Journal of Molecular Neuroscience (2020) 70:1130–1139
https://doi.org/10.1007/s12031-020-01520-z

CYP2B6 Polymorphisms Are Associated with Ischemic Stroke Risk in a Chinese Han Population

Guolong Tu[1] · Wenliang Zhan[1] · Yao Sun[2] · Jiamin Wu[2] · Zichao Xiong[2] · Jianfeng Liu[2] · Yuanwei Liu[2] · Haiyue Li[2] · Ying Xia[1]

Received: 29 April 2019 / Accepted: 26 September 2019 / Published online: 20 April 2020

Abstract

Genetic factors have been demonstrated to play an important role in the pathology of ischemic stroke (IS). This study was conducted to explore the association between *CYP2B6* polymorphisms and IS risk in a Chinese Han population. Four single-nucleotide polymorphisms (SNPs) in *CYP2B6* from 477 cases and 495 controls were genotyped using the Agena MassARRAY. The odds ratio (OR) and 95% confidence interval (CI) were calculated under genetic models and haplotype analysis to assess the association between SNPs and IS risk. We found that rs2099361 was associated with an increased IS risk (CC vs. AA: overall: OR = 1.85, 95% CI: 1.16–2.93, $P = 0.010$; age ≤ 60: OR = 1.94, 95% CI: 1.02–3.70, $P = 0.045$; male: OR = 2.17, 95% CI: 1.22–3.86, $P = 0.009$). The GT genotype of rs4803420 was associated with a reduced IS risk (OR = 0.74, 95% CI: 0.57–0.98, $P = 0.036$); the GG genotype was associated with an increased IS risk in women (OR = 2.31, 95% CI: 1.00–5.31, $P = 0.049$). The rs1038376 polymorphism was associated with reduced IS risk for age ≤ 60 years (AT vs. TT: OR = 0.63, 95% CI: 0.40–0.99, $P = 0.046$). Interestingly, there were significant differences in some clinical indicator levels between case and control groups, and genotypes of SNPs. Our results indicated that *CYP2B6* polymorphisms (rs2099361, rs4803420, and rs1038376) were associated with the risk of IS. Further studies are still needed to validate our findings with larger sample sizes.

Keywords *CYP2B6* · Ischemic stroke (IS) · Single-nucleotide polymorphisms (SNPs) · Case–control study

Introduction

Stroke is the leading cause of death and disability worldwide (Feigin et al. 2015; Hankey 2017). In China, stroke is currently the leading cause of death and contributes to a heavy disease burden. The National Epidemiological Survey of Stroke in China (NESS-China), which was conducted in 2012–2013 and involved 480,687 individuals (aged ≥20 years) from 31 provinces, reported age-standardized prevalence, incidence, and mortality rates for stroke of 1115 per 100,000 people, and 247 and 115 per 100,000 person-years, respectively (Wang et al. 2017). Stroke burden in China has increased over the past 30 years, and remains particularly high in rural areas (Wu et al. 2019). Ischemic stroke (IS) is the most common type, accounting for 80% of all cases of stroke. Epidemiological studies have shown that older age, smoking, alcohol intake, hypertension, diabetes mellitus, hyperlipidemia, atrial fibrillation, and low sex hormones are major risk factors for IS (Boehme et al. 2017; Meschia and Brott 2018). In addition to the environmental factors, genetic factors have been demonstrated to play an important role in the pathology of IS (Boehme et al. 2017). Many genes associated with IS risk have been investigated, revealing numerous significant associations, such as variants in *ALOX5* (He et al. 2016), *AHSG* (Li et al. 2018), and *MMP-2* (Niu et al. 2018).

Cytochrome P450, family 2, subfamily B, polypeptide 6 (CYP2B6) is a member of the cytochrome P450 superfamily, which plays a vital role in the degradation of various endogenous metabolites, including therapeutic drugs, carcinogens, and other environmental chemicals, as well as many endogenous genotoxic compounds (Hodgson and Rose 2007; Lamba et al. 2003; Wang et al. 2006). In addition, it was found that CYP2B6 expression is brain region-specific, and was observed in both neurons and astrocytes (Miksys et al. 2003).

✉ Ying Xia
xiaying008@163.com

[1] Department of Neurosurgery, Haikou Hospital Affiliated to Xiangya School of Medicine, Central South University, Haikou 570208, Hainan, China

[2] Key Laboratory of Resource Biology and Biotechnology in Western China, Ministry of Education, School of Medicine, Northwest University, Xi'an 710069, Shaanxi, China

One of the polymorphisms (516G > T) in exon 4 the of *CYP2B6* gene is associated with a pronounced decrease in gene expression and CYP2B6 activity in the liver (Hofmann et al. 2008). The genetic variants in *CYP2B6* are known to decrease the activity of the CYP2B6 enzyme, contributing to an increased breast cancer risk (Justenhoven et al. 2014). Daraki et al. suggested that the *CYP2B6* polymorphism (785A > G) and specific *CYP2B6* haplotypes may contribute to acute myeloid leukemia and its specific chromosomal aberrations (Daraki et al. 2014). Moreover, several studies have shown that polymorphisms of the *CYP2B6* gene were associated with the risk of prostate cancer (Kurosaki et al. 2009), Hirschsprung disease (Xu et al. 2015), and bronchopulmonary dysplasia in preterm neonates (Zachaki et al. 2017).

However, little is known about the association between the *CYP2B6* polymorphisms and the risk of IS. Therefore, we carried out a case–control study including 477 patients with IS and 495 healthy controls to investigate the relationship between *CYP2B6* polymorphisms (rs2099361, rs4803420, rs1038376, and rs12979270) and the susceptibility to IS in a Chinese Han population. The effects of the genetic polymorphisms of *CYP2B6* on risk of IS were investigated which may provide new insights into understanding the mechanisms of IS.

Materials and Methods

Study Subjects

A total of 477 patients with IS from the Haikou Hospital Affiliated to Xiangya School of Medicine, Central South University, were enrolled in the study. The IS patients had been newly diagnosed and confirmed according to the International Classification of Disease using brain computed tomography (CT) and magnetic resonance imaging (MRI) combined with history interview and neurological examination. The 495 healthy controls were randomly recruited from people who requested general health examinations in the same hospital during the same period. Inclusion and exclusion criteria of participants were as follows: All participants were of a genetically unrelated Chinese Han population; Patients with a history of other types of stroke (hemorrhagic stroke, subarachnoid hemorrhage, and transient ischemic attack), traumatic brain injuries, cerebral vein thrombosis, or brain aneurysm were excluded in this study.

Medical records were used to collect information on patient demographic characteristics (age, gender, and ethnicity) and clinical indicators including fasting serum levels of total protein, total bilirubin, direct bilirubin, indirect bilirubin, uric acid, total cholesterol, triglyceride, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), glucose, white blood cell (WBC) count, neutrophil absolute value (NEUT), eosinophils absolute value (EO), basophilic absolute value (BASO), platelet count, plateletcrit, red blood cell (RBC) count, hemoglobin (HGB), erythrocyte mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), red blood cell distribution width (RDW), and red blood cell distribution width coefficient of variation (RDW-CV) were collected from medical records review.

DNA Extraction

Vacutainers containing ethylene diamine tetraacetic acid (EDTA) were used to collected peripheral venous blood samples (5 ml) from IS patients and controls, which were then stored at −80 °C in a refrigerator for DNA extraction. We used the GoldMag-Mini Whole Blood Genomic DNA Purification Kit (GoldMag Co., Ltd., Xi'an City, China) to extract DNA from whole blood, according to the manufacturer's instructions. We used a spectrophotometer (NanoDrop 2000; Thermo Fisher Scientific, Waltham, MA, USA) to determine the purity and concentration of the extracted DNA and for sample quality control.

