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Research Paper

TIMD4 rs6882076 SNP Is Associated with Decreased Levels of Triglycerides and the Risk of Coronary Heart Disease and Ischemic Stroke

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Abstract

Background: The T-cell immunoglobulin and mucin domain 4 gene (*TIMD4*) rs6882076 single nucleotide polymorphism (SNP) has been associated with serum total cholesterol, low-density lipoprotein cholesterol and triglycerides (TG) levels, but the results are inconsistent. Moreover, little is known about such association in Chinese populations. The aim of this study was to detect the association of the *TIMD4* rs6882076 SNP and serum lipid levels and the risk of coronary heart disease (CHD) and ischemic stroke (IS) in a Southern Chinese Han population.

Methods: Genotypes of the *TIMD4* rs6882076 SNP in 1765 unrelated subjects (CHD, 581; IS, 559 and healthy controls, 625) were determined by the Snapshot Technology.

Results: The genotypic and allelic frequencies of the *TIMD4* rs6882076 SNP were different between the CHD/IS patients and controls ($P < 0.05$ for all). The subjects with CT/TT genotypes were associated with decreased risk of CHD ($P = 0.014$ for CT/TT *vs*. CC genotypes, $P = 0.010$ for T *vs*. C alleles) and IS ($P = 0.003$ for CT/TT *vs*. CC genotypes; $P = 0.016$ for T *vs*. C alleles). The T allele carriers in healthy controls were also associated with decreased levels of serum TG.

Conclusions: The results of the present study suggest that the *TIMD4* rs6882076 SNP is associated with decreased risk of CHD and IS in our study population. It is likely to decrease the CHD and IS risk by reducing serum TG levels.

Key words: T-cell immunoglobulin and mucin domain 4, single nucleotide polymorphism, coronary heart disease, ischemic stroke, serum lipids.

Introduction

Coronary heart disease (CHD) and ischemic stroke (IS) remain the leading causes of morbidity and mortality worldwide [1, 2]. More than 700,000 people die from CHD each year in China [3]. The major pathological basis of two diseases had been proved to be atherosclerosis which the essential as an ambitious inflammatory disorder. Therefore, both of diseases would be involved in the same genetic and environmental backgrounds, including gender, time to life, hypercholesterol, hypertension, diabetes, cigarette smoking, and genetic factors [4-6]. Twin and family studies have indicated that the heritable factors account for 30%–60% of the interindividual variation in the risk of CHD and IS [7]. Recently, a large number of genes and loci related with CHD [8] or IS [9] were reported in several genome-wide association studies (GWASes). In addition, some genetic variants that initially association with CHD were detected to be related to IS afterwards [10, 11].

The T-cell immunoglobulin and mucin domain 4 gene (*TIMD4*, also known as T-cell membrane protein 4, *TIM-4*) is located on chromosome 5q33.3. *TIMD4* is exclusively expressed in antigen-presenting cells, where it mediates phagocytosis of apoptotic cells and plays an important role in maintaining tolerance [12]. Blockade of *TIMD4* enhanced the risk of atherosclerosis in low-density lipoprotein (LDL) receptor-deficient mice [13]. GWASes and other studies performed in different populations have reported that the *TIMD4* variants were associated with serum lipid traits, but the findings are inconsistent [14-18]. In addition, little is known about the association of the *TIMD4* SNPs and the risk of CHD and IS. Therefore, in the current study, we aimed to detect the association of the *TIMD4* rs6882076 SNP and serum lipid levels and the susceptibility of CHD and IS in a Southern Chinese Han population.

