



A SNP in 5' untranslated region of CD40 gene is associated with an increased risk of ischemic stroke in a Chinese population: a case-control study

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Abstract

Cluster of differentiation 40 (CD40), the receptor for CD154, is a member of the tumor necrosis factor (TNF) receptor superfamily. Several studies have been conducted to investigate the effect of the CD40 rs1883832 polymorphism on atherosclerotic disease in different population; however, inconsistent results were obtained. In this study, we investigated the association of four polymorphisms (rs1883832, rs13040307, rs752118 and rs3765459) of CD40 gene and their effect on CD40 expression with the risk of ischemic stroke (IS) in a Chinese population. Three hundred and eighty patients with IS and 450 control subjects were included in the study. The CD40 polymorphisms were discriminated by Snapshot SNP genotyping assay. Serum soluble CD40 (sCD40) levels were detected by ELISA. We found that the rs1883832CT and rs1883832TT genotypes were associated with an increased risk of IS compared with the rs1883832CC genotype (OR = 1.42, 95% CI: 1.03–1.95, $p = 0.030$ and OR = 1.91, 95% CI: 1.29–2.82, $P = 0.001$, respectively), and the rs1883832T allele was associated with a significantly increased risk of IS compared with rs1883832C allele (OR = 1.40, 95% CI: 1.15–1.70, $P = 0.001$). Elevated serum sCD40 levels were observed in patients with IS compared with the control group ($P < 0.01$). Individuals carrying the rs1883832TT or rs1883832CT genotypes showed significantly higher sCD40 levels compared with the rs1883832CC genotype in the IS group [(64.8 ± 25.4 pg/mL, TT = 94); (63.9 ± 24.3 pg/mL, CT = 185) vs (53.3 ± 22.5 pg/mL, CC = 101), $P < 0.01$]. The TCCA haplotype was associated with an increased risk of IS compared with the control group (OR = 2.10, 95% CI: 1.23–3.58, $p = 0.005$). However, we did not find a significant association between the other three polymorphisms and IS risk. In conclusion, after a comprehensive comparison with other studies, we confirmed that the rs1883832T allele but not the rs1883832C allele is associated with an increased risk of IS. The rs1883832 polymorphism may exert influences on abnormal CD40 expression in IS patients among the Chinese population.

Keywords: CD40, gene, polymorphism, ischemic stroke.

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Introduction

Stroke is one of the leading causes of death and a common cause of long-term disability in the world (Feigin *et al.*, 2009, Lloyd-Jones *et al.*, 2010). In China, there are approximately 2.5 million new strokes, and more than one million people die from stroke-related causes every year (Liu *et al.*, 2011). Ischemic stroke (IS) is the most common

type of stroke, accounting for more than 80% of cases (Liu *et al.*, 2011). Previous studies have identified that age and sex are closely related to IS (Lloyd-Jones *et al.*, 2010; Turtzo and McCullough, 2010). Hypertension, smoking, alcohol abuse, diabetes mellitus, and hypercholesterolemia were demonstrated as important risk factors for IS (Sacco *et al.*, 1997). However, these risk factors together explain only about 50% of the risk (Sacco *et al.*, 1989), indicating that other factors such as immune, inflammatory and genetic factors may also be involved in the pathogenesis of IS. In the last few years, candidate genes for IS have been in-

tensely studied, and numerous susceptible candidates including CD40 gene have been found.

