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ORIGINAL RESEARCH

Association Between *HTRA1*, *GAS6* and *IFNGR2* Gene Polymorphisms and Stroke Susceptibility in the Chinese Han Population

Fan Zhang[1,*], Hao Peng[1,*], Chuanyi Fu[1], Yidong Deng[1], Mao Zhang[1], Wenan Li[1], Jian Zhong[1], Qing Zhou[1], Li Huang[1], Shuli Xiao[1], Jiannong Zhao[2]

[1]Department of Cerebrovascular Disease, Hainan Affiliated Hospital of Hainan Medical University, Hainan General Hospital, Haikou, Hainan, 570311, People's Republic of China; [2]Department of Neurosurgery, Hainan Medical University, Hainan Affiliated Hospital of Hainan Medical University, Hainan General Hospital, Haikou, Hainan, 571199, People's Republic of China

*These authors contributed equally to this work

Correspondence: Jiannong Zhao, Department of Neurosurgery, Hainan Medical University, Hainan Affiliated Hospital of Hainan Medical University, Hainan General Hospital, Haikou, 571199, Hainan, People's Republic of China, Tel/Fax +86-13876673888, Email jiannong_zhao@163.com

Background: Stroke has a high disability rate, and 30% of stroke cases have an unknown cause. Accurate diagnosis and treatment of stroke requires consideration of several rare heritable and non-heritable factors.

Objective: This study aimed to evaluate the impacts of three genetic polymorphisms (rs369149111 in *HTRA1*, rs1803628 in *GAS6* and rs9808753 in *IFNGR2*) on stroke susceptibility among the Chinese Han population.

Methods: Three single nucleotide polymorphisms (SNPs) from 623 stroke cases and 572 healthy controls were genotyped by the Agena MassARRAY platform. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated by logistic regression analysis to evaluate the associations of three SNPs with stroke susceptibility. Additionally, SNP-SNP interactions were analyzed by multifactor dimensionality reduction (MDR).

Results: As demonstrated by the overall analysis, rs9808753 in *IFNGR2* (allele: OR = 1.25, 95% CI = 1.06–1.47, $p = 0.007$; homozygous: OR = 1.59, 95% CI = 1.14–2.23, $p = 0.007$; dominant: OR = 1.31, 95% CI = 1.02–1.67, $p = 0.032$; recessive: OR = 1.42, 95% CI = 1.05–1.91, $p = 0.022$; additive: OR = 1.26, 95% CI = 1.07–1.48, $p = 0.007$) was associated with an increased susceptibility to stroke. Besides, stratification analysis suggested that rs9808753 was associated with an increased risk of stroke in subgroup aged ≤ 64 years, males and drinkers ($p < 0.05$). And rs1803628 in *GAS6* was significantly associated with an increased susceptibility to stroke in non-smokers ($p < 0.05$).

Conclusion: A risk-increasing effect of *IFNGR2* rs980875 on stroke was detected in this study, which further broadens the understanding of the relationship between genetic polymorphisms and stroke susceptibility.

Keywords: *IFNGR2*, polymorphisms, stroke, susceptibility, case-control

Introduction

Stroke, a kind of common cerebrovascular disease, is a major global health problem and the second leading cause of disability and death in the world nowadays.[1–3] The main types of stroke include ischemic stroke (87%), cerebral hemorrhage (10%) and subarachnoid hemorrhage (3%).[2] In addition, hypertension, atherosclerosis, or heart diseases possibly lead to complications such as stroke.[4] Statistically, there were 12.2 million new strokes and 101 million epidemic strokes worldwide in 2019, and the number of cases will rise in the coming years.[5] By 2050, there are projected to be approximately 200 million stroke survivors worldwide, with more than 30 million new strokes and 12 million stroke deaths each year thereafter.[6]

In fact, as a highly heterogeneous disease, stroke can be caused by extremely diverse factors. A study on the Swedish Twin Registry has concluded that stroke is significantly inherited, and monozygotic twins are more likely to develop

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strokes than dizygotic twins.[7,8] The heritability of stroke is approximately 40%, as estimated by two related studies.[9,10] Currently, increasing evidence supports the role of genetic factors in determining stroke risk,[11] and there is growing interest in identifying other genetic factors for stroke, such as single nucleotide polymorphisms (SNPs). However, few genetic loci for stroke have been found. Therefore, investigating the genetic risk factors for stroke is vital to promote the discovery of new therapeutic targets and optimize prevention strategies.[12]

