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*Original Article*

# Association of the *TTC39B* rs581080 SNP and serum lipid levels and the risk of coronary artery disease and ischemic stroke

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**Abstract:** The tetratricopeptide repeat domain protein 39B gene (*TTC39B*) single nucleotide polymorphism (SNP) of rs581080 has been associated with serum high-density lipoprotein cholesterol (HDL-C) levels. However, little is known about such association in the Chinese populations. The present study was performed to assess the association between the *TTC39B* rs581080 SNP and serum lipid levels and the risk of coronary artery disease (CAD) and ischemic stroke (IS) in the Guangxi Han population. Genotypes of the *TTC39B* rs581080 SNP in 1741 unrelated subjects (CAD, 578; IS, 537; and healthy controls; 624) were determined by the Snapshot Technology. The genotypic and allelic frequencies of the *TTC39B* rs581080 SNP were different between the CAD/IS patients and controls ($P < 0.01$ for all). The CG/GG genotypes and G allele were associated with an increased risk of CAD ($P = 0.001$ for CG/GG *vs*. CC, $P = 0.003$ for G *vs*. C) and IS ($P = 0.002$ for CG/GG *vs*. CC; $P = 0.004$ for G *vs*. C). The CG/GG genotypes in the healthy controls, but not in CAD or IS patients, were also associated with a decreased serum HDL-C concentration. These results suggest that the *TTC39B* rs581080 SNP is associated with the risk of CAD and IS in our study population. It is likely to increase the risk of CAD and IS by reducing serum HDL-C levels.

**Keywords:** The tetratricopeptide repeat domain protein 39B, single nucleotide polymorphism, coronary artery disease, ischemic stroke, lipids

## Introduction

Both coronary artery disease (CAD) and ischemic stroke (IS) have been recognized as widespread causes of death and disability for more than a century [1-3]. More than 700,000 people die from CAD each year in China [4]. The major pathophysiology mechanisms of the two diseases are atherosclerosis [5], which is a chronic process characterized by the deposition of excessive cholesterol in the arterial intima [6], and an ambitious inflammatory disorder. CAD and IS are complex and multifactorial disorders resulted from the joint effects of multiple environmental factors including gender, time to life, hyperlipidemia, hypertension, diabetes, cigarette smoking, and genetic background [7-10]. A large number of genes and loci associated with CAD [11] or IS [12] were reported in previous genome-wide association studies (GWASes). In addition, some genetic variants initially associated with CAD were detected to be related to IS soon afterwards [13, 14].

Resent GWASes have identified multiple novel loci associated with serum lipid levels in the European population [15, 16], including a single nucleotide polymorphism (SNP) of rs581080 in the tetratricopeptide repeat domain protein 39B gene (*TTC39B*). The relevant studies [15-17] have showed that the *TTC39B* rs581080 SNP can regulate serum high-density lipoprotein cholesterol (HDL-C) levels ($P = 3 \times 10^{-10}$ for genotype-HDL-C association and $P = 3 \times 10^{-8}$ for genotype-expression association). Knockdown of the mouse orthologue *TTC39B* via a viral vector, with 50% knockdown of transcript as determined by qRT-PCR, resulted in significantly higher plasma HDL-C levels at four days (19%) and seven days (14%) [15]. *TTC39B* also promotes the ubiquitination and degradation of the Liver X receptor (LXR), transcription factor

of which remains a target of interest because of its anti-atherogenic, cholesterol removal and anti-inflammatory activities [18]. Therefore, *TTC39B* may be appropriate candidate as gene to elevate HDL-C concentrations and affect the risk of CAD and IS. Studies showed that the *TTC39B* rs581080 SNP had been associated with serum HDL-C levels. However, little is known about such association in the Chinese populations. In addition, the association of the *TTC39B* rs581080 SNP with CAD and IS has never been detected before. Therefore, the purpose of the present study was to assess the association of the *TTC39B* rs581080 SNP and serum lipid levels and the risk of CAD and IS in the Chinese population.