CD40, the costimulatory receptor for CD40 ligand (CD40L/CD154), is a 48-kDa type I transmembrane protein receptor belonging to the tumor necrosis factor (TNF) receptor superfamily (Elgueta *et al.*, 2009). Overexpression of CD40 and/or its ligand CD40L have been detected in patients with atherosclerosis-related diseases such as coronary artery disease and stroke, and were suggested as potential biomarkers for predicting cardiovascular disease (Yan *et al.*, 2002; Yan *et al.*, 2004; Li *et al.*, 2015). Binding of CD40L to its receptor in vascular endothelial cells triggers the transcription of proinflammatory and proatherogenic genes, which are important components in the onset of atherosclerosis-related diseases, including stroke (Chen *et al.*, 2006). It is accepted that atherosclerosis, plaque instability and thrombus are important pathological basis of IS. *In vitro*, binding of CD40L to its receptor on the surface of endothelial cells and smooth muscle cells leads to the activation of these cells, resulting in the expression of adhesion molecules, which is an initiating step of atherogenesis (Schonbeck and Libby, 2001; Schonbeck *et al.*, 2002). Moreover, the interaction between CD40 and its ligand induces the expression of matrix metalloproteinases (MMP), resulting in the degradation of interstitial collagen and the thin fibrous cap of atheromatous plaques, and eventually leading to the instability and rupture of plaques (Schonbeck and Libby, 2001). Furthermore, the CD40-CD40L interaction promotes the expression of tissue factors on macrophage cells and endothelial cells, leading to decreased thrombomodulin expression, and favoring a local procoagulant and prothrombotic status (Aukrust *et al.*, 2004). These data indicate that CD40 might play a pathological role in IS, and it may be used as a biomarker and therapeutic target for IS. Hence, the CD40 gene is likely a potential candidate gene for IS risk.

The gene encoding CD40 is located on 20q12-q13.2 in humans, which is consisted of 9 exons and 8 introns. The rs1883832 locus, previously demonstrated to be associated with CD40 expression, is located at the -1 position within the Kozak sequence (Jacobson *et al.*, 2005; Tian *et al.*, 2010). Several studies have investigated the association between rs1883832 polymorphism and risk of atherosclerotic disease in different populations, however, the results were inconclusive (Yan *et al.*, 2010; Wang *et al.*, 2011; Ma *et al.*, 2013; Zhang *et al.*, 2013). To confirm the results, we conducted a case-control study with 380 IS patients and 450 control subjects. Moreover, three new polymorphisms (rs13040307, rs752118 and rs3765459) were added in this study. Until now, little information has addressed the effect of CD40 polymorphisms on CD40 expression and their effect on IS risk. The aim of the present study was to investigate the role of these polymorphisms in the genetic basis of IS and to assess the relationship between CD40 polymorphisms and serum level of CD40 in a Chinese population.

Materials and Methods

Study population

The study protocol was approved by the ethics committee of Affiliated Hospital of Youjiang Medical University for Nationalities, and informed consent was obtained from all the IS patients and control subjects. The study population included 380 IS patients (290 men and 90 women, mean age: 60.7 ± 13.2 years) and 450 control subjects (325 men and 125 women, mean age: 63.9 ± 10.3 years). All IS patients were recruited from the Department of Neurology of the institution from May 2013 to December 2014. The control subjects were frequency-matched with the IS group on the basis of age and sex. All the control subjects were recruited from the physical examination center of the same hospital from July 2013 to December 2013. According to thorough clinical and laboratory evaluation, none of them were found to have any medical condition other than hypertension, diabetes, hypercholesterolemia or hypertriglyceridemia. All participants were Han Chinese and were consecutively selected from the same geographic region of Guangxi, China.

IS patients were classified in accordance with the Trial of Org 10172 in Acute Stroke Treatment (TOAST) classification (Adams Jr *et al.*, 1993) as large-artery atherosclerosis (LAA), small-artery occlusion (SAO), cardioembolism or stroke of other determined etiology. Classifications were based on clinical findings, neuroimaging data [computed tomography (CT) and/or cranial magnetic resonance imaging (MRI)] and results of diagnostic studies such as duplex imaging of extracranial arteries, cardiac imaging (echocardiography) and laboratory evaluation.

Hypertension was diagnosed if the diastolic blood pressure was ≥ 90 mmHg and/or the systolic blood pressure was ≥ 140 mmHg, or if the person was currently using antihypertensive treatment. Hypercholesterolemia was defined as fasting serum total cholesterol level > 6.2 mmol/L, and hypertriglyceridemia was diagnosed if the fasting serum triglyceride level was > 2.3 mmol/L.