The high temperature requirement serine peptidase A1 (*HTRA1*) gene, belonging to the HTRA protein family and located on chromosome 10 (10q26), encodes a thermostable serine protease.[13] *HTRA1* can be involved in a variety of physiological processes, including maintenance of mitochondrial homeostasis, cell signaling, and apoptosis.[14] Meanwhile, as a serine enzyme mediating cell signaling, *HTRA1* also plays an important role in vascular integrity, skeletal development and osteogenesis.[15] Remarkably, previous studies have shown that *HTRA1* mutations are closely related to the occurrence of stroke, including small vessel ischemic stroke[16] and lacunar stroke.[17] Moreover, it has been reported that *HTRA1* mutations may contribute to stroke susceptibility.[14] However, at present, little is known about the specific role of the rs369149111 polymorphism of *HTRA1* in stroke susceptibility.

Growth-arrest specific gene 6 (*GAS6*), containing fifteen exons and spanning 43.8 kb, is located on chromosome 13q34.[18] Related studies have elucidated that *GAS6* can be expressed in vascular smooth muscle cells (VSMCs) and involved in the regulation of vascular homeostasis.[19] VSMCs are key regulators in maintaining vascular homeostasis, and VSMC dysfunction is a common cause of stroke.[20] Therefore, we speculated that *GAS6* may affect stroke susceptibility by regulating VSMC function. At present, studies have revealed the relationship between *GAS6* variants and disease risk, such as the role of rs1803628 in preeclampsia risk.[21] However, little information reveals the relationship between rs1803628 and stroke susceptibility.

Interferon gamma receptor 2(*IFNGR2*), located on chromosome 21q22.11, is the second subunit of the IFN-γ receptor. Some studies have demonstrated that *IFNGR2* gene polymorphisms are associated with the risk of many diseases, such as viremias[22] and marginal zone B-cell lymphoma.[23] And *IFNGR2* (rs9808753) has been identified to be significantly related to the risk of multiple sclerosis (MS).[24] An increased risk of stroke has been reported in MS patients. For example, one cohort study showed that the risk of stroke remained increased in the MS cohort compared with the control group after adjusting for confounding variables.[25] However, little is known about the effect of *IFNGR2* rs9808753 on stroke susceptibility, especially in the Chinese population.

Consequently, a case-control study was carried out to explore the association between *HTRA1*-rs369149111, *GAS6*-rs1803628 and *IFNGR2*-rs9808753 and stroke susceptibility, trying to provide a new perspective for the prevention, diagnosis and treatment of stroke.

Materials and Methods

Study Subjects

Power analysis was performed to determine the required sample size with the relevant parameters (Effect size=0.2, α=0.05, Power=0.9 and case/control=0.995) before the study. Based on the power analysis, the case and control groups should consist of at least 526 and 528 individuals, respectively. All of 1195 unrelated participants (623 stroke cases and 572 controls) were enrolled from Hainan General Hospital. Stroke cases were newly diagnosed based on the World Health Organization diagnosis criteria and were confirmed by professionals using cranial magnetic resonance imaging (MRI) or computed tomography. Patients with tumors, systemic inflammatory diseases or other serious illnesses were excluded. The controls were selected randomly from healthy subjects in Hainan General Hospital. At the same time, we conducted MRI for controls to exclude the asymptomatic stroke. The study was approved by the Ethics Committee of Hainan General Hospital. All participants signed informed consent forms and completed questionnaires about their basic information before entering the study.

Data Collection, DNA Extraction and SNP Genotyping

In this study, three SNPs (rs369149111 in *HTRA1*, rs1803628 in *GAS6*, and rs9808753 in *IFNGR2*) were selected for genotyping. The minor allele frequencies (MAFs) of the above SNPs from the Southern Han Chinese population in the 1000 Genomes Project (https://www.internationalgenome.org/) database were rs369149111-T = 0.186, rs1803628-A = 0.219 and rs9808753-G = 0.433, respectively, which are all greater than 0.05. The peripheral blood (5 mL) was collected from patients and healthy controls in EDTA-coated tubes. The samples were centrifuged and stored at −80°C for following analyses. The demographic and clinical symptoms were obtained from medical records and questionnaires. Primer design was performed by Agena MassARRAY Assay Design 3.0 software. The GoldMag Genomic DNA Purification kit (GoldMag Co., Ltd., Xi'an, China) was utilized to extract genomic DNA. The DNA concentration was estimated by NanoDrop 2000 (Thermo Scientific, Waltham, Massachusetts, USA). Agena MassARRAY (Agena Bioscience, San Diego, CA, USA) was applied to genotype SNPs. Ten percent of the samples were genotyped repeatedly, and the concordance rate was 100%.