## Materials and methods

### *Cases*

A total of 1,117 unrelated patients with CAD (*n* = 578) and IS (*n* = 539) were recruited from hospitalized patients in the First Affiliated Hospital, Guangxi Medical University from September 2009 to October 2011. CAD was defined as coronary angiographic findings (significant coronary stenosis (≥ 50%) in at least either one of the three main coronary arteries or their major branches (branch diameter ≥ 2 mm). Subjects with a history of hematologic, neoplastic, renal, liver, thyroid, autoimmune diseases and type I diabetes mellitus were excluded. Patients with CAD who had congenital heart disease, cardiomyopathy, valvular disease was also excluded. The group of patients with IS accepted strict neurological examination and brain magnetic resonance imaging (MRI) scan. IS was diagnosed according to the International Classification of Diseases (9$^{th}$ Revision). The patients with IS who had embolic brain infarction, transient ischemic attack, stroke caused by inflammatory diseases, cardioembolic stroke, as well as a past history of CAD were excluded from the study.

### *Controls*

A total of 624 healthy subjects matched by age, gender, and ethnic group (Han Chinese) were consecutively recruited as a control group from the First Affiliated Hospital, Guangxi Medical University during the same period when IS and CAD patients were recruited. The controls were free of IS, CAD and other diseases by questionnaires, history taking and clinical examination. All enrolled individuals were Han Chinese from Guangxi, the People's Republic of China. A standard questionnaire was used to ascertain the general information and medical history for all participants. The study protocol was approved by the Ethics Committee of the First Affiliated Hospital, Guangxi Medical University (No. Lunshen 2009-Guike-018; Jan. 7, 2009). Informed consent was obtained from all subjects after they received a full explanation of the study [19].

### *Genotyping and biochemical analysis*

Venous blood sample was obtained from all subjects after at least 12 hours of fasting. Genomic deoxyribonucleic acid (DNA) was extracted from peripheral blood leukocytes using the phenol-chloroform method. Genotyping of the *TTC39B* rs581080 SNP was performed by the Snapshot technology platform in the Center for Human Genetics Research, Shanghai Genesky Bio-Tech Co. Ltd., China. The restirction enzyme for the *TTC39B* rs581080 SNP was SAP (Promega) and Exonucleasel (Epicentre). The sense and antisense primers were 5'-TGCCTTCTCTAAGGCACCTACCA-3' and 5'-TGCCAAATTTACTTTTGTCCCTCAC-3', respectively. The levels of serum total cholesterol (TC), triglyceride (TG), HDL-C, and low-density lipoprotein cholesterol (LDL-C) in samples were determined by enzymatic methods with commercially available kits. Serum apolipoprotein (Apo) A1 and ApoB levels were detected by the immunoturbidimetric immunoassay.

### *Diagnostic criteria*

The normal values of serum TC, TG, HDL-C, LDL-C, ApoA1, ApoB levels, and the ratio of ApoA1 to ApoB in our Clinical Science Experiment Center were 3.10-5.17, 0.56-1.70, 0.91-1.81, 2.70-3.20 mmol/L, 1.00-1.78, 0.63-1.14 g/L, and 1.00-2.50; respectively [20, 21]. The individuals with TC > 5.17 mmol/L, and/or TG > 1.70 mmol/L were defined as hyperlipidemic. Hypertension was diagnosed according to the criteria of 1999 World Health Organization International Society of Hypertension Guidelines for the management of hypertension [22]. Uncontrolled hypertension was defined as a systolic blood pressure of 140 mmHg or higher and/or a diastolic blood pressure of 90 mmHg or higher. Normal weight, overweight and obesity were defined as a body mass index (BMI) < 24, 24-28, and > 28 kg/m$^2$; respectively [23].

