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Original Article

Association of the *PCSK7* rs2277287 polymorphismand serum lipid levels in the Jing and Han populations

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Abstract: The single nucleotide polymorphism (SNP) of rs142953140 near the proprotein convertase subtilisin/kexin type 7 gene (*PCSK7*) has been associated with serum low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), and triglyceride levels in a previous genome-wide association study, but the association of *PCSK7* rs2277287 SNP and serum lipid levels has not been reported previously. The aim of this study was to detect the association of the *PCSK7* rs2277287 SNP and several environmental factors with serum lipid profiles in the Chinese Jing and Han populations. Genotypes of the *PCSK7* rs2277287 SNP in 657 individuals of Jing nationality and 657 participants of Han nationality were determined by polymerase chain reaction and restriction fragment length polymorphism, and then confirmed by direct sequencing. The frequencies of CC,CT and TT genotypes were 80.21%, 17.96% and 1.83% in the Han population, and 69.41%, 27.70% and 2.89% in the Jing population (P<0.001); Respectively. The frequency of the T allele was 10.81% in the Han individuals and 16.74% in the Jing subjects (P<0.001). The T allele carriers had higher apolipoprotein (Apo) B and lower ApoA1/ApoB ratio in Han; and higher HDL-C in Jing than the T allele non-carriers. Subgroup analyses showed that the T allele carriers had lower ApoA1/ApoB ratio in Han males and females; And higher HDL-C, LDL-C and ApoB levels in Jing males (P<0.05 for all). Serum lipid parameters in the two ethnic groups were also associated with several environmental factors. These findings revealed that there may be a racial/ethnic- and/or sex-specific association between the *PCSK7* rs2277287 SNP and serum lipid parameters in some populations.

Keywords: Proprotein convertase subtilisin/kexin type 7, single nucleotide polymorphism, lipids, environmental factor

Introduction

Compelling evidence has demonstrated that serum lipid and lipoprotein concentrations are tightly associated with coronary artery disease (CAD) [1], which is the major leading causes of death and disability worldwide [2]. Dyslipidemia such as elevated serum levels of total cholesterol (TC) [3], triglyceride (TG) [4], low-density lipoprotein cholesterol (LDL-C) [5], and apolipoprotein (Apo) B [6], together with decreased levels of high-density lipoprotein cholesterol (HDL-C) [7], ApoA1 and the ApoA1/ApoB ratio [8] is strongly related to the risk of CAD. Thus, the diagnosis, treatment and prevention of dyslipidemia appear to be greatly important. It is well known that abnormal blood lipid levels are determined by multiple environmental and genetic factors [9, 10], and their interactions [11, 12]. Several twin and family studies have demenstrated that almost 40-70% of the interindividual variation in plasma lipid phenotypes can be explained by heritable factors, such as single nucleotide polymorphisms (SNPs) [13-15].

Proprotein convertase subtilisin/kexin type 7 (PCSK7), a calcium-dependent serine endoprotease [16], is a member of the subtilisin-like proprotein convertase family that processes multiple protein precursors [17], and is located near the chromosome 11 gene cluster involving *ApoA1/C3/A4/A5*, a region where several genes regulate HDL-C and TG [18]. PCSK7 is expressed to some degree in the liver and the intestine, organs that are largely involved in lipid metabolism [19]. It has also been demonstrated that PCSK7 is implicated as a mediator

of adipogenesis [20]. Furthermore, recent data show that internalization of PCSK7 from the plasma membrane is mediated by clathrin-coated vesicles [21], which are also implicated in the internalization of other cellular receptors, such as the LDL receptor and various scavenger receptors. In a previous genome-wide association study (GWAS), Peloso *et al*. [18] showed that the *PCSK7* rs142953140 SNP (c.1511G>A; P.Arg504His) was associated with serum LDL-C, HDL-C, and TG levelsin 56,000 whites and blacks. Another GWAS has also identified that genetic variant of the *PCSK7* rs508487 SNP was associated with serum TC levels [22]. However, whether the PCSK7 rs2277287 SNP is associated with serum lipid levels like the PCSK7 rs142953140 and rs508487 SNPs remains elusive.

Han nationality is the largest group among the 56 ethnic groups in China. Jing, one of the 55 official ethnic minorities in China, is a very small minority with a population of 28199 according to the sixth national census statistics of China in 2010. Most of them inhabit in the three islands of Wanwei, Wutou, and Shanxin in the Dongxing City, Guangxi Zhuang Autonomous Region, People's Republic of China, near the Sino-Vietnam border. The history of this minority can be traced back to the early 16th century [23]. Jing is the only Chinese minority for coastal fisheries and preserves their custom of intra-ethnic marriage. Therefore, there exist lots of differences between Jing and Han (as well as the other landlocked nationalities) nationality in diet custom and culture characteristics. Previous study has shown significant association of SNP and serum lipid levels in the Jing population [24]. To the best of our knowledge, the association of the *PCSK7* rs2277287 SNP and serum lipid levels has not been previously reported in this population. Therefore, this study was undertaken to assess the association of the *PCSK7* rs2277287 SNP and several environmental factors with serum lipid profiles in the Jing and Han populations.

