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Original Article

Association of *RBM5* rs2013208 SNP with serum lipid levels in two Chinese ethnic groups

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Abstract: The RNA binding motif protein 5 gene (*RBM5*) rs2013208 single nucleotide polymorphism (SNP) has been associated with high-density lipoprotein cholesterol (HDL-C) levels in a previous genome-wide association study, but little is known about such association of the *RBM5* rs2013208 SNP and serum lipid profiles in the Chinese populations. The present study was to detect the association of the *RBM5* rs2013208 SNP and several environmental factors with serum lipid levels in the Jing and Han populations. Genotyping of the *RBM5* rs2013208 SNP in 635 subjects of Jing and 648 participants of Han peoples was performed by polymerase chain reaction and restriction fragment length polymorphism, and then confirmed by direct sequencing. There were no significant differences in the genotypic and allelic frequencies of the *RBM5* rs2013208 SNP between the two ethnic groups or between males and females. The *RBM5* rs2013208G allele carriers had lower serum HDL-C levels in both Jing and Han than the G allele non-carriers. The G allele carriers in Jing had higher serum total cholesterol (TC) levels and higher apolipoprotein (Apo) A1/ApoB ratio than the G allele non-carriers ($P < 0.05$). Subgroup analysis according to sex showed that the G allele carriers had lower serum HDL-C levels in both Jing and Han females but not in males ($P < 0.05$). The G allele carriers had higher TC levels in Jing females but not in Jing males, and lower ApoA1/ApoB ratio in Jing males but not in Jing females. 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 *RBM5* rs2013208 SNP and serum lipid levels is different between the Jing and Han populations. These associations might have an ethnic- and/or sex-specificity.

Keywords: RNA binding motif protein 5 gene, single nucleotide polymorphism, rs2013208, lipids, environmental factors

Introduction

Cardiovascular diseases (CVD) are the leading causes of morbidity and mortality in most developed and developing countries [1, 2]. It is well established that dyslipidemia is a major risk factor for CVD among several conventional risk factors such as older age, positive family history, diabetes mellitus, obesity, hypertension, tobacco use, and unhealthy diet [3-5]. Metabolic abnormalities in blood lipids, in particular low-density lipoprotein cholesterol (LDL-C) elevation and high-density lipoprotein cholesterol (HDL-C) depression, are mainly involved in the development and progression of CVD [6, 7]. Epidemiological studies have consistently shown that dyslipidemia is a complex trait resulted from the joint effects of multiple genetic and environmental factors [8, 9] and their interactions [10]. Through family history and twin studies, almost 40%-70% of the inter-individual variation in plasma lipid phenotypes can be explained by genetic polymorphisms [10, 11]. Human genetic studies of lipid levels can identify targets for new therapies for cholesterol management and prevention of heart disease [12, 13].

Since 2006, genome-wide association studies (GWAS) have implicated numerous common genetic variants and respective proteins in the determination of lipid and lipoprotein levels [12, 14]. It was reported that loci associated with blood lipids, accounting for ~10-12% of the total trait variance, and variants with small effects can point to pathways and therapeutic

targets that enable clinically-important changes in blood lipids [12, 15]. A recent study has identified 157 loci associated with lipid levels at $P < 5 \times 10^{-8}$, including 62 loci not previously associated with lipid levels in humans which has mentioned that the *RBM5* rs2013208 SNP was associated with HDL-C levels for the first time [14]. Among the new 62 loci, two SNPs showing strongest association to coronary artery disease (CAD) near *RBM5* (rs2013208, $P_{HDL} = 9 \times 10^{-12}$, $P_{CAD} = 7 \times 10^{-5}$) and *CMTM6* (rs-7640978, $P_{LDL} = 1 \times 10^{-8}$, $P_{CAD} = 4 \times 10^{-4}$) [14]. *RBM5* (http://www.ncbi.nlm.nih.gov/gene) is a candidate tumor suppressor gene which encodes a nuclear RNA binding protein that is a component of the spliceosome A complex. The encoded protein plays a role in the induction of cell cycle arrest and apoptosis through pre-mRNA splicing of multiple target genes including the tumor suppressor protein p53. This gene is located within the tumor suppressor region 3p21.3, and may play a role in the inhibition of tumor transformation and progression of several malignancies including lung cancer. However, the biological function of the *RBM5* rs2013208 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 *RBM5* rs2013208 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. In the early 16th century, the Jing ancestors emigrated from Vietnam to China, now most of them live in the so called "Three Islands of Jing Nationality", Dongxing City, Guangxi Zhuang Autonomous Region, People's Republic of China [16, 17]. Jing is unique in Chinese ethnic minorities living in the nation of the sea, the way of life is single. Jing nationality is a relatively conservative and isolated minority, and preserves their custom of intra-ethnic marriage, which suggests that there are lots of differences between Jing and Han (as well as the other landlocked nationalities) nationality in diet custom and culture characteristics. To our knowledge, the association of *RBM5* rs2013208 SNP and serum lipid profiles has not been previously reported in this population. Thus, the present study was to detect the association of the *RBM5* rs2013208 SNP and serum lipid levels in the Jing and Han populations.

