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The Niemann–Pick C1-like 1 rs2073547 polymorphism is associated with type 2 diabetes mellitus in a Chinese population

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Abstract

Objective: To explore the association between Niemann–Pick C1-like 1 gene (*NPC1L1*) single nucleotide polymorphisms and type 2 diabetes mellitus (T2DM) in a Chinese population.

Methods: Using propensity score matching, 490 T2DM patients and 490 matched controls were recruited from 13 communities in Guangxi, China. *NPC1L1* rs217386 and rs2073547 genotyping was performed using a MassARRAY system.

Results: The rs2073547 genotype distribution differed significantly among patient groups. Low-density lipoprotein cholesterol levels were similar among different rs2073547 genotypes and alleles. The rs2073547 AG genotype was significantly more prevalent in patients with T2DM. After adjusting for risk or protective factors for diabetes, AG and GG+AG genotypes of rs2073547 were associated with significantly increased risks of T2DM. Compared with the AA genotype, the AG genotype was associated with a significantly higher risk of T2DM in participants with gamma-glutamyl transpeptidase (GGT) $<$45 U/L, systolic blood pressure (SBP) $\geq$140 mmHg, or triglyceride $<$1.70 mmol/L. In participants with GGT $<$45 U/L or SBP $\geq$140 mmHg, the GG+AG genotype was associated with a significantly higher T2DM risk versus the AA genotype.

Conclusions: The rs2073547 polymorphism of *NPC1L1* may be related to T2DM susceptibility in the Chinese population.

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Introduction

Globally, the number of patients with diabetes mellitus (DM) reached 451 million in 2017 and this is expected to increase to 693 million by 2045.[1] The prevalence of DM has increased rapidly in China during the past few decades.[2] Dyslipidemia is a common metabolic disorder that is also increasing in prevalence worldwide, and was recently estimated to be 42.84% in middle-aged and older Chinese individuals.[3] Adverse consequences of dyslipidemia that seriously threaten the health of patients include atherosclerosis,[4] coronary heart disease, and stroke.[5] Therefore, lipid-lowering drugs, especially those reducing low-density lipoprotein cholesterol (LDL-C), are widely prescribed. The most common lipid-lowering drugs are statins, which reduce LDL-C concentrations by inhibiting the 3-hydroxy-3-methylglutaryl-coenzyme A reductase gene (*HMGCR*). Observational studies reported that statin therapy, but not control therapy, was associated with new-onset type 2 DM (T2DM).[6,7]. However, it is unclear whether other lipid-lowering agents are associated with an increased risk of T2DM development.

Advances in technology have enabled genome-wide association studies (GWAS) to identify more than 100 risk variants associated with T2DM.[8,9] Studies of mutations in genes encoding drug targets have been useful in the prediction of both drug efficacy and adverse effects.[10,11] For instance, common single nucleotide polymorphisms (SNPs) in *HMGCR* were successfully used as genetic proxies to explore the effects of statins.[12,13] Previous genetic findings suggested that *HMGCR* alleles are associated with an increased risk of developing T2DM and a higher body mass index (BMI).[12] These studies obtained similar findings to those reported in meta-analyses of randomized clinical trials (RCTs) of statins.[14,15] Additionally, Schmidt et al. reported that variations in the proprotein convertase subtilisin/kexin type 9 gene (*PCSK9*) that were associated with a lower LDL-C were also associated with higher fasting glucose, weight gain, a larger waist-to-hip ratio, and an increased risk of T2DM.[16]

The Niemann–Pick C1-like 1 gene (*NPC1L1*) encodes a protein expressed by gastrointestinal tract epithelial cells that mediates extracellular sterol transport across the brush border membrane. NPC1L1 is also the molecular target of ezetimibe, a potent cholesterol absorption inhibitor that lowers blood cholesterol. A meta-analysis of genetic variants of *NPC1L1* reported that LDL-C-lowering alleles (rs2073547 and rs217386) were directly associated with T2DM risk in European and American populations.[17] However, equivalent data have not yet been reported for Chinese individuals. Thus, the aim of the present study was to examine the relationship between *NPC1L1* rs2073547 and rs217386 variants and T2DM in the Guangxi population in China.