Materials and methods

Subjects

A total of 657 unrelated participants of Han (262 men, 39.88% and 395 women, 60.12%) and 657 unrelated subjects of Jing (263 males, 40.03% and 394 females, 59.97%) were randomly selected from our previous stratified randomized samples [24]. All participants were rural agricultural (Han) and/or fishery workers (Jing) living in Jiangping Down, Dongxing City, Guangxi Zhuang Autonomous Region, People's Republic of China. The age of the participants ranged from 27 to 92 years, with a mean age of 56.55±13.03 years in Han and 56.75±12.62 years in Jing (P>0.05); respectively. All study subjects were essentially healthy with no history of cardiovascular disease such as CAD and stroke, diabetes, hyper- or hypo-thyroids, and chronic renal disease. Any participant had a history of taking medications known to affect serum lipid levels (lipid-lowering drugs such as statins or fibrates, beta blockers, diuretics, or hormones) was excluded before the blood sample was drawn. The present study was approved by the Ethics Committee of the First Affiliated Hospital, Guangxi Medical University. Informed consent was taken from all participants.

Epidemiological survey

The survey was carried out using internationally standardized methods, following a common protocol [25]. Information on demographics, socioeconomic status, and lifestyle factors was collected with standardized questionnaires. Alcohol consumption was categorized into groups of grams of alcohol per day: 0 (non-drinker), <25 and ≥25. Smoking status was categorized into groups of cigarettes per day: 0 (non-smoker), <20 and ≥20. In the physical examination, several parameters such as height, weight, and waist circumference were measured. Sitting blood pressure was measured three times with the use of a mercury sphygmomanometer after a 5-minute of rest, and the average of the three measurements was recorded. Systolic blood pressure was determined by the first Korotkoff sound, and diastolic blood pressure by the fifth Korotkoff sound. Body weight, to the nearest 50 grams, was measured using a portable balance scale. Subjects were weighed in a minimum of clothing with shoes off. Height was measured, to the nearest 0.5 cm, using a stadiometer. From these two measurements body mass index (BMI, kg/m^2) was calculated.

Biochemical measurements

A fasting venous blood sample of 5 mL was drawn from the participants. A part of the sam-

Table 1. Comparison of demographic, lifestyle characteristics and serum lipid levels between the Han and Jing populations

Parameter	Han	Jing	t (x^2)	P
Number	657	657		
Male/female	262/395	263/394	0.003	0.955
Age (years)*	56.55±13.03	56.75±12.62	0.290	0.722
Height (cm)*	156.25±7.88	157.08±7.63	-1.945	0.052
Weight (kg)*	56.00±9.41	57.84±9.83	-3.453	0.001
Body mass index (kg/m^2)*	22.90±3.20	23.38±3.20	-2.715	0.007
Waist circumference*	77.67±8.83	79.82±9.04	-4.375	0.000
Smoking status [*n* (%)]				
Non-smoker	558 (84.93)	565 (86.00)		
≤20 cigarettes/day	24 (3.65)	22 (3.35)		
>20 cigarettes/day	75 (11.41)	70 (10.65)	0.303	0.859
Alcohol consumption [*n* (%)]				
Non-drinker	545 (82.95)	586 (89.19)		
≤25 g/day	25 (3.81)	35 (5.33)		
>25 g/day	87 (13.24)	36 (5.48)	24.299	0.000
Systolic blood pressure (mmHg)*	131.56±18.97	131.28±21.66	0.256	0.798
Diastolic blood pressure (mmHg)*	81.09±10.23	80.10±10.09	1.766	0.078
Pulse pressure (mmHg)*	50.47±15.16	51.18±17.51	-0.777	0.437
Glucose (mmol/L)*	6.64±1.07	6.83±1.54	3.872	0.000
Total cholesterol (mmol/L)*	4.89±0.88	5.10±0.91	-4.193	0.000
Triglyceride (mmol/L)	1.32 (0.62)	1.43 (0.70)	-3.528	0.000
HDL-C (mmol/L)*	1.78±0.53	1.77±0.45	0.537	0.591
LDL-C (mmol/L)*	2.85±0.44	2.82±0.44	1.226	0.220
ApoA1 (g/L)*	1.32±0.20	1.29±0.22	2.659	0.008
ApoB (g/L)*	1.00±0.25	1.03±0.23	-2.677	0.008
ApoA1/ApoB*	1.40±0.36	1.30±0.38	4.363	0.000

HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; Apo, Apolipoprotein. *Data were shown as mean ± SD, the difference between two ethnic groups were compared by the Student's unpaired *t*-test. The value of triglyceride was presented as median (interquartile range), the difference between the two ethnic groups was determined by the Wilcoxon-Mann-Whitney test.

ple (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 tri-sodium citrate) and used to extract deoxyribonucleic acid (DNA). Measurements of serum TC, TG, HDL-C, and LDL-C levels in the samples were performed by enzymatic methods with commercially available kits (RANDOX Laboratories Ltd., Ardmore, Diamond Road, Crumlin Co. Antrim, United Kingdom, BT29 4QY; Daiichi Pure Chemicals Co., Ltd., Tokyo, Japan). Serum ApoA1 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 [26, 27].

DNA amplification and genotyping

Genomic DNA of the samples was isolated from peripheral blood leucocytes according to the phenol-chloroform method [26, 27]. The extracted DNA was stored at 4°C until analysis. Genotyping of the *PCSK7* rs2277287 SNP was performed by polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP). PCR amplification was performed using 5'-CATTGCCTAGGTATCCGGGT-3' and 5'-GGGCTTCTCATGTGGCAATC-3' (Sangon, Shanghai, People's Republic of China) as the forward

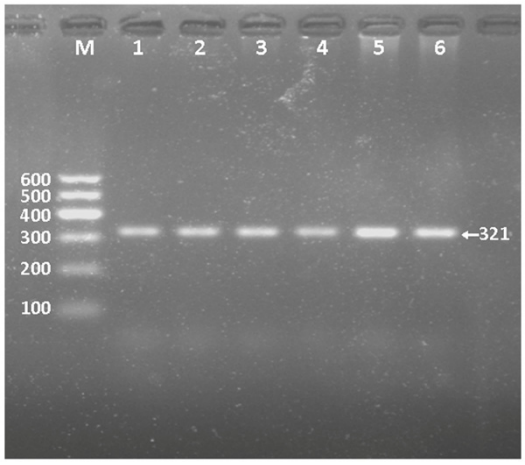

Figure 1. Electrophoresis of polymerase chain reaction products of the samples. Lane M is the 100 bp marker ladder; Lanes 1-6 are samples, the 321 bp bands are the target genes.

