ZSCAN25 and CYP2E1 Polymorphisms is Risk Factors for Ischemic Stroke in a Chinese Han Population: A Case Control Study

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Research

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Abstract

Background: We aimed to explore the relationship between *ZSCAN25* and *CYP2E1* polymorphisms and Ischemic stroke (IS) susceptibility among a Chinese Han population.

Methods: We enrolled 477 patients with IS and 480 age- and sex- matched health controls. Genotyping of the *ZSCAN25* rs10242455, *CYP2E1* rs2070672 and rs2515641 were performed by Agena MassARRAY platform. Odds ratio (OR) and 95% confidence interval (CI) were calculated by logistic regression analysis.

Results: Rs10242455 (OR = 0.56, 95% CI: 0.34–0.93, $p = 0.024$) was associated with a reduced IS susceptibility, while rs2070672 (OR = 1.40, 95% CI: 1.12–1.75, $p = 0.003$) and rs2515641 (OR = 1.29, 95% CI: 1.01–1.64, $p = 0.041$) with an increased IS occurrence. Rs2070672 was observed to correlate with IS risk (OR = 4.06, $p = 0.038$) at age > 64 years, and rs10242455 (OR = 0.45, $p = 0.021$) and rs2070672 (OR = 3.28, $p = 0.024$) affected IS risk in males. In addition, rs10242455 (OR = 1.72, $p = 0.014$) was significantly associated with hypertension in IS patients.

Conclusion: Our study firstly found that rs10242455 in *ZSCAN25*, rs2070672 and rs2515641 in *CYP2E1* were associated with the occurrence of IS in a Chinese Han population.

Introduction

Ischemic stroke (IS) is a common serious cerebrovascular disease that causes mortality and disability worldwide. Globally, there were almost 71% of 25.7 million IS survivors, 51% of 6.5 million deaths from IS, and 67% of 10.3 million new IS patients [1]. In China, stroke had been identified as the leading causes of death and disability-adjusted life-years at the national level in 2017[2]. The pathogenesis of IS still is unclear, but increasing evidence suggests that its etiology affected by various environmental and genetic factors. Observational studies have shown that age, sex, cardiovascular diseases, dyslipidemia, and hypertension advanced age are considered to be risk factors of IS [3, 4]. Moreover, genetic factors were reported to play a role in the pathophysiological process of IS. Several studies have pointed to genetic variants, such as polymorphisms in *TNIP1*, *MMP2*, *AHSG* that may contribute to the susceptibility of IS [5–7].

Zinc finger and SCAN domain containing 25 (*ZSCAN25*) gene, also known *ZNF498*, encodes a protein that bears some similarity to zinc finger proteins, which are involved in DNA binding and protein-protein interactions[8]. Seidel K et al. found that zinc finger protein might be involved in neuroprotection and was dysregulated in stroke [9]. Evidence suggested that *ZNF650* variants were associated with IS and was independent of traditional cardiovascular risk factors [10]. *ZNF208* polymorphisms were proved to associate with risk of IS [11]. So far, little is known about the detail of the *ZNF498* gene on IS.

Cytochrome P450s (CYPs) are a large family of monooxygenase enzymes which are responsible for the oxidative metabolism of endogenous and exogenous compounds[12]. CYP-derived lipid mediator has important functions in cerebral vascular function as well as their role in ischemic stroke [13]. *CYP2E1* gene encodes a member of the cytochrome P450 superfamily of enzymes, which catalyze the metabolism and synthesis of cholesterol, steroids and other lipids [14]. *CYP2E1* gene was reported to have an important effect on various diseases including coronary artery lesions, atherosclerosis and diabetes [15–17]. These studies suggested that *CYP2E1* might contribute to the pathogenesis of IS. To date, many studies have investigated the association of *CYP2E1* genetic variants with IS [18, 19], but not in Chinese Han population.

Here, our study explored the relationship between *ZSCAN25* and *CYP2E1* polymorphisms and susceptibility to IS among a Chinese Han population. We also estimated the probable effect of the confounding risk factors, including age, sex,

hypertension and coronary heart disease in this association.