Materials and methods

Subjects

A total of 648 unrelated subjects (245 males, 37.81% and 403 females, 62.19%) of Han nationality and 635 unrelated participants (244 males, 38.43% and 391 females, 61.57%) of Jing nationality were randomly selected from our previous stratified randomized samples. 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 participants' age ranged from 27 to 92 years with a mean age of 57.44±12.55 years in Han and 56.22±12.99 years in Jing, respectively. 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 [18]. 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)

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

Parameter	Han	Jing	t (x^2)	P
Number	648	635		
Male/female	245/403	244/391	0.052	0.820
Age (year)	57.44±12.55	56.22±12.99	-1.71	0.088
Height (cm)	156.21±7.75	156.94±7.73	1.68	0.093
Weight (kg)	55.50±9.37	57.87±9.89	4.42	0.000
Body mass index (kg/m^2)	22.67±3.16	23.42±3.18	4.17	0.000
Waist circumference (cm)	77.24±8.98	79.89±8.85	5.31	0.000
Cigarette smoking [n (%)]				
Non-smoker	551 (85.0)	549 (86.5)		
< 20 cigarettes/day	23 (3.5)	19 (3.0)		
≥ 20 cigarettes/day	74 (11.4)	67 (10.6)	0.600	0.741
Alcohol consumption [n (%)]				
Non-drinker	543 (83.8)	563 (88.7)		
< 25 g/day	23 (3.5)	39 (6.1)		
≥ 25 g/day	82 (12.7)	33 (5.2)	25.240	0.000
Systolic BP (mmHg)	132.28±19.25	132.13±21.65	-0.133	0.895
Diastolic BP (mmHg)	80.22±10.06	80.22±10.07	-1.55	0.12
Pulse pressure (mmHg)	51.18±15.15	51.91±17.96	0.784	0.433
Glucose (mmol/L)	6.65±1.11	7.32±0.38	14.628	0.000
Total cholesterol (mmol/L)	4.93±0.88	4.92±0.94	-0.229	0.819
Triglyceride (mmol/L)	1.32 (0.63)	1.44 (0.73)	-3.99	0.000
HDL-C (mmol/L)	1.81±0.51	1.76±0.44	-2.130	0.033
LDL-C (mmol/L)	2.86±0.44	2.82±0.45	-1.724	0.085
Apolipoprotein (Apo) A1 (g/L)	1.32±0.20	1.29±0.23	-2.432	0.015
ApoB (g/L)	1.04±0.24	1.05±0.24	0.806	0.420
ApoA1/ApoB	1.34±0.38	1.30±0.38	-2.13	0.034

BP, blood pressure; 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 were presented 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.

was calculated from the height and weight measurements.

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 total cholesterol (TC), triglyceride (TG), HDL-C and LDL-C in the samples were determined by enzymatic methods with commercially available kits. Serum apolipoprotein (Apo) A1 and ApoB levels were assessed by the immuneturbidimetric immunoassay [19, 20].

DNA amplification and genotyping

Genomic DNA was isolated from peripheral blood leukocytes using the phenol-chloroform method. The extracted DNA was stored at -20°C until analysis. Genotyping of the *RBM5* rs2013208 SNP was performed by polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP). PCR amplification was performed using 5'-CTTCGGGATTCACGCTCATC-3' and 5'-ACTCTAGGCTTGACAAAATGCA-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 45 s of denaturing at 95°C, 35 s of annealing at 58°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 *BsuR*I enzyme was added directly to the PCR products (10 μL) and digested at 37°C overnight. After restriction

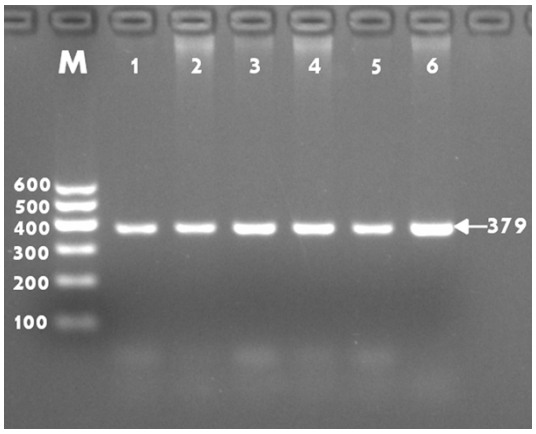

Figure 1. Electrophoresis of PCR products of the samples. Lane M, 100 bp marker ladder; lanes 1-6, samples. The 379 bp bands are the target genes.