Statistical Analysis

The statistical analysis was conducted by SPSS 22.0 statistical package (SPSS, Chicago, IL, USA). Student's *t*-test and the chi-square test were carried out to assess the differences of variables. Fisher's exact test was used to evaluate Hardy-Weinberg equilibrium (HWE) in the control group. The potential functions of three SNPs were predicted by HaploReg v4.1. The association between polymorphisms and stroke susceptibility was assessed by odds ratios (ORs) and 95% confidence intervals (CIs) using logistic regression models with PLINK 1.07 (Harvard, Boston, MA, USA). Multifactor dimensionality reduction (MDR) software (version 3.0.2) was used to assess the effect of SNP-SNP interactions on stroke susceptibility. The p-value < 0.05 was thought of statistical significance.

Results

Features of Subjects

The characteristics of all subjects are illustrated in Table 1. A total of 623 stroke patients (64.05 ± 10.58 years) and 572 healthy controls (64.12 ± 5.50 years) were included in the study. No significant differences in age (p = 0.884), gender (p = 0.111), drinking (p = 0.108), and smoking (p = 0.054) between cases and controls were found. The MAFs of all candidate SNPs were greater than 0.05 (Table 2). In addition, *GAS6*-rs1803628 and *IFNGR2*-rs9808753 were both functionally associated with SiPhy cons, Enhancer histone marks (Table 2).

Association Between Three Candidate SNPs and Stroke Susceptibility

The associations between the three SNPs and stroke susceptibility under different genetic models are presented in Table 2 and Table 3. There were no significant associations of rs369149111 and rs1803628 with stroke susceptibility under the allelic and other genetic models. By contrast, the minor allele-G of rs9808753 was related to an increased risk of stroke (OR = 1.25, 95% CI: 1.06–1.47, p = 0.007). Furthermore, rs9808753 exerted an risk-increasing effect on stroke in the Chinese Han population under the homozygous (OR = 1.59, 95% CI: 1.14–2.23, p = 0.007), dominant (OR = 1.31, 95% CI: 1.02–1.67, p = 0.032), recessive (OR = 1.42, 95% CI: 1.05–1.91, p = 0.022), and additive (OR = 1.26, 95% CI: 1.07–1.48, p = 0.007) models.

Analyses Stratified by Age, Gender, Drinking Status and Smoking Status

To further examine the effects of three SNPs on stroke susceptibility, the stratification analyses based on age, gender, drinking status and smoking status were conducted (Table 4). For subjects aged ≤ 64 years, rs9808753 was correlated with an increased susceptibility to stroke under the homozygous model (OR = 1.65, 95% CI: 1.01–2.71, p = 0.046) and additive model (OR = 1.29, 95% CI: 1.01–1.65, p = 0.042). For males, the risk-increasing effect of rs9808753 on stroke susceptibility was found under the homozygous (OR = 1.82, 95% CI: 1.17–2.84, p = 0.008), recessive (OR = 1.61, 95% CI: 1.09–2.39, p = 0.017) and additive (OR = 1.33, 95% CI: 1.07–1.65, p = 0.009) modes. For subjects who drink alcohol, rs9808753 was correlated with the rising stroke susceptibility under the homozygous (OR = 2.21, 95% CI: 1.30–

Table 1 Demographic Characteristic of the Cases with Stroke and Controls

Characteristic		Cases (%)	Controls (%)	*p*
Total		623	572	
Age	Mean ± SD (years)	64.05 ± 10.58	64.12 ± 5.50	0.884
	> 64	299 (48.0%)	222 (38.8%)	
	≤ 64	324 (52%)	350 (61.2%)	
Gender	Males	394 (63.2%)	336 (58.7%)	0.111
	Females	229 (36.8%)	236 (41.3%)	
Drinking	Yes	310 (49.8%)	258 (45.1%)	0.108
	No	313 (52.5%)	314 (54.9%)	
Smoking	Yes	331 (53.1%)	272 (47.6%)	0.054
	No	292 (49.5%)	300 (52.4%)	
ALT (U/L)	Mean ± SD	26.03 ± 32.49	25.78 ± 18.45	0.880
AST (U/L)	Mean ± SD	25.81 ± 24.38	25.88 ± 9.97	0.954
MONO (%)	Mean ± SD	7.30 ± 1.69	7.06 ± 1.80	0.291
TBA (μmol/L)	Mean ± SD	7.90 ± 8.65	5.78 ± 8.81	**< 0.001**
Cr (μmol/L)	Mean ± SD	73.10 ± 23.75	67.01 ± 12.96	**< 0.001**
UA (μmol/L)	Mean ± SD	272.90 ± 92.84	317.28 ± 76.45	**< 0.001**
TG (mmol/L)	Mean ± SD	1.59 ± 1.27	1.69 ± 1.35	0.196
CHOL (mmol/L)	Mean ± SD	3.91 ± 1.01	4.79 ± 1.02	**< 0.001**
HDL-C (mmol/L)	Mean ± SD	1.12 ± 0.28	1.15 ± 0.24	0.171
LDL-C (mmol/L)	Mean ± SD	1.94 ± 0.68	2.62 ± 0.77	**< 0.001**