Methods

Subject Recruitment

We enrolled 477 patients diagnosed with IS at the Department of Neurology of The Second Affiliated Hospital of Xi'an Jiaotong University. All of the enrolled IS patients were confirmed by combining the medical history and neuroimaging data (CT, MRI or MR angiography). Patients with hemorrhagic stroke, brain injury, stroke caused by other causes, tumors, other brain disease, inflammatory disorders or serious chronic diseases were excluded from this study. Four hundred and eighty age, sex, race and geographical area matched unrelated healthy controls were included, who were recruited from the health checkup center in the same hospital. The controls who had a history of IS, hypertension and coronary heart disease (CHD), cerebrovascular disease, arterial vascular disease, other neurological disease or inflammatory disorders were excluded. Demographic and clinical information, including age, sex, history of hypertension and CHD, total protein, total bilirubin, triglyceride, total cholesterol, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), hemoglobin, total leukocyte count (TLC), and platelets (PLT) of IS patients and controls were obtained from questionnaires and medical records. This study protocol was in accordance with the Declaration of Helsinki and permitted by the ethics committee of The Second Affiliated Hospital of Xi'an Jiaotong University, and written informed consent was obtained from all subjects.

DNA extraction and SNP Genotyping

Five mL of peripheral whole blood was collected in EDTA tubes vacutainer and stored at 4 °C before DNA isolation. The genomic DNA was extracted using GoldMag DNA Purification Kit (GoldMag Co. Ltd., Xi'an, China). The concentration and purity of DNA were determined by Nano Drop 2000 (Thermo Scientific, USA).

Three SNPs were selected with an $r^2 > 0.8$ by Haploview and with minor allele frequency (MAF) > 0.05 from 1000 Genomes Project data. Genotyping of the *ZSCAN25* rs10242455, *CYP2E1* rs2070672 and rs2515641 polymorphisms were analyzed by Agena MassARRAY platform (Agena, San Diego, CA, USA) in double-blind fashion. Detail primer sequences were presented in Supplementary Table 1. About 10% samples were randomly selected for repeat assays and the results were 100% concordant.

Data Analyses

The difference of demographic and clinical data between IS cases and health controls was tested by χ^2 test (categorical variables) or the Student's t test (continuous variables). Hardy-Weinberg equilibrium (HWE) was used to assess the genotype frequencies in the control subjects by Pearson's χ^2 test. The allele and genotype frequencies between the two groups were analyzed by χ^2 test. Odds ratio (OR) and 95% confidence interval (CI) for adjustment of age and sex were calculated by logistic regression analysis for the association of *ZSCAN25* and *CYP2E1* polymorphisms with IS susceptibility. Stratification analysis was performed to estimate potential effect of the confounding risk factors, including age, sex, hypertension and coronary heart disease on the association. Analysis of Variance (ANOVA) was applied for the relationship between genotypes and blood lipid parameters. Data analysis was performed with SPSS 20.0 software (SPSS Inc., Chicago, IL) and PLINK 2.1.7 software. *p* value was two-tailed and < 0.05 to be statistically significant.

Results

Characteristics of Study Population

Baseline demographic and clinical features were shown in Table 1. A total of 477 IS (316 males and 161 females) patients and 480 healthy controls (313 males and 167 females) were recruited for the study. The mean age was 64.13 ± 10.82 years for IS patients and 63.69 ± 6.69 years for the control subjects. The distribution of age and gender was no significant differences between two groups ($p = 0.443$ and $p = 0.735$, respectively). There were no significant differences between cases and controls in levels of triglyceride, HDL-C, hemoglobin, PLT; but significant differences for the levels of total protein, total bilirubin, total cholesterol, LDL-C and TLC were found. Cases included 340 patients (71.3%) with hypertension and 103 patients (21.6%) with coronary heart disease.

Table 1
Characteristics of patients with IS patients and controls

Variable	Cases (n = 477)	Controls (n = 480)	*p*
Age, year (mean ± SD)	64.13 ± 10.82	63.69 ± 6.69	0.443
Gender,(male/female), n	316/161	313/167	0.735
Total protein (g/L)	53.50 ± 24.36	66.40 ± 18.33	**< 0.001**
Total bilirubin (μmol/L)	12.35 ± 7.55	14.85 ± 6.99	**0.001**
Triglyceride (mmol/L)	1.61 ± 0.96	1.80 ± 1.40	0.080
Total cholesterol (mmol/L)	3.94 ± 1.02	5.50 ± 7.90	**0.020**
HDL-C (mmol/L)	1.18 ± 0.32	1.19 ± 0.30	0.696
LDL-C (mmol/L)	1.87 ± 0.60	2.69 ± 0.73	**< 0.001**
Hemoglobin (g/L)	122.87 ± 45.41	129.79 ± 37.40	0.073
TLC (10^9/L)	7.84 ± 8.27	5.72 ± 1.40	**< 0.001**
PLT (10^9/L)	152.89 ± 85.51	150.18 ± 94.77	0.763
Hypertension, n	340 (71.3%)		
Coronary heart disease, n	103 (21.6%)		