SNP Selection and Genotyping

We selected the tag SNPs of the *CYP2B6* gene with a minor allele frequency (MAF) >5% in the global population from the 1000 Genomes Project. Four SNPs (rs2099361 (C > A, intron), rs4803420 (G > T, 3′ prime UTR), rs1038376 (A > T, 3′ prime UTR), and rs12979270 (A > C, 3′ prime UTR)) were selected using a pairwise Tagger method with $r^2 > 0.8$ to capture other SNPs. We used the online software Agena Bioscience Assay Design Suite version 2.0 (https://agenacx.com/online-tools/) to design the primers. SNP genotyping was performed using the Agena MassARRAY platform with iPLEX Gold chemistry (Agena Bioscience, San Diego, CA, USA) according to the protocol described. Data management and analysis of genotyping results were conducted using Agena Bioscience Typer software (version 4.0).

Statistical Analysis

Microsoft Excel (Microsoft Corp., Redmond, WA, USA) and SPSS version 20.0 statistical software (IBM Corp., Armonk, NY, USA) were used for statistical analysis. We used the chi-squared test to evaluate the Hardy–Weinberg equilibrium of SNP genotype frequencies in the controls and the differences between allelic frequencies of these polymorphisms in the case and control groups. We evaluated the association between polymorphism genotype and IS risk by logistic regression to calculate odds ratios (ORs) and 95% confidence intervals (CIs) under four genetic models (codominant, dominant, recessive, and additive). Haploview 4.2 software was used

to conduct linkage disequilibrium (LD) haplotype block and calculate the linkage strength between each pair of SNPs with D′ and R-squared values. The association between haplotypes and IS risk was assessed by logistic regression. *P* values less than 0.05 were considered statistically significant, and all statistical tests were two-sided.

Results

Demographic and Clinical Characteristics

This case–control study recruited 477 IS patients and 495 healthy controls. The demographic and clinical characteristics of the subjects are listed in Table 1. There were no significant differences in gender between case (316 male and 161 female) and control (326 male and 169 female) study populations ($P = 0.898$). There was a significant difference in age between the IS patients (mean ± SD: 64.1 ± 10.8) and controls (mean ± SD: 60.1 ± 6.6; $P < 0.001$). There were no significant differences in HDL ($P = 0.879$) or MCV ($P = 0.658$) between patients and controls. There were also significant differences in biochemistry and blood test indicators (total protein, total bilirubin, direct bilirubin, indirect bilirubin, uric acid, total cholesterol, LDL-C, glucose, WBC, NEUT, EO, BASO, platelet count, plateletcrit, RBC, HGB, MCH, RDW, and RDW%) between cases and controls ($P < 0.05$). At the same time, we found that the mean direct bilirubin, glucose, BASO, plateletcrit, RBC, MCH, and RDW% levels in the case group were higher than the clinical reference values and those of the control group, suggesting that these factors may increase the risk of IS.

Overall Analysis of *CYP2B6* Polymorphisms and IS Risk

Basic information regarding the SNPs in the *CYP2B6* gene, including SNP ID, chromosome, position, minor and major alleles, MAF and HWE-*p*, is presented in Table 2. The call rate of genotyping was more than 95%. The genotype distribution of *CYP2B6* polymorphisms (rs2099361, rs4803420, rs1038376, rs12979270) were in accordance with HWE in the control group ($P > 0.05$). However, there were no significant differences in the allelic distribution of the four SNPs in ***CYP2B6*** between the patients and control group ($P > 0.05$). No significant association was found between *CYP2B6* polymorphisms and the risk of IS under the allele model (Table 2).

We performed genetic model analyses (codominant, dominant, recessive, and additive) to further explore the association between *CYP2B6* polymorphisms and the risk of IS (Table 2). The CC genotype of rs2099361 was significantly associated with an increased risk of IS compared with the wild homozygous AA genotype before and after adjusting for age and gender (crude OR = 1.87, 95% CI: 1.19–2.93, $P = 0.007$; adjusted OR = 1.85, 95% CI: 1.16–2.93, $P = 0.010$). In the recessive model, the CC genotype was also found to be significantly associated with an increased the risk of IS compared with the AA/CA genotype (crude OR = 1.89, 95% CI: 1.23–2.92, $P = 0.004$; adjusted OR = 1.81, 95% CI: 1.16–2.83, $P = 0.009$). The GT genotype of rs4803420 was associated with a decreased risk of IS compared with the TT genotype both before and after adjusting for age and gender (crude OR = 0.75, 95% CI: 0.57–0.98, $P = 0.033$; adjusted OR = 0.74, 95% CI: 0.57–0.98, $P = 0.036$). The other two SNPs (rs1038376 and rs12979270) were not found to be associated with IS risk under the four genetic models (Table 3).

Stratification Analysis of *CYP2B6* Polymorphisms and IS Risk

Age stratification analysis showed that rs2099361 was associated with an increased risk of IS for age ≤ 60 years (CC vs. AA: OR = 1.94, 95% CI: 1.02–3.70, $P = 0.045$; recessive CC vs. AA/CA: OR = 1.90, 95% CI: 1.03–3.53, $P = 0.041$), while rs1038376 was associated with a decreased risk of IS for age ≤ 60 years (AT vs. TT: OR = 0.63, 95% CI: 0.40–0.99, $P = 0.046$) (Table 4). In addition, we found that rs4803420 was significantly associated with reduced IS risk in those aged > 60 years (G vs. T: OR = 0.69, 95% CI: 0.50–0.93, $P = 0.015$; GT vs. TT: OR = 0.56, 95% CI: 0.37–0.86, $P = 0.008$; dominant GT/GG vs. TT: OR = 0.61, 95% CI: 0.40–0.92, $P = 0.017$). Stratification analysis based on gender indicated that rs2099361 was significantly associated with an increased risk of IS in male (CC vs. AA: OR = 2.17, 95% CI: 1.22–3.86, $P = 0.009$; recessive CC vs. AA/CA: OR = 2.05, 95% CI: 1.18–3.57, $P = 0.011$; additive: OR = 1.33, 95% CI: 1.04–1.70, $P = 0.024$). However, rs4803420 was associated with an increased risk of IS in women (GG vs. TT: OR = 2.31, 95% CI: 1.00–5.31, $P = 0.049$; GG vs. TT/GT: OR = 2.39, 95% CI: 1.06–5.37, $P = 0.035$) (Table 4). These results suggest that age and gender are important factors influencing the risk of IS.

LD and Haplotype Analysis

Linkage analysis indicated that there was strong linkage disequilibrium among the three SNPs (rs4803420, rs1038376, and rs12979270) in the *CYP2B6* gene, as shown in Fig. 1. The results of the association between the haplotypes and IS risk are listed in Table 5. However, no association was found between the haplotypes (GAC, GTA, TAA, and GAA) and risk of IS before and after adjusting for age and gender.

SNPs and Clinical Indicators

To further explore the impact of SNPs in *CYP2B6* on the risk of stroke, we compared the levels of clinical indicators under

