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Original Article

Association of the *FRMD5* rs2929282 polymorphism and serum lipid profiles in two Chinese ethnic groups

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Abstract: Little is known about the association of the single nucleotide polymorphism (SNP) of rs2929282 near the FERM domain containing 5 (*FRMD5*) and serum lipid profiles. The present study detected the association of the *FRMD5* rs2929282 SNP and several environmental factors with serum lipid profiles in the Han and Jing populations. Genotyping of the *FRMD5* rs2929282 SNP in 1065 subjects of Jing and 1061 participants of Han peoples was performed by polymerase chain reaction and restriction fragment length polymorphism, and then confirmed by direct sequencing. The genotypic and allelic frequencies of the SNP were different between Han and Jing ($P < 0.05$). The frequency of the T allele was higher in Han than in Jing (8.2% *vs.* 6.1%). The genotypic and allelic frequencies of the *FRMD5* rs2929282 SNP were significantly different between Han males and females ($P < 0.05$ for each), but not between Jing males and females. The frequency of the T allele was higher in Han females than in Han males (9.3% *vs.* 6.5%). The *FRMD5* rs2929282 T allele carriers had lower serum high-density lipoprotein cholesterol (HDL-C), apolipoprotein (Apo) A1, and ApoB levels, and higher triglyceride (TG) levels in Jing but not in Han than the T allele non-carriers. Subgroup analysis according to sex showed that the T allele carriers had higher serum TG levels in Jing females but not in males than the T allele non-carriers ($P < 0.05$). The T allele carriers had higher HDL-C levels in Han males but not in Han females, and lower HDL-C levels in Jing females but not in Jing males compared to the T allele non-carriers ($P < 0.05$). The T allele carriers had lower ApoA1 levels in Jing females but not in Jing males and lower ApoB levels in Jing males but not in Jing females than the T allele non-carriers ($P < 0.05$). Serum lipid traits were also associated with several environmental factors in the Han and Jing populations, and in males and females of the both ethnic groups. These findings indicated that there may be a racial/ethnic- and/or sex-specific association of the *FRMD5* rs2929282 SNP and serum lipid levels.

Keywords: FERM domain containing 5, single nucleotide polymorphism, rs29292982, lipids, environmental factors

Introduction

It is well-established that mortality, morbidity, disability, functional decline, and healthcare costs occurring as a result of coronary artery disease (CAD) are a critical public health concern in most developed and developing countries [1, 2]. Besides measuring blood pressure and glucose levels, assessing the lipid spectrum is the method most commonly used to identify individuals at high risk of cardiovascular disease (CVD), and many epidemiological and clinical studies have shown that dislipidemia plays a prominent role in the progression of CAD [3, 4], and it accounts for ~50% of the population attributable risk of developing CAD [3]. Prospective epidemiological evidence shows that low-density lipoprotein cholesterol (LDL-C) [5], high-density lipoprotein cholesterol (HDL-C) [6], triglyceride (TG) [7], total cholesterol (TC) [8], apolipoprotein (Apo) B [9], ApoA1 [10] and the ApoA1/ApoB ratio are heritable, modifiable, risk factors for CAD. Dyslipidemia is a distinct complex trait resulting from multiple environmental and genetic factors as well as their interactions [11, 12], therefore it is monitored as a predictor of dyslipidemia and also the main target for therapeutic intervention [13]. Studies of naturally occurring genetic variation can proceed through large-scale association analyses focused on unrelated individuals or through investigation of Mendelian forms of

dyslipidemia in families [14, 15]. In the past ten years, genome-wide association studies (GWASes) have implicated that numerous common genetic variants in multiple loci and genes influences serum lipid and lipoprotein levels [11, 16]. Common variants at these loci together explain < 10% of variation in each lipid trait. Rare variants with large individual effects may also contribute to the heritability of lipid traits [11, 17]. Recently, several GWASes have reported the association of many single nucleotide polymorphisms (SNPs) near the FERM domain containing 5 gene (*FRMD5*) located at chromosome 15q15.3 with blood lipids and related cardiovascular traits [11, 18]. However, the biological function of the *FRMD5* rs2929282 SNP on serum lipid metabolism remains unclear. Importantly, the genetic variation has different magnitudes of effect in different ethnicities but until now no GWAS has comprehensively investigated the genetic determinants of serum lipid levels in the Chinese populations.