IS; ischemic stroke; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TLC, total leukocyte count; PLT, platelets.

Bold indicate that $p < 0.05$ indicates statistical significance.

Association of ZSCAN25 and CYP2E1 polymorphism with IS risk.

Three selected SNPs were successfully genotyped, and the success rate of genotyping was > 99.2%. All genotype distribution of the studied SNPs in controls was in HWE ($p > 0.05$, Supplementary Table 2). The allelic and genotype frequencies for selected SNPs between cases and controls were shown in Table 2. The results of association analysis showed that *ZSCAN25* rs10242455 was a protect factor for IS susceptibility; while rs2070672 and rs2515641 in *CYP2E1* conferred to the increased risk for IS occurrence. For rs10242455, G allele (G vs A: OR = 0.81, 95% CI: 0.66–0.99, $p = 0.036$; and AA + AG + GG: OR = 0.80, 95% CI: 0.66–0.98, $p = 0.033$) and GG genotype (GG vs AA: OR = 0.56, 95% CI: 0.34–0.93, $p = 0.024$; and GG vs AA-AG: OR = 0.60, 95% CI: 0.36–0.97, $p = 0.039$) were related to the reduced IS risk. The significant association of rs2070672 polymorphism with IS susceptibility was found under the allele (OR = 1.40, 95% CI: 1.12–1.75, $p = 0.003$), genotype (OR = 2.81, 95% CI: 1.37–5.77, $p = 0.005$), dominant (OR = 1.38, 95% CI: 1.06–1.80, $p = 0.017$), recessive (OR = 2.58, 95% CI: 1.26–5.27, $p = 0.009$), additive (OR = 1.41, 95% CI: 1.12–1.77, $p = 0.003$) models. In

addition, we also found that rs2515641 was increased IS occurrence (T vs C: OR = 1.27, 95% CI: 1.00–1.62, $p = 0.046$; and CC + CT + TT: OR = 1.29, 95% CI: 1.01–1.64, $p = 0.041$).

Table 2
Association between ZSCAN25 and CYP2E1 polymorphisms and IS risk

SNP ID	Model	Genotype	Case	Control	Adjusted by age and gender	
					OR (95%CI)	p
ZSCAN25	Allele	A	705	668	1	
rs10242455		G	249	292	**0.81 (0.66–0.99)**	**0.036**
	Genotype	AA	256	233	1	
		AG	193	202	0.87 (0.67–1.14)	0.305
		GG	28	45	**0.56 (0.34–0.93)**	**0.024**
	Dominant	AA	256	233	1	
		AG-GG	221	247	0.81 (0.63–1.05)	0.112
	Recessive	AA-AG	449	435	1	
		GG	28	45	**0.60 (0.36–0.97)**	**0.039**
	Log-additive	—	—	—	**0.80 (0.66–0.98)**	**0.033**
CYP2E1	Allele	A	734	787	1	
rs2070672		G	220	169	**1.40 (1.12–1.75)**	**0.003**
	Genotype	AA	284	320	1	
		AG	166	147	1.28 (0.97–1.68)	0.082
		GG	27	11	**2.81 (1.37–5.77)**	**0.005**
	Dominant	AA	284	320	1	
		AG-GG	193	125	**1.38 (1.06–1.80)**	**0.017**
	Recessive	AA-AG	450	467	1	
		GG	27	11	**2.58 (1.26–5.27)**	**0.009**
	Log-additive	—	—	—	**1.41 (1.12–1.77)**	**0.003**
CYP2E1	Allele	C	762	805	1	
rs2515641		T	182	151	**1.27 (1.00–1.62)**	**0.046**
	Genotype	CC	307	336	1	
		CT	148	133	1.22 (0.92–1.62)	0.164
		TT	17	9	2.10 (0.92–4.79)	0.078
	Dominant	CC	307	336	1	
		CT-TT	165	142	1.28 (0.97–1.68)	0.080

IS; ischemic stroke; SNP, single nucleotide polymorphism; OR, odds ratio; 95% CI, 95% confidence interval.

p values were calculated by logistic regression analysis with adjustments for age and gender.

