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Original Article

TTC39B rs1407977 SNP is associated with the risk of coronary heart disease and ischemic stroke

Hui Li[1], Rui-Xing Yin[2], Jian-Hua Huang[2], Feng Huang[2], Wu-Xian Chen[2], Xiao-Li Cao[3]

[1]Clinical Laboratory of The Affiliated Cancer Hospital, Guangxi Medical University, Nanning, Guangxi, People's Republic of China; [2]Department of Cardiology, Institute of Cardiovascular Diseases, The First Affiliated Hospital, Guangxi Medical University, Nanning, Guangxi, People's Republic of China; [3]Department of Neurology, The First Affiliated Hospital, Guangxi Medical University, Nanning, Guangxi, People's Republic of China

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Abstract: Previous genome-wide association studies have showed that several tetratricopeptide repeat domain protein 39B gene (*TTC39B*) single nucleotide polymorphisms (rs581080 and rs471364) were associated with serum high-density lipoprotein cholesterol levels among populations of European ancestry, but the results are inconsistent. Furthermore, little is known about the association between *TTC39B* SNPs and the susceptibility to coronary heart disease (CHD) and ischemic stroke (IS). Therefore, this study was undertaken to detect the association between the *TTC39B* rs1407977 SNP and serum lipid levels and the risk of CHD and IS in a Southern Chinese Han population. Genotyping of the SNP in 1741 unrelated subjects (healthy controls, 624; CHD patients, 578 and IS patients, 539) was performed by the Snapshot Technology. The genotypic and allelic frequencies of the SNP were different between the control subjects and CHD patients, or between the control subjects and IS patients ($P \leq 0.001$). The T allele frequency was higher in CHD (16.2%) and IS (15.0%) patients than in controls (9.8%). The T allele carriers had higher risk of CHD (OR = 1.728, 95% CI = 1.290-2.316, $P < 0.001$) and IS (OR = 1.518, 95% CI = 1.182-2.116, $P = 0.002$) than the T allele non-carriers after controlling for potential confounders. No significant association was observed between the *TTC39B* rs1407977 SNP and all seven serum lipid traits. These results suggest that the *TTC39B* rs1407977 SNP is associated with the risk of CHD and IS in our study population and does not depend on serum lipid levels.

Keywords: Tetratricopeptide repeat domain protein 39B, single nucleotide polymorphism, coronary heart disease, ischemic stroke, risk factors

Introduction

Both coronary heart disease (CHD) and ischemic stroke (IS) remain leading causes of morbidity and mortality worldwide [1-3]. More than 2.5 and 1 million people are affected by stroke and heart attack, respectively, leading to more than 2 million deaths each year in China [4]. CHD and IS are multifactorial diseases in which multiple genes and environmental factors are involved. The pathologic basis of both diseases is atherosclerosis, a multifactorial disease consisting of a multitude of pathogenic developments, including foam cell formation, cell death, extracellular lipid accumulation, chronic inflammation, and smooth muscle cell proliferation. CHD and IS may share common pathogenesis, as well as many risk factors such as sex, age, dyslipidemia, hypertension, diabetes, cigarette smoking, and family history [4-6]. Twin and family studies have indicated that heritable factors account for 30%-60% of the interindividual variation in the risk of CHD [7, 8] and IS [9]. Some single nucleotide polymorphisms (SNPs) originally identified as influencing the risk of CHD were also subsequently associated with IS [10, 11].

The tetratricopeptide repeat domain 39B gene (*TTC39B*), also known as C9orf52, a high density lipoprotein (HDL) gene discovered in human genome wide association studies (GWASes) [12, 13] is located on Chromosome 9p22.3. It has ubiquitous expression in kidney, gall bladder and other tissues [14]. There are 27 exons in this gene. Several previous studies have showed that the *TTC39B* SNPs were associated with endometriosis (rs519664T, $P = 4.8 \times$