Methods

Study Patients

A total of 1140 unrelated patients were recruited from the hospitalized patients who were treated in the First Affiliated Hospital, Guangxi Medical University. Among them, 581 subjects suffered from CHD, and another 559 patients were diagnosed with IS. CHD was defined as including typical ischemic symptoms, plus one or more electrocardiographic changes (ST-segment depression or elevation of ≥ 0.5 mm, T-wave inversion of ≥ 3 mm in ≥ 3 leads, or left bundle branch block), in addition to increases in cardiac markers, such as creatinine kinase-MB and troponin T. Coronary angiography was carried out in patients with CHD. For the independent angiographers, two were blinded to the results of the genotypes. When coronary angiograms were performed, they were observed carefully. For a vessel to be scored, stenosis ≥ 50% had to be noted in an epicardial coronary vessel of interest or in one of its major branches. In the event of discordance of the number of vessels scored between the two reviewers, a third independent reviewer scored angiograms. The CHD subjects could be chosen for to our study when significant coronary stenosis (≥ 50%) was observed in at least one of the three main coronary arteries or their major branches (branch diameter ≥ 2 mm). In addition, the angiographic severity of disease was classified according to the number of coronary vessels with significant stenosis (luminal narrowing ≥ 50%) as one-, two-, or three-vessel disease in the three major coronary arteries [15, 19]. The definition of IS was ensured in accordance with the Trial of Org 10172 in Acute Stroke Treatment (TOAST) criteria [20] after rigorous examination, including neurological test, computed tomography, and/or magnetic resonance imaging (MRI). The IS patients entered in the study included individuals who were eligible for one of the two subtypes of TOAST criteria: large-artery atherosclerosis and small-vessel occlusion. However, if the subjects had a confirmed diagnosis of the below diseases, he/she must be excluded from our study: a history of hematologic or brain MRI revealing cerebral hemorrhage, cardio embolic stroke or unspecified stroke, neoplastic or intracranial space-occupying lesion, infection, other types of intracranial lesions, type 1 diabetes, and renal, liver, thyroid, and autoimmune diseases. The selected IS patients who had a past history of CHD or CHD patients had a past history of IS were excluded from the study.

Control Subjects

A total of 622 control subjects matched by age, gender, and ethnic group were randomly selected 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. The controls were healthy, without any CHD and IS details by questionnaires, history-taking, and clinical examination. The examination must be covered lots of items, just as physical examination, blood sampling, electrocardiography, chest X-ray, and Doppler echocardiography. All enrolled individuals were Han Chinese from Guangxi, the People's Republic of China. Trained research staff collected information on demography, socioeconomic status, medical history, and lifestyle factors with standardized questionnaires for all participants. All procedures of the investigation were carried out following the rules of the Declaration of Helsinki of 1975 (http://www.wma.net/en/30publications/10policies/b3/), revised in 2008. The study design was approved by the Ethics Committee of the First Affiliated Hospital, Guangxi Medical University (number: Lunshen-2011-KY-Guoji-001; 7 March 2011). Informed consent was obtained from all participants before the study.

Biochemical Measurements

A fasting venous blood sample of 5 ml was obtained from the participants. A part of the sample (2 mL) was collected into glass tubes and used to determine serum lipid levels. Another part of the sample (3 mL) was transferred to tubes with anticoagulants (4.80 g/ L citric acid, 14.70 g/L glucose and 13.20 g/L trisodium citrate) and used to extract deoxyribonucleic acid (DNA). Measurements of serum total cholesterol (TC), triglycerides (TG),

high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) levels in the samples were performed by enzymatic methods with commercially available kits (RANDOX Laboratories Ltd., Ardmore, Diamond Road, and Crumlin Co. Antrim, United Kingdom, BT29 4QY; Daiichi Pure Chemicals Co, Ltd., Tokyo, Japan). Serum apolipoprotein (Apo) A1 and ApoB levels were detected by the immunoturbidimetric immunoassay using a commercial kit (RANDOX Laboratories Ltd.). All determinations were performed with an auto-analyzer (Type 7170A; Hitachi Ltd., Tokyo, Japan) in the Clinical Science Experiment Center of the First Affiliated Hospital, Guangxi Medical University [21, 22].

Genotyping

Genomic DNA was extracted from peripheral blood leukocytes using the phenol-chloroform method [23-25]. Genotyping of the *TIMD4* rs6882076 SNP was performed by the Snapshot technology platform in the Center for Human Genetics Research, Shanghai Genesky Bio-Tech Co. Ltd., China [23-26]. The restriction enzyme for the *TIMD4* rs6882076 SNP was SAP (Promega) and Exonucleasel (Epicentre). The sense and antisense primers were 5'-TGACCGGACCCAGGAGTCTGT-3' and 5'-TCAC CAGGAGAAAAGGGCTCAG-3', respectively.

Diagnostic Criteria

The normal values of serum TC, TG, HDL-C, LDL-C, ApoA1, ApoB levels and the ApoA1/ApoB ratio 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 [27-31]. The individuals with TC > 5.17 mmol/L and/or TG > 1.70 mmol/L were defined as hyperlipidemic [27-31]. Hypertension was defined according to the criteria outlined by the 1999 World Health Organization-International Society of Hypertension Guidelines for the management of hypertension [32, 33]. 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 [34, 35].

