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Original Research

Association between *GABRG2* Gene Single Nucleotide Polymorphisms and Susceptibility to Ischemic Stroke in a Chinese Population

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Abstract

Background: Current evidence suggests that Gamma-aminobutyric acid (GABA) receptors are associated with the occurrence and progression of cerebrovascular diseases. The present study investigated the association between single nucleotide polymorphisms (SNPs) in the Gamma-aminobutyric acid type A receptor gamma2 subunit (*GABRG2*) gene and ischemic stroke (IS). **Methods**: A total of 120 healthy volunteers and 187 patients with IS were recruited. Patients underwent complete neurological assessment and classification with the National Institute of Health Stroke Scale (NIHSS) and the Trial of ORG 10172 in Acute Stroke Treatment (TOAST). Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) was used to analyze SNP sites in 4 different regions (*rs211037*, *rs418210*, *rs211035*, and *rs424740*) of the *GABRG2* gene. SHEsis online platform was used to assess SNP allele and genotype frequencies. Multivariate logistic regression analysis was performed to identify the risk factors for IS. **Results**: Univariate analysis showed that the *T* allele and *TT* genotype distribution for *rs211037* were significantly more frequent in the IS group compared to controls (p_{allele} = 0.01, odds ratio (OR) = 1.673, 95% confidence intervals (CI), 1.119–2.500, p_{genotype} = 0.03). Furthermore, multivariate logistic regression analysis revealed the *TT* genotype for *rs211037* was an independent risk factor for IS (p = 0.017, OR = 1.925, 95% CI, 1.122–3.303). Age was also found to be an independent risk factor, and the older the age, the higher the risk of IS (p = 0.001, OR = 1.047, 95% CI, 1.020–1.073). Finally, subgroup analysis revealed that patients with the *rs211037 TT* genotype were associated with a higher NIHSS score (p = 0.03), and that large-artery atherosclerosis (LAA) subtype was predominant in patients with the *rs211037 TT* genotype (p = 0.042). **Conclusions**: These findings suggest the rs211037 polymorphism in the *GABRG2* gene is an independent risk factor for IS in the Chinese population. GABRG2 could thus be a potential biomarker to assess the risk of IS.

Keywords: ischemic stroke; *GABRG2*; single nucleotide polymorphism; disease susceptibility

1. Introduction

Stroke is one of the common cerebrovascular diseases and can be divided into hemorrhagic stroke and ischemic stroke (IS) [1]. The morbidity and mortality from stroke have decreased with the improvement of medical care, but the absolute number of patients with this disease is still increasing yearly due to aging of the global population [2,3]. Nearly 80% of stroke patients are diagnosed with IS [1]. Although the pathogenesis of IS has not been fully elucidated, genetic susceptibility genes have been suggested as key factors in its etiology [4]. Many susceptibility factors for IS have been reported, but these only partially explain the genetic risk for IS. The remainder can be attributed to other covariates, such as blood lipids, diabetes and blood pressure [5,6]. Therefore, in-depth exploration of the genetic background of IS and the finding of new susceptibility factors has high clinical value for the early diagnosis, treatment, and prognosis of IS patients.

Gamma-aminobutyric acid (GABA) acts as an inhibitory neurotransmitter in the central nervous system. The GABA type-A receptor γ2 subunit (*GABRG2*) gene has a key role in GABA receptors [7,8] and is involved in rapid synaptic inhibition in the brain. There are 19 different subunits of the GABAA receptor. The *GABRG2* gene encodes the γ2 subunit and is located in 5q34. It has a total length of 94,898 bp and contains 11 exons [9,10]. Abnormal expression of *GABRG2* during the pathogenesis of IS has been associated with disease progression and an increased incidence of complications. Decreased expression of *GABRG2* in the brainstem of IS mouse models can inhibit the occurrence of spasms [11]. Abnormal expression of *GABRG2* in neuronal cells also affects various biological activities [12]. Studies have also found that abnormal expression of *GABRG2* can inhibit neurotransmission in the brain and increase ischemic brain injury and IS [13]. Therefore, there appears to be a strong association between *GABRG2* and the progression and risk of IS.