10^{-10}, OR = 1.29) [15], progression-free survival of epithelial ovarian cancer (rs7874043, best $P = 7 \times 10^{-5}$, HR = 1.90 and rs72700653) [16], and gallbladder disease (rs686030, $P = 6.95 \times 10^{-7}$, β = 0.271) [17]. In a recent study, we also found that the *TTC39B* rs581080 SNP was associated with the risk of CHD and IS in a Southern Chinese Han population, and this finding may be induced by reducing serum high-density lipoprotein cholesterol (HDL-C) levels [18]. However, the results of previous GWASes between the *TTC39B* SNPs and serum lipid levels are inconsistent. For example, despite sufficient power, Dumitrescu et al. [19] were unable to replicate the previous association of *TTC39B* rs471364 with HDL-C ($P = 3 \times 10^{-10}$) by Kathiresan et al. [12]. Therefore, the purpose of the present study was to assess the association of a new *TTC39B* rs1407977 SNP and serum lipid levels and the risk of CHD and IS in the Chinese population.

Materials and methods

Patients

A total of 1,117 unrelated patients (CHD, *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. The diagnosis of CHD was based on clinical manifestation, electrocardiographic change, cardiac marker increase, and coronary angiography. CHD 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) [20]. CHD patients who had congenital heart disease, cardiomyopathy, or valvular disease were excluded. The diagnosis and classification of IS was ascertained in accordance with the Trial of Org 10172 in Acute Stroke Treatment (TOAST) criteria [21] after strict neurological examination, computed tomography, or magnetic resonance imaging (MRI). Subjects with a history of hematologic or brain MRI revealing cerebral hemorrhage, cardioembolic or unspecified stroke, neoplastic or intracranial space-occupying lesion, infection, and other types of intracranial lesions, renal, liver, thyroid, autoimmune diseases, and type 1 diabetes were excluded. The IS patients who had a past history of CHD and CHD patients who had a past history of IS 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 healthy adults who underwent periodical medical check-up at the Physical Examination Center of the First Affiliated Hospital, Guangxi Medical University during the same period when CHD and IS patients were recruited. They were free of CHD and IS at time of history taking, and underwent clinical, biochemical, and image examinations such as 64-slice computed tomographic coronary angiography. 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), and conducted according to the Declaration of Helsinki. Informed consent was obtained from all subjects after they received a full explanation of the study.

Genotyping and biochemical analysis

A venous blood sample of 5 mL 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. Genotypes of the *TTC39B* rs1407977 SNP were determined by the Snapshot technology platform in the Center for Human Genetics Research, Shanghai Genesky Bio-Tech Co. Ltd., China [22-31]. The restriction enzyme was SAP (Promega) and Exonuclease I (Epicentre). The sense and antisense primers were 5'-GCATCCAGTTTTGTTGGAAAACATT-3' and 5'-TCTGGACGATCAACTTTGTGTACTTGA-3', respectively. Serum total cholesterol (TC), triglyceride (TG), HDL-C, and low-density lipoprotein cholesterol (LDL-C) in samples were assayed using common commercially available biochemical kits. Serum apolipoprotein (Apo) A1 and ApoB levels were determined by the immunoturbidimetric immunoassay [22-31]. TC, TG, ApoA1 and ApoB assay kits were purchased from RANDOX Laboratories Ltd., Antrim, UK; and HDL-C and LDL-C from Daiichi Pure Chemicals Co., Ltd., Tokyo, Japan.

Diagnostic criteria

The normal values of serum TC, TG, HDL-C, LDL-C, ApoA1, ApoB levels, and the ratio of ApoA1 to

Table 1. Comparison of general characteristics and serum lipid profiles between CHD/IS patients and control subjects

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

CHD, coronary heart disease; IS, ischemic stroke; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol. The value of triglycerides was presented as median (interquartile range), the difference between CHD/IS patients and control group was determined by the Wilcoxon-Mann-Whitney test. The remaining parameters between the CHD/IS patients and control subjects were tested by the Student's unpaired *t*-test. *P < 0.05 and **P < 0.01 in comparison with the control group.