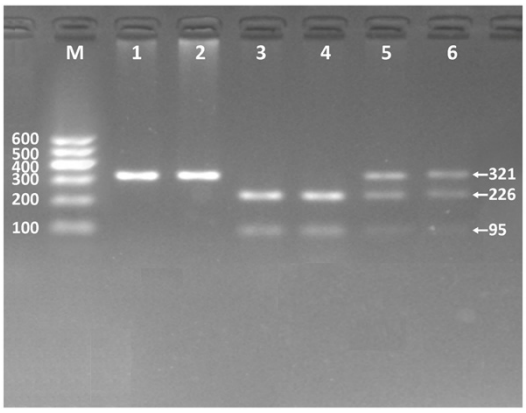

Figure 2. Genotyping of the *PCSK7* rs2277287 SNP. Lane M, 100 bp marker ladder; Lanes 1 and 2, TT genotype (321 bp); Lanes 3 and 4, CC genotype (226- and 95-bp); Lanes 5 and 6, CT genotype (321-, 226- and 95-bp).

and reverse primer pairs, respectively. Each 25 μL PCR reaction mixture consisted of 2.0 μL genomic DNA, 1.0 μL each primer (10 μmol/L), 12.5 μL of 2×*Taq* PCR Master mix (constituent: 0.1 U *Taq* polymerase/μL, 500 μM dNTP each and PCR buffer), and 8.5 μL of ddH_2O (DNase/RNase-free). PCR was performed with an initialization step of 95°C for 5 min, followed by 30 s denaturing at 95°C, 30 s of annealing at 58°C and 35 s of elongation at 72°C for 30 cycles. The amplification was completed by a final extension at 72°C for 7 min. Following electrophoresis on a 2.0% agarose gel with 0.5 μg/mL ethidium bromide, the amplification products were visualized under ultraviolet light. Subsequently, each restriction enzyme reaction was performed with 5.0 μL amplified DNA, 8.8 μL nuclease-free water, 1.0 μL of 10× buffer solution and 0.2 μL *Pvu*II restriction enzyme in a total volume of 15 μL digested at 37°C for 12 hours. After restriction enzyme digestion of the amplified DNA, genotypes were identified by electrophoresis on 2% ethidium-bromide stained agarose gels and visualized with UV illumination. Genotypes were scored by an experienced reader blinded to the epidemiological and serum lipid results. Six samples (CC, CT and TT genotypes in two; respectively) detected by the PCR-RFLP were also confirmed by direct sequencing with an ABI Prism 3100 (Applied Biosystems) 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, ApoB levels and the ApoA1/ApoB ratio 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 hyperlipidaemic [28]. Hypertension was diagnosed according to the 1999 and 2003 criteria of the World Health Organization-International Society of Hypertension Guidelines for the management of hypertension [29, 30]. 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/m^2, respectively [31].

Statistical analyses

The statistical analyses were performed with the statistical software package SPSS 17.0 (SPSS Inc., Chicago, Illinois). The quantitative variables were presented as mean ± standard deviation (serum TG levels were presented as medians and interquartile ranges). Allele frequency was determined via direct counting, and the Hardy-Weinberg equilibrium was verified with the standard goodness-of-fit test. The genotype distribution between the two groups was analyzed by the chi-square test. General characteristics between two ethnic groups

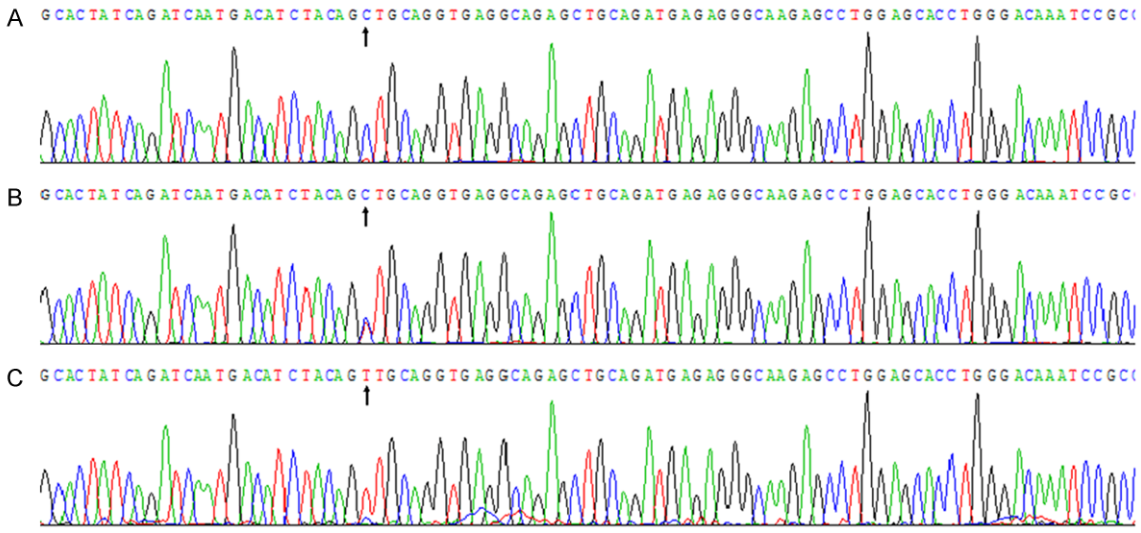

Figure 3. A part of the nucleotide sequence of the *PCSK7* rs2277287 SNP. A: CC genotype; B: CT genotype; and C: TT genotype.