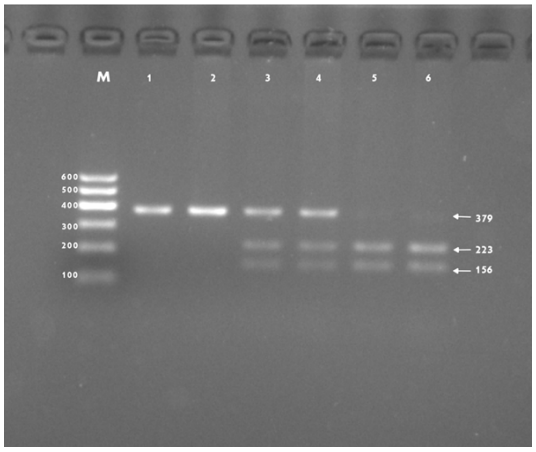

Figure 2. Genotyping of the *RBM5* rs2013208 SNP. Lane M is the 100 bp Marker Ladder; lanes 1 and 2, AA genotype (379-bp); lanes 3 and 4, AG genotype (156-, 223- and 379-bp); and lanes 5 and 6, GG genotype (156- and 223-bp).

enzyme digestion of the amplified DNA, genotypes were identified by electrophoresis on 2% ethidium-bromide stained agarose gels and visualizing with ultraviolet illumination. Genotypes were scored by an experienced reader blinded to the epidemiological and lipid results. Six samples (AA, AG and GG genotypes in two; respectively) detected by the PCR-RFLP were also confirmed by direct sequencing. The PCR product was 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. The methods of DNA amplification and genotyping were referred to our previous studies [20, 21].

Diagnostic criteria

The normal values of serum TC, TG, HDL-C, LDL-C, ApoA1 and ApoB levels, and the ratio of 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 hyperlipidemia [22, 23]. Hypertension was assessed according to the criteria outlined by the 1999 World Health Organization-International Society of Hypertension Guidelines for the management of hypertension [24, 25]. The categories of normal weight, overweight and obesity were defined as a BMI of < 24, 24-28 and > 28 kg/m^2; respectively. The diagnostic criteria of overweight and obesity were according to the Cooperative Meta-analysis Group of China Obesity Task Force [26].

Statistical analyses

Epidemiological data were recorded on a predesigned 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). Age, sex, BMI, cigarette smoking, and alcohol consumption were adjusted for the statistical analysis. Multivariable linear regression analyses with stepwise modeling were used to determine the correlation between genotypes (AA = 1, AG/GG = 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.

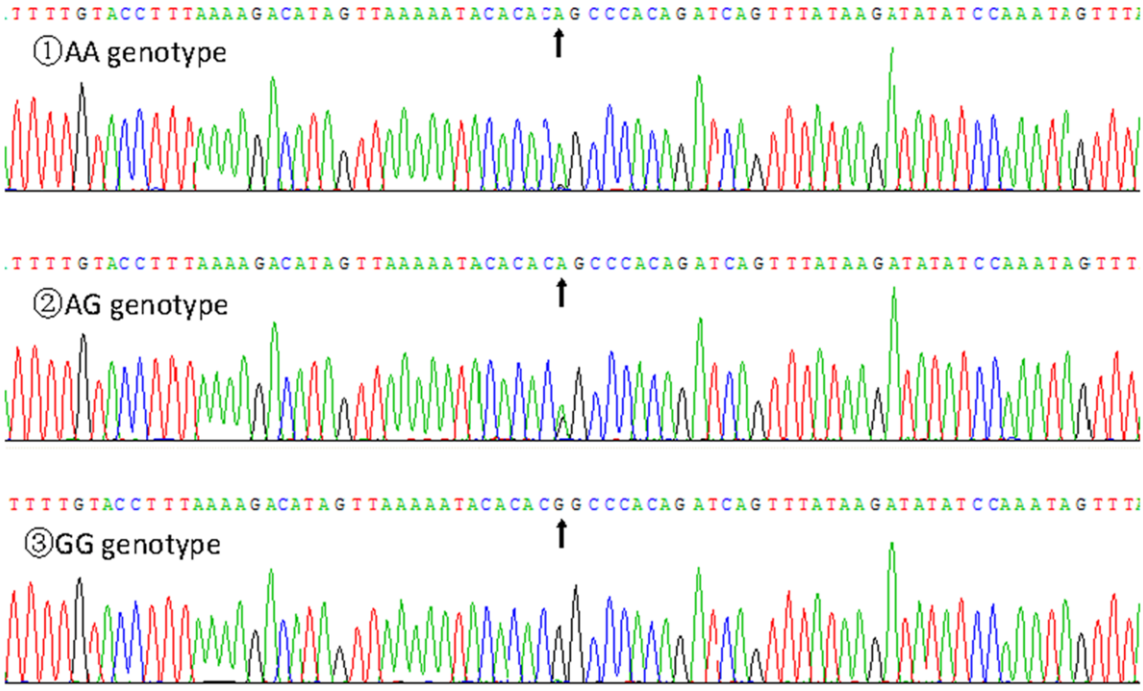

Figure 3. A part of the *RBM5* rs2013208 SNP sequence.