**Table 1.** General characteristics and serum lipid levels between the controls and patients

| Characteristic | Control (n = 624) | CAD (n = 578) | IS (n = 539) | $P_{CAD}$ | $P_{IS}$ |
|---|---|---|---|---|---|
| Male/female | 455/169 | 418/160 | 409/130 | 0.816 | 0.249 |
| Age, years | 61.63±11.99 | 61.87±10.50 | 62.60±12.35 | 0.718 | 0.178 |
| Weight, kg | 54.50±9.04 | 64.45±10.56 | 62.98±11.21 | 0.000 | 0.000 |
| Body mass index, kg/m$^2$ | 22.65±3.20 | 23.80±3.17 | 23.42±3.51 | 0.000 | 0.000 |
| Systolic blood pressure, mmHg | 127.46±19.73 | 132.97±23.32 | 147.97±21.89 | 0.000 | 0.000 |
| Diastolic blood pressure, mmHg | 81.26±13.14 | 79.17±14.16 | 83.82±12.95 | 0.008 | 0.001 |
| Pulse pressure, mmHg | 48.00±14.05 | 53.59±17.77 | 63.81±17.77 | 0.000 | 0.000 |
| Cigarette smoking, n (%) | 229 (36.7) | 263 (45.5) | 250 (46.4) | 0.002 | 0.001 |
| Alcohol consumption, n (%) | 208 (33.3) | 115 (19.9) | 223 (41.4) | 0.000 | 0.005 |
| Total cholesterol, mmol/L | 4.89±1.06 | 4.53±1.19 | 4.52±1.15 | 0.000 | 0.000 |
| Triglyceride, mmol/L | 1.00 (0.67) | 1.36 (0.96) | 1.36 (0.93) | 0.000 | 0.000 |
| HDL-C, mmol/L | 1.90±0.49 | 1.14±0.34 | 1.23±0.40 | 0.000 | 0.000 |
| LDL-C, mmol/L | 2.73±0.78 | 2.71±1.00 | 2.68±0.90 | 0.737 | 0.342 |
| Apolipoprotein (Apo) A1, g/L | 1.41±0.27 | 1.04±0.52 | 1.02±0.22 | 0.000 | 0.000 |
| ApoB, g/L | 0.90±0.21 | 0.91±0.27 | 0.89±0.24 | 0.779 | 0.442 |
| ApoA1/ApoB | 1.64±0.52 | 1.39±2.48 | 1.25±0.59 | 0.011 | 0.000 |
| Hyperlipidemia | 217 (34.8) | 320 (55.4) | 386 (71.6) | 0.000 | 0.000 |

CAD, coronary artery disease; IS, ischemic stroke; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol. $P_{CAD}$, CAD *vs*. controls; $P_{IS}$, IS *vs*. controls. The value of triglyceride was presented as median (interquartile range), the difference between CAD/IS patients and controls was determined by the Wilcoxon-Mann-Whitney test. The remaining characteristics between patients and controls were tested by the Student's unpaired *t*-test.

*Statistical analyses*

The statistical analyses were carried out using the statistical software package SPSS 22.0 (SPSS Inc., Chicago, Illinois). The standard goodness-of-fit test was used to test the Hardy-Weinberg equilibrium. A chi-square analysis was used to evaluate the difference in genotype distribution between the groups. The general characteristics between patients and controls were tested by the Student's unpaired *t*-test. The association of genotypes and serum lipid parameters was tested by analysis of covariance (ANCOVA). Sex, age, BMI, blood pressure, alcohol consumption, cigarette smoking were adjusted for the statistical analysis. Odds ratio (OR) and 95% confidence interval (CI) were calculated using unconditional logistic regression. A two-tailed *P* value less than 0.05 was considered statistically significant.