Table 1 Characteristics of case and control subjects

Variables	Case		Control		*P*
	No.	Mean ± SD	No.	Mean ± SD	
Age (years)	477	64.1 ± 10.8	495	60.1 ± 6.6	<0.001
Gender (M/F)	316/161	–	326/169	–	0.898
Total protein (g/L)	422	65.59 ± 5.80	240	70.90 ± 5.60	<0.001
Total bilirubin (umol/L)	422	13.63 ± 6.51	240	17.01 ± 5.94	<0.001
Direct bilirubin (umol/L)	422	7.72 ± 4.28	240	5.20 ± 1.95	<0.001
Indirect bilirubin (umol/L)	422	5.91 ± 3.42	240	11.81 ± 4.24	<0.001
Uric acid (umol/L)	401	284.53 ± 94.37	240	330.87 ± 80.27	<0.001
Total cholesterol (mmol/L)	407	3.90 ± 1.03	240	4.50 ± 0.92	<0.001
Triglyceride (mmol/L)	406	1.59 ± 1.06	240	1.79 ± 1.16	0.027
HDL-C (mmol/L)	408	1.09 ± 0.26	203	1.09 ± 0.23	0.879
LDL-C (mmol/l)	408	1.81 ± 0.58	202	2.56 ± 0.71	<0.001
Glucose (mmol/L)	425	6.33 ± 2.24	241	5.89 ± 1.44	0.003
WBC (*10E9/L)	420	8.22 ± 8.78	242	5.89 ± 1.49	<0.001
NEUT (*10E9/L)	419	4.77 ± 2.69	242	3.45 ± 1.18	<0.001
EO (*10E9/L)	419	0.12 ± 0.15	242	0.15 ± 0.13	0.021
BASO (*10E9/L)	419	0.11 ± 0.65	242	0.03 ± 0.02	0.015
Platelet (*10E9/L)	419	180.28 ± 66.01	242	211.47 ± 53.43	<0.001
Plateletcrit (%)	411	0.42 ± 1.55	235	0.23 ± 0.05	0.016
RBC (10E12/L)	419	7.29 ± 18.54	242	4.84 ± 0.44	0.007
Hemoglobin (g/L)	419	136.87 ± 22.77	242	147.76 ± 14.31	<0.001
MCV (fl)	419	90.70 ± 10.25	242	90.95 ± 4.48	0.658
MCH (pg)	419	35.15 ± 40.74	242	30.59 ± 1.63	0.023
RDW (fL)	419	42.89 ± 5.96	242	43.98 ± 3.07	0.002
RDW-CV (%)	419	16.53 ± 23.91	242	13.38 ± 1.68	0.008

F: female; M: male; SD: standard deviation; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; WBC: white blood cell count; NEUT: neutrophil absolute value; EO: eosinophils absolute value; BASO: basophilic absolute value; RBC: red blood cell count; MCV: erythrocyte mean corpuscular volume; MCH: mean corpuscular hemoglobin; RDW: red blood cell distribution width; RDW-CV: red blood cell distribution width coefficient of variation

$P < 0.05$ indicates statistical significance

different genotypes of *CYP2B6* polymorphisms (Table 6). We found that the level of I-BIL levels was significantly different between the three genotypes (AA: 6.28 ± 3.45 umol/L; CA: 5.37 ± 3.39 umol/L; CC: 6.36 ± 3.29 umol/L) of rs2099361 ($P = 0.025$). Relative to the three genotypes (TT, GT, and GG) of rs4803420, statistically significant differences were

Table 2 Alleles of *CYP2B6* polymorphisms and association with ischemic stroke risk

SNP ID	Chr	Position	Allele A/B	Role	HWE	MAF-case	MAF-control	OR (95% CI)	*P*
rs2099361	19	40,992,443	C/A	Intron	0.131	0.337	0.297	1.20 (0.99–1.46)	0.060
rs4803420	19	41,017,655	T/G	3 prime_UTR	0.129	0.204	0.232	0.85 (0.68–1.05)	0.138
rs1038376	19	41,018,104	T/A	3 prime_UTR	0.211	0.117	0.124	0.94 (0.71–1.23)	0.644
rs12979270	19	41,018,226	C/A	3 prime_UTR	0.143	0.288	0.280	1.04 (0.86–1.27)	0.671

SNP: single-nucleotide polymorphism; Chr: chromosome; A: minor allele; B: major allele; MAF: minor allele frequency; HWE: Hardy–Weinberg equilibrium; OR: odds ratio; 95% CI: 95% confidence interval

P values were calculated from χ^2 test (two-sided)

$P < 0.05$ was considered statistically significant

Table 3 Genetic analysis of *CYP2B6* polymorphisms and ischemic stroke risk

SNP ID	Model	Genotype	Case (%)	Control (%)	OR (95% CI)	*P*	Adjusted OR (95% CI)	*P*
rs2099361	Codominant	AA	217 (45.7)	235 (48.0)	1.00	0.015	1.00	0.031
		CA	196 (41.3)	219 (44.7)	0.97 (0.74–1.27)	0.818	1.04 (0.79–1.36)	0.796
		CC	62 (13.1)	36 (7.3)	1.87 (1.19–2.93)	0.007	1.85 (1.16–2.93)	0.010
	Dominant	AA	217 (45.7)	235 (48.0)	1.00		1.00	
		CC/CA	258 (54.3)	255 (52.0)	1.10 (0.85–1.41)	0.479	1.16 (0.89–1.50)	0.279
	Recessive	CA/AA	413 (86.9)	454 (92.7)	1.00		1.00	
		CC	62 (13.1)	36 (7.3)	1.89 (1.23–2.92)	0.004	1.81 (1.16–2.83)	0.009
	Additive	–	–	–	1.20 (0.99–1.46)	0.061	1.23 (1.01–1.50)	0.043
rs4803420	Codominant	GG	304 (63.7)	284 (57.6)	1.00	0.093	1.00	0.079
		TG	151 (31.7)	189 (38.3)	0.75 (0.57–0.98)	0.033	0.74 (0.57–0.98)	0.036
		TT	22 (4.6)	20 (4.1)	1.03 (0.55–1.92)	0.932	1.18 (0.62–2.24)	0.621
	Dominant	GG	304 (63.7)	284 (57.6)	1.00		1.00	
		TT/TG	173 (36.3)	209 (42.4)	0.77 (0.60–1.00)	0.051	0.78 (0.60–1.02)	0.071
	Recessive	TG/GG	455 (95.4)	473 (95.9)	1.00		1.00	
		TT	22 (4.6)	20 (4.1)	1.14 (0.62–2.12)	0.671	1.31 (0.69–2.48)	0.406
	Additive	–	–	–	0.85 (0.68–1.05)	0.133	0.87 (0.69–1.09)	0.216
rs1038376	Codominant	AA	372 (78.0)	376 (76.0)	1.00	0.399	1.00	0.407
		TA	98 (20.5)	115 (23.2)	0.86 (0.63–1.17)	0.338	0.88 (0.65–1.21)	0.439
		TT	7 (1.5)	4 (0.8)	1.77 (0.51–6.09)	0.366	1.97 (0.56–6.96)	0.292
	Dominant	AA	372 (78.0)	376 (76.0)	1.00		1.00	
		TT/TA	105 (22.0)	119 (24.0)	0.89 (0.66–1.20)	0.453	0.92 (0.68–1.25)	0.591
	Recessive	TA/AA	470 (98.5)	491 (99.2)	1.00		1.00	
		TT	7 (1.5)	4 (0.8)	1.83 (0.53–6.29)	0.338	2.02 (0.57–7.14)	0.273
	Additive	–	–	–	0.94 (0.71–1.24)	0.638	0.97 (0.73–1.28)	0.812
rs12979270	Codominant	AA	245 (51.4)	244 (50.5)	1.00	0.247	1.00	0.135
		CA	189 (39.6)	208 (43.1)	0.90 (0.69–1.18)	0.460	0.83 (0.63–1.10)	0.194
		CC	43 (9.0)	31 (6.4)	1.38 (0.84–2.27)	0.200	1.36 (0.81–2.27)	0.241
	Dominant	AA	245 (51.4)	244 (50.5)	1.00		1.00	
		CC/CA	232 (48.6)	239 (49.5)	0.97 (0.75–1.25)	0.793	0.90 (0.69–1.17)	0.428
	Recessive	CA/AA	434 (91.0)	452 (93.6)	1.00		1.00	
		CC	43 (9.0)	31 (6.4)	1.45 (0.89–2.34)	0.133	1.47 (0.90–2.42)	0.128
	Additive	–	–	–	1.05 (0.86–1.28)	0.668	1.00 (0.81–1.23)	0.989

SNP: single-nucleotide polymorphism; OR: odds ratio; 95% CI: 95% confidence interval

Adjusted OR and 95% CI were calculated using logistic regression adjusted for age and gender

$P < 0.05$ was considered statistically significant

found for the levels of BASO ($P = 0.009$), plateletcrit ($P = 0.030$), RBC ($P = 0.011$), MCV ($P = 0.031$), MCH ($P = 0.021$), and RDW ($P = 0.007$) in IS patients. Moreover, there was a significant difference in the distribution of patient glucose levels between the three genotypes (TT: 6.3 ± 2.13 mmol/L; AT: 6.19 ± 1.76 mmol/L; AA: 9.35 ± 7.11 mmol/L) of rs1038376 ($P = 0.001$).