Materials and Methods

Study participants

A total of 786 patients with T2DM and 1015 controls without T2DM were recruited consecutively between January 2011 and September 2012 from 13 communities in

Nanning, Guangxi, southern China. All participants met the following requirements: (a) age ≥40 years; (b) resident in Nanning for ≥5 years; and (c) not receiving ezetimibe treatment. T2DM was diagnosed according to the World Health Organization diagnostic criteria published in 1999; individuals with type 1 DM, gestational DM, and other types of DM were excluded from the study. Propensity score matching (PSM) based on age, gender, ethnicity (Han and minorities including Zhuang, Miao, Yao, Molao, Buyi, Dai, Dong, Gaoshan, Hui, Zang, Maonan, and Tujia), smoking status, drinking status, and hours of exercise per week were used to control for these potential confounders.[18] For the final analysis, 490 T2DM patients and 490 matched controls were selected. The ethics committee of the First Affiliated Hospital of Guangxi Medical University approved the study. All participants provided written informed consent before the collection of any data or samples.

Data collection

All participants completed an epidemiological questionnaire that included sociodemographic characteristics, personal history, family history, and other lifestyle habits. Trained personnel obtained anthropometric data such as height, weight, systolic blood pressure (SBP), diastolic blood pressure (DBP), heart rate, and waist circumference (WC), as well as peripheral blood samples from the participants. Serum levels of triglycerides (TG), total cholesterol (TC), high-density lipoprotein cholesterol, LDL-C, aspartate aminotransferase, alanine aminotransferase, and gamma-glutamyl transpeptidase (GGT) were measured at the First Affiliated Hospital of Guangxi Medical University. Lipid profiles were measured using the Architect C16000 autoanalyser (Abbott Diagnostics, Des Plaines, IL, USA) and blood glucose was measured using the glucose oxidase method.[19] Some continuous data were changed into binary data according to clinical significance and reference range.

DNA isolation and genotyping

Genomic DNA from all participants was manually isolated from peripheral blood using a DNA extraction kit (Tiangen Biotech, Beijing, China) according to the manufacturer's instructions. *NPC1L1* rs2073547 and rs217386 polymorphisms were genotyped by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry using the MassARRAY system (Agena Bioscience, San Diego, CA, USA). The forward and reverse primers used were 5′-ACGTTGGATGTCAGG AAGACTTCCTGGAG-3′ and 5′-ACGT TGGATGATGTG CAACCCTAGGTTG TG-3′ for rs217386 and 5′-ACGTTGGAT GTGTCCTTATTCCTTGGAGGG-3′ and 5′-ACGTTGGATGGACCAGAATGCAT CCAAGAG-3′ for rs2073547, respectively. DNA from patients with T2DM and matched controls were randomly assigned to 96-well plates and genotyped using a blinded method. Call rates for SNP genotyping were >98%.

Post-hoc calculation of sample size

The sample size for the study was calculated according to the web-based program https://ihg.gsf.de/cgi-bin/hw/power2.pl to determine whether the study would be adequately powered, based on methods described previously.[20,21] Assuming a disease prevalence of 0.1, a high-risk allele frequency of 0.05, and an alpha (type 1 error) of 0.05, the total sample size required for a power of 0.98 was calculated to be 830 for a multiplicative model, 888 for an additive model, and 955 for a dominant model. Therefore, the total sample size of 980 matched participants (490 per group) was sufficient.

Statistical analysis

Comparisons of variables between patients with T2DM and controls were carried out using the Student's t-test or paired sample t-test for continuous variables and the chi-squared test or paired chi-squared test for categorical variables. Conditional logistic regression analysis was used to identify factors associated with T2DM. To analyze genotype distributions, the Hardy–Weinberg equilibrium (HWE) for each SNP was tested using the paired chi-squared test with one degree of freedom. One-way analysis of variance and the Student's t-test were used to investigate associations between genotypes, SNP alleles, and LDL-C levels. Associations between SNP genotypes and T2DM were analyzed using conditional logistic regression under different genetic models (additive, dominant, and recessive) to adjust for potential confounders. Stratified analyses according to the important factors were performed using unconditional logistic regression. Odds ratios (ORs) and 95% confidence intervals (95% CIs) were used to evaluate the association strength between T2DM and controls. PSM and all analyses were conducted using SPSS 23.0 software (IBM Corp., Armonk, NY, USA). A two-tailed *P*-value <0.05 was considered statistically significant.