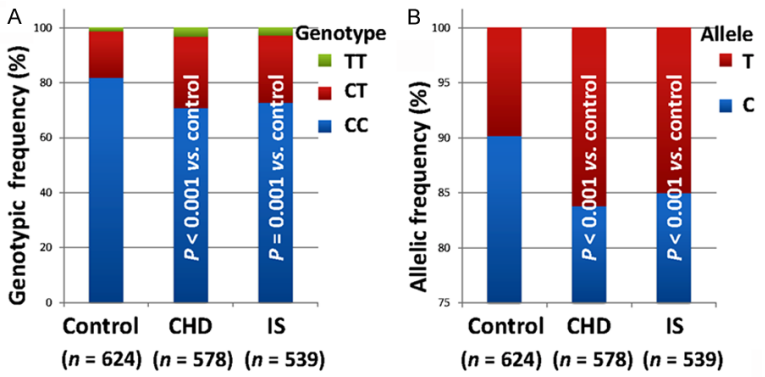

Figure 1. Genotypic and allelic frequencies of the *TTC39B* rs1407977 SNP in the controls, coronary heart disease (CHD) and ischemic stroke (IS) patients. The genotypic and allelic distribution in the three groups was analyzed by chi-square test. A. Genotypic frequency; B. Allelic frequency.

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 [22-31]. 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 [22-31]. Normal weight, overweight and obesity were defined as a body mass index (BMI) < 24, 24-28, and > 28 kg/m^2, respectively [22-31].

Statistical analyses

The statistical analyses were carried out using the statistical software package SPSS 22.0 (SPSS Inc., Chicago, Illinois). Continuous variables were expressed as mean ± SD, and percentage was calculated for categorical variables. 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

Table 2. Association between the *TTC39B* rs1407977 SNP and the risk of CHD and IS in different genetic models

Genetic model	Genotype	OR (95% CI)$_{CHD}$	P_{CHD}	OR (95% CI)$_{IS}$	P_{IS}
Codominant	CC	1		1	
	CT	1.776 (1.314-2.401)	0.000	1.660 (1.231-2.237)	0.000
	TT	1.193 (0.440-3.229)	0.729	0.682 (0.216-2.153)	0.514
Dominant	TT	1		1	
	CC+CT	0.941 (0.348-2.543)	0.904	1.614 (0.513-5.086)	0.413
Recessive	CC	1		1	
	CT+TT	1.728 (1.290-2.316)	0.000	1.518 (1.182-2.116)	0.002

CHD, coronary heart 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, hypertension.

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, and cigarette smoking were adjusted for statistical analysis. Odds ratio (OR) and 95% confidence interval (CI) were calculated using unconditional logistic regression. A two-tailed *P* value < 0.05 was considered significant.

Results

General characteristics of the subjects

Table 1 shows the general characteristics and serum lipid parameters of the participants. The mean values of body weight, BMI, SBP, pulse pressure, TG, the percentages of cigarette smoking, and the prevalence of hyperlipidemia were higher in the CHD patients than in control subjects, whereas the levels of DBP, TC, HDL-C, ApoA1, percentages of alcohol consumption, and the ratio of ApoA1 to ApoB were lower in the CHD patients than in control subjects (*P* < 0.05-0.01). There was no significant difference in the average age, sex ratio, serum LDL-C and ApoB levels between the CHD patients and control subjects.

The average levels of body weight, BMI, SBP, DBP, pulse pressure, TG, the percentages of cigarette smoking and alcohol consumption, and the prevalence of hyperlipidemia were higher in IS patients than in control subjects, whereas the levels of TC, HDL-C, ApoA1, and the ratio of ApoA1 to ApoB were lower in IS patients than in control subjects (*P* < 0.01 for all). There was no significant difference in the mean age, male to female ratio, serum LDL-C, and ApoB levels between IS patients and control subjects.

Genotypic and allelic frequencies of the participants

The genotypic and allelic distribution of the *TTC39B* rs1407977 SNP in the patients and controls is shown in **Figure 1**. The genotypic distribution was concordant with Hardy-Weinberg equilibrium in the control subjects (*P* = 0.355), and CHD (*P* = 0.235) and IS (*P* = 0.340) patients. The T allele frequency was 9.8% in controls, 16.2% in CHD patients (*P* < 0.001 *vs*. controls), and 15.0% in IS patients (*P* < 0.001 *vs*. controls). The CC, CT, and TT genotype frequencies were 81.8%, 16.9% and 1.3% in controls; 70.9%, 25.8% and 3.3% in CHD patients (*P* < 0.001 *vs*. controls); and 72.7%, 24.5% and 2.8% in IS patients (*P* = 0.001 *vs*. controls), respectively.