Statistical Analyses

The statistical analyses were performed with the statistical software package SPSS 24.0 (SPSS Inc., Chicago, Illinois). The quantitative variables were presented as mean ± standard deviation (serum TG levels were presented as medians and interquartile ranges), qualitative variables were expressed as percentages. Allelic frequency was determined via direct counting, and the Hardy-Weinberg equilibrium was verified with the standard goodness-of-fit test. The sex ratio and genotypic distribution between the two groups were analyzed by the chi-square test. General characteristics between patients and controls were compared by the Student's unpaired *t*-test. The association between genotypes and serum lipid parameters was tested by covariance analysis (ANCOVA). Unconditional logistic regression was used to assess the correlation between the risk of CHD or IS and genotypes. Gender, age, BMI, blood pressure, alcohol consumption and cigarette smoking were adjusted for the statistical analysis. Odds ratio (OR) and 95% confidence interval (CI) were calculated by 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 general characteristics of the patients and healthy controls are summarized in Table 1. The male to female ratio, mean age, serum LDL-C and ApoB levels were not different between the control and experimental groups ($P > 0.05$ for all). The body height, weight, the values of BMI, the percentage of cigarette smoking, systolic blood pressure, pulse pressure, TG and the prevalence of hypertension were higher, but diastolic blood pressure, TC, HDL-C, ApoA1, the ratio of ApoA1 to ApoB and the percentage of alcohol consumption were lower in CHD patients than in controls ($P < 0.05$). The body height, weight, the values of BMI, the percentage of cigarette smoking, systolic blood pressure, diastolic blood pressure, pulse pressure, TG, and the prevalence of hypertension were higher, whereas those of TC, HDL-C, ApoA1, the ratio of ApoA1 to ApoB and the percentage of alcohol consumption were lower in IS patients than in controls ($P < 0.05$).

Genotypic and Allelic Frequencies

The genotypic and allelic frequencies of the rs6882076 SNP are presented in Table 2. The genotypic and allelic frequencies were different between the CHD/ IS and control groups ($P < 0.05$). The C and T allele frequencies were 74.7% and 25.3% in controls, 70.1% and 29.9% in CHD, and 70.3% and 29.7% in IS patients; respectively. The CC, CT and TT genotype frequencies were 57.6%, 34.2% and 8.2% in controls; 49.6%, 41.0% and 9.5% in CHD; and 50.1%, 40.4% and 9.5% in IS patients; respectively. The genotypic distribution was in accordance with the Hardy-Weinberg equilibrium in the three groups ($P > 0.05$).

Table 1. Comparison of the Clinical Characteristics and Serum Lipid Levels between the Controls and Patients

Parameter	Control	CHD	IS	P_{CHD}	P_{IS}
Number	625	581	559		
Male/female	465/160	435/146	406/153	0.890	0.595
Age (years)	61.68±11.80	62.25±10.57	62.85±12.32	0.368	0.093
Height (cm)	155.08±7.82	164.13±6.91	161.89±15.40	0.000	0.000
Weight (kg)	54.54±9.00	64.58±10.67	64.91±17.50	0.000	0.000
Body mass index (kg/m²)	22.61±2.81	23.86±3.37	23.49±3.61	0.000	0.000
Cigarette smoking [*n* (%)]					
Non-smoker	392(61.8)	313(53.1)	316(56.2)		
≤ 20 cigarettes/day	185(29.7)	89(16.0)	176(31.5)	0.000	0.048
> 20 cigarettes/day	48(8.5)	179(30.9)	67(12.3)		
Alcohol consumption [*n* (%)]					
Non-drinker	359(56.6)	429(72.3)	401(71.2)		
≤ 25 g/day	203(32.5)	93(16.7)	123(22.1)	0.000	0.000
> 25 g/day	63(10.8)	59(11.1)	35(6.7)		
Systolic blood pressure (mmHg)	127.22±19.78	133.14±23.39	147.77±22.03	0.000	0.000
Diastolic blood pressure (mmHg)	81.21±13.33	79.26±14.12	83.73±12.89	0.013	0.001
Pulse pressure (mmHg)	48.05±13.99	53.44±18.23	63.87±18.21	0.000	0.000
Total cholesterol (mmol/L)	4.88±1.04	4.54±1.21	4.53±1.15	0.000	0.000
Triglyceride (mmol/L)	1.12(0.67)	1.36(0.96)	1.36(0.93)	0.038	0.027
HDL-C (mmol/L)	1.89±0.48	1.14±0.34	1.23±0.40	0.000	0.000
LDL-C (mmol/L)	2.72±0.77	2.71±1.02	2.68±0.90	0.870	0.379
ApoA1 (g/L)	1.41±0.27	1.04±0.53	1.02±0.22	0.000	0.000
ApoB (g/L)	0.90±0.20	0.90±0.27	0.89±0.24	0.690	0.510
ApoA1/ApoB	1.64±0.55	1.38±2.44	1.19±0.60	0.011	0.000
Hypertension [*n* (%)]	178(27.5)	264(43.6)	381(67.0)	0.000	0.000