Table 2. Comparison of the genotype and allele frequencies of PCSK7 rs2277287 SNP in the Han and Jing populations [*n* (%)]

Group	*n*	Genotype			Allele	
		CC	CT	TT	C	T
Han	657	527 (80.21)	118 (17.96)	12 (1.83)	1172 (89.19)	142 (10.81)
Jing	657	456 (69.41)	182 (27.70)	19 (2.89)	1094 (83.26)	220 (16.74)
x^2		20.362			19.492	
P		0.000			0.000	
Han						
Male	262	210 (80.15)	49 (18.70)	3 (1.15)	469 (89.50)	55 (10.50)
Female	395	317 (80.25)	69 (17.47)	9 (2.28)	703 (88.99)	87 (11.01)
x^2		1.242			0.087	
P		0.537			0.768	
Jing						
Male	263	171 (65.02)	85 (32.32)	7 (2.66)	427 (81.18)	99 (18.82)
Female	394	285 (72.34)	97 (24.62)	12 (3.04)	667 (84.64)	121 (15.36)
x^2		4.736			2.718	
P		0.097			0.099	

The genotype distribution between the two groups was analyzed by the chi-square test.

were compared by the Student's unpaired *t*-test. The association between genotypes and serum lipid parameters was tested by covariance analysis (ANCOVA). Gender, age, BMI, blood pressure, alcohol consumption and cigarette smoking were adjusted for the statistical analysis. Multivariable linear regression analyses with stepwise modeling were used to determine the correlation between the genotypes (CC=1, CT/TT=2) and several environmental factors with serum lipid levels in males and females of Han and Jing populations. Two sided *P* value <0.05 was considered statistically significant.

Results

General characteristics and serum lipid levels

The general characteristics and serum lipid levels between the Han and Jing populations are summarized in **Table 1**. The percentages of alcohol consumption, the levels of ApoA1 and the ratio of ApoA1 to ApoB were higher in Han

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								
CC	527	4.89±0.87	1.32 (0.60)	1.79±0.53	2.85±0.45	1.32±0.20	1.00±0.25	1.42±0.42
CT/TT	130	4.93±0.90	1.32. (0.72)	1.74±0.53	2.81±0.39	1.31±0.20	1.06±0.18	1.29±0.31
F		0.375	0.062	0.279	1.004	0.002	5.919	8.227
P		0.540	0.951	0.597	0.317	0.967	0.015	0.004
Jing								
CC	456	5.07±0.91	1.43 (0.73)	1.75±0.45	2.80±0.43	1.29±0.23	1.03±0.24	1.3±0.38
CT/TT	201	5.17±0.93	1.42 (0.67)	1.81±0.44	2.86±0.46	1.28±0.21	1.05±0.22	1.2±0.39
F		0.512	0.177	4.618	0.864	0.002	0.003	0.015
P		0.474	0.859	0.032	0.353	0.965	0.954	0.903

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. *Data were shown as mean ± SD, the difference between two genotypes was tested by covariance analysis (ANCOVA). The value of TG was presented as median (interquartile range), the difference between the genotypes was determined by the Wilcoxon-Mann-Whitney test.

than in Jing (P<0.05-0.001), whereas the levels of body weight, BMI, waist circumference, blood glucose, serum TC, TG and ApoB were lower in Han than in Jing (P<0.05-0.001). There were no significant differences in the gender ratio, age structure, body height, percentage of cigarette smoking, diastolic blood pressure, systolic blood pressure, pulse pressure, serum HDL-C and LDL-C between the two ethnic groups (P>0.05 for all).

Results of electrophoresis and genotyping

After the genomic DNA of the samples was amplified using PCR and visualized with 2% agarose gel electrophoresis, the products of 321 bp nucleotide sequences were observed in all samples (**Figure 1**). The genotypes identified were termed according to the presence (C allele) or absence (T allele) of the enzyme restriction sites. Thus, the CC genotype is homozygous for the presence of the site (bands at 226 bp and 95 bp), the CT genotype is heterozygous for the presence and absence of the site (bands at 321-, 226- and 95-bp) and the TT genotype is homozygous for the absence of the site (bands at 321 bp; **Figure 2**). The CC, CT and TT genotypes detected by PCR-RFLP were also confirmed by direct sequencing (**Figure 3**), respectively.

Genotypic and allelic frequencies

The genotypic and allelic frequencies of the *PCSK7* rs2277287 SNP are shown in **Table 2**. The frequencies of C and T alleles were 89.19% and 10.81% in Han, and 83.26% and 16.74% in Jing populations (P<0.001), respectively. The frequencies of CC, CT and TT genotypes were 80.21%, 17.96% and 1.83% in the Han population, and 69.41%, 27.70% and 2.89% in the Jing population (P<0.001), respectively. No differences in the genotypic and allelic frequencies between males and females in the two ethnic groups (P>0.05 for each) were identified.