TTC39B rs1407977 SNP and the risk of CHD and IS

As shown in **Table 2**, the T allele carriers had a higher risk of CHD (OR = 1.728, 95% CI = 1.290-2.316, *P* < 0.001) and IS (OR = 1.518, 95% CI = 1.182-2.116, *P* = 0.002) than the T allele non-carriers after adjustment for age, gender, BMI, cigarette smoking, alcohol consumption, and blood pressure.

Genotypes and serum lipid levels in the control subjects

As shown in **Figure 2**, no significant difference in all seven serum lipid parameters was observed between the CC and CT/TT genotypes.

Discussion

The major new finding of the present study was a significant association between the *TTC39B* rs1407977 SNP and the risk of CHD and IS in a Southern Chinese Han population.

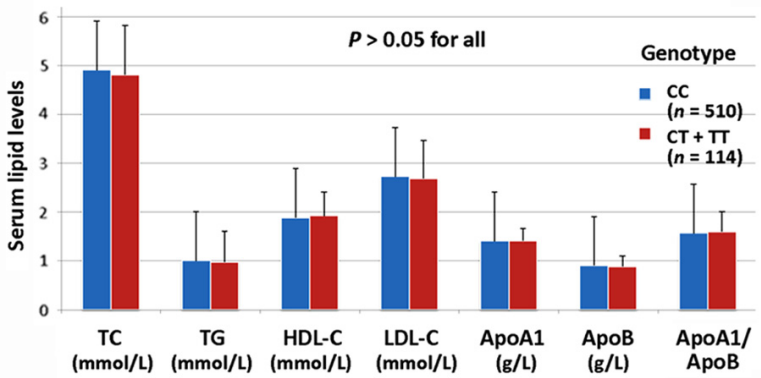

Figure 2. Association between the *TTC39B* rs1407977 genotypes and serum lipid parameters in the control group. TC, total cholesterol; TG, triglyceride; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; ApoA1, apolipoprotein A1; ApoB, apolipoprotein B; ApoA1/ApoB, the ratio of apolipoprotein A1 to apolipoprotein B. The value of TG was presented as median (interquartile range), and the difference between the genotypes was determined by the Wilcoxon-Mann-Whitney test. The association between genotypes and the remaining serum lipid parameters was tested by analysis of covariance (ANCOVA).

The rs1407977T allele frequency was higher in CHD (16.2%) and IS (15.0%) patients than in controls (9.8%, $P < 0.001$). The T allele carriers had higher risk of CHD (OR = 1.728, 95% CI = 1.290-2.316, $P < 0.001$) and IS (OR = 1.518, 95% CI = 1.182-2.116, $P = 0.002$) than the T allele non-carriers after controlling for potential confounders such as age, gender, BMI, cigarette smoking, alcohol consumption, and blood pressure.

The genotypic and allelic distribution of the *TTC39B* rs1407977 SNP in different ethnic groups is not well-known. According to the International 1000 Genomes data-base (https://www. ncbi.nlm.nih.gov/variation/tools /1000genomes/), the C and T allele frequencies of the *TTC39B* rs1407977 SNP were 81.25% and 18.75% in African Caribbeans in Barbados (ACB); 86.07% and 13.93% in Americans of African Ancestry in SW USA (ASW); 70.35% and 29.65% in Bengali from Bangladesh (BEB); 93.01% and 6.99% in Chinese Dai in Xishuangbanna, China (CDX); and 58.59% and 41.41% in Utah Residents (CEPA) with North and Western European Ancestry (CEU), respectively. The CC, CT and TT genotype frequencies were 37.2%, 49.6% and 13.2% in CEU; 81.4%, 17.4% and 1.2% in Japanese in Tokyo, Japan (JPT); 83.0%, 17.0% and 0 in Han Chinese in Beijing, China (CHB); and 75.9%, 21.4% and 2.7% in Yoruba in Ibadan, Nigeria (YRI); respectively. In the present study, we showed that the rs1407977T allele frequency was 9.8% in healthy controls, 16.2% in CHD patients and 15.0% in IS cases ($P < 0.001$ *vs.* controls). These findings revealed that the *TTC39B* rs1407977 SNP may have racial/ethnic and population specificity.