## Results

*General characteristics and serum lipid levels*

The baseline characteristics of the patients with CAD or IS and the controls are shown in **Table 1**. The average age, male to female ratio, serum LDL-C and ApoB levels were similar between the controls and CAD patients and between the controls and IS patients ($P > 0.05$). As compared with control group, more patients in CAD or IS groups had significant higher weight, pulse pressure, BMI, systolic blood pressure, the percentages of subjects who smoked cigarettes and TG levels; and lower levels of TC, HDL-C, ApoA1 and the ratio of ApoA1 to ApoB ($P < 0.05$). In addition, the CAD patients had lower levels of diastolic blood pressure, the percentages of subjects who consumed alcohol than the controls ($P < 0.05$). In contrast, the IS patients had higher levels of diastolic blood pressure, the percentages of subjects who consumed alcohol than the controls ($P < 0.05$).

*Genotypic and allelic frequencies*

The frequency of the C and G alleles was 89.2% and 10.8% in the controls, 84.9% and 15.1% in the CAD patients ($P = 0.002$ *vs*. controls), and 85.1% and 14.9% in the IS patients ($P = 0.004$ *vs*. controls); respectively (**Table 2**). The frequency of the CC, CG and GG genotypes was 78.5%, 19.6% and 1.9% in the controls, 70.1%, 28.5% and 1.4% in the CAD patients ($P = 0.001$ *vs*. controls), and 70.5%, 28.0% and 1.5% in the

**Table 2.** Genotypic and allelic frequencies of the *TTC39B* rs581080 SNP and the risk of CAD and IS [n (%)]

| Genotype/ allele | Control (n = 624) | CAD (n = 578) | IS (n = 539) | OR (95% CI)$_{CAD}$ | $P_{CAD}$ | OR (95% CI)$_{IS}$ | $P_{IS}$ |
|---|---|---|---|---|---|---|---|
| CC | 490 (78.5) | 405 (70.1) | 380 (70.5) | 1 | | 1 | |
| CG | 122 (19.6) | 165 (28.5) | 151 (28.0) | 1.636 (1.251-2.140) | 0.000 | 1.596 (1.214-2.098) | 0.001 |
| GG | 12 (1.9) | 8 (1.4) | 8 (1.5) | 0.807 (0.327-1.882) | 0.641 | 0.860 (0.348-2.124) | 0.743 |
| $X^2$ | | 13.573 | 11.638 | | | | |
| *P* | | 0.001 | 0.003 | | | | |
| CC | 490 (78.5) | 405 (70.1) | 380 (70.5) | 1 | | 1 | |
| CG/GG | 134 (21.5) | 173 (29.9) | 159 (29.5) | 1.562 (1.203-2.028) | 0.001 | 1.530 (1.173-1.996) | 0.002 |
| $X^2$ | | 11.283 | 9.882 | | | | |
| *P* | | 0.001 | 0.002 | | | | |
| C | 1102 (89.2) | 975 (84.9) | 911 (85.1) | 1 | | 1 | |
| G | 134 (10.8) | 173 (15.1) | 159 (14.9) | 1.449 (1.138-1.846) | 0.003 | 1.435 (1.122-1.836) | 0.004 |
| $X^2$ | | 9.485 | 8.349 | | | | |
| *P* | | 0.002 | 0.004 | | | | |
| $P_{HWE}$ | 0.180 | 0.052 | 0.104 | | | | |

HWE, Hardy-Weinberg equilibrium; CAD, coronary artery disease; IS, ischemic stroke; OR, odds ratio; CI, confidence interval. OR and 95% CI were obtained from unconditional Logistic regression model after adjusted for age, gender, body mass index, smoking status, alcohol consumption.

IS patients (*P* = 0.003 *vs*. controls); respectively. The genotypic distribution was concordant with Hardy-Weinberg equilibrium in both experimental groups (*P* = 0.052 for CAD and *P* = 0.104 for IS) and control group (*P* = 0.180).

