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ORIGINAL RESEARCH

TLR4 rs11536889 and rs1927914 SNPs are Associated with Ischemic Stroke Risk in a Southern Chinese Han Population

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Background: The polymorphisms of the Toll-like receptor 4 (TLR4) gene are associated with lipid levels, such as serum total cholesterol, low-density lipoprotein cholesterol (LDL-C), and triglycerides (TG). The aim of this study was to detect the association of the six polymorphisms in *TLR4* gene and serum lipid levels and the risk of ischemic stroke (IS) in a Southern Chinese Han population.
Methods: Genotypes of six polymorphisms in *TLR4* gene in 372 subjects (IS, 186 and healthy controls, 186) were determined by the Snapshot Technology. The relationship between *TLR4* polymorphisms and serum lipid levels, risk of IS were analyzed.
Results: The levels of fasting blood glucose and triglyceride were higher, and the high-density lipoprotein cholesterol (HDL-C) level was lower in IS cases than those in controls. The allelic frequencies of *TLR4* gene rs11536889 SNP (p=0.037) and rs1927914 SNP (p=0.036) were different between the IS and control groups. The rs11536889 C allele carriers had an increased risk of IS (odds ratio (OR)=1.278, 95% confidence interval (CI) =1.013–1.784, p=0.037 for C vs G alleles), and the G allele carriers of rs1927914 had a decreased risk of IS (OR=0.695, 95% CI=0.534–0.949, p=0.036 for G vs A allele) in the southern Chinese Han population.
Conclusion: The *TLR4* rs11536889 and rs1927914 SNPs may be associated with decreased risk of IS in the Chinese population.
Keywords: ischemic stroke, the toll-like receptors 4, single-nucleotide polymorphisms

Introduction

Every year, there are over 15 million new cases of stroke worldwide, and stroke causes approximately 6 million deaths each year, accounting for about 10% of the total global deaths.[1] As the process of population aging progresses, the disease and social burdens caused by strokes have become increasingly severe, and this situation is particularly prominent in regions such as sub-Saharan Africa and South Asia.[2] Stroke is a major cause of disability and death in the aging population and about 85% of stroke are ischemic.[3] The pathological process of ischemic stroke (IS), from the formation of atherosclerotic plaques to the ischemic-reperfusion injury of brain tissue, is the result of the combined effects of genetic factors and environmental factors.[4] It has been reported that single-nucleotide polymorphisms (SNPs) of toll-like receptor 4 (TLR4), tumor necrosis factor receptor-associated factor 6 (TRAF6), T-cell immunoglobulin and mucin domain 4 gene (TIMD4), and mitogen-activated protein kinase 4 (MAP2K4) are associated with the risk of IS in Southern Chinese Han Population, which indicating gene polymorphisms might have potential to predict the susceptibility of IS.[5–8]

Stroke is associated with immune response and inflammation responses, and Toll-like receptors (TLRs) play a significant role in this process. TLRs are a group of pattern recognition receptors expressed on the cell surface. When activated, they may trigger immune and inflammatory responses.[9,10] TLR4 is a major member of the TLRs family, and the gene encoding *TLR4* is located on chromosome 9q32-q33.[11] TLR4 recognizes pathogen-associated molecular patterns (such as lipopolysaccharide) and damage-associated molecular patterns (such as heat shock proteins), thereby initiating downstream inflammatory signaling pathways, activating transcription factors such as nuclear factor -κB (NF-κB), and triggering the release of pro-inflammatory cytokines, thereby participating in immune responses and

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inflammatory reactions.[12,13] The myeloid differentiation factor 88 (MyD88) dependent activation of nuclear transcription factor-κB (NF-κB) is the main TLR-mediated inflammatory response signaling pathway.[14] When TLR4 is exposed to its ligands, NF-κB is activated by MyD88, which results to the release of chemokines and cytokines, and induce adaptive immune–inflammation responses.[15] The phosphorylation and expression level of TLR4 are associated with brain ischemic injury.[16] It has been reported that *TLR4* C1196T polymorphism was correlated with the occurrence of extra-cranial large artery stroke.[17] *TLR4* gene polymorphism A119C is significantly associated with stroke.[18] The rs1927914 is located in the 5′ untranslated region (UTR) of the *TLR4* gene, and it is associated with the expression of the *TLR4* gene.[19] Gu et al discovered that *TLR4* rs1927914 polymorphism was associated with the susceptibility of IS in a Chinese population.[5] However, there are also some inconsistent research results. *TLR4* Asp299Gly and Thr399Ile polymorphisms showed lack of association with IS.[18,20,21]

Based on the literature retrieval, a total of six *TLR4* gene polymorphisms (rs11536889, rs1927914, rs7193343, rs10759932, rs11536879, and rs11536891) were found to be associated with the susceptibility of IS, but some of the research results of original studies failed to reach an agreement. One of the reasons is the association of *TLR4* SNPs and the susceptibility to IS was different among different ethnics. Currently, we conducted a case-control study to analyze the association of these 6 *TLR4* gene variants with the susceptibility to IS.