Table 2. Comparison of the genotype and allele frequencies of the *RBM5* rs2013208 SNP in the Han and Jing populations [n (%)]

Group	n	Genotype			Allele	
		AA	AG	GG	A	G
Han	648	470 (72.5)	169 (26.1)	9 (1.4)	1109 (85.6)	187 (14.4)
Jing	635	477 (75.1)	143 (22.5)	15 (2.4)	1097 (86.4)	173 (13.6)
x^2			3.587		0.346	
P			0.166		0.556	
Han						
Male	245	177 (72.2)	67 (27.3)	1 (0.4)	421 (85.9)	69 (14.1)
Female	403	293 (72.7)	102 (25.3)	8 (2.0)	688 (85.4)	118 (14.6)
x^2			2.975		0.077	
P			0.226		0.781	
Jing						
Male	244	182 (74.6)	55 (22.5)	7 (2.9)	419 (85.9)	69 (14.1)
Female	391	295 (75.4)	88 (22.5)	8 (2.0)	678 (86.7)	104 (13.3)
x^2			0.445		0.180	
P			0.800		0.671	

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 weight, waist circumference, BMI, TG, glucose were higher in Jing than in Han, but the ApoA1/ApoB ratio, the percentages of subjects consuming alcohol, the levels of ApoA1, HDL-C 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, LDL-C, TC, 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 379-bp nucleotide sequences was 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

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	470	4.93±0.87	1.33 (0.62)	1.84±0.54	2.87±0.43	1.32±0.20	1.03±0.24	1.35±0.38
AG/GG	178	4.94±0.91	1.30 (0.67)	1.74±0.43	2.86±0.44	1.32±0.20	1.06±0.25	1.31±0.36
F		0.138	-0.530	3.937	0.002	1.254	1.696	1.474
P		0.710	0.596	0.048	0.969	0.263	0.193	0.225
Jing								
AA	477	4.86±0.94	1.43 (0.76)	1.78±0.45	2.83±0.44	1.29±0.24	1.05±0.24	1.28±0.37
AG/GG	158	5.11±0.90	1.52 (0.66)	1.67±0.43	2.80±0.46	1.30±0.18	1.04±0.26	1.34±0.42
F		5.586	-1.214	4.984	2.136	0.001	2.010	6.376
P		0.018	0.225	0.026	0.144	0.972	0.157	0.012

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	177	4.81±0.86	1.32 (0.63)	1.74±0.53	2.83±0.43	1.32±0.21	1.04±0.23	1.34±0.40
AG/GG	68	4.90±0.86	1.35 (0.70)	1.71±0.53	2.90±0.45	1.31±0.20	1.09±0.26	1.26±0.35
F		0.522	-0.633	0.273	1.256	0.055	1.638	0.960
P		0.471	0.527	0.602	0.264	0.815	0.202	0.328
Han/female								
AA	293	5.00±0.87	1.35 (0.61)	1.90±0.53	2.88±0.44	1.33±0.20	1.03±0.25	1.34±0.36
AG/GG	110	4.97±0.94	1.27 (0.67)	1.76±0.36	2.84±0.44	1.33±0.19	1.05±0.25	1.39±0.35
F		0.089	-0.167	4.351	0.441	0.287	0.411	0.209
P		0.766	0.867	0.038	0.507	0.592	0.522	0.648
Jing/male								
AA	182	4.82±0.90	1.50 (0.81)	1.70±0.41	2.84±0.41	1.26±0.21	1.08±0.23	1.26±0.39
AG/GG	62	5.05±0.75	1.64 (1.08)	1.59±0.43	2.77±0.34	1.28±0.18	1.03±0.25	1.22±0.34
F		1.106	-0.735	0.869	3.072	1.387	3.123	4.698
P		0.294	0.462	0.352	0.081	0.240	0.079	0.031
Jing/female								
AA	295	4.89±0.96	1.38 (0.61)	1.83±0.46	2.83±0.46	1.31±0.25	1.04±0.24	1.31±0.36
AG/GG	96	5.15±0.99	1.52 (0.34)	1.73±0.42	2.81±0.53	1.32±0.19	1.04±0.27	1.33±0.41
F		5.488	-0.975	2.635	0.102	0.197	0.000	0.924
P		0.020	0.329	0.105	0.750	0.657	0.989	0.337

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.

the cutting site indicates the A allele cannot be cut, while its presence indicates the G allele can be cut. Thus, GG genotype is homozygote for the presence of the site (156- and 223-bp), AG genotype is heterozygote for the presence and absence of the site (156-, 223- and 379-bp), and AA genotype is homozygote for the absence of the site (379-bp; **Figure 2**).