DNA extraction and genotyping

Genomic DNA was extracted from venous blood leukocytes by using a salting-out method (John *et al.*, 1991). Genotyping method was described in detail previously (Chen *et al.*, 2015a). Briefly, SnapshotSNP genotyping assay was used to determine the genotypes of rs1883832, rs13040307, rs752118 and rs3765459 polymorphisms. PCR primers were designed in accordance with the GenBank reference sequence (accession no. NC_000020.11). Moreover, DNA sequencing method was used to confirm our genotyping results.

Serum sCD40 determination

Serum samples from IS patients and control subjects were separated from venous blood at room temperature and

stored at -70°C until use. Serum sCD40 levels were analyzed by enzyme linked immunosorbent assay (ELISA) kits (Bender Med Systems, USA) according to the protocol of the manufacturer. Developed color reaction was quantified by an ELISA reader (RT-6000, China). The concentration of serum sCD40 was determined by using a standard curve constructed with the kit's standards over the range of 0–500 pg/mL.

Statistical analysis

Statistical analyses were performed using the SPSS software program version 17.0. Continuous variables are displayed as mean \pm standard deviation (SD). If the data were normally distributed, the Student's *t*-test was used. Categorical variables are reported as proportions and compared using the chi-square test. Hardy-Weinberg equilibrium (HWE) was tested by the chi-square test. The Shi's standardized coefficient *D'* (*D'*) (Shi and He, 2005) was used to quantify the linkage disequilibrium (LD) between polymorphisms. Haplotypes and their frequencies were estimated on the basis of a Bayesian algorithm using the Phase program (Stephens *et al.*, 2001). The statistically significant criteria was assumed at $P < 0.05$ level.

Results

Clinical characteristics of the study participants

The clinical characteristics of IS patients and healthy control subjects are shown in Table 1. There were no statistically significant differences between the two groups in age, gender, hypertriglyceridemia and hypercholesterolemia ($P > 0.05$). The frequencies of abnormal LDL-cholesterol, serum total cholesterol and triglycerides, smokers, diabetes and hypertension in IS patients were significantly higher than those in the control group ($P < 0.05$). Increased levels of serum sCD40 were observed in IS patients com-

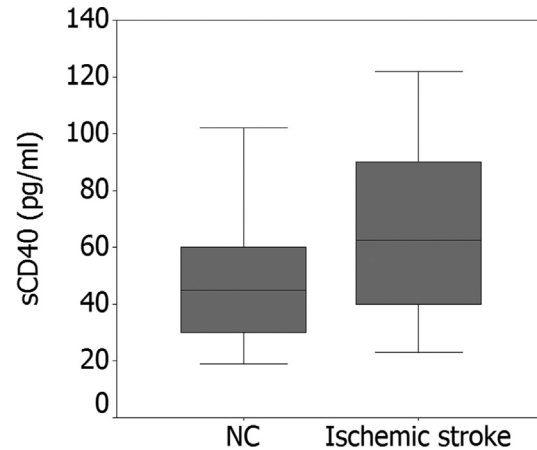


Figure 1 - The levels of sCD40 in patient with IS and the control group. Levels of sCD40 were higher in IS group than in the control group (61.3 ± 23.7 pg/mL, $n = 380$ vs. 44.5 ± 18.7 pg/mL, $n = 450$), $P < 0.001$.

pared with the control group [$(61.3 \pm 23.7$ pg/mL, $n = 380$) vs (44.5 ± 18.7 pg/mL, $n = 450$); $P < 0.001$] (Figure 1).