Materials and Methods

Study Design

Consecutive IS patients from the Department of Neurology, Meizhou People's Hospital from January 2018 to July 2018 were collected. The diagnosis of IS is based on the Chinese Stroke Association Guidelines for Clinical Management of Ischaemic Cerebrovascular Diseases[22] The presence of ischemic changes in brain tissue is indicated by a head computed tomography (CT) or magnetic resonance imaging (MRI) examination.[23] All IS cases had received rigorous examination from at least two experienced associate chief physicians from the Department of Neurology. Exclusion criteria are as follows: (1) patients with a history of unknown causes of stroke, intracranial space-occupying lesions, and infections; (2) patients with inflammatory diseases, heart diseases, liver diseases, kidney diseases, thyroid diseases and autoimmune diseases; and (3) incomplete clinical medical records.

A group of people who underwent health check-ups at Meizhou People's Hospital during the same period were selected as controls. They had no history of stroke and ruled out cerebrovascular diseases through medical history inquiry, physical examination (neck vascular ultrasound, cranial computed tomography (CT), and magnetic resonance imaging (MRI)), and related laboratory tests. CT or MRI examinations. The exclusion criteria were the same as those of the case group. At the same time, their age and gender were required to be matched with those of the case group to reduce the influence of confounding factors. Based on the case group, the gender was matched to the control group at a ratio of 1:1.2 to 1:1.3, and the age matching accuracy was ±5 years.

All enrolled IS cases and control individuals were Han Chinese from Meizhou, Guangdong province, which is in the south of China. Present study was performed under the guidance of the Declaration of Helsinki and approved by the Ethics Committee of Medicine, Meizhou People's Hospital.

Data Collection

For all IS cases and control participants, the sociodemographic information and lifestyle risk factors were collected by standardized self-reported questionnaires by trained research staff. Written informed consent was obtained from each participant before any of the study procedures were conducted.

Serum level of total cholesterol (TC) and triglycerides (TG) were measured by commercially kits (Medicalsystem Biotechnology Co., Ltd., Ningbo, China). The normal values of serum TC and TG were 3.10–5.17 mmol/L and 0.56–1.70 mmol/L. Hyperlipidemic was defined as TC > 5.17 mmol/L and/or TG > 1.70 mmol/L. Serum level of high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C), apolipoprotein (Apo) A1 and ApoB were measured using commercial kits (Zhongshan Chuangyi Biochemical Engineering Co., Ltd., Zhongshan, China), performed with AU5800 Chemistry Analyzer (Beckman). The normal values of serum HDL-C and LDL-C were

0.91–1.81 mmol/L and 2.70–3.20 mmol/L, respectively. For ApoA1, ApoB levels and the ApoA1/ApoB ratio, the normal values were 1.00–1.78 g/L, 0.63–1.14 g/L, and 1.00–2.50. All above determinations were in accordance with the manufacturer's instructions in the Clinical Laboratory Center, Meizhou People's hospital.

DNA Isolation and Genotyping

Five milliliters of peripheral venous blood from the research subjects were collected and placed in an EDTA anticoagulant tube, gently inverted and mixed the sample. The blood samples were stored in a refrigerator at 4°C and equally subdivided into three tubes on the day of blood drawn. One tube was used to extract the total DNA within 7 days of sample collection. The other two tubes were stored in a freezer at −80 °C. The total DNA was extracted with a TIANamp blood DNA kit (Tiangen Biotech, Beijing, China). A total of six polymorphisms in *TLR4* gene were selected, such as rs11536889, rs1927914, rs7193343, rs10759932, rs11536879, and rs11536891. The Sequenom Assay Designer 3.1 software was used to design the primers used in the polymerase chain reaction (PCR) amplification of the *TLR4* gene. The genotypes were analyzed using the Sequenom MassARRAY iPLEX platform (Sequenom, San Diego, USA). Five percent of the subjects were randomly selected to repeat the genotyping. The status of the cases and the control subjects were blinded to laboratory technicians.

Statistical Analysis

This study is a 1:1 matched case-control study. The sample size was calculated based on the previous research data on the association between the *TLR4* gene polymorphisms and ischemic stroke, using the Power Analysis and Sample Size (PASS) Software. The statistical parameters were set as follows: the significance level $\alpha = 0.05$ (two-sided test), the power = 0.85, the adjustment coefficient for the potential confounding effect of the matching factors was set at 1.2. After calculation, at least 180 pairs (180 cases and 180 controls) of research subjects need to be included in the study.

Quantitative variables were presented as mean ± standard deviation (SD), and compared by the unpaired Student's t-tests between IS cases and controls. Qualitative variables were expressed as percentages, and compared by chi-square test. Hardy-Weinberg equilibrium was verified with the standard goodness-of-fit test. The multiple comparisons of the association between SNPs and diseases were corrected using the Bonferroni correction method. Odds ratio (OR) and 95% confidence interval (CI) was calculated to assess the correlation between the risk of IS and genotypes by unconditional logistic regression. All the statistical analyses were conducted using the SPSS 24.0 software. $p<0.05$ is considered to be statistically significant.

Results

Clinical Characteristics of IS Cases and Controls

A total of 186 IS patients and 186 healthy control individuals were included and successfully genotyped. The characteristics of IS patients and healthy controls are shown in Table 1. The serum levels of TC, LDL-C and ApoB and ApoA1/ApoB were not different between the IS and control groups (all $p > 0.05$). The levels of fasting blood glucose ($p<0.001$) was higher, and the triglyceride ($p=0.027$), HDL-C ($p<0.001$), and Apo-A1 ($p<0.001$) levels were lower in IS cases than those in controls.