Genotypes and serum lipid levels

Tables 3 and **4** describe the association between genotypes and serum lipid levels. Serum levels of ApoB and the ratio of ApoA1 to ApoB in Han were different between the genotypes (P<0.05 for each), the T allele carriers had higher serum ApoB levels and lower the ratio of ApoA1 to ApoB than the T allele non-carriers. Serum HDL-C levels in Jing were different between the genotypes (P<0.05), the T allele carriers had higher serum HDL-C levels than the T allele non-carriers. Subgroup analyses showed that the ApoA1/ApoB ratio in Han males and females were different between the genotypes (P<0.05 for each), the T allele carriers had lower the ApoA1/ApoB ratio than the T allele non-carriers. In contrast, serum HDL-C, LDL-C and ApoB levels in Jing males but not in females were different between the genotypes (P<0.05 for all), the T allele carriers had higher serum HDL-C, LDL-C and ApoB levels than the T allele non-carriers.

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								
CC	210	4.83±0.89	1.30 (0.66)	1.72±0.54	2.85±0.45	1.33±0.21	1.00±0.25	1.41±0.46
CT/TT	52	4.85±0.80	1.37. (0.85)	1.66±0.57	2.83±0.36	1.30±0.19	1.06±0.18	1.27±0.30
F		0.088	0.604	0.156	0.100	0.203	2.160	3.987
P		0.767	0.546	0.693	0.752	0.653	0.143	0.047
Han/Female								
CC	317	4.92±0.86	1.35 (0.59)	1.84±0.51	2.86±0.42	1.31±0.98	0.98±0.25	1.42±0.40
CT/TT	78	4.98±0.96	1.31 (0.64)	1.81±0.50	2.81±0.42	1.32±0.20	1.00±0.23	1.31±0.31
F		0.316	0.556	0.076	1.189	0.141	3.529	4.138
P		0.574	0.578	0.784	0.276	0.707	0.061	0.043
Jing/Male								
CC	171	5.01±0.85	1.52 (0.92)	1.63±0.45	2.78±0.36	1.27±0.36	1.03±0.21	1.30±0.41
CT/TT	92	5.22±0.76	1.39 (0.72)	1.77±0.43	2.93±0.38	1.25±0.22	1.05±0.20	1.25±0.38
F		0.965	0.411	7.681	5.172	0.057	4.700	2.711
P		0.327	0.681	0.006	0.024	0.811	0.031	0.101
Jing/Female								
CC	285	5.10±0.94	1.40 (0.72)	1.82±0.44	2.82±0.46	1.31±0.24	1.04±0.25	1.32±0.35
CT/TT	109	5.15±1.06	1.43 (0.64)	1.84±0.45	2.81±0.53	1.30±0.21	1.01±0.23	1.36±0.40
F		0.064	0.047	0.118	0.075	0.067	2.173	1.490
P		0.801	0.963	0.731	0.784	0.797	0.141	0.223

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. *Data were shown as mean ± SD, the difference between two genotypes was tested by covariance analysis (ANCOVA). The value of TG was presented as median (interquartile range), the difference between the genotypes was determined by the Wilcoxon-Mann-Whitney test.

Relative factors for serum lipid parameters

Multiple linear regression analysis showed that serum ApoB levels and the ApoA1/ApoB ratio in Han; and HDL-C levels in Jing were correlated with the genotypes of the *PCSK7* rs2277287 SNP ($P<0.05$ for all; **Table 5**). When the correlation of serum lipid parameters and the genotypes was analyzed according to sex, we showed that the ApoA1/ApoB ratio in Han males; Serum HDL-C and LDL-C levels in Jing males were correlated with the genotypes ($P<0.05$ for all; **Table 6**). Serum lipid parameters were also associated with age, gender, BMI, waist circumference, systolic and diastolic blood pressure, pulse pressure, fasting blood glucose, cigarette smoking and alcohol consumption in both ethnic groups or in males and females ($P<0.05$-0.001; **Tables 5** and **6**).

Discussion

The SNP of rs142953140 near the *PCSK7* has been associated with blood LDL-C, HDL-C and TG levels in a previous GWAS [18]. Recently, another GWAS has also identified that genetic variant of the *PCSK7* rs508487 SNP was associated with serum TC levels [22]. To the best of our knowledge, this is the first report about the association of the *PCSK7* rs2277287 SNP and serum lipid levels. In the present study, we revealed that the genotypic and allelic frequencies of the *PCSK7* rs2277287 SNP were different between the Chinese Han and Jing populations. The frequency of the *PCSK7* rs2277287 T allele was 10.81% in Han, and 16.74% in Jing ($P<0.001$). The frequencies of CT and TT genotypes were 17.96% and 1.83% in Han, and 27.70% and 2.89% in Jing ($P<0.001$); respectively. According to the Hap Map data, the frequencies of T allele and CT, TT genotypes were 16.28%, 27.90% and 2.32% in Han Chinese in Beijing; 24.41%, 41.86% and 3.49% in Japanese; and 20.09%, 29.46% and 5.36% in European population; respectively. These results indicate that the prevalence of the *PCSK7* rs2277287 SNP may have racial/ethnic specificity.