Single nucleotide polymorphisms (SNPs) are third-generation genetic diagnostic biomarkers that have been used widely in many fields of biology and medical research [14]. SNPs in *GABAR2* have been associated with nervous system diseases, including epilepsy [15–17]. The *GABRG2*

C588T polymorphism is a potential predictor of generalized epilepsy risk [15]. The *rs211037* SNP in the *GABRG2* gene has also been associated with the risk of idiopathic generalized epilepsy [16]. The association of *GABRG2* SNPs with the risk of IS in humans has not been investigated, however. Moreover, associations between *GABRG2* SNPs and autoimmune associated diseases, including IS, have not been proven conclusively. In the present study we investigated possible associations between 4 SNPs (*rs211037*, *rs418210*, *rs211035*, and *rs424740*) in *GABRG2* and the susceptibility to IS in a Chinese population.

2. Materials and Methods

2.1 Patient Enrollment and Sample Collection

This case-control study included 187 patients with atherosclerotic acute IS. The inclusion criteria were: (1) IS confirmed by cerebral magnetic resonance imaging (MRI); (2) cases were classified as large-artery atherosclerosis (LAA), cardiogenic embolism (CE) or small artery occlusion (SAO) based on Trial of ORG 10172 in Acute Stroke Treatment (TOAST). Exclusion criteria were: (1) family history of stroke or previous stroke; (2) other type of stroke; (3) cerebral hemorrhage, severe heart and liver disease, blood or autoimmune diseases, chronic inflammatory diseases; (4) inability to perform MRI imaging; (5) patients related to each other.

During the same period, 120 healthy volunteers were recruited to our hospital for physical examination and served as the control group. None of the control volunteers had a computer tomography (CT), MRI or family history of stroke. Healthy volunteers who participated in the study had no tumors, atherosclerotic disease, immune-related diseases, infections, severe heart disease, severe liver and kidney dysfunction, or endocrine disorders. The study protocol was approved by the Medical Ethics Committee of Red Cross Hospital (Approval NO: 2023030). Patients and/or their families gave informed consent for samples to be collected and analyzed.

Data were collected for a variety of clinical characteristics including age, gender, smoking, drinking history, diabetes and hypertension. Fasting blood samples were tested for glucose, triglycerides (TG), plasma total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C).

2.2 NIHSS Score

The National Institute of Health Stroke Scale (NIHSS) evaluates 11 factors, including consciousness level, muscle strength, aphasia, ataxia, and sensation. The higher the score, the worse the neurological function. Scores represent mild (1–4), moderate (5–15), moderate to severe (16–20), or severe (21–42) neurological disability [18].

2.3 Selection of SNP Sites

The following SNPs in the *GABRG2* gene (locus gene frequency >0.05) were selected using the dbSNP database (http://www.ncbi.nlm.nih.gov/SNP): *rs211037*, *rs418210*, *rs211035*, and *rs424740*. This was based on findings from past research [19–22].

2.4 Blood DNA Extraction and Genotyping

Fasting venous blood (4 mL) was collected into an EDTA tube and centrifuged at 4 °C 1500 ×g for 20 min. The plasma was separated and stored at –80 °C. Whole blood DNA extraction kit (Cat. No. 55204, Qiagen, Hilden, North Westphalia, Germany) was used to purify genomic DNA. *GABRG2* genotyping was performed as previously described [23]. The *GABRG2* genotype was analyzed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) kit (Cat. No. 10101, Yeasen Biotechnology, Shanghai, China). The reaction conditions were as follows: a total of 50 ng of genomic DNA was mixed with 5 pmol PCR primers (Sango Biotech, Shanghai, China) in a total volume of 25 microliters, including Tris-hydrochloride (pH 8.8), 0.8% Nonidet P40, magnesium chloride, potassium chloride, deoxyribonucleotide triphosphate, and DNA polymerase. The reaction mixture was cycled in a DNA thermal circulator (Bio-Rad, Hercules, CA, USA) by denaturation at 94 °C for 3 min, then 35 cycles of 94 °C for 1 min, 55 °C for 1 min, and 72 °C for 1 min, and finally extended at 72 °C for 7 min. The PCR products were digested with Msp1 restriction enzyme (Cat. No. 511922, Bioo Scientific, Austin, TX, USA). Restriction enzyme digestions were performed in a total volume of 15 microliters containing 10 microliters of PCR product, 5 units of restriction enzyme, 1.5 microliters of 10× buffer, and 3.5 microliters of distilled water. The restricted digestion mixture was incubated overnight at 37 °C. All generated fragments were then separated by electrophoresis on an ethidium bromide-stained 3% agarose gel and observed under ultraviolet light. The primer sequences for the 4 SNP sites (*rs211037*, *rs418210*, *rs211035*, and *rs424740*) in *GABRG2* are shown in Table 1.