Genotypic and Allelic Frequencies

Genotype analyses were performed among 6 *TLR4* gene variants. The genotypic distribution of these two SNPs was presented in Table 2. The genotypic distributions of these two SNPs were in accordance with the Hardy-Weinberg equilibrium among the subjects (all $p>0.05$). Only two of them, that is rs11536889 and rs1927914, showed significantly associated with the risk of IS. For rs11536889, the GG, GC, CC genotype frequencies were 62.4%, 30.1%, and 7.5% in controls; and 51.1%, 43.5%, and 5.4% in IS cases. For rs1927914, the AA, AG, GG genotype frequencies were 40.9%, 44.1%, and 15.1% in controls; and 43.5%, 49.5%, and 7.0% in IS cases, respectively.

The G and C allele frequencies of rs11536889 were 77.4% and 22.6% in controls, 72.8% and 27.2% in IS cases; the A and G allele frequencies of rs1927914 were 62.9% and 37.1% in controls, 68.3% and 31.7% in IS cases, respectively. The

Table 1 Comparison of the Clinical Characteristics and Serum Lipid Levels Between the IS Cases and Controls

Parameter	IS (N=186)	Controls (N=186)	*p* value
Fasting blood glucose, mmol/L	6.69±3.29	5.50±1.50	<0.001 (t=−4.488)
TC, mmol/L	5.09±1.24	5.33±1.15	0.061 (t=1.883)
Triglyceride, mmol/L	1.57±0.86	1.89±1.73	0.027 (t=2.217)
LDL - C, mmol/L	3.02±0.90	3.18±1.75	0.253 (t=1.145)
HDL- C, mmol/L	1.31±0.35	1.49±0.38	<0.001 (t=3.598)
ApoA1, g/L	1.23±0.32	1.46±0.38	<0.001 (t=6.497)
ApoB, g/L	1.27±5.37	0.94±0.27	0.402 (t=−0.839)
ApoA1/ApoB	1.50±0.55	1.68±0.71	0.007 (t=2.703)

Abbreviations: IS, ischemic stroke; TC, total cholesterol; HDL -C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; ApoA1, apolipoprotein A1; ApoB, apolipoprotein B; ApoA1/ApoB, the ratio of apolipoprotein A1 to apolipoprotein B.

allelic frequencies of *TLR4* gene rs11536889 SNP (p=0.037) and rs1927914 SNP (p=0.036) were different between the IS and control groups. The G allele carriers of rs11536889 and G allele carriers of rs1927914 had a decreased risk of IS.

Genotype and Allele Frequencies of rs11536889, rs1927914, rs7193343, rs10759932, rs11536879, and rs11536891 and the Risk of IS

The rs11536889 C allele carriers had an increased risk of IS (OR=1.587, 95% CI=11.050–2.399, p=0.028 for CG/GG vs GG genotypes, Table 2; OR=1.278, 95% CI=1.013–1.784, p=0.037 for C vs G alleles, Table 3). The G allele carriers of rs1927914 had a decreased risk of IS (OR=0.436, 95% CI=0.210–0.903, p=0.025 for AG/GG vs AA genotypes, Table 2; OR=0.695, 95% CI=0.534–0.949, p=0.036 for G vs A allele, Table 3).

Genotypes and Serum Lipid Levels

As shown in Table 4, no significant differences in the serum lipid parameters were found between the GG and GC/CC genotypes of rs11536889, and GG and AA/AG genotypes of rs1927914 (all $p > 0.05$).

Discussion

IS, as one of the leading causes of death and disability worldwide, imposes a heavy burden on society and families due to its high incidence, high recurrence rate and high disability rate.[24,25] With the development of molecular genetics and genomics technologies, more and more studies have focused on the association between genetic polymorphisms and ischemic stroke, attempting to reveal the pathogenesis of the disease at the genetic level and providing theoretical basis for early prevention, precise diagnosis and individualized treatment of the disease.[26,27] Studies have shown that the *TLR4* gene is associated with the development of IS. It might be related to the fact that TLR4, as a key pattern recognition receptor in the innate immune system, plays a role in inflammatory responses and immune regulation.[28,29]

TLR4, as a key member of the pattern recognition receptor family, plays an important role in identifying pathogen-associated molecular patterns (PAMPS) and endogenous danger signals.[30,31] When polymorphic changes occur in the *TLR4* gene, it may affect the structure and function of the protein it encodes, thereby interfering with the activation of downstream inflammatory signaling pathways.[32,33] Mutations at sites such as rs4986790 and rs4986791 can enhance the sensitivity of TLR4 to ligands such as lipopolysaccharide (LPS),[34] continuously activate inflammatory signaling pathways such as NF-κB, lead to the massive release of pro-inflammatory cytokines such as IL-6 and TNF-α,[35,36] and promote the instability and rupture of atherosclerotic plaques and increase the risk of IS.[37]

The current study investigated the relationship of *TLR4* gene 6 SNPs (rs11536889, rs1927914, rs7193343, rs10759932, rs11536879, and rs11536891) with the risk of IS. The results showed that *TLR4* SNPs rs1927914 and rs11536889 are associated with IS risk in the southern Chinese Han population. The rs1927914 is located in the 5′ untranslated region (UTR) of the *TLR4* gene, and it is associated with the expression of the *TLR4* gene.[19] Gu et al showed

Table 2 Genotype Distribution of rs11536889, rs1927914, rs7193343, rs10759932, rs11536879, and rs11536891 and the Risk of IS

