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Original Article

Association of the *VLDLR* rs3780181 SNP and serum lipid levels in the Jing and Han populations

Hui Gao, Rui-Xing Yin, Qing-Hui Zhang, Ling Qiu, Eksavang Khounphinith, Duo-Shun Wang, Kai-Guang Li

Department of Cardiology, Institute of Cardiovascular Diseases, The First Affiliated Hospital, Guangxi Medical University, Nanning, Guangxi, China

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Abstract: The very low-density lipoprotein receptor gene (*VLDLR*) rs3780181 single nucleotide polymorphism (SNP) has been associated with total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C) levels in a previous genome-wide association study, but little is known about such association of the *VLDLR* rs3780181 SNP and serum lipid profiles in the Chinese populations. The present study was to detect the association of the *VLDLR* rs3780181 SNP and several environmental factors with serum lipid levels in the Jing and Han populations. Genotyping of the *VLDLR* rs3780181 SNP in 707 subjects of Jing and 707 participants of Han peoples was performed by polymerase chain reaction and restriction fragment length polymorphism, and then confirmed by direct sequencing. The *VLDLR* rs3780181 G allele frequency was lower in Jing than in Han (*P*<0.05). The G allele carriers had lower serum TC levels in Jing, and lower LDL-C levels in Han than the G allele non-carriers. Subgroup analyses showed that the G allele carriers had lower TC levels in Jing females but not in Jing males and lower LDL-C and apolipoprotein B levels in Han females but not in Han males. Serum lipid parameters were also correlated with several environmental factors in the Jing and Han populations, or in males and females in both ethnic groups. The association of the *VLDLR* rs3780181 SNP and serum lipid levels was different between the Jing and Han populations. These trends of association suggest that this SNP might have racial/ethnic or gender specificity. The differences in the serum lipid profiles between the two ethnic groups might be partly attributed to the differences in this SNP and the SNP-environmental interactions.

Keywords: Very low density lipoprotein receptor gene, single nucleotide polymorphism, rs3780181, lipids, environmental factors

Introduction

It is well-established that mortality and morbidity occurring as a result of coronary artery disease (CAD) are a critical public health concern in most developed and developing countries [1]. Low-density lipoprotein cholesterol (LDL-C) [2], high-density lipoprotein cholesterol (HDL-C) [3], triglyceride (TG) [4], total cholesterol (TC) [5], apolipoprotein (Apo) B [6], ApoA1 and the ApoA1/ApoB ratio [7] are heritable, modifiable, risk factors for CAD, therefore traditionally monitored as predictors of dyslipidemia and also the main target for therapeutic intervention [8]. Dyslipidemia is well-recognized caused by various environmental and genetic factors [8, 9], and their interactions [10]. In twin and familial studies,almost 40%-70% of the interindividual variation in plasma lipid phenotypes can be explained by genetic polymorphisms [10-12]. Therefore, the understanding of the correlation of genetic variants and serum lipid levels can prove a way to explore prevention and treatment of CAD [7, 13].

In the past few years, genome-wide association studies (GWASes) have shown great success in identified numerous common genetic variants and respective proteins associated with plasma lipid and lipoprotein levels [7, 13-16], but many new loci do not include genes implicated in lipid biology by previous literature. It was reported that loci associated with blood lipids, accounting for ~10-12% of the total trait variance [7, 17], and variants with small effects can point to pathways and therapeutic targets that enable clinically-important changes in blood lipids [7]. We predict that more new loci involving in lipid metabolism will be found through GWAS.

The low density lipoprotein receptor (LDLR) gene family consists of cell surface proteins involved in receptor-mediated endocytosis of specific ligands. The very low density lipoprotein receptor gene (*VLDLR*; https://www.ncbi.nlm.nih.gov/gene/7436), located at chromosome 7q32, encodes a lipoprotein receptor that is a member of the LDLR family and plays important roles in VLDL-TG metabolism and the reelin signaling pathway. The human *VLDLR* contains 19 exons spanning approximately 40 kilobases. Its sequence is 94% identical in humans and rabbits and 84% identical in humans and chickens, implying a conserved function. The exon-intron organization of the gene is almost the same as that of the LDLR gene, except for an extra exon that encodes an additional repeat in the ligand binding domain of the VLDLR. Mutations in this gene cause *VLDLR*-associated cerebellar hypoplasia. Alternative splicing generates multiple transcript variants encoding distinct isoforms for this gene. Yet research on the function of *VLDLR* is at the initial stage, and its functional role is still unclear. *VLDLR* is expressed primarily in muscle and adipose tissue. Scientists had long assumed that the *VLDLR* plays a crucial role in the metabolism of lipoproteins that contain ApoE [18]. The exon-intron organization of the gene is also completely conserved between mouse and human.Researchers kept balb/c mice from eating for periods of time, they found than *VLDLR* expression increased in mouse heart [19]. In contrast, *VLDLR* expression decreased progressively with fasting in membranes from epididymal fat [19]. The *VLDLR* mRNA expression was down-regulated by feeding atherogenic diet in heart and skeletal muscle in LDLR-/- mice. In contrast, *VLDLR* mRNA expression was up-regulated by atherogenic diet in adipose tissue [20].

A previous study demonstrated that *VLDLR* participate the body's hypertriglyceridemia metabolism which was required for normal lipoprotein lipase regulation *in vivo* [21]. However, others opposed it, and on the contrary, their research suggested that the *VLDLR* of the mouse is not required to maintain normal levels of plasma TG or cholesterol [22]. Further work is now required to enhance our understanding of the relationship between *VLDLR* and lipid metabolism.

In 2012, a large-scale gene-centric meta-analysis across 32 studies revealed that a locus (rs7024888) in *VLDLR* associated with LDL-C [23]. A recently study has identified 157 loci associated with lipid levels at $P<5\times10^{-8}$ which has mentioned that the *VLDLR* rs3780181 SNP was associated with TC and LDL-C levels for the first time [13]. However, the biological function of the *VLDLR* rs3780181 SNP on serum lipid metabolism remains unclear. Importantly, the genetic variation has different magnitudes of effect in the different ethnicities but until now no GWAS has comprehensively investigated the genetic determinants of serum lipid levels in the Chinese populations. Therefore, it would be necessary to characterize the relationship between the *VLDLR* rs3780181 SNP and serum lipid levels in the Chinese populations.