Discussion

This study investigated the association between SNPs in *CYP2B6* and IS risk in a Chinese Han population. The results demonstrated that the rs2099361 was associated with an increased risk of IS overall and for age ≤ 60 years and male gender. The GT genotype of rs4803420 was associated with a reduced risk of IS overall and for age > 60 years, but the GG genotype was found to be associated with increased IS risk in women. The AT genotype of rs1038376 was associated with a reduced IS risk in those aged ≤ 60 years. Notably, there was significant difference in the distribution of clinical indicator levels in patients between the case and control groups, and genotypes of each SNP.

CYP2B6, a member of the human CYP family of pharmacogenes expressed mainly in the liver, accounts for 2–10% of total hepatic CYP content and contributes to the biotransformation of many drugs and xenobiotics (Wang and Tompkins 2008; Zanger and Klein 2013). It was estimated that CYP2B6 is involved in the metabolism of 4% of the top 200 drugs (Zanger et al. 2008). CYP2B6 is also expressed in the brain and may play an important role in the metabolism of drugs by acting on the central nervous system, resulting in neurological side effects of drug treatments (Miksys and Tyndale 2004; Thorn et al. 2010). SNPs are the most common form of genetic variation in CYP genes (Daly 2004). The *CYP2B6* gene is highly polymorphic, with a large number of allelic variants (http://www.cypalleles.ki.se/cyp2b6.htm). High inter-individual variation is seen in CYP2B6 mRNA expression and activity, ranging from 20- to 250-fold, which is considered to be attributable mainly to the highly inducible

Table 4 Stratification analysis of *CYP2B6* polymorphisms and ischemic stroke risk

SNP ID	Model	Genotype	Age >60 years				Age ≤60 years			
			Case (%)	Control (%)	OR (95% CI)	*P*	Case (%)	Control (%)	OR (95% CI)	*P*
rs2099361	Allele	A	367 (67.2)	296 (70.8)	1		263 (65.1)	393 (69.9)	1	
		C	179 (32.8)	122 (29.2)	1.18 (0.90–1.56)	0.232	141 (34.9)	169 (30.1)	1.25 (0.95–1.64)	0.113
	Codominant	AA	130 (47.6)	102 (48.8)	1		87 (43.1)	133 (47.3)	1	
		CA	107 (39.2)	92 (44.0)	1.08 (0.70–1.65)	0.727	89 (44.1)	127 (45.2)	1.04 (0.70–1.54)	0.851
		CC	36 (13.2)	15 (7.2)	1.70 (0.82–3.52)	0.155	26 (12.9)	21 (7.5)	1.94 (1.02–3.70)	0.045
	Dominant	AA	130 (47.6)	102 (48.8)	1		87 (43.1)	133 (47.3)	1	
		CC/CA	143 (52.4)	107 (51.2)	1.17 (0.78–1.76)	0.439	115 (56.9)	148 (52.7)	1.16 (0.80–1.69)	0.422
	Recessive	CA/AA	237 (86.8)	194 (92.8)	1		176 (87.1)	260 (92.5)	1	
		CC	36 (13.2)	15 (7.2)	1.64 (0.81–3.30)	0.168	26 (12.9)	21 (7.5)	1.90 (1.03–3.53)	0.041
	Additive	–	–	–	1.21 (0.89–1.65)	0.226	–	–	1.25 (0.94–1.66)	0.121
rs4803420	Allele	G	444 (81.0)	313 (74.5)	1		315 (77.6)	444 (78.4)	1	
		T	104 (19.0)	107 (25.5)	0.69 (0.50–0.93)	0.015	91 (22.4)	122 (21.6)	1.05 (0.77–1.43)	0.750
	Codominant	GG	181 (66.1)	110 (52.4)	1		123 (60.6)	174 (61.5)	1	
		TG	82 (29.9)	93 (44.3)	0.56 (0.37–0.86)	0.008	69 (34)	96 (33.9)	1.02 (0.69–1.51)	0.934
		TT	11 (4.0)	7 (3.3)	1.22 (0.43–3.45)	0.706	11 (5.4)	13 (4.6)	1.04 (0.44–2.46)	0.929
	Dominant	GG	181 (66.1)	110 (52.4)	1		123 (60.6)	174 (61.5)	1	
		TT/TG	93 (33.9)	100 (47.6)	0.61 (0.40–0.92)	0.017	80 (39.4)	109 (38.5)	1.02 (0.70–1.49)	0.919
	Recessive	TG/GG	263 (96.0)	203 (96.7)	1		192 (94.6)	270 (95.4)	1	
		TT	11 (4.0)	7 (3.3)	1.53 (0.55–4.26)	0.420	11 (5.4)	13 (4.6)	1.03 (0.44–2.42)	0.939
	Additive	–	–	–	0.73 (0.51–1.04)	0.080	–	–	1.02 (0.74–1.39)	0.911
rs1038376	Allele	A	483 (88.1)	380 (89.6)	1		359 (88.4)	487 (86.0)	1	
		T	65 (11.9)	44 (10.4)	1.16 (0.77–1.74)	0.467	47 (11.6)	79 (14.0)	0.81 (0.55–1.19)	0.276
	Codominant	AA	211 (77.0)	171 (80.7)	1		161 (79.3)	205 (72.4)	1	
		TA	61 (22.3)	38 (17.9)	1.45 (0.87–2.39)	0.151	37 (18.2)	77 (27.2)	0.63 (0.40–0.99)	0.046
		TT	2 (0.7)	3 (1.4)	0.54 (0.08–3.68)	0.531	5 (2.5)	1 (0.4)	5.95 (0.68–52.19)	0.108
	Dominant	AA	211 (77.0)	171 (80.7)	1		161 (79.3)	205 (72.4)	1	
		TT/TA	63 (23.0)	41 (19.3)	1.37 (0.84–2.24)	0.208	42 (20.7)	78 (27.6)	0.71 (0.46–1.09)	0.119
	Recessive	TA/AA	272 (99.3)	209 (98.6)	1		198 (97.5)	282 (99.6)	1	
		TT	2 (0.7)	3 (1.4)	0.50 (0.07–3.40)	0.480	5 (2.5)	1 (0.4)	6.55 (0.75–57.38)	0.090
	Additive	–	–	–	1.26 (0.80–1.98)	0.320	–	–	0.82 (0.55–1.22)	0.331
rs12979270	Allele	A	377 (68.8)	292 (71.6)	1		302 (74.4)	404 (72.4)	1	
		C	171 (31.2)	116 (28.4)	1.14 (0.86–1.51)	0.355	104 (25.6)	154 (27.6)	0.90 (0.68–1.21)	0.492
	Codominant	AA	129 (47.1)	99 (48.5)	1		116 (57.1)	145 (52.0)		
		CA	119 (43.4)	94 (46.1)	0.89 (0.58–1.35)	0.575	70 (34.5)	114 (40.9)	0.80 (0.54–1.18)	0.264
		CC	26 (9.5)	11 (5.4)	1.52 (0.64–3.58)	0.343	17 (8.4)	20 (7.2)	0.92 (0.45–1.86)	0.816
	Dominant	AA	129 (47.1)	99 (48.5)	1		116 (57.1)	145 (52.0)	1	
		CC/CA	145 (52.9)	105 (51.5)	0.95 (0.63–1.43)	0.802	87 (42.9)	134 (48.0)	0.82 (0.56–1.19)	0.292
	Recessive	CA/AA	248 (90.5)	193 (94.6)	1		186 (91.6)	259 (92.8)	1	
		CC	26 (9.5)	11 (5.4)	1.61 (0.70–3.70)	0.266	17 (8.4)	20 (7.2)	1.01 (0.50–2.00)	0.989
	Additive	–	–	–	1.04 (0.75–1.45)	0.801	–	–	0.89 (0.66–1.19)	0.413
			Male				Female			
rs2099361	Allele	A	412 (65.6)	460 (70.8)	1		218 (67.7)	229 (69.4)	1	
		C	216 (34.4)	190 (29.2)	1.27 (1.00–1.61)	0.050	104 (32.3)	101 (30.6)	1.08 (0.78–1.51)	0.642
	Codominant	AA	141 (44.9)	158 (48.6)	1		76 (47.2)	77 (46.7)	1	
		CA	130 (41.4)	144 (44.3)	1.12 (0.80–1.58)	0.513	66 (41)	75 (45.5)	0.91 (0.57–1.44)	0.682
		CC	43 (13.7)	23 (7.1)	2.17 (1.22–3.86)	0.009	19 (11.8)	13 (7.9)	1.39 (0.64–3.05)	0.407
	Dominant	AA	141 (44.9)	158 (48.6)	1		76 (47.2)	77 (46.7)	1	
		CC/CA	173 (55.1)	167 (51.4)	1.27 (0.92–1.75)	0.151	85 (52.8)	88 (53.3)	0.98 (0.63–1.53)	0.935
	Recessive	CA/AA	271 (86.3)	302 (92.9)	1		142 (88.2)	152 (92.1)	1	
		CC	43 (13.7)	23 (7.1)	2.05 (1.18–3.57)	0.011	19 (11.8)	13 (7.9)	1.46 (0.69–3.09)	0.325
	Additive	–	–	–	1.33 (1.04–1.70)	0.024	–	–	1.07 (0.76–1.50)	0.700
rs4803420	Allele	G	505 (79.9)	503 (77.4)	1		254 (78.9)	254 (75.6)	1	
		T	127 (20.1)	147 (22.6)	0.86 (0.66–1.13)	0.270	68 (21.1)	82 (24.4)	0.83 (0.58–1.20)	0.315
	Codominant	GG	201 (63.6)	189 (58.2)	1		103 (64)	95 (56.5)	1	
		TG	103 (32.6)	125 (38.5)	0.79 (0.56–1.10)	0.166	48 (29.8)	64 (38.1)	0.92 (0.58–1.48)	0.738
		TT	12 (3.8)	11 (3.4)	0.94 (0.48–1.82)	0.844	10 (6.2)	9 (5.4)	2.31 (1.00–5.31)	0.049
	Dominant	GG	201 (63.6)	189 (58.2)	1		103 (64)	95 (56.5)	1	
		TT/TG	115 (36.4)	136 (41.8)	0.81 (0.58–1.12)	0.194	58 (36)	73 (43.5)	1.10 (0.70–1.71)	0.688
	Recessive	TG/GG	304 (96.2)	314 (96.6)	1		151 (93.8)	159 (94.6)	1	
		TT	12 (3.8)	11 (3.4)	1.04 (0.55–1.99)	0.896	10 (6.2)	9 (5.4)	2.39 (1.06–5.37)	0.035
	Additive	–	–	–	0.87 (0.67–1.14)	0.318	–	–	1.24 (0.88–1.74)	0.210
rs1038376	Allele	A	553 (87.5)	289 (89.8)	1		566 (86.8)	301 (89.1)	1	
		T	79 (12.5)	33 (10.2)	0.94 (0.68–1.3)	0.712	86 (13.2)	37 (10.9)	0.93 (0.57–1.53)	0.771