Results

Baseline information

A total of 786 patients with T2DM and 1015 controls without T2DM were initially enrolled. Of these, PSM selected 490 patients with T2DM and 490 matched controls. Baseline characteristics of study participants are presented in Table 1. After PSM, there were no significant differences in age, gender, ethnicity, smoking status, drinking status, or the number of exercise hours between the two groups (Table 1). However, patients with T2DM had significantly higher SBP ($\geq$140 mmHg), DBP ($\geq$90 mmHg), WC (men $\geq$90 cm, women $\geq$85 cm), TG ($\geq$1.7 mmol/L), GGT ($\geq$45 U/L), and a higher occurrence of DM family history than controls ($P<0.05$). Participants showed significantly different stratified BMI levels and educational attainment (all $P<0.05$).

Factors associated with T2DM

Factors associated with T2DM in univariate analysis were identified by conditional logistic regression analysis. This revealed that GGT $\geq$45 U/L (OR: 1.927, 95% CI: 1.188–3.126), SBP $\geq$140 mmHg (OR: 1.539, 95% CI: 1.120–2.115), TG $\geq$1.7 mmol/L (OR: 1.738, 95% CI: 1.268–2.381), and a family history of DM (OR: 1.927, 95% CI: 1.188–3.126) were independently associated with the presence of T2DM (each $P<0.05$). BMI $\geq$18.5 kg/m^2 was also a risk factor for T2DM (18.5–23.9 kg/m^2 vs. $\leq$18.5 kg/m^2, OR: 3.192, 95% CI: 1.203–8.471; 24–27.9 kg/m^2 vs. $\leq$18.5 kg/m^2, OR: 3.429, 95% CI: 1.255–9.369; 28–31.9 kg/m^2 vs. $\leq$18.5 kg/m^2, OR: 4.452, 95% CI: 1.494–13.263; $\geq$32 kg/m^2 vs. $\leq$18.5 kg/m^2, OR: 10.443, 95% CI: 2.438–44.722). Education time $\geq$12 years (vs. $\leq$6 years; OR: 0.560, 95% CI: 0.337–0.931) was a significant protective factor against T2DM ($P<0.05$) (Table 2).

Comparison of genotype distributions and allelic frequencies between T2DM and matched control groups

Genotype distributions and allelic frequencies of rs2073547 and rs217386 SNPs in *NPC1L1* are shown in Table 3. The genotype distribution of rs2073547 differed significantly between T2DM patients and matched controls ($P<0.05$), but there were no differences in the allelic frequency of rs2073547 or in the genotype distribution

Table 1. Comparisons of general characteristics between patients with type 2 diabetes mellitus and controls.