2.5 Statistical Analysis

Data visualization and analysis were performed using GraphPad Prism 6.0 (GraphPad Software, Inc., San Diego, CA, USA) and SPSS 21.0 (IBM Corp., Armonk, NY, USA). The normality of measurement data was analyzed by F test, and Student *t* test was used to evaluate differences between two groups. Comparison of numerical data between two groups was conducted using the Chi-square test. Odds ratio (OR) and 95% confidence intervals (CI) were calculated using logistic regression analysis. Genotyping results for the 4 SNPs in *GABRG2* (*rs211037*, *rs418210*, *rs211035*, and *rs424740*) were analyzed with the SHEsis online platform (http://analysis.bio-x.cn/myAnalysis.php). Hardy-Weinberg equilibrium (HWE), linkage disequilib-

Table 1. Primer sequences used for polymerase chain reaction.

SNP		Primer sequence
rs211037	sense	5′-*ACGTTGGATGTACCATCTTGGCTTCTGGTG*-3′
	anti-sense	5′-*ACGTTGGATGAGCTTCTGTCTGTCAGGTCG*-3′
rs418210	sense	5′-*ATGCAATTCTCTTTTCTGTCTAC*-3′
	anti-sense	5′-*AGTTAAATTTAGCAGTTGCATG*-3′
rs211035	sense	5′-*CTTTGCTTCATATTGGCAA*-3′
	anti-sense	5′-*CAATTACTCCTTTCCTTTTGC*-3′
rs424740	sense	5′-*ATGCAATTCTCTTTTCTGTCTAC*-3′
	anti-sense	5′-*AGTTAAATTTAGCAGTTGCATG*-3′

SNP, Single nucleotide polymorphism.

Table 2. Clinical characteristics of the control and IS groups.

Characteristic	Controls (n = 120)	IS patients (n = 187)	*p*-value
Male (n, %)	64, 53.33%	97, 51.87%	0.80
Age (years, mean ± SD)	60.03 ± 8.00	64.23 ± 10.64	<0.001
Smoking (n, %)	38, 31.67%	64, 34.22%	0.64
Drinking (n, %)	31, 25.83%	45, 24.06%	0.73
Hypertension (n, %)	52, 43.33%	109, 58.29%	0.01
Diabetes mellitus (n, %)	25, 20.83%	65, 34.76%	0.01
TG (mmol/L, mean ± SD)	1.72 ± 1.20	1.80 ± 1.14	0.58
TC (mmol/L, mean ± SD)	4.86 ± 1.15	5.13 ± 1.16	0.04
LDL-C (mmol/L, mean ± SD)	3.37 ± 0.91	3.59 ± 0.84	0.03
HDL-C (mmol/L, mean ± SD)	1.38 ± 0.46	1.29 ± 0.42	0.07

SD, Standard deviation; TG, Triglyceride; TC, Total cholesterol; HDL-C, High density lipoprotein cholesterol; LDL-C, Low density lipoprotein cholesterol; IS, Ischemic stroke.

rium and haplotype analysis were also carried out. Indicators of significant differences between the IS and control groups were taken as covariates, with the incidence of IS taken as the dependent variable. The relationship between the *rs211037* genotype and IS was analyzed using multivariate logistic regression, and $p < 0.05$ was considered to indicate statistical significance.