Note: $p < 0.05$ in bold type indicates statistical significance.
Abbreviations: SD, standard deviation; ALT, Alanine transaminase; AST, Aspartate aminotransferase; MONO, Monocytes ratio; TBA, Total bile acid; Cr, Creatinine; UA, Uric Acid; TG, Triglyceride; CHOL, Total cholesterol; HDL-C, High density lipid-cholesterol; LDL-C, Low density lipid-cholesterol.

Table 2 Basic Information About SNPs and Association with Risk of Stroke in Allele Model

SNP-ID	Chr: Position	Genes(s)	Alleles A/B	HWE	MAF		OR (95% CI)	*p*	Function
					Case	Control			
rs369149111	10:122461711	*HTRA1*	T/C	0.431	0.091	0.088	1.03 (0.77–1.36)	0.853	——
rs1803628	13:113827141	*GAS6*	A/G	0.556	0.185	0.172	1.09 (0.88–1.35)	0.418	SiPhy cons, Enhancer histone marks
rs9808753	21:33415005	*IFNGR2*	G/A	0.664	0.458	0.404	1.25 (1.06–1.47)	**0.007**	SiPhy cons, Enhancer histone marks, DNAse, Motifs changed, GRASP QTL hits, Selected eQTL hits

Notes: *p* values were calculated using Pearson's χ^2 test. $p < 0.05$ in bold type indicates statistical significance.
Abbreviations: SNP, single nucleotide polymorphism; Chr, chromosome; A, minor alleles; B, major alleles; HWE, Hardy-Weinberg equilibrium; MAF, minor allele frequency; OR, odds ratio; 95% CI, 95% confidence interval.

3.74, $p = 0.003$), dominant (OR = 1.53, 95% CI: 1.08–2.17, $p = 0.018$), recessive (OR = 1.83, 95% CI: 1.13–2.95, $p = 0.014$), and additive (OR = 1.46, 95% CI: 1.14–1.87, $p = 0.003$) modes. For non-smoking subjects, rs1803628 was correlated with an increased risk of stroke under the heterozygous (OR = 1.45, 95% CI: 1.01–2.07, $p = 0.042$), dominant (OR = 1.49, 95% CI: 1.06–2.09, $p = 0.023$) and additive (OR = 1.41, 95% CI: 1.05–1.89, $p = 0.021$) models. The analyses stratified by age, gender, drinking status and smoking status showed no significant association between rs369149111 and stroke susceptibility under all genetic models.

Table 3 Genetic Model Analyses of the Association Between Three SNPs and the Risk of Stroke (Adjusted by Age and Gender)

SNP-ID	Model	Genotype	Case (%)	Control (%)	OR (95% CI)	*p*
rs369149111 *HTRA1*	Genotype	TT	5 (0.8%)	6 (1.1%)	0.77 (0.23–2.55)	0.673
		TC	102 (16.5%)	89 (15.6%)	1.08 (0.79–1.47)	0.650
		CC	511 (82.7%)	476 (83.4%)	1.00	0.819
	Dominant	TT+TC	107 (17.3%)	95 (16.6%)	1.06 (0.78–1.43)	0.728
		CC	511 (82.7%)	476 (83.4%)	1.00	
	Recessive	TT	5 (0.8%)	6 (1.1%)	0.76 (0.23–2.52)	0.659
		TC+CC	613 (99.2%)	565 (99%)	1.00	
	Additive	/	/	/	1.03 (0.78–1.37)	0.828
rs1803628 *GAS6*	Genotype	AA	24 (3.9%)	19 (3.3%)	0.77 (0.23–2.55)	0.673
		AG	181 (29.2%)	158 (27.8%)	1.08 (0.79–1.47)	0.650
		GG	414 (66.9%)	392 (68.9%)	1.00	0.819
	Dominant	AA+AG	205 (33.1%)	177 (31.1%)	1.09 (0.85–1.39)	0.508
		GG	414 (66.9%)	392 (68.9%)	1.00	
	Recessive	AA	24 (3.9%)	19 (3.3%)	1.19 (0.64–2.19)	0.588
		AG+GG	595 (96.1%)	550 (96.7%)	1.00	
	Additive	/	/	/	1.08 (0.88–1.33)	0.455
rs9808753 *IFNGR2*	Genotype	GG	130 (20.9%)	90 (15.8%)	1.59 (1.14–2.23)	**0.007**
		GA	311 (49.9%)	280 (49.1%)	1.21 (0.94–1.57)	0.140
		AA	182 (29.2%)	200 (35.1%)	1.00	0.024
	Dominant	GG+GA	441 (70.8%)	370 (64.9%)	1.31 (1.02–1.67)	**0.032**
		AA	182 (29.2%)	200 (35.1%)	1.00	
	Recessive	GG	130 (20.9%)	90 (15.8%)	1.42 (1.05–1.91)	**0.022**
		GA+AA	493 (79.1%)	480 (84.2%)	1.00	
	Additive	/	/	/	1.26 (1.07–1.48)	**0.007**