*TTC39B rs581080 SNP and the risk of CAD and IS*

The G allele was associated with a raised risk of CAD (OR = 1.449, 95% CI = 1.138-1.846, *P* = 0.003) and IS (OR = 1.435, 95% CI = 1.122-1.836, *P* = 0.004). The CG and CG+GG genotypes were also associated with a raised risk of CAD (OR = 1.636, 95% CI = 1.252-2.140, *P* = 0.000 for CG *vs*. CC and OR = 1.562, 95% CI = 1.203-2.208, *P* = 0.001 for CG+GG *vs*. CC) and IS (OR = 1.596, 95% CI = 1.214-2.098, *P* = 0.001 for CG *vs*. CC and OR = 1.530, 95% C I = 1.173-1.996, *P* = 0.002 for CG+GG *vs*. CC; **Table 2**). Stratified analysis showed an increased risk of CAD in subjects with the CG/GG genotypes, mainly in those who belonged to one of the following subgroups: males (adjusted OR = 1.690, 95% CI = 1.251-2.285, *P* = 0.001), age > 60 years (adjusted OR = 1.686, 95% CI = 1.185-2.399, *P* = 0.004), BMI > 24 kg/m$^2$ (adjusted OR = 1.669, 95% CI = 1.093-2.550, *P* = 0.018), hypertension (adjusted OR = 2.615, 95% CI = 1.694-4.038, *P* < 0.001), smokers (adjusted OR = 1.626, 95% CI = 1.103-2.397, *P* = 0.014) and nodrinking (adjusted OR = 1.732, 95% CI = 1.273-2.358, *P* < 0.001). There was an increased risk of IS in subjects with the CG/GG genotypes, mainly in those who belonged to one of the following subgroups: males (adjusted OR = 1.615, 95% CI = 1.192-2.188, *P* = 0.002), age > 60 years (adjusted OR = 1.715, 95% CI = 1.202-2.448, *P* = 0.003), BMI > 24 kg/m$^2$ (adjusted OR = 1.772, 95% CI = 1.139-2.757, *P* = 0.011), hypertension (adjusted OR = 2.209, 95% CI = 1.460-3.342, *P* < 0.001) and non-drinkers (adjusted OR = 1.687, 95% CI = 1.203-2.358, *P* = 0.002). The rs581080 SNP was also significantly interacted with hypertension on the risk of CAD and IS (*P* < 0.01 for each; **Table 3**).

*Related risk factors for CAD and IS*

Multivariate logistic analysis showed that the incidence of CAD and IS was positively correlated with smoking, BMI, rs581080CG/GG and hypertension and negatively correlated with the alcohol consumption (**Table 4**).

*Genotypes and serum lipid levels in the controls*

As shown in **Table 5**, serum HDL-C levels were different between the CC and CG/GG genotypes (*P* = 0.009), the G allele carriers (CG/GG

**Table 3.** The *TTC39B* rs581080 SNPand the risk of coronary heart disease and ischemic stroke according to gender, age, BMI, smoking, drinking and hypertension

