical software for Windows (SPSS Inc., Chicago, IL USA). A Bonferroni correction was applied to allow for multiple comparisons amongst the groups such that a *P*-value of <0.0125 was considered statistically significant. All tests, with the exception of multiple comparisons, were two-tailed and a *P*-value of 0.05 was considered statistically significant.

Results

Distribution of the SNP rs2208454 Genotype

The genotype of SNP rs2208454 for IS patients and controls is listed in Table 2. The distribution of the genotypes between IS patients and controls showed a statistically significant difference (*P* = 0.008). No significant difference was observed between controls and IS subtypes (*P* > 0.0125). In the control group, there was no deviation from HWE in SNP rs2208454 (*P* = 0.904).

Association of the SNP rs2208454 Variant with IS

We assessed whether an rs2208454 variant was associated with IS using a logistic regression analysis. Due to possible inconsistencies caused by different modes of inheritance, the analysis was performed under dominant (TT+GT vs. GG), recessive (TT vs. GT+GG), and additive models (TT vs. GG). Genotypic association between the rs2208454 variant and total IS was significant for both the recessive and additive models (recessive model: OR = 1.79, 95% CI: 1.24–2.61, *P* = 0.002; additive model: OR = 1.80, 95% CI: 1.23–2.64, *P* = 0.003; Table 3). A statistically

Table 2 Distributions of the single nucleotide polymorphism (SNP) rs2208454 genotype among IS cases and controls

	SNP rs2208454 genotype			χ^2 value	<i>P</i> -value
	TT (%)	GT (%)	GG (%)		
Controls	49 (6.3)	294 (38.0)	431 (55.7)	9.61	0.008*
Overall IS	77 (10.8)	259 (36.4)	376 (52.8)		
Stroke subtypes					
LAA	31 (9.5)	128 (39.1)	168 (51.4)	4.00	0.135
SAO	25 (11.3)	76 (34.4)	120 (54.3)	6.39	0.041
CE	6 (11.1)	19 (35.2)	29 (53.7)	1.87	0.391

CE, cardioembolism; IS, ischemic stroke; LAA, large artery atherosclerosis; SAO, small-artery occlusion. **P* < 0.0125 versus control.

Table 3 Analysis of genotypic association of rs2208454 with IS under different models

Model	Crude OR (95% CI)	<i>P</i> -value	Adjusted OR* (95% CI)	<i>P</i> -value*
Overall IS				
Dominant	1.12 (0.92–1.38)	0.266	1.20 (0.95–1.52)	0.131
Recessive	1.79 (1.24–2.61)	0.002	1.79 (1.16–2.77)	0.008
Additive	1.80 (1.23–2.64)	0.003	1.88 (1.20–2.94)	0.006
LAA				
Dominant	1.19 (0.92–1.54)	0.190	1.32 (0.97–1.81)	0.079
Recessive	1.55 (0.97–2.48)	0.068	1.93 (1.10–3.41)	0.023
Additive	1.62 (1.00–2.63)	0.050	2.16 (1.19–3.93)	0.011
SAO				
Dominant	1.06 (0.78–1.43)	0.715	1.16 (0.82–1.63)	0.408
Recessive	1.89 (1.14–3.13)	0.014	1.75 (0.97–3.15)	0.064
Additive	1.83 (1.08–3.09)	0.023	1.80 (0.97–3.35)	0.065
CE				
Dominant	1.08 (0.62–1.88)	0.777	1.14 (0.63–2.05)	0.675
Recessive	1.85 (0.75–4.53)	0.179	1.87 (0.69–5.51)	0.215
Additive	1.82 (0.72–4.60)	0.206	1.90 (0.69–5.27)	0.217

CE, cardioembolism; IS, ischemic stroke; LAA, large artery atherosclerosis; SAO, small-artery occlusion. *Adjusted for age, sex, family history of IS, hypertension history, and history of diabetes mellitus.

significant association was still apparent after adjusting for possible risk factors, including age, gender, family history of IS, hypertension history, and history of DM (recessive model: adjusted OR = 1.79, 95% CI: 1.16–2.77, *P* = 0.008; additive model: adjusted OR = 1.88, 95% CI: 1.20–2.94, *P* = 0.006; Table 3). When further stratified by sex, the association between IS and the rs2208454 variant was evident only in men. Compared with the wild-type genotype of GG, the TT genotype was associated with a 2.19-fold increased risk (adjusted OR = 2.19, 95% CI: 1.23–3.90, *P* = 0.008) for overall stroke in men (Table 4).

Association of SNP rs2208454 with Stroke Subtypes

In the analysis of IS subgroups, the rs2208454 variant was associated with LAA (additive model: TT vs. GG: OR = 1.62, 95% CI: 1.00–2.63, *P* = 0.050; adjusted OR = 2.16, 95% CI: 1.19–3.93, *P* = 0.011; Table 3). The rs2208454 variant failed to show significant association with either the SAO or CE IS subtypes.