Table 5. Relationship between serum lipid parameters and relative factors in the Han and Jing populations

Lipid	Risk factor	B	Std.error	Beta	*t*	*P*
Han and Jing						
TC	Glucose	0.156	0.018	0.230	8.521	0.000
	Age	0.007	0.002	0.106	3.865	0.000
	Ethnic group	0.193	0.048	0.107	4.025	0.000
	Height	-0.008	0.003	-0.068	-2.538	0.011
TG	Waist circumference	0.038	0.005	0.393	7.518	0.000
	Cigarette smoking	0.302	0.038	0.220	7.968	0.000
	Glucose	0.097	0.017	0.147	5.618	0.000
	Height	-0.020	0.004	-0.178	-5.674	0.000
	Diastolic blood pressure	0.006	0.002	0.073	2.741	0.006
	Age	-0.005	0.002	-0.069	-2.564	0.010
	Body mass index	-0.028	0.014	-0.102	-2.018	0.044
HDL-C	Waist circumference	-0.016	0.001	-0.292	-10.634	0.000
	Gender	0.226	0.041	0.225	5.564	0.000
	Alcohol consumption	0.115	0.025	0.142	4.697	0.000
	Cigarette smoking	-0.055	0.024	-0.072	-2.283	0.023
	Height	0.008	0.002	0.120	3.243	0.001
	Age	0.002	0.001	0.064	2.202	0.028
LDL-C	Glucose	0.046	0.009	0.138	5.004	0.000
	Age	0.004	0.001	0.103	3.708	0.000
	Diastolic blood pressure	0.003	0.001	0.072	2.629	0.009
ApoA1	Alcohol consumption	0.053	0.010	0.151	5.518	0.000
	Weight	-0.005	0.001	-0.212	-7.727	0.000
	Glucose	-0.013	0.004	-0.080	-2.987	0.003
ApoB	Waist circumference	0.002	0.001	0.075	2.712	0.007
	Cigarette smoking	0.027	0.010	0.073	2.675	0.008
	Age	0.001	0.001	0.068	2.482	0.013
	Gender	0.031	0.013	0.065	2.367	0.018
ApoA1/ApoB	Waist circumference	-0.007	0.001	-0.156	-5.680	0.000
	Ethnic group	-0.073	0.022	-0.091	-3.326	0.001
	Gender	0.078	0.025	0.096	3.173	0.002
	Alcohol consumption	0.054	0.020	0.081	2.659	0.008
Han						
TC	Glucose	0.232	0.031	0.284	7.596	0.000
	Height	-0.009	0.004	-0.081	-2.169	0.030
TG	Waist circumference	0.028	0.004	0.276	7.360	0.000
	Glucose	0.146	0.032	0.177	4.636	0.000
	Cigarette smoking	0.256	0.053	0.188	4.789	0.000
	Height	-0.014	0.005	-0.122	-3.024	0.003
	Age	-0.006	0.003	-0.095	-2.476	0.014
HDL-C	Waist circumference	-0.014	0.002	-0.237	-6.010	0.000
	Cigarette smoking	-0.110	0.037	-0.136	-2.963	0.003
	Alcohol consumption	0.105	0.036	0.137	2.919	0.004
	Gender	0.185	0.059	0.172	3.148	0.002
	Diastolic blood pressure	0.004	0.002	0.079	2.057	0.040
	Height	0.007	0.003	0.097	1.970	0.049

LDL-C	Glucose	0.079	0.016	0.194	4.989	0.000
	Systolic blood pressure	0.003	0.001	0.127	3.269	0.001
ApoA1	Body mass index	-0.011	0.002	-0.178	-4.706	0.000
	Alcohol consumption	0.066	0.013	0.230	5.154	0.000
	Gender	0.050	0.018	0.123	2.751	0.006
ApoB	Waist circumference	0.006	0.001	0.226	5.819	0.000
	Systolic blood pressure	0.002	0.000	0.135	3.567	0.000
	Genotype	0.055	0.023	0.089	2.379	0.018
	Height	-0.005	0.002	-0.158	-3.217	0.001
	Gender	-0.063	0.024	-0.127	-2.646	0.008
ApoA1/ApoB	Waist circumference	-0.012	0.002	-0.265	-7.070	0.000
	Glucose	-0.045	0.014	-0.119	-3.157	0.002
	Genotype	-0.108	0.037	-0.106	-2.870	0.004
	Alcohol consumption	0.054	0.022	0.091	2.460	0.014
	Systolic blood pressure	-0.002	0.001	-0.085	-2.232	0.026
Jing						
TC	Glucose	0.127	0.022	0.214	5.678	0.000
	Age	0.014	0.003	0.189	4.848	0.000
	Cigarette smoking	0.192	0.062	0.133	3.110	0.002
	Gender	0.225	0.081	0.121	2.785	0.006
TG	Waist circumference	0.030	0.004	0.312	8.603	0.000
	Cigarette smoking	0.278	0.050	0.201	5.599	0.000
	Glucose	0.081	0.021	0.143	3.945	0.000
HDL-C	Waist circumference	-0.018	0.002	-0.352	-9.750	0.000
	Gender	0.184	0.035	0.199	5.177	0.000
	Alcohol consumption	0.161	0.035	0.177	4.598	0.000
	Genotype	0.073	0.035	0.075	2.089	0.037
LDL-C	Age	0.005	0.001	0.135	3.480	0.001
	Glucose	0.032	0.011	0.113	2.906	0.004
ApoA1	Waist circumference	-0.005	0.001	-0.196	-5.113	0.000
ApoB	Waist circumference	0.005	0.001	0.194	5.085	0.000
	Age	0.002	0.001	0.125	3.295	0.001
ApoA1/ApoB	Waist circumference	-0.011	0.002	-0.271	-7.245	0.000
	Age	-0.003	0.001	-0.105	-2.795	0.005

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; B, unstandardized coefficient; Beta, standardized coefficient. The correlation between serum lipid parameters and the genotypes (CC=1, CT/TT=2) and several environmental factors was determined by multivariable linear regression analyses with stepwise modeling.