Notes: *p* values were calculated by Wald test under logistic regression. $p < 0.05$ in bold type indicates statistical significance.
Abbreviations: SNP, single nucleotide polymorphism; OR, odds ratio; 95% CI, 95% confidence interval.

MDR Analysis of the Role of SNP-SNP Interaction in Stroke Susceptibility

The SNP-SNP interaction was analyzed by MDR. Figure 1 is a dendrogram about the analysis of the SNP-SNP interaction. Different colors represent the synergistic or redundant effects of SNP-SNP interaction on stroke susceptibility. The brown line indicates that candidate SNPs have no synergistic or redundant effect in regulating stroke susceptibility. The blue line indicates that candidate SNPs have a redundant effect on stroke susceptibility (Figure 1). Additionally, as presented by Table 5, the rs9808753 single locus was regarded as the best model to predict stroke risk, with the highest testing balanced accuracy of 0.529 and good cross-validation consistency (10/10).

Difference in Indicators Based on the Genotypes of Selected SNPs

We also assessed the impact of three candidate SNPs on the level of indicators under different genotypes. As shown in Table 6, the levels of alanine transaminase (ALT, $p < 0.001$), aspartate aminotransferase (AST, $p < 0.001$), and monocytes ratio (MONO, $p = 0.018$) had significant differences under different genotypes of rs1803628. Differences in total cholesterol levels (CHOL, $p = 0.014$) under different genotypes of rs9808753 were also detected. Whereas, there was no significant difference between rs369149111 and the level of indicators (Table S1).

Discussion

To our knowledge, this study is the first to assess the relationship between three selected SNPs and stroke susceptibility in the Chinese Han population. Our results showed that rs369149111 was unrelated with stroke susceptibility in overall and stratification analyses. The rs1803628 was related to stroke susceptibility only in the non-smoking group. The

Table 4 Distribution of SNPs in Different Age, Gender, Smoking Status, Drinking Status and Its Association with Risk of Stroke