China is a multiethnic country of 56 ethnic groups; the custom of every ethnic group is not identical. Han is the dominant ethnic group and Jing is a native minority existing 28199 people among the 55 minority groups according to the sixth national census statistics of China in 2010. Most of them live in the so called "Three Islands of Jing Nationality", Dongxing City, Guangxi Zhuang Autonomous Region, People's Republic of China [24]. Jing is mainly engaged in coastal fisheries. Jing is unique in Chinese ethnic minorities living in the nation of the sea; the way of life is single. It has a lot of differences between Jing and Han nationalities in diet custom and culture characteristic [24]. The Jing nationality has become a valuable subgroup for use in population genetic studies. However, there were no studies to examine the association of the *VLDLR* rs3780181 SNP and serum lipid levels in this population. Thus, the present study was to detect the association of the *VLDLR* rs3780181 SNP and serum lipid levels in the Jing and Han populations.

Materials and methods

Subjects

The study populations included 707 unrelated subjects (276 males, 39.0% and 431 females, 61.0%) of Han and 707 unrelated participants (278 males, 39.3% and 429 females, 60.7%) of Jing. They were randomly selected from our previous stratified randomized samples [24]. All participants were agricultural (Han) and/or fishery (Jing) workers from Jiangping Town, Dongxing City, Guangxi Zhuang Autonomous Region, People's Republic of China. The ages of the par-

ticipants ranged from 27 to 92 years. The mean age of Jing participants was 57.02±13.53 years, whereas that of Han subjects was 57.03± 13.08 years. All participants were healthy and had no evidence of diseases related to atherosclerosis, CAD and diabetes. None of them were using lipid-lowering medication. The present study was approved by the Ethics Committee of the First Affiliated Hospital, Guangxi Medical University (No: Lunshen-2011-KY-Guoji-001; Mar. 7, 2011). Informed consent was obtained from all participants.

Epidemiological survey

The survey was carried out using internationally standardized methods [25]. A standard questionnaire collecting the information on demographics, socioeconomic status, and lifestyle factors was obtained from all the subjects. The alcohol information included questions about the number of liangs (about 50 g) of rice wine, corn wine, rum, beer, or liquor consumed during the preceding 12 months. Alcohol consumption was classified as groups of grams of alcohol per day: <25 and ≥25. Smoking status was categorized into groups of cigarettes per day: <20 and ≥20. In the physical examination, several parameters such as blood pressure, height, weight, waist circumference were measured, and body mass index (BMI, kg/m^2) was calculated from the height and weight measurements. The methods of measuring above parameters were referred to a previous study [26].

Biochemical parameter

A fasting venous blood sample of 5 ml was drawn from the participants after an overnight (at least 12 hours) fast. A part of the sample (2 mL) was collected into glass tubes and allowed to clot at room temperature, and used to determine serum lipid levels. Another part of the sample (3 mL) was transferred to tubes with anticoagulate solution (4.80 g/L citric acid, 14.70 g/L glucose, and 13.20 g/L tri-sodium citrate) and used to extract DNA. The levels of TC, TG, HDL-C, and LDL-C in the samples were determined by enzymatic methods with commercially available kits [27]. Serum ApoA1 and ApoB concentrations were quantified by the immunoturbidimetric immunoassay using a commercial kit [27, 28]. Fasting blood glucose was determined by glucose meter. All determinations were performed with an autoanalyzer (Type 7170A; Hitachi Ltd., Tokyo, Japan) in the Clinical Science Experiment Center of the First Affiliated Hospital, Guangxi Medical University [29].

DNA amplification and genotyping

Total genomic DNA of the samples was isolated from peripheral blood leukocytes according to a standard phenol-chloroform method [30, 31]. The extracted DNA was stored at -20°C until analysis. Genotyping of the *VLDLR* rs3780181 SNP was performed by polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP). PCR amplification was performed using 5'-TTGAGTCAGTAGAGGCCTGC-3' and 5'-GCTGTGCCAACCTATGCTAC-3' (Sangon, Shanghai, People's Republic of China) as the forward and reverse primer pairs; respectively. Each amplification reaction was performed in a total volume of 25 μL, containing 10× PCR buffer (1.8 mM $MgCl_2$) 2.5 μL, 1 U *Taq* polymerase, 2.5 mmol/L of each dNTP (Tiangen, Beijing, People's Republic of China) 2.0 μL, 20 pmol/L of each primer and 50 ng of genomic DNA, processing started with 95°C for 7 min and followed by 40 s of denaturing at 95°C, 40 s of annealing at 57°C and 1 min of elongation at 72°C for 30 cycles. The amplification was completed by a final extension at 72°C for 7 min. Then 10 U of *Tas*I enzyme was added directly to the PCR products (10 μL) and digested at 65°C overnight. After restriction enzyme digestion of the amplified DNA, the genotypes were identified by electrophoresis on 2% agarose gels and visualized with ethidium-bromide staining ultraviolet illumination. The length of each digested DNA fragment was determined by comparing migration of a sample with that of standard DNA marker. Genotypes were scored by an experienced reader blinded to epidemiological data and serum lipid levels. Six samples (AA, AG and GG genotypes in two; respectively) detected by the PCR-RFLP were also confirmed by direct sequencing. The PCR products were purified by low melting point gel electrophoresis and phenol extraction, and then the DNA sequences were analyzed in Shanghai Sangon Biological Engineering Technology & Services Co., Ltd., People's Republic of China.

Diagnostic criteria

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

Table 1. Comparison of demography, lifestyle and serum lipid levels between the Jing and Han Chinese

Parameter	Han	Jing	*t* (x^2)	*P*
Number	707	707		
Male/female	276/431	278/429	0.012	0.913
Age (year)	57.03±13.08	57.02±13.53	-0.018	0.986
Height (cm)	156.12±7.82	157.02±7.71	2.174	0.030
Weight (kg)	55.50±9.35	58.04±9.97	4.947	0.000
Body mass index (kg/m²)	22.73±3.22	23.47±3.24	4.329	0.000
Waist circumference (cm)	77.15±8.99	80.22±9.19	6.355	0.000
Cigarette smoking [n (%)]				
Non-smoker	600 (84.9)	613 (86.7)		
<20 cigarettes/day	27 (3.8)	21 (3.0)		
≥20 cigarettes/day	80 (11.3)	73 (10.3)	1.210	0.546
Alcohol consumption [n (%)]				
Non-drinker	593 (83.9)	626 (88.5)		
<25 g/day	26 (3.7)	46 (6.5)		
≥25 g/day	88 (12.4)	35 (5.0)	29.286	0.000
Systolic BP (mmHg)	129.84±19.95	131.88±21.92	1.837	0.066
Diastolic BP (mmHg)	80.13±10.49	80.46±10.60	0.581	0.561
Pulse pressure (mmHg)	49.71±15.71	51.43±17.51	1.946	0.052
Glucose (mmol/L)	6.64±1.08	6.87±1.88	2.788	0.006
Total cholesterol (mmol/L)	4.93±0.86	5.08±0.93	3.104	0.002
Triglyceride (mmol/L)	1.31 (0.62)	1.42 (0.74)	-3.508	0.000
HDL-C (mmol/L)	1.79±0.52	1.79±0.45	-0.366	0.715
LDL-C (mmol/L)	2.81±0.46	2.82±0.44	0.424	0.672
Apolipoprotein (Apo) A1 (g/L)	1.32±0.20	1.29±0.23	-1.670	0.095
ApoB (g/L)	1.04±0.25	1.06±0.25	1.601	0.110
ApoA1/ApoB	1.34±0.37	1.29±0.38	-2.421	0.016