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	0.040	0.005	0.419	8.807	0.000
	Cigarette smoking	0.250	0.037	0.185	6.737	0.000
	Weight	-0.018	0.004	-0.206	-4.295	0.000
	Glucose	0.099	0.025	0.103	3.925	0.000
	Diastolic blood pressure	0.009	0.002	0.105	3.914	0.000
TC	Glucose	0.153	0.028	0.151	5.502	0.000
	Age	0.006	0.002	0.086	3.039	0.002
	Height	-0.011	0.003	-0.098	-3.525	0.000
	Diastolic blood pressure	0.007	0.002	0.082	2.982	0.003
	Genotype	0.122	0.056	0.059	2.161	0.031
HDL-C	Waist circumference	-0.016	0.002	-0.301	-10.687	0.000
	Gender	0.169	0.036	0.171	4.647	0.000
	Alcohol consumption	0.120	0.025	0.149	4.854	0.000
	Cigarette smoking	-0.073	0.024	-0.097	-3.063	0.002
	Genotype	-0.085	0.029	-0.078	-2.954	0.003
	Diastolic blood pressure	0.003	0.001	0.061	2.255	0.024
	Height	0.005	0.002	0.077	2.234	0.026
LDL-C	Glucose	0.037	0.014	0.075	2.695	0.007
	Height	-0.004	0.002	-0.070	-2.485	0.013
	Diastolic blood pressure	0.005	0.001	0.106	3.842	0.000
	Age	0.003	0.001	0.081	2.857	0.004
ApoA1	Body mass index	-0.013	0.002	-0.188	-6.940	0.000
	Alcohol consumption	0.057	0.011	0.159	5.264	0.000
	Gender	0.059	0.013	0.133	4.422	0.000
	Glucose	-0.015	0.006	-0.062	-2.298	0.022
ApoB	Waist circumference	0.006	0.001	0.223	7.933	0.000
	Age	0.001	0.001	0.078	2.658	0.008
	Height	-0.004	0.001	-0.134	-3.537	0.000
	Gender	-0.047	0.018	-0.093	-2.563	0.010
ApoA1/ApoB	Body mass index	0.072	0.032	0.601	2.264	0.024
	Glucose	-0.040	0.011	-0.093	-3.488	0.001
	Age	-0.002	0.001	-0.056	-1.934	0.053
	Waist circumference	-0.007	0.002	-0.158	-2.908	0.004
	Alcohol consumption	0.058	0.019	0.090	3.058	0.002
	Gender	0.095	0.030	0.122	3.228	0.001
	Height	0.030	0.009	0.602	3.178	0.002
	Weight	-0.036	0.013	-0.919	-2.807	0.005
Han						
TG	Waist circumference	0.030	0.006	0.319	4.731	0.000
	Cigarette smoking	0.193	0.050	0.151	3.836	0.000
	Diastolic blood pressure	0.007	0.003	0.089	2.291	0.022
	Glucose	0.124	0.028	0.165	4.394	0.000
	Weight	-0.013	0.006	-0.141	-2.062	0.040
TC	Height	-0.012	0.004	-0.103	-2.740	0.006
	Glucose	0.213	0.030	0.269	7.127	0.000
HDL-C	Waist circumference	-0.013	0.002	-0.227	-5.947	0.000

Nucleotide sequences

The results were separated into AA, AG and GG genotypes of the *RBM5* rs2013208 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 rs2013208 SNP in the both ethnic groups are shown in **Table 2**. The frequency of *RBM5* rs2013208-G allele was 14.4% in Han and 13.6% in Jing ($P > 0.05$). There was no significant difference in either genotypic or allelic frequencies between Han and Jing, or between males and females of the both ethnic groups.

Genotypes and serum lipid levels

As shown in **Tables 3** and **4**, the levels of TC and the ratio of ApoA1 to ApoB were different between the AA and AG/GG genotypes in Jing ($P < 0.05$ for each) but not in Han, the G allele carriers had higher TC levels and ApoA1/ApoB ratio than the G allele non-carriers. The G allele carriers had lower serum HDL-C levels than the G allele non-carriers in both Han and Jing. Subgroup analyses showed that the G allele carriers in Han females but not in

	Gender	0.124	0.049	0.118	2.558	0.011
	Alcohol consumption	0.121	0.035	0.160	3.417	0.001
	Cigarette smoking	-0.112	0.037	-0.142	-3.039	0.002
LDL-C	Glucose	0.061	0.015	0.155	3.922	0.000
	Systolic blood pressure	0.003	0.001	0.112	2.848	0.005
	Height	-0.004	0.002	-0.079	-2.066	0.039
ApoA1	Body mass index	-0.012	0.002	-0.186	-4.936	0.000
	Alcohol consumption	0.075	0.013	0.253	5.809	0.000
	Gender	0.064	0.018	0.155	3.571	0.000
	Glucose	-0.015	0.007	-0.080	-2.128	0.034
ApoB	Waist circumference	0.006	0.001	0.220	5.536	0.000
	Systolic blood pressure	0.001	0.000	0.115	2.966	0.003
	Height	-0.007	0.002	-0.208	-4.201	0.000
	Gender	-0.077	0.024	-0.152	-3.133	0.002
ApoA1/ApoB	Waist circumference	-0.007	0.003	-0.156	-2.016	0.044
	Body mass index	0.093	0.043	0.780	2.176	0.030
	Glucose	-0.037	0.013	-0.109	-2.911	0.004
	Systolic blood pressure	-0.002	0.001	-0.092	-2.388	0.017
	Alcohol consumption	0.076	0.023	0.136	3.225	0.001
	Gender	0.160	0.040	0.206	3.994	0.000
	Height	0.040	0.012	0.817	3.186	0.002
	Weight	-0.045	0.017	-1.113	-2.573	0.010
Jing						
TG	Waist circumference	0.047	0.007	0.463	6.529	0.000
	Cigarette smoking	0.303	0.059	0.214	5.114	0.000
	Height	-0.029	0.006	-0.251	-5.003	0.000
	Diastolic blood pressure	0.010	0.003	0.117	3.111	0.002
	Body mass index	-0.040	0.019	-0.143	-2.122	0.034
	Gender	-0.184	0.089	-0.100	-2.058	0.040
TC	Age	0.015	0.003	0.205	4.818	0.000
	Genotype	0.204	0.084	0.094	2.422	0.016
	Diastolic blood pressure	0.009	0.004	0.098	2.464	0.014
	Pulse pressure	-0.006	0.002	-0.121	-2.843	0.005
	Height	-0.022	0.006	-0.182	-3.512	0.000
	Cigarette smoking	0.149	0.061	0.100	2.461	0.014
	Weight	0.010	0.005	0.106	2.135	0.033
HDL-C	Waist circumference	-0.016	0.002	-0.327	-8.753	0.000
	Gender	0.148	0.036	0.162	4.068	0.000
	Alcohol consumption	0.144	0.036	0.159	4.001	0.000
	Genotype	-0.084	0.038	-0.082	-2.218	0.027
LDL-C	Age	0.004	0.001	0.128	3.258	0.001
	Diastolic blood pressure	0.004	0.002	0.101	2.563	0.011
ApoA1	Weight	-0.005	0.001	-0.204	-5.253	0.000
ApoB	Waist circumference	0.005	0.001	0.179	4.603	0.000
	Age	0.002	0.001	0.105	2.693	0.007
ApoA1/ApoB	Waist circumference	-0.007	0.003	-0.155	-2.381	0.018
	Genotype	0.085	0.034	0.096	2.513	0.012
	Age	-0.003	0.001	-0.091	-2.386	0.017
	Body mass index	-0.017	0.008	-0.139	-2.114	0.035