SNP-ID	Model	Genotype	Case	Control	OR (95% CI)	*p*	Case	Control	OR (95% CI)	*p*
Smoking status			**Smoking**				**Non-smoking**			
rs1803628	Genotype	AA	10 (3%)	10 (3.7%)	0.79 (0.32–1.94)	0.606	14 (4.8%)	9 (3%)	1.83 (0.77–4.34)	0.168
GAS6		AG	82 (24.9%)	78 (28.8%)	0.80 (0.56–1.16)	0.239	99 (34.1%)	80 (26.9%)	1.45 (1.01–2.07)	**0.042**
		GG	237 (72%)	183 (67.5%)	1.00	0.463	177 (61%)	209 (70.1%)	1.00	0.068
	Dominant	AA+AG	92 (28%)	88 (32.5%)	0.80 (0.56–1.14)	0.215	113 (39%)	89 (29.9%)	1.49 (1.06–2.09)	**0.023**
		GG	237 (72%)	183 (67.5%)	1.00		177 (61%)	209 (70.1%)	1.00	
	Recessive	AA	10 (3%)	10 (3.7%)	0.84 (0.34–2.05)	0.695	14 (4.8%)	9 (3%)	1.63 (0.69–3.83)	0.263
		AG+GG	319 (97%)	261 (96.3%)	1.00		276 (95.2%)	289 (97%)	1.00	
	Additive	/	/	/	0.83 (0.62–1.13)	0.236	/	/	1.41 (1.05–1.89)	**0.021**
Age			**Age ≤ 64**				**Age > 64**			
rs9808753	Genotype	GG	70 (21.6%)	58 (16.7%)	1.65 (1.01–2.71)	**0.046**	60 (20.1%)	32 (14.4%)	1.50 (0.86–2.6)	0.152
IFNGR2		GA	164 (50.6%)	167 (48%)	1.31 (0.88–1.93)	0.181	147 (49.2%)	113 (50.9%)	1.04 (0.69–1.57)	0.849
		AA	90 (27.8%)	123 (35.3%)	1.00	0.125	92 (30.8%)	77 (34.7%)	1.00	0.316
	Dominant	GG+GA	234 (72.2%)	225 (64.7%)	1.40 (0.97–2.03)	0.076	207 (69.2%)	145 (65.3%)	1.14 (0.77–1.68)	0.504
		AA	90 (27.8%)	123 (35.3%)	1.00		92 (30.8%)	77 (34.7%)	1.00	
	Recessive	GG	70 (21.6%)	58 (16.7%)	1.41 (0.91–2.17)	0.122	60 (20.1%)	32 (14.4%)	1.46 (0.89–2.39)	0.132
		GA+AA	254 (78.4%)	290 (83.3%)	1.00		239 (79.9%)	190 (85.6%)	1.00	
	Additive	/	/	/	1.29 (1.01–1.65)	**0.042**	/	/	1.19 (0.91–1.55)	0.201
Gender			**Females**				**Males**			
rs9808753	Genotype	GG	48 (21%)	43 (18.3%)	1.33 (0.79–2.24)	0.285	82 (20.8%)	47 (14%)	1.82 (1.17–2.84)	**0.008**
IFNGR2		GA	111 (48.5%)	109 (46.4%)	1.21 (0.80–1.83)	0.366	200 (50.8%)	171 (51%)	1.22 (0.88–1.70)	0.232
		AA	70 (30.6%)	83 (35.3%)	1.00	0.509	112 (28.4%)	117 (34.9%)	1.00	0.029
	Dominant	GG+GA	159 (69.4%)	152 (64.7%)	1.24 (0.84–1.83)	0.271	282 (71.6%)	218 (65.1%)	1.35 (0.99–1.85)	0.059
		AA	70 (30.6%)	83 (35.3%)	1.00		112 (28.4%)	117 (34.9%)	1.00	
	Recessive	GG	48 (21%)	43 (18.3%)	1.19 (0.75–1.88)	0.465	82 (20.8%)	47 (14%)	1.61 (1.09–2.39)	**0.017**
		GA+AA	181 (79%)	192 (81.7%)	1.00		312 (79.2%)	288 (86%)	1.00	
	Additive	/	/	/	1.16 (0.89–1.49)	0.257	/	/	1.33 (1.07–1.65)	**0.009**

Drinking status			Drinking				Non-drinking			
rs9808753	Genotype	GG	58 (18.7%)	29 (11.3%)	2.21 (1.30–3.74)	**0.003**	72 (23%)	61 (19.5%)	1.30 (0.83–2.03)	0.249
IFNGR2		GA	161 (51.9%)	128 (49.8%)	1.38 (0.95–1.98)	0.089	150 (47.9%)	152 (48.6%)	1.08 (0.75–1.56)	0.667
		AA	91 (29.4%)	100 (38.9%)	1.00	0.012	91 (29.1%)	100 (31.9%)	1.00	0.507
	Dominant	GG+GA	219 (70.7%)	157 (61.1%)	1.53 (1.08–2.17)	**0.018**	222 (70.9%)	213 (68%)	1.15 (0.81–1.61)	0.437
		AA	91 (29.4%)	100 (38.9%)	1.00		91 (29.1%)	100 (31.9%)	1.00	
	Recessive	GG	58 (18.7%)	29 (11.3%)	1.83 (1.13–2.95)	**0.014**	72 (23%)	61 (19.5%)	1.24 (0.84–1.82)	0.278
		GA+AA	252 (81.3%)	228 (88.7%)	1.00		241 (77%)	252 (80.5%)	1.00	
	Additive	/	/	/	1.46 (1.14–1.87)	**0.003**	/	/	1.14 (0.91–1.41)	0.261

Notes: *p* values were calculated by Wald test under logistic regression with adjustment for gender and age. $p < 0.05$ in bold type indicates statistical significance.
Abbreviations: SNP, single nucleotide polymorphism; OR, odds ratio; 95% CI, 95% confidence interval.