TC, total cholesterol; TG, triglyceride; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; ApoA1, apolipoprotein A1; ApoB, apolipoprotein B. The quantitative variables were presented as mean ± standard deviation and their difference between the groups was determined by the *t*-test. The values of triglyceride werepresented as median (interquartile range), and the difference between the groups was determined by the Wilcoxon-Mann-Whitney test. The difference in percentage of cigarette smoking and alcohol consumption between the groups was determined by Chi-square-test.

ApoA1 to ApoB in our Clinical Science Experiment Center were 3.10-5.17, 0.56-1.70, 1.16-1.42, 2.70-3.10 mmol/L, 1.20-1.60, 0.80-1.05 g/L, and 1.00-2.50; respectively. The individuals with TC>5.17 mmol/L and/or TG>1.70 mmol/L were defined as hyperlipidemic [32, 33]. Hypertension was assessed according to the criteria outlined by the 1999 World Health Organization-International Society of Hypertension Guidelines for the management of hypertension [34]. The diagnostic criteria of overweight and obesity were according to the Cooperative Meta-analysis Group of China Obesity Task Force. Normal weight, overweight and obesity were defined as a BMI<24, 24-28, and >28 kg/m²; respectively [35].

Statistical analyses

Epidemiological data were recorded on a pre-designed form and managed with Excel software. Data analysis was performed using the software SPSS version 16.0 (SPSS Inc., Chicago, Illinois). Qualitative variables are expressed as raw counts and percentages. Quantitative variables are presented as the mean ± standard deviation, except serum TG levels, which were presented as medians and interquartile ranges. Allele frequency was determined via direct counting, and the standard goodness-of-fit test was used to test the Hardy-Weinberg equilibrium. Difference in genotype distribution between the groups was obtained using the chi-square test. The difference in general characteristics between Jing and Han was tested by the Student's unpaired *t*-test. The association between 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. In order to evaluate the association of serum lipid levels and genotypes (AA=1, AG/GG=2) or several environmental factors, multivariable linear regression analysis with stepwise modeling was also performed in the combined population of Jing and Han, Jing, Han, males, and females; respectively. A *P* value (two-tailed) of less than 0.05 was considered statistically significant.

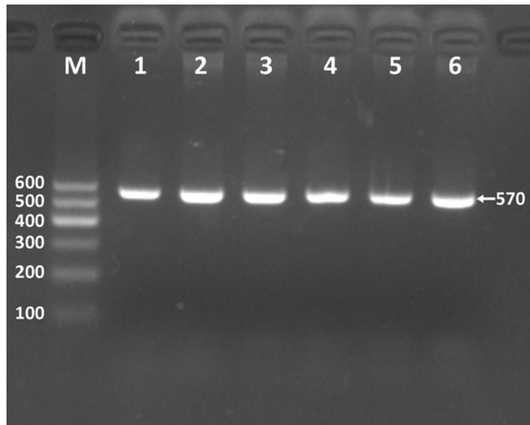

Figure 1. Electrophoresis of PCR products of the samples. Lane M, 100 bp marker ladder; lanes 1-6, samples. The 570 bp bands are the target genes.

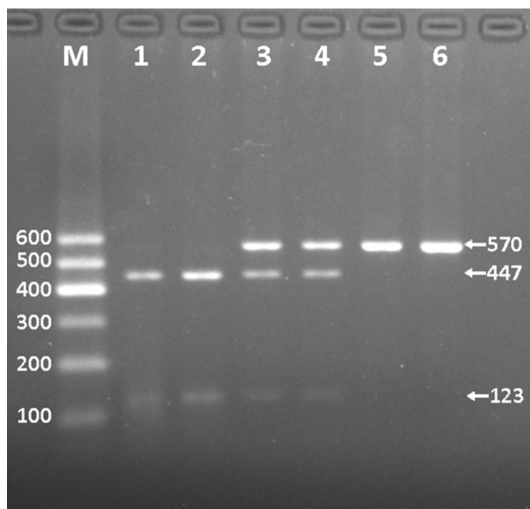

Figure 2. Genotyping of the *VLDLR* rs3780181 SNP. Lane M is the 100 bp Marker Ladder; lanes 1 and 2, AA genotype (447- and 123-bp); lanes 3 and 4, AG genotype (123-, 447- and 570-bp); and lanes 5 and 6, GG genotype (570-bp).

Results

General and biochemical characteristics of the subjects

Table 1 shows the general characteristics and serum lipid levels between the Jing and Han populations. The levels of height, weight, waist circumference, BMI, TC, TG, glucose were higher in Jing than in Han, but theratioof ApoA1/ApoB, the percentages of subjects consuming alcohol were lower in Jing than in Han (P<0.05-0.001). The values of gender ratio, age, systolic blood pressure, diastolic blood pressure, pulse pressure, HDL-C, LDL-C, ApoA1, ApoB and the percentages of smoking were not different between the two ethnic groups (P>0.05 for all).

Results of genotyping

After the genomic DNA of the samples was amplified by PCR and imaged by 2% agarose gel electrophoresis, the purpose gene of 570-bp nucleotide sequences were seen in all samples (**Figure 1**). The genotypes identified were named according to the presence or absence of the enzyme restriction sites. The absence of the cutting site indicates the G allele; while its presence indicates the A allele (can be cut). AA genotype is homozygote for the presence of the site (123- and 447-bp), AG genotype is heterozygote for the presence and absence of the site (123-, 447- and 570-bp), and GG genotype is homozygote for the absence of the site (570 bp; **Figure 2**).

Nucleotide sequences

The results were separated into AA, AG and GG genotypes of the rs3780181 SNP by PCR-RFLP and the genotypes were further confirmed by direct sequencing (**Figure 3**); respectively.