	Unmatched			Matched		
Variable	Control (*N*=1015)	T2DM (*N*=786)	*P*	Control (*N*=490)	T2DM (*N*=490)	*P*
Age (years)	59.8±9.7	60.7±9.9	**0.039**	61.1±8.9	61.1±8.9	1.0
Gender (male/female)	381 (37.5)	302 (38.4)	0.701	136 (27.8)	136 (27.8)	1.0
Ethnicity			0.48			1.0
Han	755 (74.4)	573 (72.9)		393 (80.2)	393 (80.2)	
Minorities	260 (25.6)	213 (27.1)		97 (19.8)	97 (19.8)	
Current smoker (yes)	150 (14.8)	123 (15.6)	0.61	42 (8.6)	42 (8.6)	1.0
Current alcohol drinker (yes)	117 (11.5)	84 (10.7)	0.57	21 (4.3)	21 (4.3)	1.0
Exercise ≥3.5 hours/week (yes)	286 (28.2)	224 (28.5)	0.88	111 (22.7)	111 (22.7)	1.0
SBP ≥140 mmHg	377/1004 (37.5)	386/775 (49.8)	**<0.001**	195/485 (40.2)	251/486 (51.6)	**<0.001**
DBP ≥90 mmHg	166/1004 (16.5)	183/775 (23.6)	**<0.001**	75/485 (15.5)	114/486 (23.5)	**0.002**
WC (men ≥90 cm, women ≥85 cm)	318/988 (32.2)	359/768 (46.7)	**<0.001**	159/482 (33.0)	225/480 (46.9)	**<0.001**
BMI (kg/m^2)			**<0.001**			**<0.001**
<18.5	38 (3.8)	11 (1.4)		24 (5.0)	6 (1.2)	
18.5–23.99	471 (47.4)	287 (37.3)		234 (48.4)	192 (39.8)	
24–27.99	392 (39.5)	333 (43.2)		181 (37.5)	198 (41.2)	
28–32	80 (8.1)	109 (14.2)		39 (8.1)	69 (14.3)	
≥32	12 (1.2)	30 (3.9)		5 (1.0)	17 (3.5)	
LDL-C ≥3.4 mmol/L)	366/1015 (36.1)	310/785 (39.5)	0.136	198/490 (40.4)	200/490 (40.8)	0.894
HDL-C <1.04mmol/L	220/1015	176/785	0.705	79/490	98/490	0.115
TC ≥5.2 mmol/L	505/1015 (49.8)	442/784 (56.4)	**0.005**	270/490 (55.1)	291/490 (59.4)	0.171
TG ≥1.7 mmol/L	256/1015 (25.2)	347/780 (44.5)	**<0.001**	128/490 (26.1)	208/488 (42.6)	**<0.001**
AST ≥80 U/L	2/1013 (0.2)	3/783 (0.4)	0.459	0/490 (0.0)	2/490 (0.4)	0.471
ALT ≥80 U/L	2/912 (0.2)	3/742 (0.4)	0.662	0/447 (0.0)	2/464 (0.4)	0.471
GGT ≥45 U/L	89/1013 (8.8)	147/784 (18.8)	**<0.001**	36/488 (7.4).	79/490 (16.1)	**<0.001**
Family history of DM (yes)	150 (14.8)	149 (19.0)	**0.02**	69 (14.1)	100 (20.4)	**0.010**
Educational attainment (years)			**0.001**			**0.017**
≤6	196 (19.4)	196 (25.0)		105 (21.5)	136 (27.9)	
7–9	347 (34.3)	283 (36.1)		176 (36.1)	171 (35.1)	
10–12	335 (33.1)	215 (27.5)		147 (30.1)	129 (26.5)	
≥12	134 (13.2)	89 (11.4)		60 (12.3)	51 (10.5)	
Residential pattern			0.117			0.941
living with children and spouse	584 (57.9)	446 (57.0)		280 (57.6)	278 (57.2)	
living with children	247 (24.5)	217 (27.7)		120 (24.7)	134 (27.5)	
living with spouse	126 (12.5)	74 (9.5)		65 (13.4)	40 (8.2)	
living alone	51 (5.1)	45 (5.8)		21 (4.3)	35 (7.2)	

Data are presented as the mean ± standard deviation or *n* (%). Some missing data are presented as *n*/total (%). ALT: alanine aminotransferase; AST: aspartate aminotransferase; BMI: Body mass index; DM: diabetes mellitus; DBP: diastolic blood pressure; GGT: gamma-glutamyl transpeptidase; LDL-C: low-density lipoprotein cholesterol; HDL-C: high-density lipoprotein cholesterol; SBP: systolic blood pressure; T2DM: type2 diabetes mellitus; TC: total cholesterol; TG: triglycerides; WC: waist circumference.

or allelic frequency of rs217386 between groups. Of the two SNPs tested, rs2073547 was consistent with the HWE, indicating that the population was well represented. However, rs217386 showed significant deviations from HWE assumptions in the control group (P=0.002), so was excluded from subsequent analysis.

Associations between genotypes, alleles of rs2073547, and LDL-C levels

The association between rs2073547 genotypes, alleles, and serum LDL-C levels was investigated (Table 4). No significant differences in LDL-C levels were detected among different genotypes or alleles of rs2073547 in the two groups or total population.

Table 2. Conditional logistic regression analysis of the clinical factors associated with type 2 diabetes mellitus.

Variable	OR	95%CI		*P*
Educational attainment (vs. ≤6) years				
7–9	0.846	0.560	1.178	0.428
10–12	0.664	0.426	1.037	0.072
≥12	0.560	0.337	0.931	**0.046**
BMI (vs. <18.5 kg/m^2)				
18.5–23.9	3.192	1.203	8.471	**0.020**
24–27.9	3.429	1.255	9.369	**0.016**
28–31.9	4.452	1.494	13.263	**0.007**
≥32	10.443	2.438	44.722	**0.002**
GGT ≥45 U/L	1.927	1.188	3.126	**0.008**
SBP ≥140 mmHg	1.539	1.120	2.115	**0.008**
DBP ≥90 mmHg	1.320	0.876	1.991	0.184
WC ≥90 cm (men) or ≥85 cm (women)	1.335	0.927	1.922	0.120
TG ≥1.7 mmol/L	1.738	1.268	2.381	**0.001**
Family history of DM (yes)	1.927	1.188	3.126	**0.008**

95%CI: 95% confidence interval; OR: odds ratio; BMI: Body mass index; DM: diabetes mellitus; DBP: diastolic blood pressure; GGT: gamma-glutamyl transpeptidase; SBP: systolic blood pressure; TG: triglycerides; WC: waist circumference.