3. Results

3.1 Baseline Data of IS Patients and Control Participants

A total of 307 participants were recruited to the study, comprising 187 patients with IS and 120 healthy control volunteers. The demographic characteristics for these two groups are summarized in Table 2. No significant differences in gender, smoking habits, alcohol consumption, TG and HDL-C levels were found between the IS and control groups ($p > 0.05$). The IS group had a significantly higher incidence of hypertension, diabetes, TC and LDL-C ($p < 0.05$).

3.2 HWE Test

The genotype distribution of the 4 *GABRG2* SNP loci did not differ significantly from HWE ($p > 0.05$) in both the patient and control groups (Table 3). In other words, the gene frequency observed in the IS study population was representative of the gene distribution observed in the general population.

3.3 GABRG2 SNP Allele and Genotype Frequencies

GABRG2 SNP allele and genotype frequencies were compared between the control and IS groups (Table 4). The allele and genotype frequencies for *rs211037* were significantly different between the two groups ($p < 0.05$). Based on this result, the *rs211027* genotype was included as an independent variable in subsequent multivariate analysis to determine whether it was a genetic factor in the susceptibility for IS.

3.4 Linkage Disequilibrium of SNPs in GABRG2 and Associations between Haploid Type and IS

Linkage disequilibrium analysis was conducted on the 4 *GABRG2* SNPs. The D' values between the 4 SNPs were all <0.8, indicating the absence of a strong linkage relationship (Table 5). The 4 sites were next analyzed for haploid types. After dismissing haplotypes with a frequency of <3%, a total of 16 haploid types were identified. These haploid types were compared between the IS and control groups (Table 6). One haploid type, *TCAG*, was different between the IS and control groups ($p < 0.05$), with a significantly lower frequency in IS cases compared to the controls. Hence the *TCAG* haplotype was considered protective for IS.

Table 3. HWE analysis of SNP genotypes in the control and IS groups.

SNP	Genotype	Controls (n, %)	*p*-value	IS patients (n, %)	*p*-value
rs211037	*CC*	68 (56.67)	0.90	134 (71.66)	0.11
	TT	7 (5.83)		8 (4.28)	
	CT	45 (37.50)		45 (24.06)	
rs418210	*CC*	30 (25.00)	0.26	56 (29.95)	0.91
	TT	24 (20.00)		39 (20.86)	
	CT	66 (55.00)		92 (49.19)	
rs211035	*AA*	16 (13.33)	0.53	28 (14.97)	0.57
	GG	44 (36.67)		75 (40.11)	
	AG	60 (50.00)		84 (44.92)	
rs424740	*AA*	6 (5.00)	0.49	9 (4.81)	0.44
	GG	78 (65.00)		122 (65.24)	
	AG	36 (30.00)		56 (29.95)	

HWE, Hardy-Weinberg equilibrium; SNP, Single nucleotide polymorphism; IS, Ischemic stroke.

Table 4. Allele and genotype comparison between control and IS groups.

	Allele (n, %)		*p*-value	OR (95% CI)	Genotype (n, %)			*p*-value
rs211037	*C*	*T*			*CC*	*TT*	*CT*	
Control	181 (75.42)	59 (24.58)	0.01	1.673 [1.119∼2.500]	68 (56.67)	7 (5.83)	45 (37.50)	0.03
IS	313 (83.69)	61 (16.31)			134 (71.66)	8 (4.28)	45 (24.06)	
rs418210	*C*	*T*			*CC*	*TT*	*CT*	
Control	126 (52.50)	114 (47.50)	0.62	1.086 [0.784∼1.503]	30 (25.00)	24 (20.00)	66 (55.00)	0.56
IS	204 (54.55)	170 (45.45)			56 (29.95)	39 (20.86)	92 (49.19)	
rs211035	*A*	*G*			*AA*	*GG*	*AG*	
Control	92 (38.33)	148 (61.67)	0.82	0.963 [0.689∼1.344]	16 (13.33)	44 (36.67)	60 (50.00)	0.68
IS	140 (37.43)	234 (62.57)			28 (14.97)	75 (40.11)	84 (44.92)	
rs424740	*C*	*G*			*CC*	*CG*	*GG*	
Control	48 (20.00)	192 (80.00)	0.95	0.987 [0.657∼1.481]	6 (5.00)	78 (65.00)	36 (30.00)	0.99
IS	74 (19.79)	300 (80.21)			9 (4.81)	122 (65.24)	56 (29.95)	

OR, Odds ratio; CI, Confidence intervals; IS, Ischemic stroke.