Genotypic and allelic frequencies

The genotypic and allelic frequencies of the rs3780181 SNP in the both ethnic groups are shown in **Table 2.** The genotypic distribution was followed Hardy-Weinberg equilibrium (HWE). The allele frequency but not genotypic frequency of the *VLDLR* rs3780181 SNP was different between Jing and Han (P<0.05), the frequency of minor G allele was lower in Jing than in Han. The genotypic frequency but not allele frequency of the *VLDLR* rs3780181 SNP was different between males and females in Jing but not in Han (P<0.05). The genotype and allele frequencies of the *VLDLR* rs3780181 SNP were not different between Han males and females (P<0.05).

Genotypes and serum lipid levels

As shown in **Tables 3** and **4**, the levels of TC were different between the AA and AG/GG genotypes in Jing (P<0.001) but not in Han, the G allele carriers had lower TC levels than the G allele non-carriers. The levels of LDL-C in Han but not in Jing were different between the AA

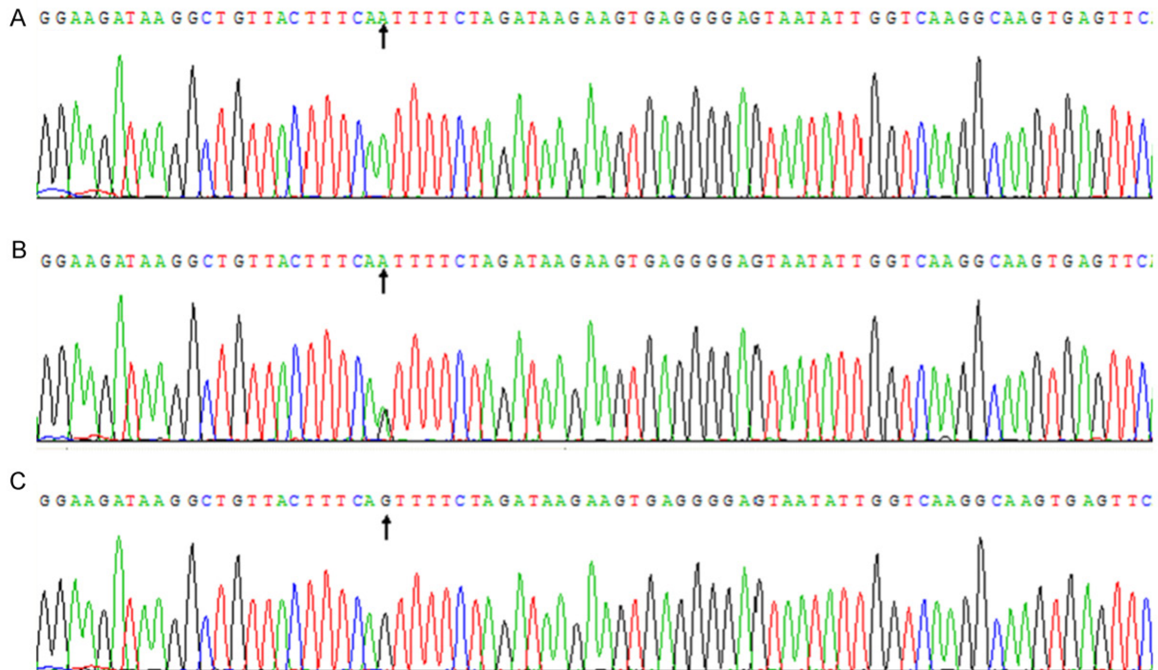

Figure 3. A part of the *VLDLR* rs3780181 SNP sequence. A. AA genotype; B. AG genotype; and C. GG genotype.

Table 2. Comparison of the genotype and allele frequencies of the *VLDLR* rs3780181 SNP in the Han and Jing populations [n (%)]

Group	n	Genotype			Allele	
		AA	AA	GG	A	G
Han	707	566 (80.1)	133 (18.8)	8 (1.1)	1265 (89.5)	149 (10.5)
Jing	707	594 (84.0)	109 (15.4)	4 (0.6)	1297 (91.7)	117 (8.3)
x^2			4.389		4.249	
P			0.111		0.039	
Han						
Male	276	228 (82.6)	44 (15.9)	4 (1.4)	500 (90.6)	52 (9.4)
Female	431	338 (78.4)	89 (20.6)	4 (0.9)	765 (88.7)	97 (11.3)
x^2			2.840		1.199	
P			0.229		0.274	
Jing						
Male	278	235 (84.5)	39 (14.0)	4 (1.6)	509 (91.5)	47 (8.5)
Female	429	359 (83.7)	70 (16.3)	0 (0)	788 (91.8)	70 (8.2)
x^2			6.240		0.039	
P			0.034		0.844	

and AG/GG genotypes (*P*<0.001), the G allele carriers had lower LDL-C levels than the G allele non-carriers. In the subgroup analyses, the G allele carriers in Han females but not in Han males had lower LDL-C and ApoB levels than the G allele non-carriers (*P*<0.05). The G allele carriers in Jing females but not in Jing males had lower TC levels than the G allele non-carriers (*P*<0.001). There was no significant difference in the remaining serum lipid parameters bet-ween the genotypes in Jing, Han, males, or females (*P*>0.05 for all).

Risk factors for serum lipid parameters

The risk factors for serum lipid parameters in Jing and Han are shown in **Tables 5** and **6**. Multiple linear regression analyses showed that serum TC and LDL-C levels in Jing and Han, LDL-C levels in Han, and TC levels in Jing were correlated with genotypes (*P*<0.05), respectively. When serum lipid data were analyzed according to gender, LDL-C and ApoB levels in Han, and TC levels in Jing were associated with the genotypes only in females but not in males. Several environmental factors such as age, gender, height, weight, waist circumference, alcohol consumption and cigarette smok-

Table 3. Comparison of the genotypes and serum lipid levels in the Han and Jing populations

Genotype	n	TC (mmol/L)	TG (mmol/L)	HDL-C (mmol/L)	LDL-C (mmol/L)	ApoA1 (g/L)	ApoB (g/L)	ApoA1/ApoB
Han								
AA	566	4.92±0.89	1.32 (0.63)	1.80±0.56	2.86±0.45	1.32±0.20	1.04±0.25	1.34±0.38
AG/GG	141	4.95±0.73	1.29 (0.62)	1.79±0.35	2.57±0.44	1.30±0.20	1.01±0.23	1.34±0.36
F		0.047	-0.527	0.133	60.464	1.496	1.208	0.001
P		0.829	0.598	0.715	0.000	0.222	0.272	0.972
Jing								
AA	594	5.14±0.94	1.42 (0.76)	1.79±0.45	2.82±0.45	1.30±0.22	1.06±0.25	1.29±0.37
AG/GG	113	4.74±0.85	1.44 (0.66)	1.76±0.41	2.80±0.40	1.30±0.29	1.06±0.26	1.29±0.39
F		16.631	-0.485	1.061	0.016	0.041	0.457	0.501
P		0.000	0.628	0.303	0.900	0.840	0.499	0.479

TC, total cholesterol; TG, triglyceride; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein; ApoA1, apolipoprotein A1; ApoB, apolipoprotein B; ApoA1/ApoB, the ratio of apolipoprotein A1 to apolipoprotein B. The value of triglyceride was presented as median (interquartile range); the difference between the genotypes was determined by the Wilcoxon-Mann-Whitney test.