SNP_ID	Model	Genotype	IS	Control	Adjusted OR (95% CI)*	*p* values	SNP_ID	Model	Genotype	IS	Control	Adjusted OR (95% CI)*	*p* values
rs11536889							rs10759932						
	Codominant	G/G	95(51.1%)	116(62.4%)	1			Codominant	T/T	112(60.2%)	105(56.5%)	1	
		G/C	81(43.5%)	56(30.1%)	1.766(1.143–2.730)	0.010			T/C	70(37.6%)	67(36.0%)	0.979(0.638–1.503)	0.924
		C/C	10(5.4%)	14(7.5%)	0.872(0.371–2.052)	0.754			C/C	4(2.2%)	14(7.5%)	0.268(0.085–0.840)	0.204
	Dominant	G/G	95(51.1%)	116(62.4%)	1			Dominant	T/T	112(60.2%)	105(56.5%)	1	
		G/C+C/C	91(48.9%)	70(37.6%)	1.587(1.050–2.399)	0.028			T/C+C/C	74(39.8%)	81(43.5%)	0.856(0.567–1.294)	0.462
	Recessive	G/G+G/C	176(94.6%)	172(92.5%)	1			Recessive	T/T+T/C	182(97.8%)	172(92.5%)	1	
		C/C	10(5.4%)	14(7.5%)	0.698(0.302–1.614)	0.401			C/C	4(2.2%)	14(7.5%)	0.270(0.087–0.836)	0.023
rs1927914							rs11536879						
	Codominant	A/A	81(43.5%)	76(40.9%)	1			Codominant	A/A	150(80.6%)	149(80.1%)	1	
		A/G	92(49.5%)	82(44.1%)	1.053(0.683–1.621)	0.816			A/G	34(18.3%)	33(17.7%)	1.023(0.602–1.739)	0.932
		G/G	13(7.0%)	28(15.1%)	0.436(0.210–0.903)	0.025			G/G	2(1.1%)	4(2.2%)	0.497(0.090–2.753)	0.423
	Dominant	A/A	81(43.5%)	76(40.9%)	1			Dominant	A/A	150(80.6%)	149(80.1%)	1	
		A/G+G/G	105(56.5%)	110(59.1%)	0.896(0.593–1.352)	0.600			A/G+G/G	36(19.4%)	37(19.9%)	0.966(0.579–1.612)	0.896
	Recessive	A/A+A/G	173(93.0%)	158(84.9%)	1			Recessive	A/A+A/G	184(98.9%)	182(97.8%)	1	
		G/G	13(7.0%)	28(15.1%)	0.424(0.212–0.847)	0.015			G/G	2(1.1%)	4(2.2%)	0.495(0.089–2.734)	0.420
rs7193343							rs11536891						
	Codominant	T/T	85(45.7%)	99(53.2%)	1			Codominant	T/T	151(81.2%)	150(80.6%)	1	
		T/C	78(41.9%)	77(41.4%)	1.180(0.769–1.810)	0.449			T/C	35(18.8%)	34(18.3%)	1.023(0.606–1.726)	0.933
		C/C	23(12.4%)	10(5.4%)	2.679(1.207–5.944)	0.015			C/C	0(0)	2(1.1%)	–	0.999
	Dominant	T/T	85(44.1)	99(53.2%)	1			Dominant	T/T	151(81.2%)	150(80.6%)	1	
		T/C+C/C	101(55.9)	87(46.8%)	1.352(0.899–2.033)	0.147			T/C+C/C	35(18.8%)	36(19.4%)	0.966(0.576–1.620)	0.895
	Recessive	T/T+T/C	163(87.2)	176(94.6%)	1			Recessive	T/T+T/C	186(100.0%)	184(98.9%)	1	
		C/C	23(12.8)	10(5.4%)	2.483(1.147–5.376)	0.021			C/C	0(0)	2(1.1%)	–	0.999

Notes: The genotype distribution of rs11536889, rs1927914, rs7193343, rs10759932, rs11536879, and rs11536891 conformed to the Hardy-Weinberg equilibrium among IS patients with *p* values of 0.168, 0.053, 0.441, 0.065, 0.962, and 0.157, respectively. The genotype distribution of rs11536889, rs1927914, rs7193343, rs10759932, rs11536879, and rs11536891 conformed to the Hardy-Weinberg equilibrium among controls with *p* values of 0.058, 0.450, 0.314, 0.471, 0.193, and 0.962, respectively.

Abbreviations: IS, ischemic stroke; OR, odds ratio; CI, confidence interval.

Table 3 Allele Frequencies of rs11536889, rs1927914, rs7193343, rs10759932, rs11536879, and rs11536891 and the Risk of IS

SNP_ID	Allele	IS	Control	OR (95% CI)	*p* value
rs11536889					
	G	271(72.8%)	288(77.4%)	1	
	C	101(27.2%)	84(22.6%)	1.278(1.013–1.784)	0.037
rs1927914					
	A	254(68.3%)	234(62.9%)	1	
	G	118(31.7%)	138(37.1%)	0.695(0.534–0.949)	0.036
rs7193343					
	T	248(66.7%)	275(73.9%)	1	
	C	124(33.3%)	97(26.1%)	1.418(1.1033–1.945)	0.051
rs10759932					
	T	294(79.0%)	277(74.5%)	1	
	C	78(21.0%)	95(25.5%)	0.774(0.550–1.088)	0.082
rs11536879					
	A	334(89.8%)	331(89.0%)	1	
	G	38(10.2%)	41(11.0%)	0.919(0.576–1.465)	0.406
rs11536891					
	T	337(90.6%)	334(89.8%)	1	
	C	35(9.4%)	38(10.2%)	0.913(0.563–1.480)	0.403