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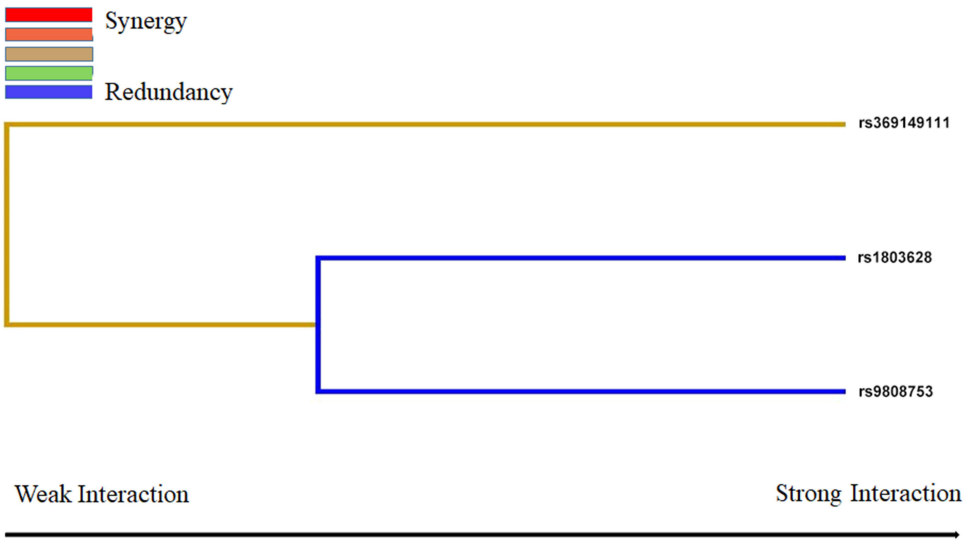

Figure 1 Dendrogram about the analysis of SNP-SNP interactions. Colors in the dendrogram indicate synergies or redundancies. Red indicates a high degree of synergistic interaction; Orange indicates a lesser degree, whereas brown represents the midpoint; blue represents the highest level of redundancy, followed by green. The shorter the distance between nodes, the stronger the interaction between them.

rs9808753 was significantly associated with a higher stroke susceptibility under the allelic, dominant, recessive and additive models, and the relationship between rs9808753 and stroke susceptibility was age-, gender- and drinking dependent. Collectively, the results broadened our knowledge on the effects of SNPs on stroke susceptibility, and provided new clues for the screening of high-risk groups and early detection and diagnosis of the disease.

The occurrence of stroke is related to many factors. In our results, rs1803628 was related to an increased stroke susceptibility only in the non-smoking subgroup. This is inconsistent with previous studies that has identified smoking as a risk factor for stroke.[26] This may be related to the random selection of the study population. In addition, we also noticed that rs9808753 was significantly associated with a higher stroke susceptibility under the allelic, dominant, recessive and additive models. Particularly, the effect of rs9808753 on stroke susceptibility might be correlated with age, gender and drinking, according to the results of stratification analyses. It has been reported that age is a major risk factor for stroke occurrence, and the older the age, the higher the stroke risk.[2] However, our results suggested that rs9808753 was associated with an increased susceptibility of stroke in people younger than 64 years of age, which may be related to study participants and their lifestyles. It could also mean that stroke is becoming more common at a younger age. Similar to our findings, Mao et al have pointed out that gene polymorphisms are associated with stroke risk in Chinese males[27] and Caucasian males.[28] In addition, males are historically considered to be more susceptible to stroke than females,[29] which is consistent with our findings. This is likely because of the higher risk of dyslipidemia, diabetes, myocardial infarction and peripheral artery disease in males, as well as their unhealthy living habits, such as smoking and alcohol consumption. Just as Millwood et al have found that alcohol consumption is a risk factor for stroke.[30] All in all, these findings may suggest that genetic susceptibility to stroke differs by age, gender, drinking and smoking status in genetic association studies.

The detection of stroke-related clinical indicators is very important for the prevention, diagnosis and treatment of stroke. Our study found a significant difference in CHOL level under the different genotypes of rs9808753. As elucidated

Table 5 Summary of SNP-SNP Interactions on the Risk of Stroke Analyzed by MDR Method

Model	Training Bal. Acc	Testing Bal. Acc	CVC	OR (95% CI)	*p*
rs9808753*	0.529	0.529	10/10	1.31 (1.02–1.68)	**0.036**
rs369149111, rs9808753	0.534	0.504	10/10	1.39 (1.07–1.82)	**0.015**
rs369149111, rs1803628, rs9808753	0.540	0.490	10/10	1.38 (1.09–1.76)	**0.008**

Notes: *p* values were calculated using χ^2 test. $p < 0.05$ in bold type indicates statistical significance. *The best model in MDR analysis.
Abbreviations: MDR, multifactor dimensionality reduction; Bal. Acc, balanced accuracy; CVC, cross-validation consistency; OR, odds ratio; 95% CI, 95% confidence interval.