| Factor | OR (95% CI)$_{CC}$ | OR (95% CI)$_{CG/GG}$ | *P* | $P_I$ |
|---|---|---|---|---|
| CAD | | | | |
| Sex/male | 1 | 1.690 (1.251-2.285) | 0.001 | |
| Sex/female | 1 | 1.240 (0.731-2.104) | 0.426 | 0.921 |
| Age/≤ 60 years | 1 | 1.426 (0.962-2.114) | 0.077 | |
| Age > 60 years | 1 | 1.686 (1.185-2.399) | 0.004 | 0.941 |
| BMI/≤ 24 kg/m$^2$ | 1 | 1.406 (0.996-1.995) | 0.053 | |
| BMI/> 24 kg/m$^2$ | 1 | 1.669 (1.093-2.550) | 0.018 | 0.517 |
| Smoking/No | 1 | 1.395 (0.973-1.999) | 0.070 | |
| Smoking/Yes | 1 | 1.626 (1.103-2.397) | 0.014 | 0.562 |
| Drinking/No | 1 | 1.732 (1.273-2.358) | 0.000 | |
| Drinking/Yes | 1 | 1.124 (1.537-3.842) | 0.666 | 0.742 |
| Hypertension/No | 1 | 1.102 (0.783-1.550) | 0.577 | |
| Hypertension/Yes | 1 | 2.615 (1.694-4.038) | 0.000 | 0.005 |
| IS | | | | |
| Sex/male | 1 | 1.615 (1.192-2.188) | 0.002 | |
| Sex/female | 1 | 1.236 (0.708-2.160) | 0.456 | 0.612 |
| Age/≤ 60 years | 1 | 1.307 (0.869-1.966) | 0.199 | |
| Age > 60 years | 1 | 1.715 (1.202-2.448) | 0.003 | 0.372 |
| BMI/≤ 24 kg/m$^2$ | 1 | 1.388 (0.952-1.879) | 0.093 | |
| BMI/> 24 kg/m$^2$ | 1 | 1.772 (1.139-2.757) | 0.011 | 0.426 |
| Smoking/No | 1 | 1.462 (0.993-2.137) | 0.052 | |
| Smoking/Yes | 1 | 1.444 (0.986-2.454) | 0.059 | 0.664 |
| Drinking/No | 1 | 1.687 (1.203-2.358) | 0.002 | |
| Drinking/Yes | 1 | 1.300 (1.840-2.010) | 0.239 | 0.327 |
| Hypertension/No | 1 | 1.059 (0.688-1.632) | 0.793 | |
| Hypertension/Yes | 1 | 2.209 (1.460-3.342) | 0.000 | 0.006 |

OR, odds ratio; CI, confidence interval; CAD, coronary artery disease; IS, ischemic stroke. OR and 95% CI were obtained from unconditional Logistic regression model after adjusted for age, gender, body mass index, smoking status, alcohol consumption, hypertension. $P_I$, the *P* value of interaction between the SNP and risk factor.

**Table 4.** The relative risk factors for CAD and IS

| Factor | OR (95% CI)$_{CAD}$ | $P_{CAD}$ | OR (95% CI)$_{IS}$ | $P_{IS}$ |
|---|---|---|---|---|
| Nonsmoking | 1 | | 1 | |
| Smoking | 2.043 (1.580-2.641) | 0.000 | 2.121 (1.593-2.824) | 0.000 |
| Nondrinking | 1 | | 1 | |
| Drinking | 0.373 (0.280-0.500) | 0.000 | 0.997 (0.744-1.336) | 0.983 |
| BMI < 24 kg/m$^2$ | 1 | | 1 | |
| BMI ≥ 24 kg/m$^2$ | 2.224 (1.731-2.856) | 0.000 | 1.460 (1.114-1.192) | 0.006 |
| Rs581080 CC | 1 | | 11 | |
| Rs581080 CG/GG | 1.401 (1.063-1.846) | 0.017 | 1.389 (1.036-1.864) | 0.028 |
| Normal blood lipids | 1 | | 1 | |
| Hyperlipidemia | 1.357 (1.058-1.740) | 0.016 | 4.641 (3.777-6.021) | 0.000 |

OR, odds ratio; CI, confidence interval; CAD, coronary artery disease; IS, ischemic stroke. OR and 95% CI were obtained from unconditional Logistic regression model after adjusted for age, gender, body mass index, smoking status, alcohol consumption, hypertension.

genotypes) had lower HDL-C levels than the G allele non-carriers (CC genotype). No significant difference in the remaining serum lipid parameters was observed between the CC and CG/GG genotypes.

## Discussion

Despite therapeutic advances that control many risk factors, such as LDL-C, to levels lower than previously possible, death from cardiovascular disease continues to increase worldwide. Statins have reduced the risk of complications and death from cardiovascular causes by only approximately one third, leaving the remaining two thirds of patients unprotected [24]. Accordingly, the quest for pharmacologic agents that target other steps in atherogenesis has intensified in recent years. The *TTC39B* has been considered to be a promising drug target for therapeutic intervention against hyperlipidemia and atherosclerosis. However, whether the *TTC39B* inhibition will serve as an effective drug target for controlling atherosclerosis is under debate. Therefore, the genetic evidences for an association between the *TTC39B* SNP and atherosclerosis in humans still needs to been clarified.