The potential association of the *TTC39B* rs1407977 SNP and serum lipid levels has not been previously reported in different racial/ethnic groups. Two previous GWASes have identified that the *TTC39B* rs471364 ($P = 3 \times 10^{-10}$) [12] and rs581080 ($P = 3 \times 10^{-12}$) [13] SNPs were associated with serum HDL-C levels in the European population. Recently, we also showed that serum HDL-C levels were different between the rs581080CC and CG/GG genotypes ($P = 0.009$). In healthy controls, the rs581080G allele carriers had lower HDL-C levels than the G allele non-carriers [18]. Hsieh et al. [32] showed that *TTC39B* mRNA was highly expressed in liver and small intestine of chow-fed wild type (WT) mice and was reduced by > 90% in *TTC39B*$^{-/-}$ mice. HDL-C levels were increased by ~22% in chow-fed *TTC39B*$^{-/-}$ mice compared to WT while non-HDL-C and TG levels were unchanged. *TTC39B*$^{-/-}$ mice challenged with 3 weeks of the high fat/high cholesterol/bile salt diet had a 42% increase in HDL-C levels, a 45% increase in ApoA1, the major protein component of HDL particles, decreased very low density lipoprotein /chylomicron cholesterol levels and no difference in plasma TG levels. However, previous findings on the association of the *TTC39B* SNPs with the alterations in serum lipid levels are inconsistent. Dumitrescu et al. [19] failed to replicate *TTC39B* rs471364 previously associated with HDL-C concentrations ($P = 3 \times 10^{-10}$) by Kathiresan et al. [12]. In the current study, we also failed to find a significant genetic effect of the *TTC39B* rs1407977 SNP on serum HDL-C concentrations and other serum lipid traits in the healthy controls. These findings suggest that the increased CHD and IS risk of *TTC39B* rs1407977 SNP in our study

population may not be induced by reducing serum HDL-C levels.

The exact mechanism of the *TTC39B* rs1407977 SNP on the risk of CHD and IS is unknown. Hsieh et al. [32] discovered that the *TTC39B* promotes the ubiquitination and degradation of liver X receptor (LXR). Decreasing the ubiquitination and increasing the abundance of endogenous LXR protein can activate anti-atherogenic cholesterol removal while inhibiting the lipogenesis that leads to steatosis. The LXR transcription factor remains a target of interest because of its anti-atherogenic, cholesterol removal and anti-inflammatory activities. Thus, by ameliorating cardiovascular disease and non-alcoholic fatty liver disease, *TTC39B* inhibition could offer a new therapeutic approach to tackle two globally prevalent chronic diseases.

Several potential limitations should be acknowledged in this study. Firstly, as compared with many previous GWASes and replication studies, our sample size was relatively small. Thus, larger samples in different populations are needed to confirm our findings in the future studies. Secondly, there were significant differences in the general characteristics and serum lipid profiles between the CHD/IS patients and control subjects. Although several confounders such as sex, age, BMI, cigarette smoking, alcohol consumption and blood pressure have been adjusted for the statistical analyses, we could not completely eliminate the potential influences of these factors on the results. Thirdly, since many CHD/IS patients were taking lipid-lowering drugs, it was inappropriate to analyze the association of the SNP and serum lipid levels in these patients. Finally, this study only detected a *TTC39B* rs1407977 SNP and its association with the risk of CHD and IS; other genetic variants were not detected and analyzed together. Thus, there are still many unmeasured environmental and genetic factors and their interactions.

Conclusions

The results of the present study showed that there was significant association between the *TTC39B* rs1407977 SNP and the risk of CHD and IS. The *TTC39B* rs1407977T allele carriers have higher risk for CHD and IS than the T allele non-carriers. No significant association was noted between the *TTC39B* rs1407977 SNP and all seven serum lipid traits. These results suggest that the association between the *TTC39B* rs1407977 SNP and the risk of CHD and IS in our study population may not be achieved by reducing serum HDL-C levels.

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Disclosure of conflict of interest

None.

Address correspondence to: Rui-Xing Yin, Department of Cardiology, Institute of Cardiovascular Diseases, The First Affiliated Hospital, Guangxi Medical University, 22 Shuangyong Road, Nanning 530021, Guangxi, People's Republic of China. E-mail: yinruixing@163.com

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