Bold indicate that $p < 0.05$ means the data is statistically significant.

	Recessive	CC-CT	455	469	1	
		TT	17	9	1.98 (0.87–4.49)	0.103
	Log-additive	—	—	—	**1.29 (1.01–1.64)**	**0.041**

IS; ischemic stroke; SNP, single nucleotide polymorphism; OR, odds ratio; 95% CI, 95% confidence interval.

p values were calculated by logistic regression analysis with adjustments for age and gender.

Bold indicate that $p < 0.05$ means the data is statistically significant.

Stratification analysis by age and sex for the association of ZSCAN25 and CYP2E1 variants with IS risk

Considering age and sex as a potential risk factor for IS, stratification analysis by age and sex was performed to estimate potential effect of age and gender on the association between *ZSCAN25* and *CYP2E1* variants and IS risk (Table 3). Among the subgroup with age > 64 years, *CYP2E1* rs2070672 GG genotype was observed to correlate with the risk of IS compared with AA genotype (OR = 4.06, 95% CI: 1.08–15.26, $p = 0.038$) or AA-AG genotype (OR = 3.90, 95% CI: 1.05–14.55, $p = 0.043$). In the subgroup at age ≤ 64 years, *ZSCAN25* rs10242455 also had a protective effect on IS susceptibility (G vs A: OR = 0.75, 95% CI: 0.57–0.98, $p = 0.035$; and AA + AG + GG: OR = 0.74, 95% CI: 0.55–0.99, $p = 0.045$). In addition, we observed an increased IS risk for *CYP2E1* rs2070672 under the allele (OR = 1.41, 95% CI: 1.05–1.90, $p = 0.023$), dominant (OR = 1.49, 95% CI: 1.02–2.17, $p = 0.038$), and additive (OR = 1.46, 95% CI: 1.06–2.02, $p = 0.021$) models at age ≤ 64 years.

Table 3

Association between ZSCAN25 and CYP2E1 polymorphisms and IS risk according to the stratification by gender and age

SNP ID	Model	Genotype	Case	Control	OR (95%CI)	*p*	Case	Control	OR (95%CI)	*p*
Age			**> 64 years**				**≤ 64 years**			
ZSCAN25	Allele	A	330	270	1		375	398	1	
rs10242455		G	128	120	0.87 (0.65–1.17)	0.368	121	172	**0.75 (0.57–0.98)**	**0.035**
	Genotype	AA	118	96	1		138	137	1	
		AG	94	78	0.90 (0.59–1.38)	0.639	99	142	0.74 (0.50–1.08)	0.113
		GG	17	21	0.58 (0.28–1.21)	0.148	11	24	0.54 (0.25–1.18)	0.125
	Dominant	AA	118	96	1		138	137	1	
		AG-GG	111	99	0.83 (0.56–1.25)	0.376	110	148	0.71 (0.49–1.02)	0.062
	Recessive	AA-AG	212	174	1		237	261	1	
		GG	17	21	0.61 (0.30–1.24)	0.170	11	24	0.62 (0.29–1.33)	0.221
	Log-additive	—	—	—	0.82 (0.60–1.11)	0.199	—	—	**0.74 (0.55–0.99)**	**0.045**
CYP2E1	Allele	A	355	321	1.00		379	466	1	
rs2070672		G	103	67	1.39 (0.99–1.96)	0.059	117	102	**1.41 (1.05–1.90)**	**0.023**
	Genotype	AA	139	130	1		145	190	1	
		AG	77	61	1.13 (0.73–1.75)	0.586	89	86	1.41 (0.95–2.08)	0.086
		GG	13	3	**4.06 (1.08–15.26)**	**0.038**	14	8	2.43 (0.93–6.32)	0.070
	Dominant	AA	139	130	1		145	190	1	
		AG-GG	90	64	1.27 (0.83–1.93)	0.275	103	94	**1.49 (1.02–2.17)**	**0.038**

IS; ischemic stroke; SNP, single nucleotide polymorphism; OR, odds ratio; 95% CI, 95% confidence interval.

p values were calculated by logistic regression analysis with adjustments for age and gender.