Abbreviations: IS, ischemic stroke; OR, odds ratio; CI, confidence interval.

that *TLR4* gene rs1927914 polymorphism was associated with the risk of IS in males.[5] Our research also revealed that rs1927914 is significantly associated with the risk of IS in both men and women. Song et al showed that *TLR4* rs1927911 was associated with atherosclerotic cerebral infarction (ACI) in a Han Chinese population.[38]

A previous study conducted in the Guangxi Han population showed that the *T-cell immunoglobulin and mucin domain 4 (TIMD4)* gene rs6882076 SNP was strongly associated with the risk of CHD and IS and was associated with serum TG levels.[8] In present study, the *TLR4* gene polymorphisms were not associated with serum lipid levels. Zhu et al found that the *TLR4* rs1928295 polymorphism was associated with the dietary patterns and blood lipid levels of Chinese Han children.[39] A study among young college students in Mexico found that *TLR4* gene polymorphisms were associated with changes in lipid profiles.[40] The relationship between *TLR4* gene polymorphism and blood lipid levels is consistent with the theory of the interaction between inflammation and lipid metabolism. TLR4, as a key node connecting innate immunity and lipid metabolism, its gene polymorphism can indirectly affect the lipid metabolism process by regulating the inflammatory response[41] The variation of *TLR4* gene polymorphism may change the affinity of TLR4 protein for ligands, over-activate the NF-κB signaling pathway, promote lipid uptake and foam cell formation in macrophages, and thereby lead to an increase in LDL-C levels.[42,43] Meanwhile, inflammatory factors such as TNF-α can inhibit the degradation of apolipoprotein B in the liver and delay the clearance of LDL,[44,45] while IL-6 may interfere with the activity of lipoprotein enzymes and affect the metabolism of triglycerides, ultimately leading to abnormal lipid profiles.[46]

The pathogenesis of IS involves multiple systems. Atherosclerosis is the most important pathological basis for it. By inducing vascular lumen stenosis, thrombosis and plaque detachment, it becomes the "initiating factor" for the occurrence of ischemic stroke. These processes may be closely related to inflammatory responses, immune activation, and vascular homeostasis.[47–49] However, in the field of molecular biology, its potential etiological mechanism has not yet been clarified. Genetic factors play an important role in the pathogenesis of stroke, and IS is highly hereditary.[50,51] A number of studies have focused on the role of genetic factors in the risk assessment of IS, and have achieved certain research results.[51–53] The present study proves that the G allele carriers of rs11536889 and G allele carriers of rs1927914 had a decreased risk of IS in the southern Chinese Han population. This research analysis provides a valuable supplement to the genetic risk assessment of ischemic stroke to a certain extent. Of course, further research should be conducted, especially by using group analysis in terms of gender to verify these findings.

Table 4 Genotype Distribution of rs11536889 and rs1927914 and the Risk of IS

SNP_ID	Genotype	TC, mmol/L		TG, mmol/L		LDL - C, mmol/L		HDL- C, mmol/L		ApoA1, g/L		ApoB, g/L		ApoA1/ApoB	
		IS	Control	IS	Control	IS	Control	IS	Control	IS	Control	IS	Control	IS	Control
rs11536889															
	G/G	5.23±1.20	5.33±1.24	1.61±0.92	1.81±1.69	3.13±0.86	3.27±2.15	1.30±0.32	1.45±0.34	1.22±0.31	1.46±0.34	0.90±0.25	0.95±0.29	1.45±0.54	1.69±0.77
	G/C+C/C	4.96±1.27	5.32±0.99	1.54±0.80	2.01±1.79	2.90±0.92	3.05±0.71	1.33±0.38	1.45±0.44	1.24±0.33	1.47±0.43	1.65±7.67	0.93±0.23	1.55±0.56	1.66±0.59
	p value	0.135	0.962	0.608	0.444	0.073	0.411	0.624	0.923	0.612	0.931	0.342	0.658	0.206	0.819
rs1927914															
	A/A+A/G	5.05±1.22	5.32±1.18	1.57±0.85	1.89±1.54	3.00±0.89	3.21±1.88	1.31±0.34	1.44±0.38	1.23±0.31	1.46±0.39	1.30±5.57	0.94±0.28	1.51±0.56	1.67±0.73
	G/G	5.65±1.39	5.41±0.97	1.70±1.08	1.90±2.58	3.31±0.94	3.06±0.65	1.35±0.45	1.49±0.35	1.23±0.41	1.50±0.32	0.94±0.25	0.92±0.23	1.34±0.43	1.72±0.60
	p value	0.092	0.683	0.592	0.968	0.225	0.684	0.715	0.515	0.973	0.597	0.819	0.713	0.292	0.700

Notes: The lipid levels are expressed as mean ± SD.

Abbreviations: IS, ischemic stroke; TC, total cholesterol; TG, triglyceride; LDL-C, low - density lipoprotein cholesterol; HDL -C, high-density lipoprotein cholesterol; ApoA1, apolipoprotein A1; ApoB, apolipoprotein B; ApoA1/ApoB, the ratio of apolipoprotein A1 to apolipoprotein B.