Table 5. Linkage disequilibrium between *GABRG2* SNPs (D' values).

SNP	*rs418210*	*rs211035*	*rs424740*
rs211037	0.094	0.035	0.030
rs418210	—	0.020	0.066
rs211035	—	—	0.119

SNP, Single nucleotide polymorphism.

3.5 Multifactorial Analysis of the rs211037 SNP in GABRG2 Gene and IS

In view of the significant differences observed between the IS and control groups for some clinical characteristics (age, hypertension, diabetes, TC, LDL-C), multivariate logistic regression analysis was conducted to exclude confounding variables (Table 7). The dependent variable was acute IS, while the included independent variables were age, hypertension, diabetes, TC, LDL-C and the *rs211037* genotype. The *TT* genotype of *rs211037* was found to be an independent risk factor for IS (OR = 1.925, 95% CI, 1.122–3.303, $p = 0.017$). Age was also an independent risk factor for IS, with older age being associated with a higher risk for IS (OR = 1.047, 95% CI, 1.020–1.073, $p = 0.001$).

3.6 Association of GABRG2 rs211037 Genotype with NIHSS and TOAST Classification of IS Patients

We next analyzed the relationship between *GABRG2 rs211037* genotype and the patients' NIHSS score. Higher HIHSS scores were observed in *TT* genotype patients (Table 8). According to the TOAST classification, the *TT* genotype was significantly more frequent in LAA than the SAO and CE groups.

4. Discussion

This study was conducted to investigate possible associations between *GABRG2* (*rs211037*, *rs418210*, *rs211035*, and *rs424740*) genotypes and the onset of IS. Univariate analysis showed significant differences in the distribution of the *rs211037* genotype between the control and IS groups. Our results indicate that patient age and the

Table 6. Haploid type analysis in *GABRG2*.

Haplotype	IS (freq)	Control (freq)	*p*-value	OR (95% CI)
C C A A	12.94 (0.035)	12.92 (0.054)	0.258894	0.637 [0.289∼1.402]
C C A G	50.26 (0.134)	23.10 (0.096)	0.140473	1.480 [0.877∼2.497]
C C G A	14.30 (0.038)	3.78 (0.016)	0.103146	2.514 [0.799∼7.902]
C C G G	90.74 (0.243)	50.64 (0.211)	0.323549	1.218 [0.823∼1.804]
C T A A	13.60 (0.036)	10.09 (0.042)	0.742100	0.870 [0.379∼1.998]
C T A G	43.39 (0.116)	19.24 (0.080)	0.139160	1.527 [0.869∼2.686]
C T G A	14.64 (0.039)	11.85 (0.049)	0.560834	0.793 [0.362∼1.737]
C T G G	73.12 (0.196)	49.37 (0.206)	0.807565	0.951 [0.633∼1.427]
T C A G	6.07 (0.016)	13.57 (0.057)	0.006102	0.278 [0.105∼0.735]
T C G A	7.74 (0.021)	6.52 (0.027)	—	—
T C G G	17.93 (0.048)	15.47 (0.064)	0.396196	0.739 [0.366∼1.490]
T T A G	9.58 (0.026)	13.08 (0.055)	0.067925	0.461 [0.197∼1.079]
T T G A	6.63 (0.018)	2.83 (0.012)	—	—
T T G G	8.90 (0.024)	7.52 (0.031)	0.585925	0.761 [0.284∼2.038]
T C A A	4.00 (0.011)	0.00 (0.000)	—	—
T T A A	0.14 (0.000)	0.00 (0.000)	—	—
Global result			0.041351	

IS, Ischemic stroke; OR, Odds ratio; CI, Confidence Interval.