Bold indicate that $p < 0.05$ means the data is statistically significant.

SNP ID	Model	Genotype	Case	Control	OR (95%CI)	*p*	Case	Control	OR (95%CI)	*p*
	Recessive	AA-AG	216	191	1		234	276	1	
		GG	13	3	**3.90 (1.05–14.55)**	**0.043**	14	8	2.15 (0.83–5.55)	0.113
	Log-additive	—	—	—	1.36 (0.94–1.97)	0.098	—	—	**1.46 (1.06–2.02)**	**0.021**
Sex			**Male**				**Female**			
ZSCAN25	Allele	A	474	446	1		231	222	1	
rs10242455		G	158	180	0.83 (0.64–1.06)	0.133	91	112	0.78 (0.56–1.09)	0.144
	Genotype	AA	172	162	1		84	71	1	
		AG	130	122	1.00 (0.72–1.39)	0.983	63	80	0.66 (0.42–1.05)	0.077
		GG	14	29	**0.45 (0.23–0.89)**	**0.021**	14	16	0.71 (0.32–1.56)	0.391
	Dominant	AA	172	162	1		84	71	1	
		AG-GG	144	151	0.9 (0.66–1.23)	0.501	77	96	0.67 (0.43–1.04)	0.072
	Recessive	AA-AG	302	284	1		147	151	1	
		GG	14	29	**0.45 (0.23–0.87)**	**0.018**	14	16	0.86 (0.41–1.84)	0.705
	Log-additive	—	—	—	0.82 (0.64–1.06)	0.129	—	—	0.76 (0.54–1.07)	0.120
CYP2E1	Allele	A	495	518	1.00		239	269	1	
rs2070672		G	137	104	**1.38 (1.04–1.83)**	**0.026**	83	65	1.44 (0.99–2.08)	0.053
	Genotype	AA	194	212	1		90	108	1	
		AG	107	94	1.24 (0.89–1.75)	0.207	59	53	1.33 (0.84–2.13)	0.225

IS; ischemic stroke; SNP, single nucleotide polymorphism; OR, odds ratio; 95% CI, 95% confidence interval.

p values were calculated by logistic regression analysis with adjustments for age and gender.

Bold indicate that $p < 0.05$ means the data is statistically significant.

SNP ID	Model	Genotype	Case	Control	OR (95%CI)	*p*	Case	Control	OR (95%CI)	*p*
		GG	15	5	**3.28 (1.17–9.21)**	**0.024**	12	6	2.37 (0.85–6.59)	0.098
	Dominant	AA	194	212	1		80	108	1	
		AG-GG	122	99	1.35 (0.97–1.87)	0.076	71	59	1.44 (0.92–2.25)	0.109
	Recessive	AA-AG	301	306	1		149	161	1	
		GG	15	5	**3.05 (1.10–8.51)**	**0.033**	12	6	2.14 (0.78–5.85)	0.140
	Log-additive	—	—	—	**1.40 (1.05–1.86)**	**0.024**	—	—	1.42 (0.98–2.06)	0.060

IS; ischemic stroke; SNP, single nucleotide polymorphism; OR, odds ratio; 95% CI, 95% confidence interval.

p values were calculated by logistic regression analysis with adjustments for age and gender.

Bold indicate that $p < 0.05$ means the data is statistically significant.

Stratified by sex, *ZSCAN25* rs10242455 GG genotype had the protective effect on IS risk in males compared with AA genotype (OR = 0.45, 95% CI: 0.23–0.89, $p = 0.021$) or AA-AG genotype (OR = 0.45, 95% CI: 0.23–0.87, $p = 0.018$), but not females. The contribution of *CYP2E1* rs2070672 to the increased IS occurrence was observed for various genetic models (G vs A: OR = 1.38, 95% CI: 1.04–1.83, $p = 0.026$; GG vs AA: OR = 3.28, 95% CI: 1.17–9.21, $p = 0.024$; GG vs AA-AG: OR = 3.05, 95% CI: 1.10–8.51, $p = 0.033$; and AA + AG + GG: OR = 1.40, 95% CI: 1.05–1.86, $p = 0.024$) in males.