Table 4 (continued)

SNP ID	Model	Genotype	Age >60 years				Age ≤60 years			
			Case (%)	Control (%)	OR (95% CI)	*P*	Case (%)	Control (%)	OR (95% CI)	*P*
	Codominant	AA	240 (75.9)	132 (82)			243 (74.5)	133 (78.7)		
		TA	73 (23.1)	25 (15.5)	0.98 (0.67–1.43)	0.923	80 (24.5)	35 (20.7)	0.70 (0.39–1.25)	0.225
		TT	3 (0.9)	4 (2.5)	1.11 (0.21–5.8)	0.903	3 (0.9)	1 (0.6)	4.38 (0.48–40.42)	0.192
	Dominant	AA	240 (75.9)	132 (82)	1		243 (74.5)	133 (78.7)	1	0.423
		TT/TA	76 (24.1)	29 (18)	0.99 (0.68–1.43)	0.941	83 (25.5)	36 (21.3)	0.80 (0.46–1.39)	
	Recessive	TA/AA	313 (99.1)	157 (97.5)	1		323 (99.1)	168 (99.4)	1	
		TT	3 (0.9)	4 (2.5)	1.11 (0.21–5.81)	0.898	3 (0.9)	1 (0.6)	4.67 (0.51–42.95)	0.173
	Additive	–	–	–	0.99 (0.7–1.41)	0.966	–	–	0.93 (0.57–1.51)	0.757
rs12979270	Allele	A	457 (72.3)	222 (68.9)	1		458 (71.3)	238 (73.5)	1	
		C	175 (27.7)	100 (31.1)	0.95 (0.75–1.22)	1.250	184 (28.7)	86 (26.5)	1.25 (0.89–1.75)	0.205
	Codominant	AA	164 (51.9)	81 (50.3)	1		158 (49.2)	86 (53.1)	1	
		CA	129 (40.8)	60 (37.3)	0.79 (0.56–1.10)	0.166	142 (44.2)	66 (40.7)	0.92 (0.58–1.48)	0.738
		CC	23 (7.3)	20 (12.4)	0.94 (0.48–1.82)	0.844	21 (6.5)	10 (6.2)	2.31 (1.00–5.31)	0.049
	Dominant	AA	164 (51.9)	81 (50.3)	1		158 (49.2)	86 (53.1)	1	
		CC/CA	152 (48.1)	80 (49.7)	0.81 (0.58–1.12)	0.194	163 (50.8)	76 (46.9)	1.10 (0.70–1.71)	0.688
	Recessive	CA/AA	293 (92.7)	141 (87.6)	1		300 (93.5)	152 (93.8)	1	
		CC	23 (7.3)	20 (12.4)	1.04 (0.55–1.99)	0.896	21 (6.5)	10 (6.2)	2.39 (1.06–5.37)	0.035
	Additive	–			0.87 (0.67–1.14)	0.318			1.24 (0.88–1.74)	0.210

SNP: single-nucleotide polymorphism; OR: odds ratio; 95% CI: 95% confidence interval

OR and 95% CI were calculated using logistic regression adjusted for age and gender

$P < 0.05$ was considered statistically significant

and polymorphic nature of this gene (Code et al. 1997; Lamba et al. 2003; Lang et al. 2001).

Our study is first to find that rs2099361, rs4803420, and rs1038376 are significantly associated with the risk of IS.