Table 7. Multivariate logistic regression analysis of risk factors for IS.

Factor	*p*-value	OR (95% CI)
Age	0.001	1.047 [1.020∼1.073]
Hypertension	0.095	0.622 [0.356∼1.087]
Diabetes mellitus	0.076	0.556 [0.291∼1.064]
TC	0.566	1.073 [0.842∼1.368]
LDL-C	0.094	1.317 [0.954∼1.818]
rs211037		
CC	0.044	—
TT	0.017	1.925 [1.122∼3.303]
CT	0.960	0.969 [0.288∼3.259]

TC, Total cholesterol; LDL-C, Low density lipoprotein cholesterol; OR, Odds ratio; CI, Confidence Intervals.

TT genotype of *rs211037* may be independent risk factors for susceptibility to IS. In addition, IS patients with the *rs211037 TT* genotype had a higher NIHSS score, with the cause of stroke in these patients being mostly atherosclerosis of the large arteries. These findings suggest potential factors for the identification of IS-susceptible individuals in the population, while also providing a theoretical basis for the etiology of stroke.

SNPs in *GABRG2* have been associated with certain diseases. For example, the *rs211037* polymorphism in the *GABRG2* gene has been linked to an increased risk of idiopathic generalized epilepsy [16]. A meta-analysis demonstrated that *GABRG2 rs211037* was associated with susceptibility to epilepsy [10]. A study of Indian epilepsy patients reported the *TC* genotype frequency of the *rs418210* locus in *GABRG2* was significantly different between patients and controls ($p = 0.011$) [22]. Yin *et al.* [20] reported that individuals with the GA haplotype for the *rs211035* and *rs211034* SNPs were less likely to be suicidal. Another study by the same group also found the SNP *rs424740* was associated with major depression [24]. However, to our knowledge there have been no published reports on whether *GABRG2* SNPs are genetic susceptibility factors for IS. The current study investigated 4 SNPs in *GABRG2* based on previous work: *rs211037*, *rs418210*, *rs211035*, and *rs424740*. Univariate analysis revealed that only *rs211037* showed different allele and genotype frequencies between control and IS groups. Clinical risk factors for stroke were also recorded in this study. The incidence of hypertension, diabetes, TC and LDL-C were significantly different between IS patients and healthy controls. We performed multivariate analysis to exclude the impact of these clinical factors on the evaluation of *rs211037* genotype with regard to IS. The *TT* genotype of *rs211037* was found to be an independent risk factor for IS. These results suggest the *GABRG2 rs211037* SNP is an IS-related risk factor. Another study reported similar results [16], whereas other studies have suggested that *GABRG2 rs211037* SNP does not influence susceptibility to epilepsy in different populations [25]. The *GABRG2* SNPs *rs211035*, *rs424740*, *rs418210*, *rs211034*, and *rs424740* may not be susceptibility factors for epilepsy in Indian patients [22]. The diversity of findings in these studies may be due to factors such as ethnic differences, study design, sample size, and disease species.

The pathogenesis and risk of IS also associated with a variety of clinical factors or features in addition to genetic factors [26]. For example, Egan *et al.* [27] found that blood pressure and increased morbidity and mortality of stroke were associated with hypertension. A Mendelian randomization study also found that type 2 diabetes was a risk factor

Table 8. Association of *rs211037* SNP with NIHSS and TOAST classification in IS patients.