China is a multiethnic country of 56 ethnic groups; the customs of every ethnic group are not identical. Han is the dominant ethnic group and Jing is a native minority with 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 [19]. Jing is mainly engaged in coastal fisheries. Jing is unique in Chinese ethnic minorities living in the area of the sea; the way of life is unique. There are many differences between Jing and Han (as well as other landlocked nationalities) nationalities in diet custom and culture characteristics. Owing to a variety of lifestyles and environments in our population living in Guangxi, the effect of genetic variation may be further modified. Since the Jing population has different lifestyles and customs, it tends to be more genetically isolated. However, there are no studies to examine the association of the *FRMD5* rs2929282 SNP and serum lipid levels in this population. Therefore, it is necessary to characterize the relationship between the *FRMD5* rs2929282 SNP and several environmental factors with serum lipid traits in the Jing and Han populations.

Methods

Subjects

The study populations included 1061 unrelated subjects (451 males, 42.51% and 610 females, 57.49%) of Han and 1065 unrelated participants (458 males, 43.00% and 607 females, 57.00%) of Jing. They were randomly selected from our previously stratified randomized samples. 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 participants ranged from 27 to 92 years. The mean age of Jing participants was 56.62 ± 13.39 years, whereas that of Han subjects was 57.68 ± 12.86 years. All participants were healthy and had no evidence of diseases related to atherosclerosis, CAD, or 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. Informed consent was obtained from all participants.

Epidemiological survey

The survey was carried out using internationally standardized methods, following a common protocol [20]. Demographic data, socioeconomic status, and lifestyle factors were collected by standardized questionnaires. 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 categorized into groups of grams of alcohol per day: < 25 and ≥ 25. Smoking status was categorized into groups of cigarettes per day: < 20 and ≥ 20. Sitting blood pressure was measured three times with the use of a mercury sphygmomanometer after the subjects had a 5-minute rest, and the average of the three measurements was used for the level of blood pressure. Systolic blood pressure was determined by the first Korotkoff sound, and diastolic blood pressure was determined by the fifth Korotkoff sound. Body weight, to the nearest 50 grams, was measured by using a portable balance scale. Subjects were weighed without shoes and minimum of clothing. Height was measured, to the nearest 0.5 cm, using a portable measuring device. From these two measurements, BMI (kg/m^2) was calculated.

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

Parameter	Han	Jing	t (x^2)	P
Number	1061	1065		
Male/female	451/610	458/607	0.054	0.817
Age (year)	57.68 ± 12.86	56.62 ± 13.39	-1.866	0.062
Height (cm)	156.32 ± 8.27	157.39 ± 7.78	3.069	0.002
Weight (kg)	55.70 ± 9.65	58.18 ± 9.94	5.834	0.000
Body mass index (kg/m^2)	22.76 ± 3.43	23.42 ± 3.19	4.583	0.000
Waist circumference (cm)	77.24 ± 9.16	80.12 ± 9.09	7.268	0.000
Cigarette smoking [n (%)]				
Non-smoker	883 (83.2)	913 (85.7)		
< 20 cigarettes/day	41 (3.9)	35 (3.3)		
≥ 20 cigarettes/day	137 (12.9)	117 (11.0)	2.542	0.281
Alcohol consumption [n (%)]				
Non-drinker	872 (82.2)	941 (88.4)		
< 25 g/day	38 (3.6)	60 (5.6)		
≥ 25 g/day	151 (14.2)	64 (6.0)	42.762	0.000
Systolic BP (mmHg)	132.35 ± 19.02	132.06 ± 22.04	-0.319	0.750
Diastolic BP (mmHg)	81.16 ± 10.47	80.50 ± 10.18	-1.480	0.139
Pulse pressure (mmHg)	51.19 ± 15.45	51.57 ± 17.98	0.519	0.604
Glucose (mmol/L)	6.42 ± 0.31	6.80 ± 1.48	8.119	0.000
Total cholesterol (mmol/L)	4.90 ± 0.88	5.11 ± 0.92	5.255	0.000
Triglyceride (mmol/L)	1.31 (0.63)	1.45 (0.74)	-6.123	0.000
HDL-C (mmol/L)	1.77 ± 0.50	1.76 ± 0.45	-0.510	0.610
LDL-C (mmol/L)	2.86 ± 0.44	2.82 ± 0.44	-1.976	0.048
Apolipoprotein (Apo) A1 (g/L)	1.32 ± 0.20	1.29 ± 0.23	-3.176	0.002
ApoB (g/L)	1.04 ± 0.25	1.05 ± 0.24	1.410	0.159
ApoA1/ApoB	1.34 ± 0.38	1.30 ± 0.37	-2.825	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. 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.