In the present study, we also found that the association of the *PCSK7* rs2277287 SNP and serum lipid parameters was different between the two ethnic groups. Serum ApoB levels and the ApoA1/ApoB ratio in Han were different between the genotypes, the T allele carriers had higher ApoB levels and lower ApoA1/ApoB ratio than the T allele non-carriers. Serum HDL-C levels in Jing were different between the genotypes, the T allele carriers had higher HDL-C levels than the T allele non-carriers. Subgroup analyses according to sex showed that the ApoA1/ApoB ratio in Han males and females were different between the genotypes, the T allele carriers had lower the ApoA1/ApoB ratio than the T allele non-carriers. Serum HDL-C, LDL-C and ApoB levels in Jing males but not in females were different between the genotypes, the T allele carriers had higher HDL-C, LDL-C and ApoB levels than the T allele non-

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*
Han/Male						
TC	Glucose	0.255	0.044	0.333	5.761	0.000
	Diastolic blood pressure	0.012	0.005	0.143	2.475	0.014
TG	Waist circumference	0.052	0.013	0.426	3.934	0.000
	Cigarette smoking	0.232	0.068	0.205	3.430	0.001
	Glucose	0.163	0.053	0.183	3.086	0.002
	Age	-0.016	0.005	-0.215	-3.351	0.001
	Weight	-0.033	0.013	-0.289	-2.569	0.011
	Diastolic blood pressure	0.013	0.006	0.138	2.364	0.019
HDL-C	Waist circumference	-0.014	0.004	-0.212	-3.450	0.001
	Diastolic blood pressure	0.008	0.003	0.153	2.492	0.013
LDL-C	Glucose	0.106	0.023	0.278	4.721	0.000
	Diastolic blood pressure	0.006	0.002	0.147	2.500	0.013
ApoA1	Alcohol consumption	0.071	0.013	0.323	5.497	0.000
	Waist circumference	-0.006	0.001	-0.231	-3.943	0.000
ApoB	Waist circumference	0.006	0.002	0.214	3.580	0.000
	Systolic blood pressure	0.002	0.001	0.155	2.560	0.011
	Glucose	0.027	0.012	0.132	2.197	0.029
ApoA1/ApoB	Waist circumference	-0.013	0.003	-0.255	-4.324	0.000
	Alcohol consumption	0.095	0.028	0.202	3.414	0.001
	Systolic blood pressure	-0.005	0.002	-0.187	-3.036	0.003
	Genotype	-0.131	0.062	-0.121	-2.113	0.036
	Age	0.005	0.002	0.158	2.532	0.012
	Glucose	-0.052	0.023	-0.137	-2.294	0.023
Han/Female						
TC	Glucose	0.196	0.043	0.229	4.535	0.000
	Age	0.008	0.004	0.108	2.141	0.033
TG	Waist circumference	0.025	0.004	0.289	6.074	0.000
	Glucose	0.130	0.037	0.168	3.539	0.000
HDL-C	Body mass index	-0.032	0.007	-0.216	-4.384	0.000
LDL-C	Glucose	0.059	0.022	0.138	2.699	0.007
	Age	0.006	0.002	0.160	3.136	0.002
ApoA1	Body mass index	-0.010	0.003	-0.174	-3.510	0.001
ApoB	Waist circumference	0.007	0.001	0.258	5.326	0.000
	Height	-0.007	0.002	-0.184	-3.638	0.000
	Age	0.003	0.001	0.160	3.215	0.001
ApoA1/ApoB	Age	-0.003	0.002	-0.107	-2.099	0.036
	Waist circumference	-0.009	0.004	-0.217	-2.152	0.032
	Glucose	-0.043	0.018	-0.115	-2.396	0.017
	Body mass index	0.176	0.059	1.551	2.980	0.003
	Height	0.068	0.018	1.115	3.736	0.000
	Weight	-0.081	0.026	-1.874	-3.148	0.002
Jing/Male						
TC	Glucose	0.083	0.031	0.162	2.693	0.008
	Cigarette smoking	0.161	0.058	0.173	2.782	0.006
	Age	0.014	0.004	0.216	3.204	0.002

	Pulse pressure	-0.010	0.003	-0.184	-2.816	0.005
	Body mass index	0.036	0.016	0.132	2.185	0.030
TG	Waist circumference	0.046	0.006	0.439	7.444	0.000
	Cigarette smoking	0.286	0.067	0.244	4.306	0.000
	Glucose	0.136	0.035	0.212	3.836	0.000
	Height	-0.038	0.010	-0.238	-3.939	0.000
	Age	-0.015	0.005	-0.186	-3.091	0.002
HDL-C	Waist circumference	-0.018	0.003	-0.399	-7.205	0.000
	Alcohol consumption	0.171	0.035	0.270	4.879	0.000
	Genotype	0.143	0.052	0.152	2.765	0.006
LDL-C	Genotype	0.135	0.047	0.175	2.869	0.004
ApoA1	Waist circumference	-0.006	0.001	-0.296	-4.977	0.000
	Alcohol consumption	0.050	0.017	0.171	2.884	0.004
ApoB	Waist circumference	-0.006	0.001	-0.296	-4.977	0.000
	Alcohol consumption	0.050	0.017	0.171	2.884	0.004
ApoA1/ApoB	Weight	-0.014	0.002	-0.346	-5.956	0.000
Jing/Female						
TC	Glucose	0.169	0.031	0.260	5.421	0.000
	Age	0.021	0.004	0.259	4.990	0.000
	Pulse pressure	-0.006	0.003	-0.110	-2.115	0.035
TG	Waist circumference	0.026	0.004	0.298	6.161	0.000
	Cigarette smoking	1.166	0.316	0.175	3.687	0.000
	Height	-0.027	0.006	-0.213	-4.370	0.000
HDL-C	Waist circumference	-0.018	0.003	-0.344	-7.011	0.000
	Diastolic blood pressure	0.005	0.002	0.104	2.111	0.035
LDL-C	Age	0.008	0.002	0.207	4.221	0.000
	Glucose	0.048	0.016	0.149	3.032	0.003
ApoA1	Body mass index	-0.011	0.004	-0.158	-3.165	0.002
ApoB	Age	0.004	0.001	0.188	3.826	0.000
	Body mass index	0.011	0.004	0.153	3.123	0.002
ApoA1/ApoB	Body mass index	-0.023	0.005	-0.204	-4.146	0.000
	Age	-0.004	0.001	-0.131	-2.670	0.008
	Glucose	-0.024	0.012	-0.099	-2.003	0.046