Genotype and allele frequencies of the four polymorphisms

All the four polymorphisms showed three genotypes according to sequencing results. The distribution of the genotype and allele frequencies of the rs1883832, rs13040307, rs752118 and rs3765459 polymorphisms in IS patients and control subjects are presented in Table 2. The genotype distribution of the four polymorphisms among IS patients and control subjects were in HWE. The frequencies of the rs1883832CC, rs1883832CT and rs1883832TT genotypes were 26.6, 48.7 and 24.7% in IS patients and 36.0, 46.4 and 17.6% in the control group, respectively. There were statistically significant differences in the genotype and allele frequencies of the rs1883832 polymorphism between IS patients and the control group ($P < 0.01$). The

Table 1 - Clinical characteristics of the study participants.

Variable	Control subjects $n = 450$ (%)	Stroke patients $n = 380$ (%)	<i>P</i> value
Age (mean \pm SD)	63.9 ± 10.3	60.7 ± 13.2	0.102
Sex (M/F)	325 / 125	290 / 90	0.180
Smokers	203 (45.1)	212 (55.8)	0.002
Hypertension	165 (36.7)	210 (55.3)	< 0.001
Diabetes	53 (11.8)	76 (20.0)	0.001
Hypercholesterolemia	50 (11.1)	55 (14.5)	0.147
Hypertriglyceridemia	62 (13.8)	46 (12.1)	0.476
Total cholesterol (mmol/L)	4.86 ± 1.08	5.29 ± 1.22	0.023
Triglycerides (mmol/L)	1.54 ± 0.97	2.09 ± 1.56	0.001
HDL-cholesterol (mmol/L)	1.69 ± 0.46	1.31 ± 0.36	0.038
LDL-cholesterol (mmol/L)	2.31 ± 0.98	2.98 ± 0.93	0.002

Table 2 - Distribution of the genotype and allele frequencies four polymorphisms of the CD40 gene in ischemic stroke (IS) patients and control subjects.

Polymorphisms	Control subjects n = 450 (%)	IS patients n = 380 (%)	OR (95% CI)	χ^2	P value
rs1883832					
CC	162 (36.0)	101 (26.6)	1.00		
CT	209 (46.4)	185 (48.7)	1.42 (1.03 - 1.95)	4.692	0.030
TT	79 (17.6)	94 (24.7)	1.91 (1.29 - 2.82)	10.175	0.001
C	533 (59.2)	387 (50.9)	1.00		
T	367 (40.8)	373 (49.1)	1.40 (1.15 - 1.70)	11.493	0.001
rs13040307					
CC	265 (58.9)	224 (58.9)	1.00		
CT	161 (35.8)	130 (34.2)	0.96 (0.71 - 1.28)	0.095	0.758
TT	24 (5.3)	26 (6.8)	1.28 (0.72 - 2.30)	0.699	0.403
C	691 (76.8)	578 (76.1)	1.00		
T	209 (23.2)	182 (23.9)	1.04 (0.83 - 1.31)	0.120	0.729
rs752118					
CC	276 (61.3)	236 (62.1)	1.00		
CT	148 (32.9)	127 (33.4)	1.00 (0.75 - 1.35)	0.001	0.981
TT	26 (5.8)	17 (4.5)	0.77 (0.41 - 1.44)	0.688	0.407
C	700 (77.8)	599 (78.8)	1.00		
T	200 (22.2)	161 (21.2)	0.94 (0.74 - 1.19)	0.261	0.610
rs3765459					
GG	249 (55.3)	223 (58.7)	1.00		
GA	171 (38.0)	134 (35.3)	0.88 (0.66 - 1.17)	0.818	0.366
AA	30 (6.7)	23 (6.1)	0.86 (0.48 - 1.52)	0.284	0.594
G	669 (74.3)	580 (76.3)	1.00		
A	231 (25.7)	180 (23.7)	0.90 (0.72 - 1.13)	0.869	0.351

rs1883832CT and rs1883832TT genotypes were associated with an increased risk of IS compared with the rs1883832CC genotype (OR = 1.42, 95% CI: 1.03–1.95, $p = 0.030$ and OR = 1.91, 95% CI: 1.29–2.82, $P = 0.001$, respectively). Comparing with the rs1883832C allele, the rs1883832T allele was associated with an increased risk of IS (OR = 1.40, 95% CI: 1.15–1.70, $P = 0.001$). However, there was no significant association between IS patients and the control group in the genotype and allele frequencies of the rs13040307, rs752118 and rs3765459 polymorphisms ($P > 0.05$).