Table 3. Genotype distributions, allelic frequencies, and Hardy–Weinberg equilibrium analysis of the two single nucleotide polymorphisms.

SNP	T2DM group (*N*=490)	Control group (*N*=490)	*P*	*Pa**
rs2073547				0.265
AA	208 (42.8)	249 (51.1)	**0.034**	
AG	227 (46.7)	192 (39.3)		
GG	51 (10.5)	47 (9.6)		
G allele	329 (33.6)	690 (29.1)	0.078	
rs217386				**0.002**
GG	454 (93.8)	456 (93.4)	0.223	
GA	30 (6.2)	29 (5.9)		
AA	0 (0.0)	3 (0.6)		
G allele	938 (95.7)	941 (96.0)	0.550	

*Pa**: *P*-value of the Hardy–Weinberg equilibrium test in the control group; SNP: single nucleotide polymorphism; T2DM: type2 diabetes mellitus. Values in brackets represent the percentage of the sample population.

Associations between genotypes, alleles of rs2073547, and T2DM

We evaluated the association between *NPC1L1* rs2073547 and the risk of T2DM under different inheritance models (Table 5). After adjusting for educational attainment, BMI, GGT, SBP, TG, and a family history of DM, we found that rs2073547 AG and GG+AG genotypes were associated with a significantly greater risk of T2DM than the AA genotype (AG vs. AA: OR: 1.347, 95%

Table 4. Association between genotypes, alleles of rs2073547, and LDL-C levels.

	Genotype					Allele			
LDL-C (mmol/L)	AA	AG	GG	*F*	*P*	A	G	*t*	*P*
T2DM	3.2±1.0	3.3±0.9	3.3±1.0	0.566	0.568	3.2±1.0	3.3±1.0	−1.039	0.970
Control	3.1±0.9	3.1±0.9	3.2±0.7	0.329	0.720	3.1±0.9	3.2±0.9	−0.675	0.500
Total	3.2±1.0	3.2±0.9	3.3±0.9	0.920	0.399	3.2±0.9	3.2±0.9	−1.321	0.187

LDL-C: low-density lipoprotein cholesterol; T2DM: type2 diabetes mellitus.

Table 5. Associations between rs2073547 and type 2 diabetes mellitus.

SNP	Model	Crude OR (95%CI)	Crude *P*	Adjusted OR (95%CI)	Adjusted *P**
rs2073547					
	AG vs. AA	1.424 (1.087–1.865)	**0.010**	1.347 (1.019–1.791)	**0.015**
	GG vs. AA	1.316 (0.842–2.058)	0.229	1.322 (0.739–2.120)	0.234
	GG vs. AG+AA	1.098 (0.719–1.676)	0.666	1.198 (0.763–1.879)	0.432
	GG+AG vs. AA	1.404 (1.085–1.817)	**0.010**	1.593 (1.179–2.152)	**0.002**
	G vs. A	1.150 (0.985–1.343)	0.078	1.275 (1.038–1.566)	0.723

95%CI: 95% confidence interval; OR: odds ratio; SNP: single nucleotide polymorphism. * Adjusted for educational attainment, body mass index, gamma-glutamyl transpeptidase, systolic blood pressure, triglycerides and family history of diabetes mellitus.

CI: 1.019–1.791, P=0.015; GG+AG vs. AA: OR: 1.593, 95% CI: 1.179–2.152, P=0.002).

Stratified analysis of the association between rs2073547 genotypes and T2DM

We also carried out an analysis of the association of rs2073547 genotypes and T2DM stratified by BMI, GGT, SBP, and TG using different inheritance models. As shown in Table 6, the AG genotype was associated with a significantly greater risk of T2DM than the AA genotype (GGT <45 U/L group: OR: 1.408, 95% CI: 1.060–1.871, P=0.018; SBP ≥140 mmHg group: OR: 1.584, 95% CI: 1.063–2.360, P=0.024; TG <1.70 mmol/L group: OR: 1.447, 95% CI: 1.039–2.015, P=0.029). The odds of T2DM in GG+AG carriers were significantly greater than for AA carriers in the GGT <45 U/L group (OR: 1.349, 95% CI: 1.031–1.766, P=0.029) and the SBP ≥140 mmHg group (OR: 1.565, 95% CI: 1.072–2.285, P=0.020). The odds of T2DM in the group of patients with SBP ≥140 mmHg were greater for G allele carriers than for A allele carriers (OR: 1.340, 95% CI: 1.006–1.786, P=0.046). However, there were no significant effects of rs2073547 variants on T2DM susceptibility in other subgroups.