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Although this research yielded some valuable results, it still has certain limitations. Firstly, as a complex disease, the occurrence of IS is the result of the interaction between genetic factors and environmental factors. This study did not include other potential factors that might affect the occurrence of IS for evaluation. Secondly, the sample size was relatively small, all patients came from a single center, and there was no validation cohort for verification, which may lead to insufficient statistical power and selection bias. Especially for the analysis of those loci with a low frequency of secondary alleles, the results need to be interpreted with caution. Thirdly, this study did not conduct classification analysis of different IS subtypes. In the future, more cases need to be included to analyze the risk factors of different IS subtypes. Finally, this study did not conduct functional validation experiments, and it cannot directly prove the influence of polymorphic loci on the function of TLR4 protein. Further verification needs to be conducted through combined cell models or animal experiments.

Conclusion

TLR4 rs11536889 and rs1927914 SNPs are associated with the risk of IS in the southern Chinese Han population. This result suggests that the polymorphism of the *TLR4* gene may be a genetic susceptibility marker for IS. It has expanded the reference data related to IS risk prediction as well as individualized prevention and treatment. In the future, multi-center, large-sample, and cross-ethnic validation studies need to be conducted, and in combination with functional experiments, the molecular mechanism by which the *TLR4* gene regulates the occurrence of IS should be further explored to further improve the genetic etiology theory of IS.

Data Sharing Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Ethics Approval and Consent to Participate

All participants were informed on the study procedures and goals and the study obtained written informed consent from all the participants. The study was performed under the guidance of the Declaration of Helsinki and approved by the Ethics Committee of Medicine, Meizhou People's Hospital.

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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.

References

1. Strong K, Mathers C, Bonita R. Preventing stroke: saving lives around the world. *Lancet Neurol*. 2007;6(2):182–187. doi:10.1016/S1474-4422(07)70031-5
2. Kim JS. Stroke in Asia: a global disaster. *Int J Stroke*. 2014;9(7):856–857. doi:10.1111/ijs.12317