Genotype and allele distribution of the rs1883832 polymorphism in different populations

Considering the importance of the CD40 rs1883832 polymorphism in the etiology of IS, we then performed a comparison of the genotype distribution of the rs1883832 polymorphism in different populations (Table 3), and found that the genotype distribution of rs1883832 polymorphism in our current study was significantly different from HapMap-CEU, HapMap-HCB, HapMap-JPT, HapMap-YRI, HapMap-ASW, HapMap-GIH, HapMap-LWK, HapMap-MKK and HapMap-TSI populations ($P < 0.05$). However, no significant difference was found when comparing with HapMap-CHB and HapMap-CHD populations ($P > 0.05$).

Haplotype analysis of the four polymorphisms

We further performed a haplotype analysis, and the possible seven haplotypes are listed in Table 4. The results showed that the rs1883832 polymorphism was in strong linkage disequilibrium (LD) with the rs13040307 ($D' = 0.873$), rs752118 ($D' = 0.895$) and rs3765459 ($D' = 0.898$) polymorphisms. Also, the rs13040307 polymorphism was in strong LD with the rs752118 ($D' = 0.946$) and rs3765459 ($D' = 0.937$) polymorphisms. Moreover, the rs752118 polymorphism was in strong LD with the rs3765459 ($D' = 0.942$) polymorphism. CCCG and TCCG were the two major haplotypes, and accounted for 27.1 and 44.1%, and 30.6 and 42.0% in both IS patients and control subjects, respectively. As shown in Table 4, the TCCA haplotype was associated with an increased risk of IS compared with the control group (OR = 2.10, 95% CI: 1.23–3.58, $P = 0.005$).

Association between CD40 polymorphisms and serum sCD40 levels

The rs1883832 polymorphism was significantly associated with serum sCD40 levels in patients with IS. Individuals carrying the rs1883832TT (64.8 ± 25.4 pg/mL, $n = 94$) or rs1883832CT genotype (63.9 ± 24.3 pg/mL, $n = 185$) showed significantly higher sCD40 levels compared with the rs1883832CC genotype (53.3 ± 22.5 pg/mL, $n = 101$, P

Table 3 - Comparison of the rs1883832 polymorphism in different populations.

Population	sample size	Genotypes (%)			Minor allele (%)		Ethnicity
		CC	CT	TT	T		
Our data	450	162 (36.0)	209 (46.4)	79 (17.6)	367 (40.8)		Guangxi China
HapMap-CEU*	226	124 (54.9)	94 (41.6)	8 (3.5)	55 (24.3)		European
HapMap-HCB*	86	40 (46.5)	44 (51.2)	2 (2.3)	24 (27.9)		Asian
HapMap-JPT*	170	40 (23.5)	90 (53.0)	40 (23.5)	85 (50.0)		Asian
HapMap-YRI*	226	224 (99.1)	2 (0.9)	-	2 (0.4)		African
HapMap-ASW*	98	94 (95.9)	4 (4.1)	-	4 (2.0)		African
HapMap-CHB	82	40 (48.8)	30 (36.6)	12 (14.6)	27 (32.9)		Asian
HapMap-CHD	170	72 (42.4)	68 (40.0)	30 (17.6)	64 (37.6)		Asian
HapMap-GIH*	176	98 (55.7)	66 (37.5)	12 (6.8)	45 (25.6)		Asian
HapMap-LWK*	180	166 (92.2)	14 (7.8)	-	7 (3.9)		Asian
HapMap-MKK*	286	244 (85.3)	40 (14.0)	2 (0.7)	22 (7.7)		African
HapMap-TSI*	176	68 (38.6)	104 (59.1)	4 (2.3)	56 (31.8)		European