Discussion

T2DM is a global health problem, and its complex pathogenesis is not yet fully understood. However, GWAS have identified several genetic variants that help explain some of the individual variations in T2DM susceptibility.[8,22] Because multiple genetic and environmental factors affect T2DM incidence, a combination of PSM and multivariate logistic regression analysis were adopted in this study to minimize the confounding effects of clinical factors

Table 6. Stratified analysis of rs2073547 by BMI, GGT, SBT, and TG.

	T2DM/Control			AG/ AA		GG/AA		GG VS. AG+AA			GG+AG VS. AA		G VS. A	
Variables	AA	AG	GG	*P*	OR (95%CI)	*P*	OR (95%CI)	*P*	OR (95%CI)		*P*	OR (95%CI)	*P*	OR (95%CI)
BMI (kg/m^2)														
<18.5	3/12	2/8	1/4	1.000	1.000 (0.135–7.392)	1.000	1.000 (0.080–12.557)	1.000	1.000 (0.091–11.028)	1.000		1.000 (0.167–5.985)	1.000	1.000 (0.261–3.826)
18.5–23.9	82/116	88/92	20/24	0.145	1.353 (0.901–2.032)	0.624	1.179 (0.611–2.275)	0.952	1.020 (0.545–1.909)	0.161		1.317 (0.896–1.937)	0.276	1.176 (0.879–1.572)
24–27.9	83/91	92/76	23/14	0.192	1.327 (0.868–2.030)	0.113	1.801 (0.870–3.730)	0.206	1.568 (0.781–3.149)	0.103		1.401 (0.934–2.102)	0.071	1.327 (0.976–1.805)
28–31.9	32/23	31/13	5/3	0.209	1.714 (0.740–3.972)	0.817	1.198 (0.260–5.523)	0.949	0.952 (0.215–4.221)	0.237		1.617 (0.729–3.585)	0.365	1.340 (0.711–2.525)
≥32	6/3	9/1	1/1	0.236	4.500 (0.374–54.16)	0.661	0.500 (0.023–11.088)	0.385	0.267 (0.014–5.267)	0.382		2.500 (0.320–19.53)	0.798	1.222 (0.263–5.682)
GGT (U/L)														
<45	179/232	188/173	40/46	**0.018**	1.408 (1.060–1.871)	0.615	1.127 (0.707–1.797)	0.856	0.960 (0.614–1.500)	**0.029**		1.349 (1.031–1.766)	0.113	1.180 (0.961–1.448)
≥45	29/17	39/17	11/1	0.482	1.345 (0.589–3.073)	0.087	6.448 (0.764–54.417)	0.110	5.500 (0.682–44.383)	0.235		1.628 (0.728–3.644)	0.096	1.688 (0.911–3.127)
SBP (mmHg)														
<140	104/145	105/116	24/28	0.211	1.262 (0.876–1.818)	0.561	1.195 (0.655–2.179)	0.817	1.070 (0.602–1.902)	0.208		1.249 (0.883–1.766)	0.286	1.154 (0.887–1.501)
≥140	102/101	120/75	27/18	**0.024**	1.584 (1.063–2.360)	0.238	1.485 (0.770–2.865)	0.589	1.189 (0.634–2.229)	**0.020**		1.565 (1.072–2.285)	**0.046**	1.340 (1.006–1.786)
TG (mmol/L)														
<1.70	85/63	96/55	26/10	**0.029**	1.447 (1.039–2.015)	0.917	1.030 (0.591–1.797)	0.597	0.866 (0.508–1.477)	0.057		1.358 (0.991–1.860)	0.222	1.161 (0.914–.475)
≥1.70	122/186	130/137	25/37	0.278	1.294 (0.813–2.059)	0.107	1.927 (0.867–4.284)	0.177	1.695 (0.789–3.644)	0.145		1.391 (0.893–2.168)	0.086	1.343 (0.960–1.879)