Genotype	NIHSS		TOAST		
	Minor (n = 47)	Moderate (n = 140)	LAA (n = 68)	SAO (n = 70)	CE (n = 49)
CC	30, 63.8%	104, 74.3%	52, 76.5%	50, 71.4%	32, 65.3%
TT	0, 0.0%	8, 5.7%	6, 8.8%	1, 1.4%	1, 2.0%
CT	17, 36.2%	28, 20.0%	10, 14.7%	19, 27.2%	16, 32.7%
p-value	0.030		0.042		

NIHSS, National Institute of Health Stroke Scale; TOAST, Trial of ORG 10172 in Acute Stroke Treatment; LAA, Large-artery atherosclerosis; SAO, Small artery occlusion; CE, Cardiogenic embolism.

for IS in both large and small arteries [28]. This was also confirmed in the present study, where in addition to the *TT* genotype of *rs211037*, patient age was shown to be an independent risk factor for IS. Hence, clinical features may also assist in the treatment of stroke. For example, some investigators reported that thrombus permeability in patients was a potential neuroimaging marker for predicting the risk of distal embolism in mechanical thrombectomy [29]. Therefore, a combined analysis of *rs211037* SNP and patient age may improve the accuracy of prediction for IS risk.

Subgroup analysis has been a core problem in the clinical classification of IS and other diseases. This type of analysis can reduce the influence of phenotypic heterogeneity on disease diagnosis. A multicenter review found that large vessel occlusion was more suitable for thrombectomy compared to other subgroups [30]. The change of gene polymorphism was also closely related to the region, race and age of persons with IS. The *IL-6 rs1800795* polymorphism is significantly correlated with IS depending on ethnicity and geography [31]. Subgroup analysis showed that the negative effect of the circular (circ)-*STAT3 rs2293152 GG* genotype was more pronounced in female and older patients [32]. The NIHSS score is a standard classification method for IS patients. SNPs in Nitric Oxide Synthase 1 show a negative effect in IS patients in the codominant and dominant categories [33]. With the TOAST classification, some researchers reported the 1082 *A/G* polymorphism of *IL-10* was significantly associated with the risk of IS for large vessel disease (LVD) and small vessel disease (SVD) patients [34]. Similar to these results, we also found that patients with the *TT* genotype of *rs211037* had higher NIHSS scores. This was the predominant genotype in the LAA subgroup of IS patients. In previous studies on LAA and other IS subtypes, researchers generally believed that etiological differences between subtypes were directly related to clinical characteristics [35]. In the present study, the *rs211037* SNP was also regarded as a risk factor for the LAA subtype.

There are several limitations to this study. First, the number of patients and healthy volunteers was small, and the findings need to be verified by study of a larger sample size. Second, the study participants were all of Chinese population, hence it is not possible to extrapolate the significance of the results to other populations. Further analysis of different populations may clarify the actual significance of this genetic polymorphism. Third, only four polymorphic loci in *GABRG2* were studied, and future work should study investigate other polymorphisms in the *GABRG2* gene for possible associations with stroke. Finally, this study was focused on clinical and genetic risk factors for IS and did not evaluate possible associations with prognosis. This may be a worthwhile future research direction.

5. Conclusions

In conclusion, univariate analysis showed the genotype distribution of the *GABRG2 rs211037* SNP was significantly different between IS patients and healthy controls. Older patient age and the *TT* genotype of *rs211037* may be independent risk factors for susceptibility to IS. In subgroup analysis, IS patients with the *TT* genotype of *rs211037* were found to have higher NIHSS scores. The *TT* polymorphism of *rs211037* was also the predominant genotype in the LAA subtype of IS. Our findings indicate the *rs211037* SNP in *GABRG2* may be a genetic susceptibility factor for IS in the Chinese population. The results of this study may help to transform genetic discoveries into disease prediction biomarkers. These results also provide useful information for understanding the complex causes of IS.

Availability of Data and Materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Author Contributions

MM and JC conceived and designed the study, revised the draft. MM, JZ and DX conducted the experiments. JC wrote the first draft and revised the draft. MM and JZ led statistical analysis and revised the draft. JC led the revision of the draft. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors had complete access to all research data and assume complete responsibility for the data integrity and accuracy of the data analysis.

Ethics Approval and Consent to Participate

The experiments were approved by the Medical Ethics Committee of Red Cross Hospital. The ethical statement

No. is 2023030. All subjects gave written informed consent in accordance with the Declaration of Helsinki.

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Not applicable.

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Conflict of Interest

The authors declare no conflict of interest.

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