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 anticoagulation 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 measured according to standard enzymatic methods. Serum ApoA1 and ApoB levels were detected by the immunoturbidimetric immunoassay. All determinations were performed by an autoanalyzer (Type 7170A; Hitachi Ltd., Tokyo, Japan) in the Clinical Science Experiment Center of the First Affiliated Hospital, Guangxi Medical University.

DNA amplification and genotyping

Genomic DNA was isolated from peripheral blood leukocytes using the phenol-chloroform method [21]. The extracted DNA was stored at -20°C until analysis. Genotyping of the *FRMD5* rs2929282 SNP was performed by polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP). PCR amplification was performed using 5'-TCACAACCACTTCCCTCACA-3' and 5'-AGGATCAAGAAACCTGGGAAC-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, followed by 35 s of denaturing at 95°C, 40 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 *RsaI* enzyme was added directly to the PCR products (10 μL) and digested at 37°C overnight. After restriction enzyme digestion of the amplified DNA, the genotypes were identified by electrophoresis on 2% agarose

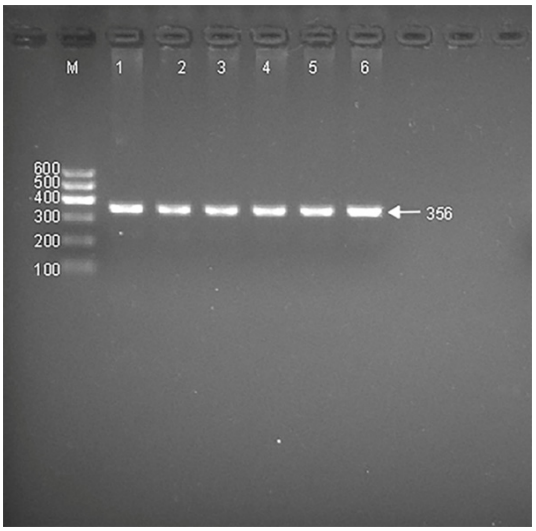

Figure 1. Electrophoresis of PCR products of the samples. Lane M, 100 bp marker ladder; lanes 1-6, samples. The 356 bp bands are the target genes.

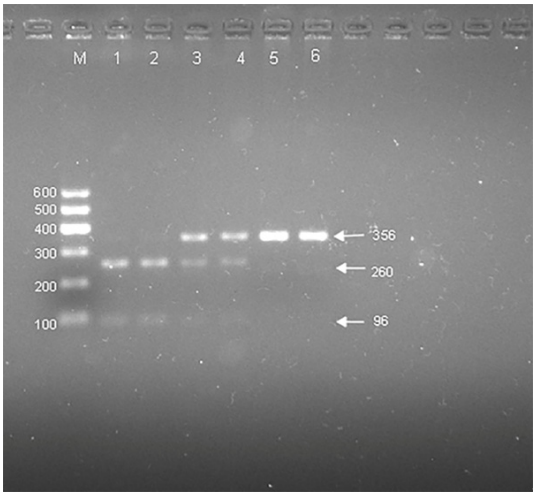

Figure 2. Genotyping of the *FRMD5* rs2929282 SNP. Lane M is the 100 bp marker ladder; lanes 1 and 2, AA genotype (96- and 260-bp); lanes 3 and 4, AT genotype (96-, 260- and 356-bp); and lanes 5 and 6, TT genotype (356-bp).