Table 4 Relative risk of overall ischemic stroke (IS) for rs2208454 TT/GT versus GG genotype in male (female)

Sex	TT versus GG		GT versus GG	
	OR*	<i>P</i> -value*	OR*	<i>P</i> -value*
Male	2.19 (1.23–3.90)	0.008	1.12 (0.81–1.54)	0.497
Female	1.39 (0.67–2.86)	0.376	1.01 (0.67–1.53)	0.962

*Adjusted for age, family history of IS, hypertension history, and history of diabetes mellitus.

Discussion

To our knowledge, this is the first study to demonstrate an association between SNP rs2208454 and IS. Our results showed that, under either the recessive or the additive model, the TT genotype for the rs2208454 variant was significantly correlated with IS. This correlation suggests that the TT genotype increases the overall risk for IS. The risk for IS in carriers with the TT genotype was increased 1.79-fold when compared with carriers with the GG+TT genotype and 1.80-fold when compared with carriers with the GG genotype. Under the additive model, the rs2208454 variant was also significantly correlated with the LAA stroke subtype. The variant did not show a statistically significant association with other IS subtypes under any model applied.

The rs2208454 SNP variant is located within the third intron of the MACRO domain containing 2 (*MACROD2*) gene and in the downstream region of fibronectin leucine-rich transmembrane protein 3 (*FLRT3*) gene. The function of the protein encoded by *MACROD2* is currently unclear. *MACROD2* contains a portion of a MACRO domain, which binds to adenosine diphosphate (ADP)-ribose. The presence of a MACRO domain suggests that *MACROD2* may participate in the process of ADP-ribosylation. ADP-ribosylation is a posttranslational modification that allows ADP-ribose to bind to a receptor protein using nicotinamide adenine dinucleotide (NAD) as a modified donor. ADP-ribosylation exists in most cell types and has been shown to be one of the most important ways to modify the structure and function of a protein. Many critical cellular events such as DNA repair, transcriptional activation and gene expression are dependent on ADP-ribosylation. Recent research has shown that polymorphisms in *MACROD2* are associated with autism [11,12]. Other studies have demonstrated that SNPs in *MACROD2* are also associated with hypertension [13] and dementia [14].

The *FLRT3* gene is located upstream of the rs2208454 variant and is the nearest gene to rs2208454 besides *MACROD2*. *FLRT3* encodes for a protein expressed in various tissues including the brain. *FLRT3* regulates homophilic adhesion between cells and up-regulates fibroblast growth factor signaling. *FLRT3* may participate in the formation of vessels and nerve development. Experiments in animal models have provided evidence that *FLRT3* promotes axon regeneration after axonal injury.

Genome-wide studies using high-throughput genotyping technologies have been implemented to examine hundreds of thousands of markers to identify genetic variants and high-risk haplotypes associated with complex disease [15]. Recently, a GWAS [9] on a population of MRI-defined silent brain infarcts found that the rs2208454 variant was associated with silent infarcts, though further validation in different populations was still necessary. MRI-defined silent brain infarcts share similar pathophysiology with SAO. Thus, we anticipated that SAO would also be associated with the rs2208454 SNP. Surprisingly, there was no significant association between the SAO subtype and the TT variant, which suggests a fundamental difference in the

pathophysiological mechanisms underlying MRI-defined silent brain infarcts and SAO.

Our results have shown that rs2208454 was associated with IS and that the TT genotype may confer an increased risk for IS. In our analysis of IS subgroups, the TT genotype showed an increased risk for LAA. The association of the TT genotype with an increased risk for IS differs from the GWAS results described above. In the original GWAS, the T allele was shown to be protective against silent brain infarcts. This contradiction raises the possibility that carriers with the T allele suffered from symptomatic IS rather than a silent infarct. Conversely, carriers lacking the T allele do not show outward clinical symptoms of IS and therefore present with silent brain infarcts. This possibility should be tested in future case-control studies where the control group was designed to include people with silent infarcts. We hypothesize that changes in expression of the two genes (*MACROD2* and *FLRT3*) proximal to the rs2208454 SNP may promote the different outcomes we observed. This hypothesis, however, warrants functional studies to establish how the genotype of the rs2208454 SNP affects IS and silent brain infarcts.

In our study, the association between IS and the rs2208454 variant was evident only in men. When compared with the wild-type genotype of GG, the TT genotype was associated with a 2.19-fold increased risk in men (adjusted OR = 2.19, 95% CI: 1.23–3.90, $P = 0.008$) for overall stroke. Thus, we hypothesize that the TT genotype may be an independent risk factor for IS in men though the exact mechanism remains to be elucidated.

There are limitations in the present study. First, our study population was limited to southern Chinese individuals. Our findings need to be validated in other ethnic populations. Second, our study sample size was relatively small, though it should be noted that the recruitment of >1000 individuals from an ethnically homogeneous population is sufficient to yield reliable data. Third, the biological and molecular mechanisms that drive the genetic association remain unclear.

In conclusion, we found that the rs2208454 SNP on chromosome 20p12 was significantly associated with an increased incidence of IS in a southern Chinese Han population. This association was confined to LAA when stroke subtypes were examined. Further research will be required to explore the role of the rs2208454 SNP in the pathogenesis of IS.

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Conflict of Interest

The authors declare no conflict of interest.

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