CHD, coronary heart disease; IS, ischemic stroke; 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; P_{CHD}: CHD *vs*. control; P_{IS}: IS *vs*. control. The value of triglyceride was presented as median (interquartile range); the difference between CHD/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.

TIMD4 rs6882076 SNP and the Risk of CHD or IS

The T allele carriers had a decreased risk of CHD and IS (CHD: OR = 0.73, 95% CI = 0.57-0.94, P = 0.014 for CT/TT *vs*. CC genotypes; OR = 0.79, 95% CI = 0.66-0.94, P = 0.010 for T *vs*. C alleles; IS: OR = 0.65, 95% CI = 0.50-0.86, P = 0.003 for CT/TT *vs*. CC genotypes; OR = 0.80, 95% CI = 0.67-0.96, P = 0.016 for T *vs*. C alleles; Table 2) after adjusting for age, gender, BMI, smoking status, alcohol consumption and hypertension.

Genotype and the Risk of CHD or IS

Stratified analysis showed a decreased risk of CHD in subjects with the CT/TT genotypes, mainly in those who belonged to one of the following subgroups: males (adjusted OR = 0.60, 95% CI = 0.45–0.80, P = 0.001), age ≤ 60 years (adjusted OR = 0.59, 95% CI = 0.41-0.85, P = 0.019), BMI ≥ 24 kg/m² (adjusted OR = 0.52, 95% CI = 0.34-0.79, P = 0.002), nonsmoking (adjusted OR = 0.76, 95% CI = 0.54-1.08, P = 0.018), and nondrinking (adjusted OR = 0.72, 95% CI = 0.54-0.98, P = 0.037).

There was a decreased risk of IS in subjects with the CT/TT genotypes, mainly in those who belonged to one of the following subgroups: males (adjusted OR = 0.58, 95% CI = 0.43-0.77, P = 0.000), age ≤ 60 years (adjusted OR = 0.60, 95% CI = 0.42-0.85, P = 0.005), BMI ≥ 24 kg/m² (adjusted OR = 0.60, 95% CI = 0.39-0.91, P = 0.046), nonsmoking (adjusted OR =0.66, 95% CI = 0.48-0.90, P = 0.010) and nondrinking (adjusted OR = 0.69, 95% CI = 0.47-1.03, P = 0.018) (Table 3). No significant interaction was detected between the genotypes and these factors.

Related Risk Factors for CHD and IS

As shown in Table 4, multivariate logistic analysis showed that the incidence of CHD and IS was positively correlated with alcohol consumption, high BMI (≥ 24 kg/m²), CT/TT genotypes and hyperlipidemia, whereas it was negatively associated between the incidence of CHD and hypertension, but not between the incidence of IS and hypertension. There was also a positive association between the incidence of CHD and cigarette smoking, but not between the incidence of IS and cigarette smoking.