Association of ZSCAN25 and CYP2E1 variants with hypertension and CHD in IS patients

We next evaluated the association of *ZSCAN25* and *CYP2E1* variants with hypertension and CHD in IS patients (Table 4). When IS parents were divided into two subgroups by with hypertension or without hypertension, *ZSCAN25* rs10242455 was significantly associated with hypertension in IS patients (AG vs AA: OR = 1.72, 95% CI: 1.12–2.64, $p = 0.014$ and AG-GG vs AA: OR = 1.55, 95% CI: 1.03–2.32, $p = 0.035$). Moreover, to evaluate the combined effect of IS and CHD, patients were divided into two groups by with CHD or without CHD. However, there was not significantly associated with CHD in IS patients.

Table 4
Association between *ZSCAN25* rs10242455 and IS patients with and without hypertension or CHD

SNP ID	Model	Genotype	IS patients with/without HYP				IS patients with/without CHD			
			With HYP	**Without HYP**	**OR (95%CI)**	p	**With CHD**	**Without CHD**	**OR (95%CI)**	p
ZSCAN25	Allele	A	494	211	1		147	558	1	
rs10242455		G	186	63	1.26 (0.91–1.75)	0.165	59	190	1.18 (0.84–1.66)	0.349
	Genotype	AA	172	84	1		49	207	1	
		AG	150	43	**1.72 (1.12–2.64)**	**0.014**	49	144	1.4 (0.88–2.21)	0.151
		GG	18	10	0.82 (0.36–1.88)	0.640	5	23	0.8 (0.28–2.24)	0.667
	Dominant	AA	172	84	1		49	207	1	
		AG-GG	168	53	**1.55 (1.03–2.32)**	**0.035**	54	167	1.31 (0.84–2.05)	0.234
	Recessive	AA-AG	322	127	1		98	351	1	
		GG	18	10	0.66 (0.29–1.49)	0.315	5	23	0.68 (0.25–1.87)	0.458
	Log-additive	—	—	—	1.27 (0.90–1.78)	0.174	—	—	1.13 (0.79–1.63)	0.496

IS; ischemic stroke; HYP, hypertension; CHD, coronary heart disease; SNP, single nucleotide polymorphism; OR, odds ratio; 95% CI, 95% confidence interval.

p values were calculated by logistic regression analysis with adjustments for age and gender.

Bold indicate that $p < 0.05$ means the data is statistically significant.

The relationship between genotypes and blood lipid parameters in IS Patients

Next, the association of clinical characteristics with different genotypes of *ZSCAN25* and *CYP2E1* polymorphisms among IS patients was evaluated, as shown in Table 5. The results showed that the rs10242455 genotype was significantly associated with PLT level ($p = 0.004$). Moreover, the rs2070672-GG carrier had a hemoglobin level ($p = 0.031$) than the AA or AG carrier in IS cases. However, no statistically association was observed between *ZSCAN25* and *CYP2E1* polymorphisms and serum lipid level, including triglyceride, total cholesterol, HDL-C and LDL-C ($p > 0.05$).

Table 5
Association of clinical characteristics with different genotypes of *ZSCAN25* and *CYP2E1* polymorphisms among IS patients