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.

Han males had lower HDL-C levels than the G allele non-carriers ($P < 0.05$). The G allele carriers in Jing females but not in Jing males had higher TC levels than the G allele non-carriers ($P < 0.001$). The G allele carriers in Jing males but not in Jing females had lower ApoA1/ApoB ratio than the G allele non-carriers ($P < 0.05$). There was no significant difference in the remaining serum lipid parameters between 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 HDL-C levels in Jing and Han, HDL-C, TC levels and ApoA1/ApoB ratio in Jing were correlated with genotypes ($P < 0.05$), respectively. When serum lipid data were analyzed according to gender, serum TC levels in Jing and HDL-C levels in Han were associated with the genotypes only in females but not in males. The ApoA1/ApoB ratio in Jing was associated with the genotypes only in males but not in females. Several environmental factors such as age, gender, height, weight, waist

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	Diastolic blood pressure	0.025	0.006	0.300	4.008	0.000
	Age	0.016	0.004	0.260	3.705	0.000
	Cigarette smoking	0.167	0.064	0.171	2.620	0.009
	Systolic blood pressure	-0.009	0.004	-0.190	-2.418	0.016
TG	Waist circumference	0.042	0.007	0.396	6.136	0.000
	Cigarette smoking	0.252	0.072	0.214	3.482	0.001
	Height	-0.033	0.010	-0.214	-3.301	0.001
	Diastolic blood pressure	0.014	0.006	0.139	2.321	0.021
	Age	-0.010	0.005	-0.135	-2.111	0.036
LDL-C	Diastolic blood pressure	0.006	0.002	0.154	2.435	0.016
	Glucose	-0.131	0.061	-0.136	-2.143	0.033
HDL-C	Waist circumference	-0.017	0.003	-0.385	-6.583	0.000
	Alcohol consumption	0.147	0.034	0.249	4.275	0.000
	Glucose	-0.125	0.060	-0.121	-2.085	0.038
ApoA1	Waist circumference	-0.006	0.001	-0.285	-4.609	0.000
	Alcohol consumption	0.038	0.018	0.131	2.116	0.035
ApoB	Body mass index	0.018	0.005	0.234	3.737	0.000
ApoA1/ApoB	Weight	-0.013	0.002	-0.338	-5.573	0.000
	Genotype	0.115	0.056	0.125	2.061	0.040
Jing/female						
TG	Waist circumference	0.028	0.005	0.291	5.992	0.000
	Cigarette smoking	0.913	0.299	0.146	3.049	0.002
	Height	-0.028	0.007	-0.207	-4.230	0.000
TC	Age	0.018	0.004	0.227	4.628	0.000
	Genotype	0.276	0.111	0.122	2.476	0.014
LDL-C	Age	0.008	0.002	0.207	4.164	0.000
HDL-C	Waist circumference	-0.017	0.003	-0.309	-6.414	0.000
ApoA1	Body mass index	-0.011	0.004	-0.152	-3.029	0.003
ApoB	Body mass index	0.011	0.004	0.146	2.953	0.003
	Age	0.003	0.001	0.162	3.273	0.001
ApoA1/ApoB	Body mass index	-0.024	0.006	-0.211	-4.305	0.000
	Age	-0.003	0.001	-0.111	-2.236	0.026
	Glucose	-0.108	0.050	-0.106	-2.151	0.032
Han/male						
TG	Waist circumference	0.041	0.013	0.374	3.257	0.001
	Cigarette smoking	0.190	0.064	0.185	2.972	0.003
	Diastolic blood pressure	0.015	0.005	0.177	2.915	0.004
	Age	-0.014	0.005	-0.182	-2.765	0.006
	Glucose	0.171	0.052	0.201	3.304	0.001
	Weight	-0.026	0.013	-0.243	-2.068	0.040
TC	Diastolic blood pressure	0.012	0.005	0.153	2.525	0.012
	Glucose	0.242	0.048	0.305	5.035	0.000
LDL-C	Diastolic blood pressure	0.006	0.003	0.155	2.527	0.012
	Glucose	0.103	0.025	0.255	4.161	0.000

circumference, alcohol consumption and cigarette smoking, 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 ($P < 0.05$-0.001, **Tables 5** and **6**).