Table 2. Genotypic and Allelic Frequencies of the *TIMD4* rs6882076 SNP and the Risk of CHD and IS [*n* (%)]

Genotype or allele	Control	CHD	IS	CHD		IS	
	n=625	*n*=581	*n*=559	OR(95%CI)	P_{CHD}	OR(95%CI)	P_{IS}
CC	360(57.6)	288(49.5)	280(50.1)	1		1	
CT	214(34.2)	238(41.0)	226(40.4)	0.77(0.49-1.21)	0.264	0.75(0.46-1.23)	0.263
TT	51(8.2)	55(9.5)	53(9.5)	1.07(0.67-1.70)	0.778	1.18(0.71-1.97)	0.515
x^2		7.830	6.708				
P		0.020	0.035				
CC	360(57.6)	288(49.6)	280(50.1)	1		1	
CT+TT	265(42.4)	293(50.4)	279(49.9)	0.73(0.57-0.94)	0.014	0.65(0.50-0.86)	0.003
x^2		7.810	6.702				
P		0.005	0.010				
C	934(74.7)	814(70.1)	786(70.3)	1		1	
T	316(25.3)	348(29.9)	332(29.7)	0.79(0.66-0.94)	0.010	0.80(0.67-0.96)	0.016
x^2		6.578	5.790				
P		0.010	0.016				

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, and hypertension.

Table 3. The *TIMD4* rs6882076 SNP and the Risk of CHD and IS According to Gender, Age, Body Mass Index, Smoking Status and Alcohol Consumption

Factor	Genotype	OR(95%CI)$_{CHD}$	P_{CHD}	P_I	OR(95%CI)$_{IS}$	P_{IS}	P_I
Gender	CC	1			1		
Male	CT+TT	0.60(0.45-0.80)	0.001		0.58(0.43-0.77)	0.000	
	CC	1		0.225	1		0.189
Female	CT+TT	0.89(0.53-1.51)	0.690		0.90(0.54-1.50)	0.700	
Age	CC	1			1		
≤ 60 years	CT+TT	0.59(0.41-0.85)	0.019		0.60(0.42-0.85)	0.005	
	CC	1		0.548	1		0.173
> 60 years	CT+TT	0.86(0.61-1.19)	0.215		0.77(0.55-1.08)	0.132	
BMI	CC	1			1		
< 24 kg/m²	CT+TT	0.83(0.61-1.12)	0.232		0.72(0.54-0.97)	0.066	
	CC	1		0.697	1		0.371
≥ 24 kg/m²	CT+TT	0.52(0.34-0.79)	0.002		0.60(0.39-0.91)	0.046	
Smoking	CC	1			1		
Nonsmoking	CT+TT	0.76(0.54-1.08)	0.018		0.66(0.48-0.90)	0.010	
	CC	1		0.380	1		0.089
Smoking	CT+TT	0.78(0.54-1.12)	0.189		0.78(0.54-1.14)	0.204	
Drinking	CC	1			1		
Nondrinking	CT+TT	0.72(0.54-0.98)	0.037		0.69(0.47-1.03)	0.018	
	CC	1		0.256	1		0.529
Drinking	CT+TT	0.75(0.50-1.12)	0.164		0.69(0.39-1.22)	0.224	

OR, odds ratio; CI, confidence interval; CHD, coronary heart disease; IS, ischemic stroke; BMI, body mass index. OR and 95% CI were obtained from unconditional logistic regression model after adjusting for age, gender, body mass index, smoking status, alcohol consumption, hypertension. P_I, the value of interaction between the SNP and factors.

Table 4. The Relative Risk Factors for CHD and IS

Factor	OR(95%CI)$_{CHD}$	P_{CHD}	OR(95%CI)$_{IS}$	P_{IS}
Nonsmoking	1		1	
Smoking	10.87(5.76-20.50)	0.000	1.10(0.56-2.17)	0.768
Nondrinking	1		1	
Drinking	10.09(5.07-20.08)	0.000	2.83(1.41-5.07)	0.003
BMI < 24 kg/m²	1		1	
BMI ≥ 24 kg/m²	0.58(0.39-0-86)	0.007	0.64(0.41-0.99)	0.046
rs6882076CC	1		1	
rs6882076CT/TT	0.73(0.57-0.94)	0.014	0.65(0.50-0.86)	0.003
Normotensive	1		1	
Hypertension	0.60(0.34-1.07)	0.088	0.50(0.32-0.79)	0.003
Normal blood lipids	1		1	
Hyperlipidemia	0.58(0.35-0.96)	0.033	0.49(0.29-0.83)	0.008

OR, odds ratio; CI, confidence interval; CHD, coronary heart disease; IS, ischemic stroke; BMI, body mass index. 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 and Serum Lipid Levels

As shown in Table 5, serum TG levels were different between the CC and CT/TT genotypes in the controls (P = 0.011), but not in the CHD and IS patients. The T allele carriers (CT/TT genotypes) in controls had lower serum TG levels than the T allele non-carriers (CC genotypes). No significant differences in the remaining serum lipid parameters between the CC and CT/TT genotypes (P > 0.05 for all) were found.