Characteristics	**rs10242455**			
	AA	**AG**	**GG**	**p**
Total protein (g/L)	52.93 ± 25.27	53.82 ± 23.16	56.98 ± 24.25	0.733
Total bilirubin (mmol/L)	12.77 ± 7.95	11.64 ± 6.40	13.22 ± 10.47	0.287
Triglyceride (mmol/L)	1.61 ± 0.88	1.59 ± 1.02	1.78 ± 1.27	0.684
Total cholesterol (mmol/L)	3.99 ± 1.01	3.86 ± 1.04	4.06 ± 1.11	0.390
HDL-C (mmol/L)	1.21 ± 0.32	1.14 ± 0.31	1.08 ± 0.28	0.054
LDL-C (mmol/L)	1.91 ± 0.59	1.80 ± 0.62	1.93 ± 0.61	0.202
Hemoglobin (g/L)	123.83 ± 43.91	122.82 ± 47.08	113.98 ± 48.65	0.600
TLC (10^9/L)	7.97 ± 7.84	7.39 ± 7.73	9.77 ± 14.09	0.395
PLT (10^9/L)	165.57 ± 83.92	137.30 ± 85.76	139.85 ± 82.36	**0.004**
Characteristics	**rs2070672**			
	AA	**AG**	**GG**	p
Total protein (g/L)	53.76 ± 24.23	53.43 ± 24.38	51.23 ± 26.57	0.889
Total bilirubin (mmol/L)	12.54 ± 8.28	11.67 ± 6.29	14.43 ± 6.05	0.207
Triglyceride (mmol/L)	1.66 ± 1.06	1.55 ± 0.80	1.49 ± 0.64	0.450
Total cholesterol (mmol/L)	3.95 ± 1.07	3.92 ± 0.93	4.03 ± 1.04	0.876
HDL-C (mmol/L)	1.18 ± 0.32	1.17 ± 0.31	1.18 ± 0.35	0.959
LDL-C (mmol/L)	1.87 ± 0.61	1.85 ± 0.58	1.89 ± 0.68	0.898
Hemoglobin (g/L)	124.94 ± 43.87	123.17 ± 45.25	99.46 ± 56.45	**0.031**
TLC (10^9/L)	7.60 ± 6.92	8.57 ± 10.64	5.89 ± 2.20	0.263
PLT (10^9/L)	157.97 ± 85.29	144.65 ± 85.07	150.42 ± 90.12	0.325
Characteristics	**rs2515641**			
	TT	**TC**	**CC**	p
Total protein (g/L)	46.44 ± 27.88	53.69 ± 24.37	53.57 ± 24.38	0.582
Total bilirubin (mmol/L)	13.36 ± 3.85	12.33 ± 6.73	12.31 ± 8.10	0.887
Triglyceride (mmol/L)	1.19 ± 0.53	1.59 ± 0.81	1.65 ± 1.04	0.238
Total cholesterol (mmol/L)	3.67 ± 1.04	3.95 ± 0.95	3.95 ± 1.06	0.619

SNP, single nucleotide polymorphism; IS; ischemic stroke; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TLC, total leukocyte count; PLT, platelets.

Bold indicate that $p < 0.05$ indicates statistical significance.

Characteristics	rs10242455			
	AA	AG	GG	p
HDL-C (mmol/L)	1.22 ± 0.43	1.18 ± 0.33	1.17 ± 0.31	0.846
LDL-C (mmol/L)	1.64 ± 0.46	1.85 ± 0.56	1.88 ± 0.61	0.356
Hemoglobin (g/L)	110.07 ± 52.74	122.55 ± 46.52	124.00 ± 44.38	0.528
TLC (10^9/L)	5.26 ± 1.81	8.95 ± 11.09	7.45 ± 6.71	0.115
PLT (10^9/L)	127.5 ± 97.06	141.28 ± 79.16	159.36 ± 87.90	0.075
SNP, single nucleotide polymorphism; IS; ischemic stroke; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TLC, total leukocyte count; PLT, platelets.				
Bold indicate that $p < 0.05$ indicates statistical significance.				

Discussion

In this study, we firstly evaluated the relationship between three SNPs (rs10242455 in *ZSCAN25*, rs2070672 and rs2515641 in *CYP2E1*) and the risk of IS in a Chinese Han population. Our results displayed that rs10242455 was associated with a reduced IS susceptibility; while rs2070672 and rs2515641 were correlated with an increased IS occurrence. Gender-stratified analyses showed that rs10242455 and rs2070672 polymorphisms affected IS risk in males but not in females. In addition, rs10242455 was significantly associated with hypertension in IS patients. These findings suggested that *ZSCAN25* and *CYP2E1* genetic variants might participate in the etiology of IS.

ZSCAN25 gene is located in human chromosome 7q22.1. The downregulation of *ZNF498* increased the malignant proliferation of ovarian cancer[20]. Previously, some studies provided evidence for the correlation of *ZNF498* polymorphisms with anthropometric traits or disease. Ozren Polašek et.al reported that *ZSCAN25* polymorphism was associated with body weight, hip circumference, and brachial circumference [21]. In addition, *ZSCAN25* polymorphism is known to be related to epilepsy susceptibility[22]. However, it is uncertain for the correlation between the *ZSCAN25* polymorphism and IS occurrence. Our results displayed that rs10242455 in *ZSCAN25* was a protective effect for the decreased risk of IS. The present study firstly provided evidence on the association between *ZSCAN25* variants and IS susceptibility.