Teslovich et al. [15] performed a GWAS of plasma lipids in more than 100,000 individuals of

**Table 5.** Association between the *TTC39B* rs581080 SNP and serum lipid levels in controls

| Genotype | n | TC (mmol/L) | TG (mmol/L) | HDL-C (mmol/L) | LDL-C (mmol/L) | ApoA1 (g/L) | ApoB (g/L) | ApoA1/ ApoB |
|---|---|---|---|---|---|---|---|---|
| CC | 490 | 4.88±0.99 | 1.00 (0.68) | 1.96±0.48 | 2.72±0.78 | 1.44±0.28 | 0.90±0.21 | 1.65±0.55 |
| CG/GG | 134 | 4.92±1.26 | 0.98 (0.63) | 1.85±0.50 | 2.76±0.77 | 1.41±0.24 | 0.92±0.22 | 1.63±0.42 |
| *F* | | 0.317 | 0.398 | 6.942 | 0.690 | 3.078 | 0.778 | 0.653 |
| *P* | | 0.573 | 0.690 | 0.009 | 0.406 | 0.080 | 0.378 | 0.419 |

TC, total cholesterol; TG, triglyceride; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; ApoA1, apolipoprotein A1; ApoB, apolipoprotein B. The value of triglyceride was presented as median (interquartile range), and the difference between the two genotype subgroups was determined by the Wilcoxon-Mann-Whitney test. The association of genotypes and the remaining serum lipid parameters was tested by analysis of covariance (ANCOVA).

European ancestry and identified rs581080, a SNP in the *TTC39B* on chromosome 9q22, as having an effect on HDL-C concentrations with an effect size of -0.65 mg per deciliter and a *P* value of $3 \times 10^{-12}$. In liver, the allele associated with decreased expression and correlated with increased HDL-C. Consistent with aforementioned discovery, knockdown of the mouse orthologue *TTC39B via* a viral vector, with 50% knockdown of transcript as determined by q RT-PCR, resulted in significantly higher plasma HDL-C levels at four days (19%) and seven days (14%). In the present study, we found that the rs581080G allele carriers had lower HDL-C levels in heathy controls. These data suggest that the *TTC39B* variants may be associated with increased TTC39B protein expression and influence cellular cholesterol efflux, thereby playing an important role in atherosclerosis progression. Thus, we infer that the *TTC39B* rs581080 SNP is associated with the risk of CAD and IS. As far as we know, the association between the *TTC39B* rs581080 SNP and the risk of atherosclerosis has not been reported previously.

The prevalence of the *TTC39B* rs581080G allele may be different in diverse racial/ethnic groups. The information in the International HapMap Project's database showed that the rs581080G allele frequency was 19.1% in Europeans, 6.6% in Han Chinese in Beijing (HCB), 9.1% in Japanese, and 60.0% in Sub-Saharan African. In the present study, we showed that the *TTC39B* rs581080G allele frequency was higher in our study subjects (control, 10.8%; CAD, 15.1%; and IS, 14.9%) than in HCB (6.6%). The reason for these differences is not well known, a reasonable explanation is different genetic background between the HCB and Han Chinese in Guangxi. These inconsistent results, however, also suggest that the prevalence of the *TTC39B* rs581080 variation may have a racial/ethnic specificity. The prevalence of the rs581080G allele was higher in Europeans than in Chinese. All of these findings would be a reasonable explanation for the distinct prevalence of CAD between European and Chinese.