Table 4. Comparison of the genotypes and serum lipid levels between males and females in the Han and Jing populations

Ethnic/Genotype	n	TC (mmol/L)	TG (mmol/L)	HDL-C (mmol/L)	LDL-C (mmol/L)	ApoA1 (g/L)	ApoB (g/L)	ApoA1/ApoB
Han/male								
AA	228	4.82±0.89	1.33 (0.70)	1.69±0.57	2.84±0.44	1.32±0.19	1.04±0.24	1.34±0.40
AG/GG	48	4.93±0.75	1.27 (0.65)	1.78±0.37	2.89±0.39	1.30±0.24	1.08±0.22	1.26±0.35
F		0.945	-1.254	1.717	0.122	0.593	1.326	2.808
P		0.390	0.210	0.191	0.727	0.442	0.251	0.095
Han/female								
AA	338	4.99±0.89	1.30 (0.61)	1.86±0.54	2.88±0.45	1.33±0.21	1.04±0.26	1.34±0.36
AG/GG	93	4.97±0.72	1.35 (0.61)	1.80±0.35	2.41±0.37	1.30±0.19	0.98±0.23	1.39±0.35
F		1.816	-0.289	1.813	96.528	1.055	4.874	1.638
P		0.164	0.773	0.179	0.000	0.305	0.028	0.201
Jing/male								
AA	235	5.13±0.87	1.50 (0.89)	1.73±0.46	2.82±0.38	1.28±0.23	1.07±0.23	1.26±0.39
AG/GG	43	4.90±0.75	1.52 (1.59)	1.66±0.39	2.72±0.40	1.25±0.20	1.09±0.24	1.22±0.34
F		2.835	-0.942	1.506	2.496	1.375	0.620	1.493
P		0.093	0.346	0.221	0.115	0.242	0.432	0.223
Jing/female								
AA	359	5.15±0.98	1.40 (0.66)	1.83±0.46	2.82±0.49	1.31±0.21	1.05±0.25	1.31±0.36
AG/GG	70	4.63±0.89	1.37 (0.51)	1.82±0.41	2.84±0.40	1.33±0.34	1.05±0.26	1.33±0.41
F		13.767	-0.004	0.173	1.065	0.219	0.126	0.005
P		0.000	0.997	0.678	0.303	0.640	0.723	0.946

TC, total cholesterol; TG, triglyceride; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein; ApoA1, apolipoprotein A1; ApoB, apolipoprotein B; ApoA1/ApoB, the ratio of apolipoprotein A1 to apolipoprotein B. The value of triglyceride was presented as median (interquartile range); the difference between the genotypes was determined by the Wilcoxon-Mann-Whitney test.

ing, and traditional cardiovascular risk factors such as BMI, fasting blood glucose and blood pressure levels were also correlated with serum lipid parameters in the Han and Jing populations and in males and females of both ethnic groups ($P<0.05$, **Tables 5** and **6**).

Table 5. The risk factors for serum lipid parameters in the Han and Jing populations

Lipid	Risk factor	B	Std.error	Beta	*t*	*P*
Han and Jing						
TG	Waist circumference	0.037	0.005	0.383	7.613	0.000
	Cigarette smoking	0.312	0.037	0.221	8.403	0.000
	Glucose	0.089	0.015	0.153	6.110	0.000
	Height	-0.020	0.003	-0.173	-5.780	0.000
	Systolic blood pressure	0.007	0.002	0.080	3.141	0.002
	Age	-0.005	0.002	-0.075	-2.915	0.004
	Body mass index	-0.026	0.013	-0.096	-1.962	0.050
TC	Glucose	0.115	0.015	0.196	7.460	0.000
	Age	0.009	0.002	0.135	4.753	0.000
	Height	-0.012	0.003	-0.100	-3.791	0.000
	Genotype	-0.173	0.060	-0.073	-2.870	0.004
	Pulse pressure	-0.011	0.003	-0.208	-3.975	0.000
	Systolic blood pressure	0.006	0.002	0.144	2.783	0.005
HDL-C	Waist circumference	-0.015	0.001	-0.286	-10.891	0.000
	Gender	0.199	0.039	0.200	5.175	0.000
	Alcohol consumption	0.108	0.024	0.131	4.486	0.000
	Cigarette smoking	-0.051	0.023	-0.067	-2.216	0.027
	Height	0.007	0.002	0.109	3.078	0.002
	Age	0.003	0.001	0.069	2.467	0.014
LDL-C	Genotype	-0.162	0.030	-0.138	-5.323	0.000
	Glucose	0.030	0.008	0.101	3.836	0.000
	Age	0.003	0.001	0.098	3.710	0.000
	Systolic blood pressure	0.003	0.001	0.079	3.024	0.003
ApoA1	Weight	-0.005	0.001	-0.207	-7.644	0.000
	Alcohol consumption	0.053	0.010	0.144	5.443	0.000
	Glucose	-0.013	0.004	-0.095	-3.636	0.000
	Systolic blood pressure	0.001	0.001	0.053	1.995	0.046
ApoB	Waist circumference	0.006	0.001	0.236	8.911	0.000
	Age	0.002	0.000	0.121	4.640	0.000
	Height	-0.002	0.001	-0.075	-2.797	0.005
ApoA1/ApoB	Waist circumference	-0.008	0.002	-0.198	-3.785	0.000
	Age	-0.002	0.001	-0.064	-2.278	0.023
	Alcohol consumption	0.071	0.018	0.112	3.974	0.000
	Glucose	-0.017	0.006	-0.070	-2.715	0.007
	Body mass index	0.068	0.028	0.587	2.379	0.017
	Gender	0.082	0.028	0.106	2.892	0.004
	Height	0.027	0.009	0.565	3.199	0.001
	Weight	-0.033	0.012	-0.853	-2.822	0.005
Han						
TG	Waist circumference	0.027	0.004	0.280	7.719	0.000
	Cigarette smoking	0.261	0.052	0.194	5.058	0.000
	Glucose	0.129	0.029	0.159	4.389	0.000
	Height	-0.013	0.004	-0.120	-3.058	0.002
	Pulse pressure	-0.004	0.002	-0.081	-2.205	0.028
TC	Glucose	0.228	0.029	0.284	7.891	0.000
	Gender	0.168	0.064	0.095	2.638	0.009