3. Kim J, Olaiya MT, De Silva DA, et al. Global stroke statistics 2023: availability of reperfusion services around the world. *Int J Stroke*. 2024;19(3):253–270. doi:10.1177/17474930231210448
4. Feigin VL, Lawes CM, Bennett DA, Barker-Collo SL, Parag V. Worldwide stroke incidence and early case fatality reported in 56 population-based studies: a systematic review. *Lancet Neurol*. 2009;8(4):355–369. doi:10.1016/S1474-4422(09)70025-0
5. Gu L, Huang J, Liang B, et al. TLR4 polymorphisms affect stroke risk and inflammatory response in Chinese ischemic stroke patients. *Neurol Sci*. 2018;39(1):127–133. doi:10.1007/s10072-017-3151-y
6. Gu L, Wu Y, Hu S, et al. Analysis of association between MAP2K4 gene polymorphism rs3826392 and IL-1b serum level in Southern Chinese han ischemic stroke patients. *J Stroke Cerebrovasc Dis*. 2016;25(5):1096–1101. doi:10.1016/j.jstrokecerebrovasdis.2015.12.035
7. Su L, Chen Z, Yan Y, et al. Association between TRAF6 gene polymorphisms and susceptibility of ischemic stroke in Southern Chinese han population. *J Mol Neurosci*. 2015;57(3):386–392. doi:10.1007/s12031-015-0580-z
8. Khounphinith E, Yin RX, Cao XL, Huang F, Wu JZ, Li H. TIMD4 rs6882076 SNP is associated with decreased levels of triglycerides and the risk of coronary heart disease and ischemic stroke. *Int J Med Sci*. 2019;16(6):864–871. doi:10.7150/ijms.31729
9. Beutler B. Innate immune responses to microbial poisons: discovery and function of the toll-like receptors. *Annu Rev Pharmacol Toxicol*. 2003;43:609–628. doi:10.1146/annurev.pharmtox.43.100901.135729
10. Mogensen TH. Pathogen recognition and inflammatory signaling in innate immune defenses. *Clin Microbiol Rev*. 2009;22(2):240–273. doi:10.1128/CMR.00046-08
11. Feng A-C, Thomas BJ, Purbey PK, et al. The transcription factor NF-κB orchestrates nucleosome remodeling during the primary response to toll-like receptor 4 signaling. *Immunity*. 2024;57(3):462–477.e469. doi:10.1016/j.immuni.2024.02.004
12. Triantafilou M, Triantafilou K. Heat-shock protein 70 and heat-shock protein 90 associate with Toll-like receptor 4 in response to bacterial lipopolysaccharide. *Biochem Soc Trans*. 2004;32(Pt 4):636–639. doi:10.1042/BST0320636
13. Tsan MF, Gao B. Endogenous ligands of toll-like receptors. *J Leukoc Biol*. 2004;76(3):514–519. doi:10.1189/jlb.0304127
14. Wu C, Deng H, Li D, et al. Ctenopharyngodon idella tollip regulates MyD88-induced NF-κB activation. *Dev Comp Immunol*. 2021;123:104162. doi:10.1016/j.dci.2021.104162
15. Verstrepen L, Bekaert T, Chau TL, Tavernier J, Chariot A, Beyaert R. TLR-4, IL-1R and TNF-R signaling to NF-kappaB: variations on a common theme. *Cell Mol Life Sci*. 2008;65(19):2964–2978. doi:10.1007/s00018-008-8064-8
16. Wang L, Li Z, Zhang X, et al. Protective effect of shikonin in experimental ischemic stroke: attenuated TLR4, p-p38MAPK, NF-κB, TNF-α and MMP-9 expression, up-regulated claudin-5 expression, ameliorated BBB permeability. *Neurochem Res*. 2014;39(1):97–106. doi:10.1007/s11064-013-1194-x
17. Das S, Kaul S, Jyothy A, Munshi A. Role of TLR4 (C1196T) and CD14 (C-260T) polymorphisms in development of ischemic stroke, its subtypes and hemorrhagic stroke. *J Mol Neurosci*. 2017;63(3–4):300–307. doi:10.1007/s12031-017-0979-9
18. Lin YC, Chang YM, Yu JM, Yen JH, Chang JG, Hu CJ. Toll-like receptor 4 gene C119A but not Asp299Gly polymorphism is associated with ischemic stroke among ethnic Chinese in Taiwan. *Atherosclerosis*. 2005;180(2):305–309. doi:10.1016/j.atherosclerosis.2004.12.022
19. Ling Y, Xiong F. Associations of TLR4 gene polymorphisms with the risk of age-related macular degeneration in a Chinese Han population. *Medicine*. 2019;98(19):e15583. doi:10.1097/MD.0000000000015583
20. Reismann P, Lichy C, Rudofsky G, et al. Lack of association between polymorphisms of the toll-like receptor 4 gene and cerebral ischemia. *J Neurol*. 2004;251(7):853–858. doi:10.1007/s00415-004-0447-7
21. Yuan M, Xia J, Ma L, Xiao B, Yang Q. Lack of the toll-like receptor 4 gene polymorphisms Asp299Gly and Thr399ile in a Chinese population. *Int J Neurosci*. 2010;120(6):415–420. doi:10.3109/00207451003778736
22. Liu L, Li Z. Chinese stroke association guidelines for clinical management of ischaemic cerebrovascular diseases: executive summary and 2023 update. *Stroke Vasc Neurol*. 2023;8(6):e3. doi:10.1136/svn-2023-002998
23. Liu L, Chen W, Zhou H, et al. Chinese Stroke Association guidelines for clinical management of cerebrovascular disorders: executive summary and 2019 update of clinical management of ischaemic cerebrovascular diseases. *Stroke Vasc Neurol*. 2020;5(2):159–176. doi:10.1136/svn-2020-000378
24. Tu WJ, Wang LD. China stroke surveillance report 2021. *Mil Med Res*. 2023;10(1):33. doi:10.1186/s40779-023-00463-x
25. Shahjouei S, Sadighi A, Chaudhary D, et al. A 5-decade analysis of incidence trends of ischemic stroke after transient ischemic attack: a systematic review and meta-analysis. *JAMA Neurol*. 2021;78(1):77–87. doi:10.1001/jamaneurol.2020.3627
26. Alyousfi D, Baralle D, Collins A. Gene-specific metrics to facilitate identification of disease genes for molecular diagnosis in patient genomes: a systematic review. *Brief Funct Genomics*. 2019;18(1):23–29. doi:10.1093/bfgp/ely033
27. Dozmorov MG. Disease classification: from phenotypic similarity to integrative genomics and beyond. *Brief Bioinform*. 2019;20(5):1769–1780. doi:10.1093/bib/bby049
28. Kim HJ, Kim H, Lee JH, Hwangbo C. Toll-like receptor 4 (TLR4): new insight immune and aging. *Immun Ageing*. 2023;20(1):67. doi:10.1186/s12979-023-00383-3
29. Wang L, Geng G, Zhu T, et al. Progress in research on TLR4-mediated inflammatory response mechanisms in brain injury after subarachnoid hemorrhage. *Cells*. 2022;11(23):3781. doi:10.3390/cells11233781
30. Zhang P, Yang M, Chen C, Liu L, Wei X, Zeng S. Toll-like receptor 4 (TLR4)/opioid receptor pathway crosstalk and impact on opioid analgesia, immune function, and gastrointestinal motility. *Front Immunol*. 2020;11:1455. doi:10.3389/fimmu.2020.01455
31. Vallés PG, Gil Lorenzo AF, Garcia RD. Toll-like receptor 4 in acute kidney injury. *Int J Mol Sci*. 2023;24(2):1415. doi:10.3390/ijms24021415
32. Moura EL, Santos IFD, Freitas PP, et al. Polymorphisms in toll-like receptors genes changes the host's immune response and is associated with cervical cancer. *Immunobiology*. 2022;227(2):152187. doi:10.1016/j.imbio.2022.152187
33. Prakasam P, Abdul Salam AA. The pathogenic effect of SNPs on structure and function of human TLR4 using a computational approach. *J Biomol Struct Dyn*. 2023;41(21):12387–12400. doi:10.1080/07391102.2023.2166998
34. Liu R, Mo YY, Wang HL, et al. The relationship between toll like receptor 4 gene rs4986790 and rs4986791 polymorphisms and sepsis susceptibility: a meta-analysis. *Sci Rep*. 2016;6:38947. doi:10.1038/srep38947
35. Hu N, Wang C, Dai X, et al. Phillygenin inhibits LPS-induced activation and inflammation of LX2 cells by TLR4/MyD88/NF-κB signaling pathway. *J Ethnopharmacol*. 2020;248:112361. doi:10.1016/j.jep.2019.112361
36. Cai Q, Zhao C, Xu Y, et al. Qingda granule alleviates cerebral ischemia/reperfusion injury by inhibiting TLR4/NF-κB/NLRP3 signaling in microglia. *J Ethnopharmacol*. 2024;324:117712. doi:10.1016/j.jep.2024.117712