* $P < 0.05$ comparing with our present data; **CEU**: Utah residents with northern and western European ancestry; **HCB**: Han Chinese in Beijing, China; **JPT**: Japanese in Tokyo, Japan; **YRI**: Yoruba in Ibadan, Nigeria. **ASW**: African ancestry in Southwest USA; **CHB**: Han Chinese in Beijing, China; **CHD**: Chinese in Metropolitan Denver, Colorado; **GIH**: Gujarati Indians in Houston, Texas; **LWK**: Luhya in Webuye, Kenya; **MKK**: Maasai in Kinyawa, Kenya; **TSI**: Tuscans in Italy;

< 0.01). Nevertheless, no significant difference was found in the serum sCD40 levels between rs1883832TT and rs1883832CT genotypes (Figure 2). Furthermore, there was no significant association between the CD40 rs13040307, rs752118 and rs3765459 polymorphisms and serum sCD40 levels ($P > 0.05$).

Clinical and biochemical values in IS patients with different CD40 genotypes

No significant difference was found in the genotype frequencies of the CD40 rs1883832, rs13040307, rs752118 and rs3765459 polymorphisms after stratification of IS by age, smoking status, gender, and the absence or presence of hypertriglyceridemia, hypertension and hypercholesterolemia (data not shown). Moreover, no significant difference was found between different genotypes of the CD40 polymorphisms and laboratory values (TG, TC, LDL-C and HDL-C) (data not shown).

Association between CD40 polymorphisms and different subtypes of IS

We did not find any association between the CD40 polymorphisms rs1883832, rs13040307, rs752118 and rs3765459 and IS subtypes (data not shown).

Discussion

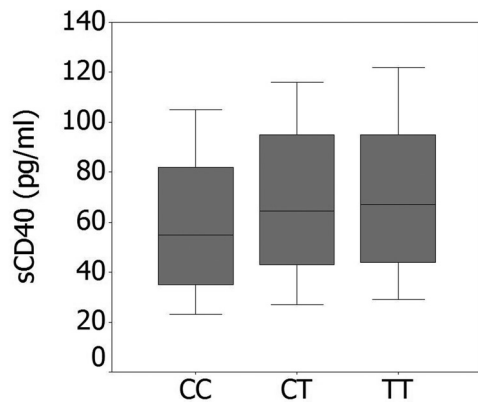
In this study, we analyzed the effect of four polymorphisms in CD40 gene on IS risk and their effect on CD40 expression in a Chinese population. Our results indicated that the rs1883832 polymorphism was associated with an increased risk of IS. The increased risk was also found in haplotype analysis. Moreover, we found that the rs1883832 polymorphism was associated with increased CD40 expression compared with the control group. The

statistical power of the study was calculated to be 82% to detect the association between rs1883832 polymorphism and IS risk in a sample size of 830 participants (380 in IS group and 450 in the control group) assuming an OR of 1.5 and α of 0.05 (NCSS PASS 11 software, version 11.0.7). Therefore, this finding indicates that the rs1883832 polymorphism may play a crucial role in the etiology of IS.