In the present study, we showed that the frequencies of the CG/GG genotypes and G allele were associated with an increased risk of CAD and IS. Multivariate analysis showed that known risk factors, such as hypertension, diabetes and hyperlipidemia, were independently associated with CAD and IS. Additionally, the *TTC39B* rs581080 CG/GG genotypes were also associated with an increased risk of CAD and IS after adjusting for potential confounding factors. In the stratified analysis, an increased risk of CAD and IS in subjects with the CG/GG genotype was mainly observed in males, aged > 60 years, non-drinkers, BMI > 24 kg/m$^2$ and those with hypertension. In addition, an increased risk of CAD in subjects with the CG/GG genotype was also noted in smokers. Significant interaction between the SNP and hypertension on the risk of CAD and IS was also observed, suggesting gene-environment interactions contributed to the risk of CAD and IS. A previous study showed that the *TTC39B*-knockout mice displayed increased HDL-C levels and increased LXR protein [18]. It is well known that HDL-C is able to extract excess cholesterol from peripheral tissues and transfer this cholesterol to the liver for biliary excretion and is a protective factor for CAD. In addition, LXR activity is critical for physiologic lipid metabolism and transport. Elimination of LXR activity in bone marrow-derived cells mimicked many aspects of Tangier disease, a human high density lipoprotein deficiency, including aberrant regulation of cholesterol transporter expression, lipid accumulation in macrophages,

splenomegaly, and increased atherosclerosis. Another study proved that the *TTC39B* rs581080 allele associated with decreased expression correlated with increased HDL-C in liver. In the present study, we showed that the rs581080G allele carriers had lower serum HDL-C levels than the G allele non-carriers. These data infer that the rs581080 mutation may increase the TTC39B expression resulting in serum HDL-C decrease, LXR activity increase, and add the risk of atherosclerotic disease.

As multifactorial diseases, both CAD and IS shared common risk factors, such as hypertension, dyslipidemia, metabolic syndrome [25], and the common pathophysiologic mechanisms atherosclerosis [24]. Atherosclerosis is a very complex disease process with a number of important cellular contributors, including endothelial cells, smooth muscle cells, and immune cells (monocyte and T cells) [26-28]. Traditional risk factors result in atherosclerosis by disrupting the function of these cells [28, 29]. Many hypotheses have been proposed to explain the underlying mechanisms of the initiation, progression and rupture of atherosclerotic plaque. The major points of these hypotheses include the following: (i) lipoprotein retention; (ii) endothelial dysfunction; (iii) immune and inflammation response of the artery; (iv) vascular smooth muscle cell proliferation; (v) lipid absorption by macrophages and vascular smooth muscle cells and the formation of foam cells; and (vi) platelet activation and thrombosis [30, 31]. Epidemiological studies in families and twins have showed genetic factor to the risk of CAD and IS in humans, the gene variants involved in these pathways of atherosclerosis could have an effect on the risk of cardiovascular disease. More than 300 candidate genes for CAD has been found in the CAD gene database [30]. While a single gene or gene region only explains a small part of the risk of atherosclerotic disease, more large association studies, including more target genes, are needed to assess the risk of cardiovascular disease.

Several potential limitations cannot be ignored. First, compared to many GWASes and replication studies, our sample numbers were relatively small. With these situations, larger sample numbers are needed to determine the consequences in future studies. Significant distinctions from demography were observed between the control and patient groups. For the sake of statistical analysis accuracy, we adjusted for several environmental exposures, including time to life, sex, BMI, cigarette smoking, and alcohol drinking, but the potential influence of these factors on serum lipid concentrations and the risk of CAD and IS could not be completely eliminated. In addition, because many subjects were taking lipid-lowering drugs, it was not proper to analyze the association of the SNP and serum lipid levels in the CAD and IS groups.

## Conclusions

The results of the present study showed that there was significant association between the *TTC39B* rs581080 SNP and the risk of CAD and IS. The *TTC39B* rs581080G allele carriers conferred a decreased serum HDL-C levels and an increased risk of CAD and IS. The CG/GG genotypes interacted with hypertension to contribute the risk of CAD and IS. Our findings should be important to help clarifying the role of the *TTC39B* SNP in atherosclerosis.

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## Disclosure of conflict of interest

None.

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