37. Becker K, Kohen R, Lee R, et al. Poststroke fatigue: hints to a biological mechanism. *J Stroke Cerebrovasc Dis*. 2015;24(3):618–621. doi:10.1016/j.jstrokecerebrovasdis.2014.10.008
38. Song Y, Liu H, Long L, Zhang N, Liu Y. TLR4 rs1927911, but not TLR2 rs5743708, is associated with atherosclerotic cerebral infarction in the Southern Han population: a case-control study. *Medicine*. 2015;94(2):e381. doi:10.1097/MD.0000000000000381
39. Zhu Q, Qian B, Xue K. TT genotype of TLR4 rs1928295 is a risk factor of overweight/obesity in han Chinese children aged 7-12 years and can interact with dietary patterns to affect the incidence of central obesity and lipid profile, systolic blood pressure levels. *Nutrients*. 2023;15(15):3441. doi:10.3390/nu15153441
40. Rodríguez-García R, Vazquez-Villamar M, Aparicio-Ozores G, Parra-Rojas I, Radilla-Vázquez RB, Castro-Alarcón N. TLR4 polymorphism and haplotype are associated with obesity and lipid profile in young population: a pilot study. *J Endocrinol Invest*. 2023;46(5):903–913. doi:10.1007/s40618-022-01950-z
41. Li B, Leung JCK, Chan LYY, Yiu WH, Tang SCW. A global perspective on the crosstalk between saturated fatty acids and toll-like receptor 4 in the etiology of inflammation and insulin resistance. *Prog Lipid Res*. 2020;77:101020. doi:10.1016/j.plipres.2019.101020
42. Li Z, Zou X, Lu R, et al. Arsenic trioxide alleviates atherosclerosis by inhibiting CD36-induced endocytosis and TLR4/NF-κB-induced inflammation in macrophage and ApoE(-/-) mice. *Int Immunopharmacol*. 2024;128:111452. doi:10.1016/j.intimp.2023.111452
43. Li Y, Zhang L, Ren P, et al. Qing-Xue-Xiao-Zhi formula attenuates atherosclerosis by inhibiting macrophage lipid accumulation and inflammatory response via TLR4/MyD88/NF-κB pathway regulation. *Phytomedicine*. 2021;93:153812. doi:10.1016/j.phymed.2021.153812
44. Khalil H, Abd El Maksoud AI, Alian A, et al. Interruption of autophagosome formation in cardiovascular disease, an evidence for protective response of autophagy. *Immunol Invest*. 2020;49(3):249–263. doi:10.1080/08820139.2019.1635619
45. Liu X, Liu J, Li Y, Zhang H. The correlation between the inflammatory effects of activated macrophages in atherosclerosis and aortic dissection. *Ann Vasc Surg*. 2022;85:341–346. doi:10.1016/j.avsg.2022.03.027
46. Martínez-Ramírez OC, Salazar-Piña DA, de Lorena RM, et al. Association of NFκβ, TNFα, IL-6, IL-1β, and LPL polymorphisms with type 2 diabetes mellitus and biochemical parameters in a Mexican population. *Biochem Genet*. 2021;59(4):940–965. doi:10.1007/s10528-021-10047-w
47. Yang YD, Li ZX, Hu XM, et al. Insight into crosstalk between mitophagy and apoptosis/necroptosis: mechanisms and clinical applications in ischemic stroke. *Curr Med Sci*. 2022;42(2):237–248. doi:10.1007/s11596-022-2579-3
48. Shen Z, Xiang M, Chen C, et al. Glutamate excitotoxicity: potential therapeutic target for ischemic stroke. *Biomed Pharmacother*. 2022;151:113125. doi:10.1016/j.biopha.2022.113125
49. Bai M, Sun R. Monocyte-related cytokines/chemokines in cerebral ischemic stroke. *CNS Neurosci Ther*. 2023;29(12):3693–3712. doi:10.1111/cns.14368
50. Boehme AK, Esenwa C, Elkind MS. Stroke risk factors, genetics, and prevention. *Circ Res*. 2017;120(3):472–495. doi:10.1161/CIRCRESAHA.116.308398
51. Ernst J, Ehrenreich H, Weissenborn K. Unraveling mechanisms of cryptogenic stroke at the genetic level: a systematic literature review. *J Am Heart Assoc*. 2023;12(15):e029843. doi:10.1161/JAHA.123.029843
52. Liu Y, Wang W. Exploring genetic associations of 3 types of risk factors with ischemic stroke: an integrated bioinformatics study. *Stroke*. 2024;55(6):1619–1628. doi:10.1161/STROKEAHA.123.044424
53. Ekkert A, Šliachtenko A, Grigaitė J, Burnytė B, Utkus A, Jatužis D. Ischemic stroke genetics: what is new and how to apply it in clinical practice? *Genes*. 2021;13(1):48. doi:10.3390/genes13010048