Table 6 Clinical Characteristics of Stroke Patients Based on the Genotypes of Selected SNPs

Characteristics	*GAS6* rs1803628				*IFNGR2* rs9808753			
	AA	**AG**	**GG**	***p***	**AA**	**AG**	**GG**	***p***
ALT (U/L)	64.47 ± 112.44	21.88 ± 11.81	25.69 ± 27.73	**< 0.001**	27.81 ± 33.81	26.76 ± 37.25	21.66 ± 11.93	0.328
AST (U/L)	58.29 ± 95.53	22.02 ± 10.05	25.65 ± 17.49	**< 0.001**	26.50 ± 23.67	26.16 ± 27.17	23.97 ± 17.50	0.706
MONO (%)	5.12 ± 0.31	6.79 ± 1.63	7.71 ± 1.62	**0.018**	0.42 ± 0.17	0.41 ± 0.21	0.44 ± 0.20	0.636
TBA (μmol/L)	10.54± 9.62	8.16 ± 6.52	7.64 ± 9.42	0.376	8.08 ± 11.70	8.06 ± 7.17	7.21 ± 6.20	0.701
Cr (μmol/L)	77.75 ± 24.83	72.46 ± 28.81	73.16 ± 21.23	0.678	72.72 ± 21.71	72.79 ± 20.43	74.48 ± 33.08	0.832
UA (μmol/L)	245.23 ± 85.00	278.45 ± 95.22	272.15 ± 91.83	0.279	277.04 ± 97.29	271.04 ± 92.25	271.59 ± 88.34	0.800
TG (mmol/L)	1.51 ± 1.18	1.58 ± 1.56	1.59 ± 1.11	0.961	1.53 ± 0.79	1.70 ± 1.58	1.38 ± 0.88	0.113
CHOL (mmol/L)	3.97 ± 1.08	3.93 ± 1.05	3.89 ± 0.99	0.890	3.87 ± 0.98	4.03 ± 1.02	3.67 ± 0.98	**0.014**
HDL-C (mmol/L)	1.25 ± 0.33	1.13 ± 0.26	1.11 ± 0.28	0.083	1.08 ± 0.22	1.15 ± 0.30	1.13 ± 0.29	0.065
LDL-C (mmol/L)	1.92 ± 0.76	2.01 ± 0.71	1.91 ± 0.67	0.399	1.92 ± 0.65	2.00 ± 0.70	1.82 ± 0.67	0.096

Note: $p < 0.05$ in bold type indicates statistical significance.

Abbreviations: ALT, Alanine transaminase; AST, Aspartate aminotransferase; MONO, Monocytes ratio; TBA, Total bile acid; Cr, Creatinine; UA, Uric Acid; TG, Triglyceride; CHOL, Cholesterol; HDL-C, High density lipid-cholesterol; LDL-C, Low density lipid-cholesterol.

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by Wang et al, CHOL value is associated with an elevated risk of incident stroke and ischemic stroke, which is partly consistent with our results.[31] Furthermore, we found the levels of ALT, AST and MONO had significant differences under the different genotypes of rs1803628. However, no significant associations of AST and ALT with stroke were found,[32] which may be related to the fact that rs1803628 was a risk factor for stroke only in non-drinking subjects.

It should be noted that this study has several limitations. First, all subjects were enrolled from the same hospital, so there is a selection bias. Second, due to missing information on smoking and alcohol consumption in some subjects, only age and gender were selected for adjustment in this study. Finally, the association of *IFNGR2* rs9808753 with increased stroke susceptibility in this study was not functionally tested. In future studies, we will expand the sample size, improve the sample information, adjust the risk factors, and perform corresponding functional tests to further analyze the relationship between *IFNGR2* rs9808753 and stroke susceptibility to make our study more convincing.

Conclusion

In summary, we found that *IFNGR2*-rs9808753 is significantly associated with an increased risk of stroke in the Han Chinese population, suggesting that *IFNGR2* variants may be biomarkers for the early detection and diagnosis of stroke.

Data Sharing Statement

All data generated or analyzed during this study are included in this published article and its Supplementary Information.

Ethics Approval and Consent to Participate

This study was approved by the Ethics Committee of Hainan General Hospital and conformed to the ethical principles of the Declaration of Helsinki. All participants signed informed consent forms before participating in this study.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors declare that they have no competing interests in this work.

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