Atherosclerosis is a major cause of cardiovascular diseases, including stroke. It is accepted that interactions between CD40 and its ligand CD40L are closely involved in the pathogenesis of inflammation, atherosclerosis and thrombosis (Anand *et al.*, 2003, Antoniadis *et al.*, 2009). Upregulation of CD40 and/or CD40L have been detected in serum/plasma/cell surface of patients with IS by several studies, and were suggested to be useful predictors and biomarker for stroke (Cha *et al.*, 2003; Garlich *et al.*, 2003; Ishikawa *et al.*, 2005; Davi *et al.*, 2009; Zhang *et al.*, 2014; Li *et al.*, 2015; Wang *et al.*, 2015). However, the exact mechanism of how CD40 is up regulated, and how the upregulated CD40 affects IS patients have not been fully elucidated. Garlich *et al.* (2003) reported that patients with acute cerebral ischemia show upregulating CD40-CD40L system expression and may contribute to a proinflammatory, proatherogenic and prothrombotic milieu. Very recently, Wang *et al.* (2015) investigated the association between sCD40L and carotid plaque in patients with acute ischemic stroke and found that sCD40L levels were significantly associated with carotid plaque formation and instability, suggesting that sCD40L may be a useful predictor for plaque formation and instability in patients with acute ischemic stroke. In this study, we demonstrated that serum sCD40 levels were significantly elevated in patients with IS compared with the control group. Moreover, we found that the rs1883832 polymorphism was associated with an increased serum sCD40 levels in patients with IS compared

Table 4 - Haplotype analysis of the four polymorphism between ischemic stroke (IS) patients and control subjects.

Haplotypes of CD40 polymorphisms (rs1883832/rs13040307/rs752118/ rs3765459)	Controls 2n = 900 (%)	IS patients 2n = 760 (%)	OR (95% CI)	P value
TCCA	22 (2.4)	38 (5.0)	2.10 (1.23-3.58)	0.005
CCCG	275 (30.6)	206 (27.1)	0.84 (0.68-1.05)	0.123
TCCG	378 (42.0)	335 (44.1)	1.09 (0.90-1.32)	0.394
CTTA	190 (21.1)	148 (19.5)	0.90 (0.71-1.15)	0.409
CTCG	10 (1.1)	13 (1.7)	1.55 (0.68-3.55)	0.298
CTTG	16 (1.8)	11 (1.4)	0.81 (0.37-1.76)	0.596
CTCA	9 (1.0)	9 (1.2)	1.19 (0.47-3.00)	0.718

**Figure 2** - Association between the CD40 rs1883832 polymorphism and sCD40 levels in IS patients. sCD40 levels were significantly lower in IS patients with rs1883832CC genotype than in rs1883832TT and rs1883832CT genotypes. However, there were no significant differences in sCD40 levels between the rs1883832CT and TT genotypes.

with the control group. Genotypes carrying the rs1883832T allele were found to be associated with increased serum sCD40 levels compared with the rs1883832CC genotype in patients with IS. We speculated that the rs1883832 polymorphism may be associated with IS by up-regulating CD40 expression, which has been demonstrated to play a major role in atherosclerosis formation, plaque destabilization, thrombosis and the initiation of inflammatory response.

Several studies have been conducted to investigate the effect of the CD40 rs1883832 (-1C/T) polymorphism on atherosclerotic disease, however, the results were controversial. Yan *et al.* (2010) reported that the rs1883832CC genotype and the rs1883832C allele in the acute coronary syndrome (ACS) group were significantly higher than those in the control group, and the rs1883832C allele was found to associate with an increased risk of ACS when compared with the control group (OR = 1.88, 95%CI: 1.40–2.52). Similarly, a case-control study conducted by Wang *et al.* (2011) found that the frequency of rs1883832C allele in the ACS group was significantly higher than those in the control group (OR = 1.55, 95%CI: 1.15–2.11, $P < 0.05$), and individuals carrying the rs1883832CC genotype

showed a significantly increased CD40 and CD40L expression compared with the rs1883832CT and rs1883832TT genotypes carriers ($P < 0.05$). In contrast, Ma *et al.* (2013) reported that the frequencies of CT and TT/CT genotypes of rs1883832 polymorphism in the IS group were significantly higher than those in the control group, and the rs1883832T allele was found to be associated with a significantly increased risk of IS (OR = 1.27, 95% CI = 1.02–1.59). Moreover, they also found that the frequency of genotypes carrying the rs1883832T allele was higher in patients with history of stroke compared with those without (for TT: OR = 6.54, 95%CI = 1.66–25.83; for TT/CT: OR = 3.47, 95%CI = 1.03–11.67). Similarly, Zhang *et al.* (2013) reported that the frequencies of TT genotype and T allele of the CD40 rs1883832 polymorphism were significantly higher in patients with cerebral infarction than those in the control group ($P < 0.05$). The rs1883832TT genotype was suggested to be associated with an increased CD40 mRNA expression and CD40L plasma concentration ($P < 0.01$). Subsequently, a meta-analysis was conducted (Yun *et al.*, 2014) and found that rs1883832C allele was significantly associated with an increased risk of coronary artery disease, ACS and atherosclerosis, whereas the rs1883832C allele was demonstrated to be associated with a decreased risk of IS.