HDL-C	Waist circumference	-0.015	0.002	-0.251	-6.663	0.000
	Alcohol consumption	0.100	0.035	0.129	2.883	0.004
	Gender	0.201	0.056	0.187	3.574	0.000
	Cigarette smoking	-0.103	0.036	-0.128	-2.895	0.004
	Systolic blood pressure	0.004	0.002	0.077	2.084	0.038
	Height	0.007	0.003	0.103	2.173	0.030
LDL-C	Glucose	0.085	0.015	0.199	5.573	0.000
	Genotype	-0.310	0.041	-0.269	-7.585	0.000
	Systolic blood pressure	0.005	0.002	0.104	2.911	0.004
ApoA1	Alcohol consumption	0.069	0.013	0.231	5.419	0.000
	Body mass index	-0.011	0.002	-0.183	-5.018	0.000
	Gender	0.054	0.018	0.131	3.079	0.002
ApoB	Waist circumference	0.006	0.001	0.235	6.277	0.000
	Systolic blood pressure	0.001	0.000	0.120	3.248	0.001
	Height	-0.005	0.001	-0.151	-3.191	0.001
	Gender	-0.048	0.024	-0.095	-2.029	0.043
ApoA1/ApoB	Waist circumference	-0.012	0.002	-0.286	-7.878	0.000
	Alcohol consumption	0.057	0.020	0.102	2.853	0.004
	Glucose	-0.040	0.013	-0.115	-3.143	0.002
	Systolic blood pressure	-0.001	0.001	-0.076	-2.040	0.042
Jing						
TG	Waist circumference	0.033	0.004	0.331	8.841	0.000
	Cigarette smoking	0.381	0.054	0.258	7.108	0.000
	Glucose	0.071	0.017	0.146	4.230	0.000
	Height	-0.019	0.004	-0.161	-4.256	0.000
TC	Age	0.019	0.003	0.272	6.867	0.000
	Glucose	0.086	0.018	0.171	4.718	0.000
	Genotype	-0.376	0.090	-0.147	-4.160	0.000
	Pulse pressure	-0.008	0.002	-0.148	-3.822	0.000
	Body mass index	0.074	0.018	0.256	4.087	0.000
	Waist circumference	-0.018	0.007	-0.172	-2.690	0.007
	Cigarette smoking	0.204	0.061	0.136	3.330	0.001
	Gender	0.184	0.081	0.096	2.264	0.024
HDL-C	Waist circumference	-0.016	0.002	-0.336	-9.499	0.000
	Alcohol consumption	0.155	0.035	0.169	4.457	0.000
	Gender	0.125	0.035	0.137	3.600	0.000
LDL-C	Age	0.004	0.001	0.127	3.379	0.001
	Glucose	0.019	0.009	0.080	2.121	0.034
ApoA1	Weight	-0.004	0.001	-0.184	-4.886	0.000
	Glucose	-0.012	0.005	-0.100	-2.692	0.007
	Alcohol consumption	0.039	0.018	0.081	2.170	0.030
ApoB	Body mass index	0.016	0.003	0.212	5.813	0.000
	Age	0.003	0.001	0.149	4.075	0.000
ApoA1/ApoB	Waist circumference	-0.011	0.001	-0.269	-7.391	0.000
	Age	-0.003	0.001	-0.113	-3.118	0.002
	Alcohol consumption	0.063	0.028	0.081	2.238	0.026

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.

Table 6. Relationship between serum lipid parameters and relative factors in the males and females of the Han and Jing populations

Lipid	Risk factor	B	Std.error	Beta	*t*	*P*
Jing/male						
TC	Waist circumference	0.045	0.006	0.408	7.322	0.000
	Cigarette smoking	0.263	0.069		3.821	0.000
	Height	-0.044	0.010	-0.264	-4.532	0.000
	Age	-0.019	0.005	-0.245	-4.131	0.000
	Glucose	0.102	0.027	0.203	3.822	0.000
TG	Height	-0.015	0.009	-0.115	-1.690	0.092
	Pulse pressure	-0.015	0.003	-0.288	-4.634	0.000
	Body mass index	0.126	0.034	0.462	3.771	0.000
	Age	0.014	0.004	0.241	3.595	0.000
	Cigarette smoking	0.126	0.059	0.128	2.142	0.033
	Glucose	0.071	0.023	0.181	3.061	0.002
	Waist circumference	-0.028	0.011	-0.326	-2.463	0.014
	Alcohol consumption	0.151	0.070	0.123	2.165	0.031
LDL-C	Pulse pressure	-0.005	0.001	-0.193	-3.157	0.002
	Height	-0.009	0.004	-0.153	-2.501	0.013
	Body mass index	0.018	0.007	0.144	2.453	0.015
HDL-C	Waist circumference	-0.017	0.002	-0.369	-6.714	0.000
	Alcohol consumption	0.165	0.036	0.254	4.619	0.000
ApoA1	Waist circumference	-0.006	0.001	-0.244	-4.188	0.000
	Alcohol consumption	0.055	0.019	0.169	2.905	0.004
ApoB	Body mass index	0.020	0.004	0.273	4.715	0.000
ApoA1/ApoB	Waist circumference	-0.013	0.002	-0.328	-5.779	0.000
	Alcohol consumption	0.096	0.032	0.172	3.032	0.003
Jing/female						
TG	Waist circumference	0.026	0.004	0.290	6.248	0.000
	Cigarette smoking	1.138	0.323	0.160	3.526	0.000
	Height	-0.025	0.006	-0.190	-4.077	0.000
	Glucose	0.051	0.022	0.108	2.366	0.018
TC	Pulse pressure	-0.006	0.003	-0.117	-2.395	0.017
	Age	0.021	0.004	0.272	5.610	0.000
	Glucose	0.135	0.028	0.221	4.852	0.000
	Genotype	-0.456	0.120	-0.171	-3.791	0.000
LDL-C	Age	0.008	0.002	0.212	4.521	0.000
	Glucose	0.039	0.014	0.133	2.846	0.005
HDL-C	Waist circumference	-0.017	0.002	-0.332	-7.037	0.000
	Diastolic blood pressure	0.004	0.002	0.095	2.011	0.045
ApoA1	Body mass index	-0.010	0.003	-0.140	-2.915	0.004
ApoB	Body mass index	0.013	0.004	0.175	3.725	0.000
	Age	0.004	0.001	0.196	4.190	0.000
ApoA1/ApoB	Body mass index	-0.025	0.005	-0.224	-4.779	0.000
	Age	-0.004	0.001	-0.139	-2.977	0.003
Han/male						
TG	Waist circumference	0.050	0.012	0.419	4.062	0.000
	Cigarette smoking	0.217	0.064	0.196	3.380	0.001
	Age	-0.018	0.005	-0.239	-3.790	0.000