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; B, unstandardized coefficient; Beta, standardized coefficient. The correlation between serum lipid parameters and the genotypes (CC=1, CT/TT=2) and several environmental factors was determined by multivariable linear regression analyses with stepwise modeling.

carriers. These findings suggest that there may be a racial/ethnic- and/or sex-specific association between the *PCSK7* rs2277287 SNP and serum lipid parameters in our study populations. Jing ethnic group belongs to coastal minority. About 1511, the ancestors of this ethnic group emigrated from Vietnam to China and first settled on the three abovementioned lands. The unique customs such as intraethnic marriages are still completely conserved to the present day. Therefore, it is considered that the hereditary characteristics and genotypes of certain lipid metabolism-related genes in this population may be different from those in the Han Chinese.

It is well known that environmental factors such as dietary patterns, lifestyle and physical inactivity are all strongly related with serum lipid levels [32]. In the present study, multivariate linear regression analysis also showed that serum lipid parameters were correlated to age, sex, waist circumference, BMI, blood pressure, blood glucose, alcohol consumption, and ciga-

rette smoking in both ethnic groups. These findings suggest that the environmental factors also play a key role in determining serum lipid levels in our study populations. The dietary habits are different between the Han and Jing populations. Rice is the Jing people's staple food supplemented with corn, sweet potato, taro and other grains. Jing people prefer to eat sweet food such as sweet glutinous rice porridge, mung bean syrup, because they believe sweet food is a symbol for happiness. This preference of sugariness may be related to the higher blood glucose levels, weight, BMI and waist circumference in Jing than in Han people. Jing nationality, as the only Chinese minority for coastal fisheries, eats lots of seafood like fish, shrimp, crabs, shellfish and sandworm. Nuoc-mam ruoc, a kind of special fish sauce in Jing, almost is used in their every meal, which contains 17 amino acids (8 essential amino acids included of course). Such a diet pattern is very similar with the Mediterranean diet, which can produce beneficial effects on serum lipid levels [33]. Many studies have proved that diet alone can account for the variability on serum lipid levels [34-36].

In addition, we also noticed that the percentages of alcohol consumption were lower in Jing than in Han nationalities ($P<0.001$). Several case-control and cohort studies have described a J- or U-shaped association between alcohol intake and atherogenesis [37]. A moderate intake of alcohol when taken on a regular basis has been showed to protect against CAD death, which has been ascribed to the changes in serum HDL-C, TG and ApoA1 levels [38]. However, alcohol consumption was also associated with worse hematological values of TC and LDL-C levels. Results from the Italian Longitudinal Study on Aging showed that in elderly men (65-84 years) alcohol consumption increases serum LDL-C levels [39]. Onat *et al*. [40] also showed that alcohol consumption is positively associated with TG, LDL-C, and ApoB levels in males and negatively correlated with TG and/or not correlated with LDL-C and ApoB levels in females. Nevertheless, another research indicated that the effects of alcohol consumption on LDL-C appear to vary by specific patient types or patterns of alcohol intake, and sex as well as genetic variants [40]. Therefore, the results of exposure to different lifestyle and environmental factors probably further modify the association of genetic variations and serum lipid levels in our study populations.

Limitations

There are several potential limitations in our study. First, we were not able to alleviate the effect of diet and several environmental factors during the statistical analysis. Second, we could not completely exclude asymptomatic disorders such as atherosclerosis which may create a potentially significant bias due to poor field study condition. Third, although we observe significant association of the *PCSK7* rs2277287 SNP and serum lipid levels, there are still many unmeasured environmental and genetic factors that needed to be considered. The interactions of gene-gene, gene-environment, and environment-environment on serum lipid levels are remained to be determined. What's more, the relevance of this finding has to be defined in further high caliber of studies including incorporating the genetic information of the *PCSK7* rs2277287 SNP and *in vitro* functional studies to confirm the impact of a varianton a molecular level.

Conclusions

The present study showed that the genotypic and allelic frequencies of the *PCSK7* rs2277287 SNP were different between the Han and Jing populations. The levels of ApoB and the ratio of ApoA1 to ApoB in Han; HDL-C in Jing; The ratio of ApoA1 to ApoB in Han males and females; and HDL-C, LDL-C and ApoB in Jing males were different between the T allele carriers and T allele non-carriers. These findings suggest that the association between the *PCSK7* rs2277287 SNP and serum lipid levelsmight have a racial/ethnic- and/or sex-specificity.

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Disclosure of conflict of interest

None.

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