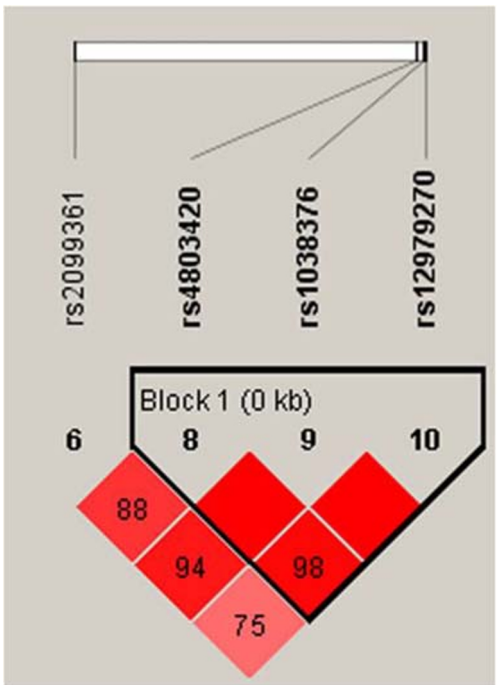

Fig. 1 Haplotype block map for the four SNPs in *CYP2B6*. A standard color frame is used to show LD pattern. One block in the figure shows higher LD. The value in each cell is the value of D′ (multiplied by 100). Bright red represents very strong LD. Pink represents intermediate LD

Interestingly, we found that the mean levels of direct bilirubin, glucose, BASO, plateletcrit, RBC, MCH, and RDW-CV in the case group were higher than those in the control group and the normal values. Moreover, the mean levels of I-BIL, BASO, plateletcrit, RBC, MCV, MCH, and RDW, and the glucose levels showed statistically significant differences between the genotypes of rs2099361, rs4803420, and rs1038376. Bilirubin is the ultimate product of heme metabolism, and when accumulated with high concentrations in tissues, it becomes highly toxic. Perlstein et al. demonstrated that higher total serum bilirubin levels were associated with reduced stroke prevalence and improved stroke outcomes (Perlstein et al. 2008). However, Arsalan et al. found that higher serum bilirubin levels were associated with increased stroke severity and poor prognosis in patients (Arsalan et al. 2011). Blood glucose is often elevated in acute stroke, and higher admission glucose levels are associated with larger lesions, greater mortality, and poorer functional outcome (Olsen 2009). One study reported that plateletcrit was significantly correlated with poor outcome of acute ischemic stroke (Mohamed et al. 2019). Panwar et al. demonstrated that lower and higher hemoglobin concentrations were associated with a higher risk of incident stroke in women (Panwar et al. 2016). RDW was found to be associated with the risk of IS and outcome in patients (Lappegard et al. 2016; Turcato et al. 2017). Therefore, these findings should be confirmed with detailed association studies in larger samples.

Although the results are promising, some limitations of our study should be mentioned. First, the small sample size in our

Table 5 Association between *CYP2B6* haplotypes and ischemic stroke risk

SNP ID	Haplotype	Case frequency	Control frequency	OR (95% CI)	*P*	Adjusted OR (95% CI)	*P*
rs4803420| rs1038376| rs12979270	GAC	0.288	0.277	1.06 (0.87–1.29)	0.575	1.02 (0.83–1.25)	0.879
	GTA	0.117	0.127	0.91 (0.69–1.20)	0.511	0.94 (0.71–1.25)	0.661
	TAA	0.796	0.766	1.20 (0.96–1.49)	0.107	1.17 (0.93–1.47)	0.176
	GAA	0.610	0.640	0.88 (0.73–1.06)	0.180	0.88 (0.72–1.06)	0.176

SNP: single-nucleotide polymorphism; OR: odds ratio; 95% CI: 95% confidence interval

OR and 95% CI were calculated using logistic regression adjusted for age and gender

P value <0.05 indicates statistical significance

study may result in insufficient power to detect the association of the *CYP2B6* polymorphisms with IS risk. Therefore, further detailed association studies in larger samples are needed to confirm these findings. Second, this study did not take into

Table 6 Distribution differences of clinical indicators in different genotypes

Indicators	rs2099361			rs4803420			rs1038376		
	Genotype	No. (mean ± SD)	*P*	Genotype	No. (mean ± SD)	*P*	Genotype	No. (mean ± SD)	*P*
Total protein (g/L)	AA	193 (65.85 ± 5.62)	0.684	TT	267 (65.56 ± 5.77)	0.883	TT	330 (65.49 ± 5.71)	0.677
	CA	170 (65.32 ± 6.07)		GT	136 (65.51 ± 5.95)		AT	84 (65.79 ± 6.22)	
	CC	56 (65.63 ± 5.57)		GG	18 (66.23 ± 5.27)		AA	7 (67.26 ± 4.91)	
Total bilirubin (umol/L)	AA	193 (14 ± 5.71)	0.555	TT	267 (13.35 ± 5.69)	0.402	TT	330 (13.67 ± 6.74)	0.892
	CA	170 (13.25 ± 7.54)		GT	136 (14.26 ± 8.13)		AT	84 (13.6 ± 5.67)	
	CC	56 (13.65 ± 5.84)		GG	18 (13.17 ± 3.56)		AA	7 (12.49 ± 5.61)	
Direct bilirubin (umol/L)	AA	193 (7.72 ± 3.41)	0.678	TT	267 (7.58 ± 3.42)	0.557	TT	330 (7.76 ± 4.53)	0.866
	CA	170 (7.88 ± 5.24)		GT	136 (8.05 ± 5.78)		AT	84 (7.64 ± 3.3)	
	CC	56 (7.3 ± 3.79)		GG	18 (7.45 ± 1.7)		AA	7 (6.94 ± 2.8)	
Indirect bilirubin (umol/L)	AA	193 (6.28 ± 3.45)	0.025	TT	267 (5.77 ± 3.23)	0.477	TT	330 (5.91 ± 3.42)	0.954
	CA	170 (5.37 ± 3.39)		GT	136 (6.21 ± 3.84)		AT	84 (5.95 ± 3.51)	
	CC	56 (6.36 ± 3.29)		GG	18 (5.72 ± 2.97)		AA	7 (5.54 ± 3.06)	
Uric acid (umol/L)	AA	180 (279.11 ± 92.35)	0.525	TT	257 (280.54 ± 91.58)	0.447	TT	312 (280.62 ± 93.04)	0.141
	CA	164 (289.85 ± 97.77)		GT	127 (291.68 ± 99.53)		AT	82 (302.67 ± 99.16)	
	CC	54 (290.15 ± 91.8)		GG	16 (300 ± 95.18)		AA	6 (261.83 ± 66.86)	
Total cholesterol (mmol/L)	AA	186 (3.93 ± 0.99)	0.880	TT	254 (3.9 ± 1.03)	0.859	TT	321 (3.92 ± 1.05)	0.618
	CA	164 (3.87 ± 1.05)		GT	134 (3.9 ± 1.07)		AT	78 (3.8 ± 0.95)	
	CC	54 (3.91 ± 1.08)		GG	18 (3.77 ± 0.64)		AA	7 (3.85 ± 0.55)	
Triglyceride (mmol/L)	AA	185 (1.49 ± 0.74)	0.176	TT	253 (1.57 ± 0.97)	0.829	TT	321 (1.59 ± 1.09)	0.355
	CA	164 (1.64 ± 1.21)		GT	134 (1.61 ± 1.23)		AT	78 (1.52 ± 0.8)	
	CC	54 (1.75 ± 1.39)		GG	18 (1.46 ± 0.67)		AA	6 (2.15 ± 1.38)	
HDL-C (mmol/L)	AA	186 (1.10 ± 0.27)	0.848	TT	255 (1.09 ± 0.25)	0.365	TT	321 (1.09 ± 0.26)	0.461
	CA	165 (1.08 ± 0.24)		GT	134 (1.10 ± 0.27)		AT	79 (1.07 ± 0.26)	
	CC	54 (1.09 ± 0.25)		GG	18 (1.01 ± 0.21)		AA	7 (1.18 ± 0.15)	
LDL-C (mmol/l)	AA	186 (1.87 ± 0.61)	0.231	TT	255 (1.8 ± 0.57)	0.818	TT	321 (1.83 ± 0.6)	0.328
	CA	165 (1.77 ± 0.57)		GT	134 (1.83 ± 0.62)		AT	79 (1.76 ± 0.53)	
	CC	54 (1.76 ± 0.5)		GG	18 (1.76 ± 0.41)		AA	7 (1.54 ± 0.29)	
Glucose (mmol/L)	AA	193 (6.34 ± 2.42)	0.739	TT	268 (6.34 ± 2.42)	0.591	TT	333 (6.3 ± 2.13)	0.001
	CA	173 (6.25 ± 1.98)		GT	138 (6.36 ± 2)		AT	84 (6.19 ± 1.76)	
	CC	56 (6.52 ± 2.45)		GG	18 (5.79 ± 1.21)		AA	7 (9.35 ± 7.11)	
WBC (*10E9/L)	AA	187 (8.04 ± 8.61)	0.905	TT	270 (8.02 ± 7.91)	0.098	TT	327 (8.26 ± 9.55)	0.781
	CA	173 (8.46 ± 9.49)		GT	131 (8.05 ± 8.31)		AT	86 (8.26 ± 5.43)	
	CC	57 (8.24 ± 7.28)		GG	18 (12.6 ± 18.97)		AA	6 (5.72 ± 2.29)	
NEUT (*10E9/L)	AA	187 (4.59 ± 2.5)	0.403	TT	269 (4.81 ± 2.71)	0.174	TT	326 (4.65 ± 2.47)	0.067
	CA	172 (4.89 ± 2.86)		GT	131 (4.84 ± 2.73)		AT	86 (5.31 ± 3.41)	
	CC	57 (5.07 ± 2.78)		GG	18 (3.61 ± 1.74)		AA	6 (3.54 ± 1.52)	
EO (*10E9/L)	AA	187 (0.13 ± 0.19)	0.216	TT	269 (0.12 ± 0.14)	0.805	TT	326 (0.12 ± 0.14)	0.987
	CA	172 (0.11 ± 0.1)		GT	131 (0.13 ± 0.19)		AT	86 (0.12 ± 0.21)	
	CC	57 (0.14 ± 0.18)		GG	18 (0.14 ± 0.15)		AA	6 (0.12 ± 0.13)	
BASO (*10E9/L)	AA	187 (0.09 ± 0.6)	0.818	TT	269 (0.09 ± 0.55)	0.009	TT	326 (0.12 ± 0.7)	0.750
	CA	172 (0.13 ± 0.72)		GT	131 (0.09 ± 0.61)		AT	86 (0.07 ± 0.5)	
	CC	57 (0.09 ± 0.62)		GG	18 (0.57 ± 1.63)		AA	6 (0.02 ± 0.02)	
Platelet (*10E9/L)	AA	187 (179.93 ± 66.43)	0.980	TT	269 (176.98 ± 64.04)	0.165	TT	326 (180.06 ± 68.36)	0.963