	Glucose	0.166	0.050	0.192	3.333	0.001
	Weight	-0.032	0.012	-0.280	-2.618	0.009
	Diastolic blood pressure	0.013	0.005	0.133	2.349	0.020
TC	Glucose	0.243	0.043	0.320	5.654	0.000
	Diastolic blood pressure	0.013	0.005	0.153	2.703	0.007
LDL-C	Glucose	0.101	0.022	0.267	4.630	0.000
	Diastolic blood pressure	0.006	0.002	0.147	2.542	0.012
HDL-C	Waist circumference	-0.014	0.004	-0.212	-3.551	0.000
	Diastolic blood pressure	0.008	0.003	0.154	2.576	0.011
ApoA1	Waist circumference	-0.006	0.001	-0.244	-4.307	0.000
	Alcohol consumption	0.074	0.013	0.335	5.906	0.000
ApoB	Waist circumference	0.006	0.002	0.220	3.792	0.000
	Systolic blood pressure	0.002	0.001	0.160	2.703	0.007
	Glucose	0.024	0.012	0.118	2.006	0.046
ApoA1/ApoB	Waist circumference	-0.013	0.003	-0.276	-4.824	0.000
	Alcohol consumption	0.092	0.025	0.214	3.710	0.000
	Age	0.004	0.002	0.147	2.417	0.016
	Systolic blood pressure	-0.004	0.001	-0.189	-3.150	0.002
	Glucose	-0.042	0.020	-0.123	-2.096	0.037
Han/female						
TG	Waist circumference	0.024	0.004	0.288	6.294	0.000
	Glucose	0.115	0.035	0.150	3.282	0.001
TC	Glucose	0.216	0.039	0.260	5.575	0.000
LDL-C	Age	0.006	0.002	0.152	3.443	0.001
	Glucose	0.058	0.020	0.126	2.849	0.005
	Genotype	-0.489	0.050	-0.423	-9.869	0.000
HDL-C	Body mass index	-0.036	0.007	-0.242	-5.160	0.000
ApoA1	Body mass index	-0.011	0.003	-0.192	-3.974	0.000
	Glucose	-0.023	0.009	-0.118	-2.453	0.015
	Diastolic blood pressure	0.002	0.001	0.105	2.131	0.034
ApoB	Age	0.003	0.001	0.167	3.519	0.000
	Waist circumference	0.007	0.001	0.252	5.504	0.000
	Height	-0.006	0.002	-0.154	-3.205	0.001
	Genotype	-0.059	0.028	-0.097	-2.134	0.033
ApoA1/ApoB	Body mass index	-0.032	0.005	-0.299	-6.603	0.000
	Age	-0.004	0.001	-0.158	-3.413	0.001
	Glucose	-0.034	0.016	-0.095	-2.043	0.042

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.

Discussion

CAD, one of the most common cardiovascular disease, is associated with high morbidity and mortality and remains one of the most common causes of death globally [36-38]. It is well recognized that dyslipidemia is a multifactorial and complicated origin which combined by genetic factors with environmental factors and is a major risk factor for CAD [39]. Thus, human genetic studies of lipid levels can provide new therapies to discover lipid-lowering medicines target which is a necessary for comprehensive prevention of CAD [7, 40]. Recently, large numbers of new candidate genes were claimed to be related to dyslipidemia by GWASes mainly in European population and replicating these results in independent populations have generally been necessary. Jing nationality is a relatively conservative and isolated minority in

China that retains its regional and special customs [24]. Strict intra-ethnic marriages have been performed in this population from time immemorial. Intermarriage is not accepted among the Jing and they seldom marry with someone with the same last name. All marriages were monogamous among Jing and cross-cousin marriage is also strictly forbidden [24]. Therefore, we hypothesized that the hereditary characteristics and genotypes of some lipid metabolism-related genes in this population may be different from those in Han Chinese.

The genotypic and allelic frequencies of the *VLDLR* rs3780181 SNP in diverse racial/ethnic groups are inconsistent. According to the 1000 genomes project data, the frequency of the rs3780181 G allele was 7.77% in Han Chinese from Beijing, 7.62% in Southern Han Chinese, 11.54% in Japanese from Tokyo, 6.59% in British in England and Scotland, 4.04% in Finnish in Finland, 5.14% in Iberian population in Spain. However, in African ancestry, the frequency of the rs3780181 G allele was 21.31% in Americans of African ancestry in SW USA, 19.70% of Esan in Nigeria, 23.89% in Gambian in Western Divisions in the Gambia. Apparently, the minor G allele frequency was lower in the Western than African ancestry populations. In the present study, we showed that the *VLDLR* rs3780181 G allele frequency was lower in Jing than in Han (8.3% *vs*. 10.5%, $P<0.05$), which was similar to Han Chinese from Beijing (7.77%) reported in the 1000 genomes project data. On gender subgroup analysis, the genotype frequencies between males and females were different in Jing but not in Han ($P<0.05$). These results indicated that the prevalence of the *VLDLR* rs3780181 G allele may have racial/ethnic as well as gender specificity.

Recently, a newly study identify and annotate 157 loci associated with lipid levels obtained from Joint GWAS and Metabochip Meta-analysis ($P<5\times10^{-8}$) including 62 loci not previously associated with lipid levels in humans which referred to the association between the *VLDLR* rs3780181 SNP and LDL-C and TC levels [13]. Besides this, rare studies have previously reported the direct effect of the *VLDLR* rs3780181 SNP on serum lipid levels. More work is needed to be done to actually confirm the findings because the effects of newly identified loci were generally smaller than in earlier GWAS [13]. In the present study, we found that the G allele carriers had lower TC levels than the G allele non-carriers in Jing but not in Han. The G allele carriers were had lower LDL-C levels than the G allele non-carriers in Han but not in Jing. Subgroup analyses according to gender showed thatthe G allele carriers in Han females but not in Han males had lower LDL-C and ApoB levels than the Gallele non-carriers. The G allele carriers in Jing femalesbut not in Jing males had lower TC levels than the G allele non-carriers. These results suggest that there may be an ethnic and/or sex specific-association of the *VLDLR* rs3780181 SNP and serum lipid parameters.