CYP2E1 gene is located on chromosome 10q26.3. CYP2E1 induction that promotes oxidative damage was reported to be involved in liver damage by induced hyperlipidemia and acute cerebral ischemia/reperfusion [23, 24], suggesting that it might play an important role in IS. *CYP2E1* rs3813867 polymorphism was found to be associated with IS risk in the Korean population[18]. Similarly, a significant association was observed between *CYP2E1**5B and the occurrence of stroke in Turkish population [19]. However, the correlation of *CYP2E1* variants (rs2070672 and rs2515641) with IS risk has not been reported in previous studies. Here, our results firstly suggested that rs2070672 (g.4682A > G) and rs2515641 (c.1263C > T) in *CYP2E1* were risk-effect factor for the susceptibility to IS in the Chinese Han population. Our finding further supported that the *CYP2E1* gene might play an important role in pathogenesis of IS.

The mortality rate for IS was increased with age rising, especially over the age of 65 years accounting for two-thirds of IS patients [25, 26]. Stratified by age, we found that rs10242455 might be a protective effect on IS susceptibility at age ≤ 64 years, suggested that the risk association of the polymorphisms might be age dependent. Stroke is a sex-specific disease and the incidence of stroke is higher in men than in women [27]. Stratification analysis by sex showed that the contribution of rs10242455 to the reduced IS risk and rs2070672 to the increased IS occurrence in males but not females,

which indicated that the influence of genetic factors on IS risk might present gender difference. Han TS et al. reported hypertension is a conventional risk factor for stroke, and stroke patients could be saved with appropriate management of hypertension [28]. Given that hypertension is the risk factor of IS, we evaluated the association of *ZSCAN25* and *CYP2E1* variants with hypertension in IS patients. Our results displayed that rs10242455 was significantly associated with hypertension in IS patients. In addition, the correlation of these variants with CHD in IS patients was further investigated. However, no significant association was observed. These results are needed to affirm in a larger sample set.

Our study provided some interesting findings that *ZSCAN25* and *CYP2E1* polymorphisms might affect the occurrence of IS, but there were several potential limitations. First, our study was a hospital-based case-control study based on a Chinese Han population, so the selection bias could be unavoidable and the results might not apply to other populations. Second, data of some IS risk factors (e.g., smoking, alcohol and BMI) was insufficient which limited our further analysis of the gene–environment interaction. Third, functional studies were needed to elucidate the biologic mechanisms of *ZSCAN25* and *CYP2E1* polymorphisms on IS.

Conclusion

In conclusion, we firstly found that rs10242455 in *ZSCAN25*, rs2070672 and rs2515641 in *CYP2E1* were associated with the occurrence of IS in a Chinese Han population. Our finding increased our knowledge regarding the effect of *ZSCAN25* and *CYP2E1* gene on the process of IS, and also provided some data for future explorations of the relationship between *ZSCAN25* and *CYP2E1* and IS risk in different populations. However, well-designed multicenter studies are needed to verify our results.

Declarations

Ethics approval and consent to participate

This study protocol was in accordance with the Declaration of Helsinki and permitted by the ethics committee of The Second Affiliated Hospital of Xi'an Jiaotong University, and written informed consent was obtained from all subjects.

Consent for publication

Not applicable.

Availability of data and material

All the data regarding the findings are available within the manuscript. Anyone who is interested in the information should contact the corresponding author.

Competing interests

The authors declare that they have no conflict of interest.

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Author Contributions

The work presented here was carried out in collaboration between all authors. Haozheng Yuan carried out the molecular genetic studies and drafted the manuscript. Pei Fan and Li Yao designed the methods and experiments, performed the statistical analyses and interpreted the results. Yuying Lv designed primers and performed the SNP genotyping experiments. Haidong Wei and Juan Zheng collected clinical information about patients and performed the SNP genotyping experiments. Xinsheng Han conceived of the study, worked on associated data collection and their interpretation, participated in the design and coordination of the study, and funded the study. All authors read and approved the final manuscript.

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