Table 6 (continued)

Indicators	rs2099361			rs4803420			rs1038376		
	Genotype	No. (mean ± SD)	*P*	Genotype	No. (mean ± SD)	*P*	Genotype	No. (mean ± SD)	*P*
	CA	172 (180.63 ± 66.66)		GT	131 (188.39 ± 68.56)		AT	86 (179.5 ± 56.93)	
	CC	57 (178.64 ± 64.16)		GG	18 (165.07 ± 71.23)		AA	6 (187.17 ± 58.73)	
Plateletcrit (%)	AA	186 (0.38 ± 1.44)	0.787	TT	263 (0.37 ± 1.38)	0.030	TT	319 (0.45 ± 1.66)	0.756
	CA	166 (0.48 ± 1.79)		GT	129 (0.39 ± 1.47)		AT	85 (0.32 ± 1.15)	
	CC	56 (0.36 ± 1.15)		GG	18 (1.36 ± 3.42)		AA	6 (0.2 ± 0.04)	
RBC (10E12/L)	AA	187 (6.83 ± 16.42)	0.871	TT	269 (6.55 ± 14.88)	0.011	TT	326 (7.63 ± 19.93)	0.771
	CA	172 (7.86 ± 20.96)		GT	131 (7.07 ± 18.94)		AT	86 (6.22 ± 12.97)	
	CC	57 (7.19 ± 18.05)		GG	18 (20.06 ± 45.08)		AA	6 (4.58 ± 0.27)	
Hemoglobin (g/L)	AA	187 (136.61 ± 22.93)	0.598	TT	269 (137.99 ± 21.25)	0.191	TT	326 (135.83 ± 23.19)	0.085
	CA	172 (136.05 ± 23)		GT	131 (135.47 ± 23.85)		AT	86 (141.24 ± 21.05)	
	CC	57 (139.55 ± 22.04)		GG	18 (128.97 ± 33.91)		AA	6 (127.17 ± 15.79)	
MCV (fl)	AA	187 (90.38 ± 10.17)	0.826	TT	269 (91.12 ± 9.45)	0.031	TT	326 (90.42 ± 10.62)	0.454
	CA	172 (91.05 ± 10.83)		GT	131 (90.7 ± 9.45)		AT	86 (91.91 ± 8.92)	
	CC	57 (90.75 ± 9.06)		GG	18 (84.54 ± 21.24)		AA	6 (89.15 ± 8.2)	
MCH (pg)	AA	187 (33.76 ± 35.66)	0.805	TT	269 (33.93 ± 35.33)	0.021	TT	326 (35.91 ± 43.49)	0.749
	CA	172 (36.59 ± 45.29)		GT	131 (34.12 ± 39.87)		AT	86 (32.87 ± 30.31)	
	CC	57 (35.67 ± 43.44)		GG	18 (61.25 ± 90.71)		AA	6 (27.72 ± 2.4)	
RDW (fL)	AA	187 (42.77 ± 5.29)	0.475	TT	269 (42.85 ± 5.59)	0.007	TT	326 (42.76 ± 6.31)	0.592
	CA	172 (43.28 ± 6.18)		GT	131 (43.55 ± 5.88)		AT	86 (43.46 ± 4.65)	
	CC	57 (42.23 ± 7.35)		GG	18 (38.87 ± 9.7)		AA	6 (42.07 ± 1.67)	
RDW-CV (%)	AA	187 (15.71 ± 19.66)	0.812	TT	269 (15.75 ± 21.22)	0.074	TT	326 (17.03 ± 25.86)	0.723
	CA	172 (17.24 ± 25.93)		GT	131 (16.44 ± 24.11)		AT	86 (14.89 ± 15.86)	
	CC	57 (17.26 ± 30.45)		GG	18 (29.04 ± 48.39)		AA	6 (13.35 ± 1.59)	

HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; WBC: white blood cell count; NEUT: neutrophil absolute value; EO: eosinophils absolute value; BASO: basophilic absolute value; RBC: red blood cell count; MCV: erythrocyte mean corpuscular volume; MCH: mean corpuscular hemoglobin; RDW: red blood cell distribution width; RDW-CV: red blood cell distribution width coefficient of variation

$P < 0.05$ indicates statistical significance

consideration the association of conventional IS risk factors, such as smoking, alcohol intake, hypertension, or diabetes mellitus, with *CYP2B6* polymorphisms. Third, other SNPs in *CYP2B6* were not assessed for their potential association with IS risk. Finally, this study did not elucidate the specific mechanism of the *CYP2B6* polymorphisms that affect the risk of IS.

Conclusion

In conclusion, our results indicated that polymorphisms (rs2099361, rs4803420, and rs1038376) in *CYP2B6* were significantly associated with the risk of IS in a Chinese Han population. The results provide evidence that CYP2B6 polymorphisms may be a genetic determinant of IS susceptibility. However, this is the first study to report this phenomenon, and it is unclear whether these findings are reproducible in other populations. Therefore, further detailed association and functional studies in larger samples are needed to validate the association between *CYP2B6* polymorphisms and risk of IS.

Acknowledgements This study was supported by the National Natural Science Foundation of China (No. 81760234). We thank all the individuals for their participation and all the neurological physicians in the Haikou Hospital Affiliated to Xiangya School of Medicine, Central South University for collecting samples.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

Ethical Statement The study protocol was approved by the ethical committee of the Haikou Hospital Affiliated to Xiangya School of Medicine, Central South University, and was conducted in accordance with the principles of the Declaration of Helsinki. Written informed consent was obtained from all individual participants included in the study.