Differences in lipid profiles and metabolism between men and women have been well documented [41-43]. It is well-known that women were proved to have more favorable plasma lipid profiles than males, with lower levels of TG, TC and LDL-C, and higher HDL-C levels than age-matched men, especially in women before menopause [44-47]. Serum lipid concentrations and lipoprotein particle concentrations, subclass distribution, sizes and lipoprotein kinetics were also found different between male and female [47-50]. The traditional view suggests that sex hormone is important regulators of plasma lipid kinetics and is responsible for sexual dimorphism in the plasma lipid profile. It was reported thata small series of men with deficient estrogen action (due to mutations in the genes for estrogen receptor or aromatase) developed early-onset atherosclerosis, increased visceral adipose tissue, hyperinsulinemia, and the constellation of risk factors known as metabolic syndrome [51]. The previous studies reported that newer hormones, including leptin, adiponectin, and vitamin D, have also been linked to different phases of vascular dysfunction [51-53]. It has been postulated that the described alterations in hormone alterations especially sex hormone may cause these cardiovascular abnormalities [51, 54]. Previous studies have suggested sex-specific heritability of lipid traits [55]. More than 12 loci exhibiting sex heterogeneity were identified in a previous GWAS [7], since then more gender specific loci were found and replicated in the other studies [26, 56-58]. However, the molecular basis of sexual dimorphism in lipid metabolism is poorly understood. It is likely that an underlying mechanism is differential

gene regulation in males and females, particularly in sex steroid responsive genes. Genetic studies that ignore sex-specific effects in their design and interpretation could fail to identify a significant proportion of the genes that contribute to risk for complex diseases [59]. The sex-specific association of the *VLDLR* rs3780181 SNP was firstly reported in the present study. Thus, sex-specific genetic associations between SNPs and serum lipid levels may help to study the sexual dimorphism in the plasma lipid profile and treatment strategy for dyslipidemia.

VLDLR is known to bind ApoE-rich lipoproteins such as VLDL [60]. The binding of these ligands is stimulated by lipoprotein lipase, and it is thought that this process is important in the uptake and degradation of fatty acids and TG-rich particles by monocytes, which may accelerate foam cell formation in atherosclerotic lesions [61, 62]. A previous study detecting *VLDLR*-deficient families found that 50% of affected patients were underweight, with a BMI of <18.5 kg/m^2 [63]. Yagyu *et al*. [21] also demonstrated that *VLDLR* participate the body's hypertriglyceridemia metabolism which is required for normal lipoprotein lipase regulation *in vivo*. One tagSNP (SNP 1226; rs1454626) located in the 5' flanking region of *VLDLR* was associated with CAD, BMI, and LDL-associated ApoB [62]. In 2012, a large-scale gene-centric meta-analysis across 32 studies revealed that a locus (rs7024888) in the *VLDLR* associated with LDL-C [23]. A recently study has identified 157 loci associated with lipid levels at $P<5\times 10^{-8}$ which has mentioned that the *VLDLR* rs3780181 SNP was associated with TC and LDL-C levels [13]. In the present study, we also found that serum TC and LDL-C levels in the combined population of Jing and Han, LDL-C levels in Han, and TC levels in Jing were correlated with genotypes ($P<0.05$), respectively. Subgroup analyses according to gender showed that LDL-C and ApoB levels in Han females and TC levels in Jing females were associated with genotypes. To the best of our knowledge, however, the association of the *VLDLR* rs3780181 SNP and serum ApoB levels has not been previously explored. Further studies with larger sample size are still needed to confirm this association. *VLDLR* is categorized by Gene Ontology [64] in the retinoid × nuclear receptor (RXR) activation pathway, which also includes genes (*ApoB, ApoE, CYP7A1, ApoA1, HNF1A, HNF4A*) in previously implicated lociassociated with lipid levels [7, 13]. This leads us to suspect that the *VLDLR* rs3780181 SNP may have linkage disequilibrium with the other genes. The interactions of gene-gene on serum lipid levels are remained to be determined.

In the present study, we alsofound that serum lipid parameters were associated with age, gender, alcohol consumption, cigarette smoking, BMI, fasting blood glucose levels and blood pressure in both Jing and Han, or males and females in both ethnic groups. We also showed that the levels of height, weight, waist circumference, BMI, TC, TG, glucose were higher in Jing than in Han but the ratio of ApoA1/ApoB, the percentages of subjects who consumed alcohol were lower in Jing than in Han ($P<0.05$-0.001). Xue *et al*. [65] showed that older subjects and females in Jing were more susceptible with a high prevalence of hypertension, diabetes, hyperlipidemia, hyperuricaemia, metabolic syndrome. These results suggest that the environmental factors also play a crucial role in determining the serum lipid profile in our populations. Jing is the only Chinese minority for coastal fisheries, meanwhile is the only sea people in China. In this case, it has a very special lifestyle and dietary habits compared with the other landlocked nationalities [24]. It is widely accepted that dietary composition remains an important, modifiable predictor of dyslipidemia [66]. It is well known that high-fat diets, particularly those contain rich saturated fatty acids are related with the serum cholesterol concentrations as well as with the prevalence and the management of dyslipidemia [67]. Diet alone could account for up to 2.5% of the variability on serum lipid levels [68, 69]. Although rice and corn are the staple foods in both ethnic groups, the people of Jing nationality like to eat seafood like fish, shrimp, crabs, shellfish and sandworm. They prefer sweet food such as sweet glutinous rice porridge, mung bean syrup, because they believe sweet food is a symbol for happiness and they also like to eat a kind of fish sauce called nuoc-mam which contains 17 amino acids (8 essential amino acids included of course) [24]. Although the omega-3 fatty acids from fish lower TG levels, overconsumption of any form of dietary energy may replace overconsumption of saturated fat as the primary factor that increases lipid and

lipoprotein levels [70, 71]. Hata *et al*. [72] conducted a meta-analysis including Japanese living in the different area of Japan, then found that TC was lower by a mean of 10 mg/dl (0.26 mmol/L) in fishing villages than in cities, and TG lower by a mean of 15 mg/dl (0.17 mmol/L) in fishing villages than in cities and agricultural villages. HDL-C was 5 mg/dl (0.13 mmol/L) higher in agricultural villages and 3 mg/dl (0.08 mmol/L) higher in fishing villages than in cities. In addition, our previous studies also documented that BMI and alcohol consumption may interact with certain lipid-related gene variants to modify the serum lipid levels in Bai Ku Yao and Han ethnic groups [73, 74]. These results suggested that such environmental factors also have a key role in determining the serum lipid levels in these study populations.

Conclusions

The association of the *VLDLR* rs3780181 SNP and serum lipid levels is different between the Jing and Han populations. These trends of association suggest that this SNP might have racial/ethnic or gender specificity. The differences in the association of the *VLDLR* rs3780181 SNP and serum lipid levels between the two ethnic groups might partly result from the differences in gene-environmental interactions.

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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, Guangxi, China. E-mail: yinruixing@163.com

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