95% CI: 95% confidence interval; OR: odds ratio; BMI: body mass index; GGT: glutamyl transpeptidase; SBP: systolic blood pressure; TG: triglycerides.

known to be associated with T2DM. Previous studies suggested that elevated TG,[23] GGT,[24] BMI,[25] and a family history of DM[26] are associated with T2DM risk. Although less well studied, elevated SBP may also be a risk factor for T2DM.[27] Borrell et al. reported that educational attainment was inversely associated with the prevalence of DM among certain racial/ethnic groups.[28] In the present study, similar results were obtained in a community-based population. To more clearly elucidate the relationship between *NPC1L1* rs2073547 and rs217386 and T2DM, the above independent risk factors were adjusted for to obtain more accurate estimations of the true effect.

NPC1L1 is associated with cholesterol metabolism, and *NPC1L1* variants were previously shown to be associated with dyslipidemia.[29–31] Naturally occurring inactivating mutations in *NPC1L1* were also reported to be linked with reduced plasma LDL-C levels and a lowered risk of coronary heart disease.[32] Additionally, Zhang et al. found that *NPC1L1* variants were associated with hepatitis C virus (HCV) infection and the biochemical characteristics of HCV-infected individuals in Yunnan, China.[33] Furthermore, a recent study conducted in Shanghai, China reported that the G allele of *NPC1L1* rs2072183 may be a risk factor for gallstone disease.[34] *In vitro* experiments also revealed that high concentrations of glucose resulted in increased expression of *NPC1L1* in cells which affected the transportation and metabolism of intestinal cholesterol.[35] However, little is known about the effect of *NPC1L1* variants on T2DM.

In the present study, we investigated the association between rs2073547 and rs217386 genotypes, rs2073547 alleles, and serum LDL-C levels. LDL-C levels were found not to be associated with rs2073547, indicating that the rs2073547 polymorphism of *NPC1L1* does not significantly affect blood LDL-C levels in the Chinese population. This finding has not previously been reported, so further research is required to confirm this. We also showed that the AG and GG+AG genotypes of *NPC1L1* rs2073547 were significantly associated with an increased T2DM risk (AG vs. AA: OR: 1.347, 95% CI: 1.019–1.791, P=0.015; GG+AG vs. AA: OR: 1.593, 95% CI: 1.179–2.152, P=0.002). These findings are inconsistent with the results of a meta-analysis carried out by Lotta et al. in populations of European ancestry where the rs2073547-G allele was associated with a lower risk of diabetes.[17] This previous study had a larger sample size and hence more statistical power than our own, but it is possible that racial heterogeneity between the studies may have caused the inconsistencies.

To our knowledge, our study is the first to investigate the association between *NPC1L1* polymorphisms rs2073547 and rs217386 and T2DM in a Chinese population. However, although we found significant associations with the AG and GG+AG rs2073547 genotypes and T2DM among certain subgroups in the stratified analysis, the underlying mechanisms remain unclear.

Nevertheless, our findings may provide a new insight into ezetimibe-based monotherapy or combination therapy in Chinese patients with dyslipidemia. Although the reduction of cardiovascular events by LDL-C-lowering drugs is believed to be beneficial despite increased risks of new-onset DM, our results suggest more focus on personalized and precision therapies is warranted to avoid some of the adverse effects of these drugs.

There are a number of limitations associated with our study. It had a cross-sectional design and was a single-center study, so the generalizability of the data to the entire Chinese population remains unknown. Moreover, only two *NPC1L1*

SNPs were investigated, and we did not implement a Mendelian randomization approach. Finally, the sample was rather small for stratified analysis, and the large number of sub-groups resulted in a wide range of 95% CIs for the OR. Additional, large-scale studies are therefore needed to extend our observations and clarify the association of *NPC1L1* SNPs with T2DM.

In conclusion, our study suggests a possible role for the *NPC1L1* rs2073547 polymorphism in increasing susceptibility to T2DM in the Chinese population. Our findings may provide a basis for future studies to reveal the mechanism underlying the association between *NPC1L1* inhibition and T2DM. Future clinical RCTs and SNP studies with larger samples are needed to confirm our findings among different ethnicities in the Chinese population.

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Declaration of conflicting interest

The authors declare that there is no conflict of interest.

Ethical approval and informed consent

The ethics committee of the First Affiliated Hospital of Guangxi Medical University approved the study. All participants provided written informed consent before the collection of any data or samples.

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