Discussion

Serum TG levels in atherosclerotic cardiovascular disease (ASCVD) have been renewed on the basis of evidence from epidemiologic, genetic, and clinical studies. Epidemiologic studies have shown that increased TG levels are correlated with an increased risk of cardiovascular diseases [36, 37] and the American Heart Association has long recognized that increased TGs are an important marker of cardiovascular risk [38]. More recently studies have shown in GWASes [16, 38-41], genetic [42-47] and mendelian randomization [48-50] that have suggested a causal role for TGs as a modifiable risk factor in the development and progression of ASCVD. Analyses from clinical data have demonstrated that lower on-treatment TGs correlate with reduced cardiovascular risk [51, 52]. The real-world analysis of administratively derived data from > 20 000 patients in the Optum Research Database identified statin-treated patients with high TGs and a diagnosis of diabetes mellitus and/or ASCVD to be at a 34.9% higher risk of major cardiovascular events than a comparator cohort of patients with TGs < 1.69 mmol/L (< 150 mg/dL) and HDL-C > 1.04 mmol/L (> 40 mg/dL) while controlling for other comorbidities. Reflective of the higher risk of major cardiovascular events, high TGs (2.26–5.64 mmol/L) were also associated with significantly higher medical costs and resource use. This is consistent with a prior observational analysis that found TGs in the range 2.26 to 5.64 mmol/L to be associated with significantly higher total medical costs than in patients with TGs < 1.69 (P < 0.001) [53].

The prevalence of the *TIMD4* rs6882076T allele may be different in diverse racial/ethnic groups. The information in the International HapMap Project's database (https://www.ncbi.nlm.nih.gov/variation/tools/1000genomes/) showed that the rs6882076T allele frequency was 31.5% in Europeans, 24.4% in Han Chinese in Beijing (HCB), 15.9% in Japanese, and 68.3% in Sub-Saharan African. In the present study, we showed that the *TIMD4* rs6882076T allele frequency was lower in our study subjects (control, 8.2%; CHD, 9.5%; and IS, 9.5%) than in HCB (24.4%). 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 *TIMD4* rs6882076 variation may have a racial/ethnic specificity. The prevalence of the rs6882076T allele was higher in Europeans or in African than in Asian. All of these findings would be a reasonable explanation for the distinct prevalence of CHD between European or African and Chinese.

Table 5. Association between the rs6882076 SNP and Serum Lipid Levels in Controls and CHD and IS Patients

Genotype	*n*	TC (mmol/L)	TG (mmol/L)	HDL-C (mmol/L)	LDL-C (mmol/L)	ApoA1 (g/L)	ApoB (g/L)	ApoA1 /ApoB
Control								
CC	360	4.91±0.97	1.29(0.57)	1.87±0.46	2.73±0.79	1.41±0.27	0.90±0.21	1.63±0.46
CT/TT	265	4.85±1.16	0.97(0.59)	1.93±0.51	2.71±0.76	1.42±0.26	0.91±0.20	1.65±0.57
F		1.047	6.535	3.141	0.414	0.007	0.004	0.190
P		0.307	0.011	0.077	0.520	0.932	0.949	0.663
CHD								
CC	288	4.56±1.16	1.40(0.96)	1.15±0.35	2.71±0.99	1.06±0.68	0.90±0.29	1.59±3.50
CT/TT	293	4.49±1.22	1.32(0.97)	1.13±0.32	2.70±1.01	1.01±0.28	0.91±0.25	1.17±0.41
F		0.797	0.159	0.393	0.060	0.988	0.005	3.695
P		0.372	0.690	0.531	0.806	0.321	0.942	0.055
IS								
CC	280	4.55±1.25	1.75(1.19)	1.22±0.46	2.69±0.95	1.02±0.21	0.89±0.25	1.20±0.71
CT/TT	279	4.47±1.01	1.35(0.91)	1.22±0.33	2.64±0.82	1.01±0.22	0.88±0.23	1.19±0.48
F		0.053	1.153	0.027	0.057	0.064	0.043	1.150
P		0.819	0.284	0.869	0.812	0.801	0.835	0.284

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).