Consistent with the results of Ma *et al.* (2013) and Zhang *et al.* (2013), in this study we found that the frequencies of the T allele, the TT and CT genotypes of CD40 rs1883832 polymorphism predicted a significantly higher IS risk compared with the control group (Table 2). A possible explanation for the inconsistencies between the rs1883832 polymorphism and different types of atherosclerotic diseases is that this polymorphism might have different genetic effects on different diseases. Similar results can be seen in the studies of Chen *et al.* (2015b) and Yi *et al.* (2014), in which they found that the impacts of genetic polymorphisms may be different according to the types of cancer.

Until now, very little information has been reported on the association of CD40 rs752118 and rs3765459 polymorphisms and disease susceptibility. Moreover, no data has been reported on the association between the rs13040307 polymorphism and disease susceptibility. Pre-

vious studies conducted by Wu *et al.* (2016) and Wagner *et al.* (2015) have tried to assess the association of rs752118 polymorphism with systemic lupus erythematosus and multiple sclerosis, respectively, but failed to obtain a positive result. Regarding the rs3765459 polymorphism, data from Shuang *et al.* (2011) demonstrated that the rs3765459A allele was higher in patients with breast cancer compared with the control group (OR = 1.22, 95%CI: 1.03–1.45, $P = 0.025$). However, the study conducted by Burdon *et al.* (2006) found that the rs3765459 polymorphism was significantly associated with a decreased risk of coronary artery calcification in diabetic families. Furthermore, rs3765459 has also been assessed in relation to systemic lupus erythematosus (Wu *et al.*, 2016) and asthma (Hsieh *et al.*, 2009), but a significant association was not detected.

In this study, we investigated the association between rs13040307, rs752118 and rs3765459 polymorphisms and risk of IS, but found no significant association. The reason for these negative results remain unknown, but two possibilities should be considered. First, it may be because of genetic trait differences, as we know that genetic polymorphisms in human genes are distinct in different ethnicities, populations and geographic regions. Data from Table 3 can support this viewpoint, as it shows that the genotype distribution of rs1883832 polymorphism in our study were significantly different from HapMap-CEU, HapMap-HCB, HapMap-JPT, HapMap-YRI, HapMap-ASW, HapMap-GIH, HapMap-LWK, HapMap-MKK and HapMap-TSI populations, but similar with HapMap-CHB and HapMap-CHD populations. In addition, IS is a multi-factorial disease, thus, individual exposure to diverse environmental factors and genetic background may cause different results.

Several limitations should be considered in our study. First, a relatively small sample size of the study may have limited the statistical power of the analysis. Second, as our study population was all Chinese, the results cannot be directly applied to other ethnic groups. Third, our study was designed as a hospital-based and case-control study, and the control subjects were not selected from general population, thus, we cannot exclude the possibility of selection bias. Finally, the limitation of the assay kit may also contribute to the distinction of the results.

Conclusions

After a comprehensive comparison with other studies, we found that the rs1883832T allele but not the rs1883832C allele is associated with an increased risk of IS. Moreover, we demonstrated for the first time that the rs13040307, rs752118 and rs3765459 polymorphisms in CD40 gene were not associated with IS in the Chinese population. Further study with a larger sample size is needed to confirm our results, especially in different ethnic groups.

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