References

Arsalan IM, Khattak MB, Khan F, Anwar MJ, Murtaza Z, Khan A (2011) Prognostic significance of serum bilirubin in stroke. Journal of Ayub Medical College, Abbottabad: JAMC 23:104–107

Boehme AK, Esenwa C, Elkind MS (2017) Stroke Risk Factors, Genetics, and Prevention. Circ Res 120:472–495. https://doi.org/10.1161/CIRCRESAHA.116.308398

Code EL, Crespi CL, Penman BW, Gonzalez FJ, Chang TK, Waxman DJ (1997) Human cytochrome P4502B6: interindividual hepatic expression, substrate specificity, and role in procarcinogen activation. Drug Metabolism and Disposition: The Biological Fate of Chemicals 25:985–993

Daly AK (2004) Pharmacogenetics of the cytochromes P450. Curr Top Med Chem 4:1733–1744

Daraki A et al (2014) The G(5)(1)(6)T CYP2B6 germline polymorphism affects the risk of acute myeloid leukemia and is associated with specific chromosomal abnormalities. PLoS One 9:e88879. https://doi.org/10.1371/journal.pone.0088879

Feigin VL et al (2015) Update on the Global Burden of Ischemic and Hemorrhagic Stroke in 1990-2013: The GBD 2013. Study Neuroepidemiology 45:161–176. https://doi.org/10.1159/000441085

Hankey GJ (2017) Stroke Lancet 389:641–654. https://doi.org/10.1016/S0140-6736(16)30962-X

He J et al (2016) Association analysis of ALOX5 gene polymorphisms with stroke risk: a case-control study in a Chinese Han population. Int J Clin Exp Pathol 9:4432–4437

Hodgson E, Rose RL (2007) The importance of cytochrome P450 2B6 in the human metabolism of environmental chemicals. Pharmacol Ther 113:420–428. https://doi.org/10.1016/j.pharmthera.2006.10.002

Hofmann MH, Blievernicht JK, Klein K, Saussele T, Schaeffeler E, Schwab M, Zanger UM (2008) Aberrant splicing caused by single nucleotide polymorphism c.516G>T [Q172H], a marker of CYP2B6*6, is responsible for decreased expression and activity of CYP2B6 in liver. J Pharmacol Exp Ther 325:284–292. https://doi.org/10.1124/jpet.107.133306

Justenhoven C et al (2014) CYP2B6*6 is associated with increased breast cancer risk. Int J Cancer 134:426–430. https://doi.org/10.1002/ijc.28356

Kurosaki T et al (2009) Polymorphism of cytochrome P450 2B6 and prostate cancer risk: a significant association in a Japanese population. International journal of urology: Official Journal of the Japanese Urological Association 16:364–368

Lamba V et al (2003) Hepatic CYP2B6 expression: gender and ethnic differences and relationship to CYP2B6 genotype and CAR (constitutive androstane receptor) expression. J Pharmacol Exp Ther 307:906–922. https://doi.org/10.1124/jpet.103.054866

Lang T et al (2001) Extensive genetic polymorphism in the human CYP2B6 gene with impact on expression and function in human liver. Pharmacogenetics 11:399–415

Lappegard J et al (2016) Red cell distribution width is associated with future risk of incident stroke. The Tromso Study Thrombosis and haemostasis 115:126–134. https://doi.org/10.1160/TH15-03-0234

Li G, Zhu L, Bai M, Wang L, Yuan D (2018) The impact of the AHSG genetic polymorphism on the risk of ischemic stroke: a case-control study. Int J Clin Exp Pathol 11:5094–5100

Meschia JF, Brott T (2018) Ischaemic stroke. Eur J Neurol 25:35–40. https://doi.org/10.1111/ene.13409

Miksys S, Lerman C, Shields PG, Mash DC, Tyndale RF (2003) Smoking, alcoholism and genetic polymorphisms alter CYP2B6 levels in human brain. Neuropharmacology 45:122–132

Miksys S, Tyndale RF (2004) The unique regulation of brain cytochrome P450 2 (CYP2) family enzymes by drugs and genetics. Drug Metab Rev 36:313–333. https://doi.org/10.1081/DMR-120034149

Mohamed AB, Elnady HM, Alhewaig HK, Moslem Hefny H, Khodery A (2019) The mean platelet volume and plateletcrit as predictors of short-term outcome of acute ischemic stroke. The Egyptian Journal of Neurology, Psychiatry and Neurosurgery 55:4. https://doi.org/10.1186/s41983-018-0035-x

Niu F, Wei B, Yan M, Li J, Ouyang Y, Jin T (2018) Matrix metalloproteinase-2 gene polymorphisms are associated with ischemic stroke in a Hainan population. Medicine 97:e12302. https://doi.org/10.1097/MD.0000000000012302

Olsen TS (2009) Blood glucose in acute stroke. Expert Rev Neurother 9:409–419. https://doi.org/10.1586/14737175.9.3.409

Panwar B, Judd SE, Warnock DG, McClellan WM, Booth JN 3rd, Muntner P, Gutierrez OM (2016) Hemoglobin Concentration and Risk of Incident Stroke in Community-Living Adults. Stroke 47:2017–2024. https://doi.org/10.1161/STROKEAHA.116.013077

Perlstein TS, Pande RL, Creager MA, Weuve J, Beckman JA (2008) Serum total bilirubin level, prevalent stroke, and stroke outcomes: NHANES 1999-2004. Am J Med 121:781–788 e781. https://doi.org/10.1016/j.amjmed.2008.03.045

Thorn CF, Lamba JK, Lamba V, Klein TE, Altman RB (2010) PharmGKB summary: very important pharmacogene information for CYP2B6. Pharmacogenet Genomics 20:520–523

Turcato G et al (2017) Red Blood Cell Distribution Width Is an Independent Predictor of Outcome in Patients Undergoing Thrombolysis for Ischemic Stroke. Semin Thromb Hemost 43:30–35. https://doi.org/10.1055/s-0036-1592165

Wang H, Tompkins LM (2008) CYP2B6: new insights into a historically overlooked cytochrome P450 isozyme. Curr Drug Metab 9:598–610

Wang J, Sonnerborg A, Rane A, Josephson F, Lundgren S, Stahle L, Ingelman-Sundberg M (2006) Identification of a novel specific CYP2B6 allele in Africans causing impaired metabolism of the HIV drug efavirenz. Pharmacogenet Genomics 16:191–198. https://doi.org/10.1097/01.fpc.0000189797.03845.90

Wang W et al (2017) Prevalence, Incidence, and Mortality of Stroke in China: Results from a Nationwide Population-Based Survey of 480 687 Adults. Circulation 135:759–771. https://doi.org/10.1161/CIRCULATIONAHA.116.025250

Wu S et al (2019) Stroke in China: advances and challenges in epidemiology, prevention, and management. The Lancet Neurology 18:394–405. https://doi.org/10.1016/S1474-4422(18)30500-3

Xu C et al (2015) Associations Between CYP2B6 rs707265, rs1042389, rs2054675, and Hirschsprung Disease in a Chinese Population. Dig Dis Sci 60:1232–1235. https://doi.org/10.1007/s10620-014-3450-6

Zachaki S, Daraki A, Polycarpou E, Stavropoulou C, Manola KN, Gavrili S (2017) GSTP1 and CYP2B6 Genetic Polymorphisms and the Risk of Bronchopulmonary Dysplasia in Preterm Neonates. Am J Perinatol 34:729–734. https://doi.org/10.1055/s-0036-1597994

Zanger UM, Klein K (2013) Pharmacogenetics of cytochrome P450 2B6 (CYP2B6): advances on polymorphisms, mechanisms, and clinical relevance. Front Genet 4:24. https://doi.org/10.3389/fgene.2013.00024

Zanger UM, Turpeinen M, Klein K, Schwab M (2008) Functional pharmacogenetics/genomics of human cytochromes P450 involved in drug biotransformation. Anal Bioanal Chem 392:1093–1108. https://doi.org/10.1007/s00216-008-2291-6