Discussion

In the present study, we showed that the levels of weight, waist circumference, BMI, TG, glucose were higher in Jing than in Han, but the ratio of ApoA1/ApoB, the percentages of subjects consuming alcohol, the levels of ApoA1 and HDL-C were lower in Jing than in Han ($P < 0.05$-0.001). There were no significant differences in the levels of LDL-C, TC, ApoB between the two ethnic groups. These differences in serum lipid profiles between the two ethnic groups may result from the combined action of genetic including lipid-associated gene variants, environmental factors including diet, alcohol consumption, cigarette smoking, obesity, exercise, hypertension and their interactions [23, 27, 28]. Jing is the only Chinese minority for coastal fisheries

HDL-C	Waist circumference	-0.017	0.004	-0.267	-4.250	0.000
	Alcohol consumption	0.107	0.038	0.187	2.825	0.005
	Diastolic blood pressure	0.006	0.003	0.128	2.041	0.042
	Cigarette smoking	-0.121	0.038	-0.207	-3.175	0.002
ApoA1	Waist circumference	-0.005	0.001	-0.213	-3.576	0.000
	Alcohol consumption	0.078	0.013	0.350	5.865	0.000
ApoB	Waist circumference	0.006	0.002	0.219	3.522	0.001
	Systolic blood pressure	0.002	0.001	0.173	2.782	0.006
ApoA1/ApoB	Waist circumference	-0.013	0.003	-0.279	-4.559	0.000
	Alcohol consumption	0.085	0.026	0.199	3.255	0.001
	Systolic blood pressure	-0.003	0.001	-0.138	-2.238	0.026
Han/female						
TG	Waist circumference	0.021	0.004	0.246	5.174	0.000
	Glucose	0.125	0.033	0.182	3.817	0.000
TC	Glucose	0.206	0.038	0.261	5.410	0.000
LDL-C	Glucose	0.047	0.019	0.121	2.404	0.017
	Age	0.003	0.002	0.091	1.736	0.083
	Height	-0.007	0.004	-0.109	-2.128	0.034
HDL-C	Body mass index	-0.031	0.007	-0.209	-4.307	0.000
	Genotype	-0.140	0.054	-0.126	-2.593	0.010
	Pulse pressure	-0.003	0.002	-0.111	-2.293	0.022
ApoA1	Body mass index	-0.011	0.003	-0.192	-3.948	0.000
	Glucose	-0.022	0.009	-0.125	-2.562	0.011
ApoB	Age	0.003	0.001	0.146	2.952	0.003
	Waist circumference	0.006	0.001	0.231	4.786	0.000
	Height	-0.008	0.002	-0.195	-3.888	0.000
ApoA1/ApoB	Weight	-0.077	0.024	-1.856	-3.201	0.001
	Body mass index	0.147	0.056	1.332	2.619	0.009
	Age	-0.004	0.001	-0.142	-2.945	0.003
	Glucose	-0.040	0.015	-0.122	-2.627	0.009
	Height	0.062	0.017	1.083	3.695	0.000

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.

and 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. Jing nationality is a relatively conservative and isolated minority in China that retains its regional and special customs. Their marriages were family-arranged in the old days when they sing antiphonal songs to look for the other half. After antiphonal singing, if the boy's into the girl he would kick sand toward her while approaching her. If the girl feel the same she would kick back, which means engagement. Jing stays endogamy, intermarriage with Han or Zhuang people is seldom happened. Jing people don't get married with someone sharing the same lastname,alsocross-cousin marriage is strictly forbidden. Therefore, we deduced that some hereditary characteristics and genotypes of specific lipid metabolism-associated genes in this population may be different from those in the Han people.

The genotypic and allelic frequencies of the *RBM5* rs2013208 SNP in diverse racial/ethnic groups are significantly different. According to the 1000 genomes project data, the frequency of the rs2013208G allele was 13.59% in Han Chinese from Beijing, 10.48% in Southern Han Chinese, 19.23% in Japanese from Tokyo, 52.75% in British in England and Scotland, 45.96% in Finnish in Finland, 52.34% in Iberian population in Spain. However, in African ancestry, the frequency of the rs2013208G allele was 46.72% in Americans of African ancestry in SW USA, 34.85% of Esan in Nigeria, 41.15% in Gambian in Western Divisions in the Gambia. In the present study, we showed that the frequencies of G alleles were 14.4% in Han and 13.6% in Jing ($P > 0.05$); respectively. Apparently, the minor allele frequency was lower in Asian than the Western populations. There were no conspicuous differences in the genotypic and allelic frequencies of the rs2013208 SNP between the Jing and Han populations, or between males and females in the both ethnic groups. As compared with the data in the 1000 genomes project data, we found that the frequencies of the G allele in our study populations (14.4% in Han and 13.6% in

Jing, $P > 0.05$) were higher than those in Southern Han Chinese (10.48%), which may be caused by different sample sizes and regions.