Another important finding in the current study was that the *TIMD4* rs6882076 SNP was strongly associated with the risk of CHD and IS in the Guangxi Han population. The CT/TT genotypes and T allele were associated with a decreased risk of CHD and IS after adjusting for potential confounding factors. Multivariate analysis showed that the known factors, such as cigarette smoking, alcohol consumption, high BMI (≥ 24 kg/m^2), hyperlipidemia and the CT/TT genotypes were dependently associated with CHD. Meanwhile, the occurrence of IS was positively correlated with alcohol consumption, high BMI (≥ 24 kg/m^2), hypertension, hyperlipidemia and the CT/TT genotypes. CHD was negatively correlated with hypertension and IS was negatively correlated with cigarette smoking. In the stratified analysis, the decreased risk of CHD and IS in subjects with the CT and TT genotypes was mainly observed in males, age ≤ 60 years, BMI ≥ 24 kg/m^2, nonsmokers and nondrinkers. No significant interactions between the *TIMD4* rs6882076 SNP and environmental factors on the risk of CHD or IS. The subjects with CT/TT genotypes of the *TIMD4* rs6882076 SNP contributed to the decreased risk of CHD and IS. In a previous study, we have reported that two *TIMD4-HAVCR1* SNPs (rs1501918 and rs2036402) interacted with alcohol consumption to influence serum HDL-C levels. Two SNPs (rs1501918 and rs12522248) interacted with BMI ≥ 24 kg/m^2 to modulate serum TC levels. The haplotypes of G-T-T and C-C-C interacted with smoking to increase the risk of CHD. The haplotypes of C-T-T, G-T-T, C-C-C, and G-C-T in BMI ≥ 24 kg/m^2 were associated with an increased risk for CHD and IS. The rs12522248TC/CC genotypes interacted with BMI ≥ 24 kg/m^2 to increase the risk of CHD. It is well known that heavy alcohol intake, smoking and obesity have an unfavourable effect on lipid profiles and atherosclerotic disease [18].

Several previous studies have reported the association of many SNPs in the *TIMD4* with one or more lipid traits [24, 27-29]. However, not all researches have consistent findings. A previous GWAS showed that the *TIMD4* rs6882076 SNP was associated with LDL-C [17]. In the present study, we found that the *TIMD4* rs6882076 SNP was only associated with serum TG levels. The genotypic and allelic frequencies of the *TIMD4* rs6882076 SNP were different between the CHD/IS patients and controls ($P < 0.05$ for all). The CT/TT genotypes and T allele were associated with a decreased risk of CHD ($P = 0.014$ for CT/TT *vs*. CC, $P = 0.010$ for T *vs*. C) and IS ($P = 0.003$ for CT/TT *vs*. CC; $P = 0.016$ for T *vs*. C). The CT/TT genotypes in the healthy controls, but not in CHD or IS patients, were also associated with a decreased serum TG concentration. It may be owing to the impact of other uncertain variants and the different genetic background, lifestyle and diet in different ethnic groups. Another possible reason is that the sample size may not be enough to detect the exact association. Therefore, further investigations with larger sample size are needed to confirm our findings.

Several potential limitations cannot be ignored. Firstly, the number of involved patients was relatively small compared to many previous GWASes and replication studies. 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 CHD and IS could not be completely eliminated. Secondly, a number of patients in CHD or IS groups took anti-atherosclerotic

drugs, such as statins, angiotensin-converting enzyme inhibitors, beta-blockers, and aspirin when they were enrolled in the study, but not in the control group. However, the drug information was missing for some IS and CHD patients. It was not proper to analyze the association of the SNP and serum lipid levels in the CHD and IS groups. Finally, only one *TIMD4* SNP was studied in this study. Therefore, the observed associations need further replications to avoid spurious associations.

Conclusions

The *TIMD4* rs6882076 SNP was associated with serum TG levels and the susceptibility of CHD and IS in a Southern Chinese Han population. The T allele carriers had a decreased risk of CHD and IS. The T allele carriers in healthy controls had lower serum TG levels than the T allele non-carriers. These findings suggest that the *TIMD4* rs6882076 SNP is likely to decrease the risk of CHD and IS by reducing serum TG levels.

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Competing Interests

The authors have declared that no competing interest exists.

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