Recently, a newly study identifies and annotates 157 loci associated with lipid levels obtained from Joint GWAS and Metabochip Meta-analysis ($P < 5 \times 10^{-8}$) including 62 loci not previously associated with lipid levels in humans which referred to the association between the *RBM5* rs2013208 SNP and HDL-C levels [14]. Besides this, rare studies have previously reported the direct effect of the *RBM5* rs2013208 SNP on serum lipid levels. Because the effects of newly identified loci were generally smaller than in earlier GWAS, more work is needed to be done to actually confirm the findings. In the present study, the levels of TC and the ratio of ApoA1 to ApoB were different between the AA and AG/GG genotypes in Jing ($P < 0.05$) but not in Han, the G allele carriers had higher TC levels and ApoA1/ApoB ratio than the G allele non-carriers. The G allele carriers had lower serum HDL-C levels than the G allele non-carriers in both Han and Jing. In the subgroup analyses, the G allele carriers in Han females but not in Han males had lower HDL-C levels than the G allele non-carriers ($P < 0.05$). The G allele carriers in Jing females but not in Jing males had higher TC levels than the G allele non-carriers ($P < 0.001$). The G allele carriers in Jing males but not in Jing females had lower ApoA1/ApoB ratio than the G allele non-carriers ($P < 0.05$). There was no significant difference in the remaining serum lipid parameters between the genotypes in Jing, Han, males, or females ($P > 0.05$ for all). These results suggest that there may be an ethnic and/or sex specific-association of the *RBM5* rs2013208 SNP and serum lipid parameters. As far as we know, our study is the first replication of GWAS signals about the association of *RBM5* rs2013208 SNP with serum lipid levels in the Chinese populations. Therefore, further studies with larger sample size are still needed to confirm this association.

In the present study, multiple linear regression analysis showed 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. These data suggest that the environmental factors also play an important role in determining lipid profiles in our study 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. 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. Fishery is the major source of income for Jing population and fish is appeared most frequently dish on their tables. A kind of fish sauce called nuoc-mam is also popular on Jing people's dinner table, which contains 17 amino acids (8 essential amino acids included of course). It has been reported that consuming fish or fish oil containing the n-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) is associated with decreased cardiovascular death [29]. In a previous meta-analysis of 11 prospective cohort studies (encompassing 222 364 persons with an average of 11.8 y of follow-up) indicated that each 20-g/d increase in fish intake was associated with a 7% lower risk of coronary heart disease mortality [30]. Jing people also prefer 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 lead to the higher glucose level in Jing than in Han people.

In the present study, we also found that the levels of weight, waist circumference, and BMI were higher and the percentages of subjects consuming alcohol were lower in Jing than in Han. A recent study in Japan showed that high BMI (> 26) was associated with higher systolic blood pressure (SBP), LDL-C, fasting blood glucose (FBG), and TG in both sexes. An increase ≥ 1.1 BMI units in 5 years was associated with increased diastolic blood pressure (DBP), LDL-C, TG, HbA1c, and FBG and decreased HDL-C. In contrast, decreased BMI was associated with decreased blood pressure and LDL-C and increased HDL-C in both sexes, and decreased TG in men and FBG in women [31]. Their researches showed that maintaining a desirable weight or losing weight may help prevent hypertension and metabolic syndrome (MS), even in non-obese individuals. Alcohol intake has a significant influence on the human serum lipid metabolism. Many studies showed that

moderate alcohol intake has been associated with reduced cardiovascular events which were attributed in great part to the increase in HDL-C caused by alcohol consumption [32, 33]. In contrast, heavy alcohol consumption was robustly positively associated with serum TG, LDL-C levels and blood pressure [32, 34, 35]. 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 [36]. Consequently, the joint effects of different dietary habits, lifestyles, and environmental factors probably further modify the association of genetic variations and serum lipid levels in our study populations.

Limitations

There are some potential limitations in our study. First, this is the first time to report the sex-specific association of the *RBM5* rs2013208 SNP and no previous evidence to support our findings and the number of subjects in our study is moderate, the statistical power is relatively reliable. Thus, further studies with larger samples are needed to replicate our findings in other populations. Second, we only measured serum TC, TG, HDL-C, LDL-C, ApoA1, ApoB levels, and the ratio of ApoA1 to ApoB, but the subclasses lipoproteins such as HDL2, HDL3, small dense LDL, and large buoyant LDL were not detected their associations with rs2013208 SNP. Third, we were not able to alleviate the effect of diet during the statistical analysis since the diet intake was self-reported and difficult to classify.

Conclusions

Our study showed that the association of the *RBM5* rs2013208 SNP and serum lipid levels was different between the Jing and Han populations, and between males and females in the both ethnic groups. These findings suggest that there may be an ethnic- and/or sex-specific association between the *RBM5* rs2013208 SNP and serum lipid levels in different populations.

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Disclosure of conflict of interest

None.

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