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 the standard DNA marker. Genotypes were scored by an experienced reader blinded to epidemiological data and serum lipid levels. Six samples (AA, AT and TT 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, 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. Individuals with TC > 5.17 mmol/L, and/or TG > 1.70 mmol/L were defined as hyperlipidemic [22]. Hypertension was diagnosed according to the criteria of 1999 World Health Organization-International Society of Hypertension Guidelines for the management of hypertension. Hypertension was defined as an average systolic blood pressure (SBP) ≥ 140 mmHg, and/or an average diastolic blood pressure (DBP) ≥ 90 mmHg, and/or self-reported current treatment for hypertension with antihypertensive medication [23]. 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 [24].

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 by 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 analysis of covariance (ANCOVA) was performed to estimate the association of genotypes (AA = 1, AT/TT = 2) and serum lipid parameters. Factors that may influence serum lipid concentrations such as sex, age, BMI, blood pressure, alco-

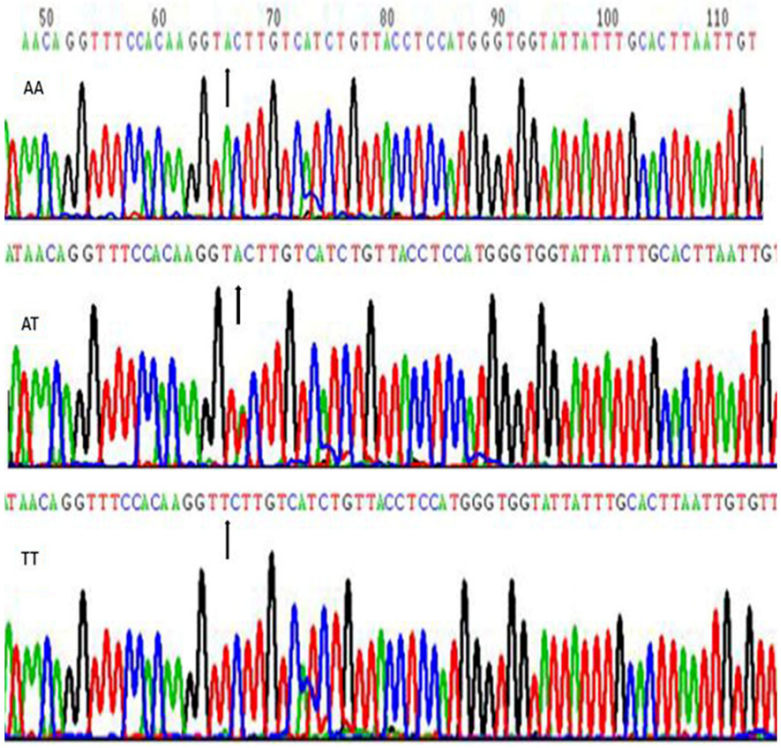

Figure 3. A part of the *FRMD5* rs2929282 SNP sequence. AA, AA genotype; AT, AT genotype; and TT, TT genotype.

Table 2. Comparison of the genotype and allele frequencies of the *FRMD5* rs2929282 SNP in the Han and Jing populations [n (%)]

Group	n	Genotype			Allele	
		AA	AT	TT	A	T
Han	1061	892 (84.1)	165 (15.6)	4 (0.4)	1949 (91.8)	173 (8.2)
Jing	1065	940 (88.3)	120 (11.3)	5 (0.5)	2000 (93.9)	130 (6.1)
x^2			8.517		6.746	
P			0.011		0.009	
Han						
Male	451	394 (87.4)	55 (12.2)	2 (0.4)	843 (93.5)	59 (6.5)
Female	610	498 (81.6)	110 (18.0)	2 (0.3)	1106 (90.7)	114 (9.3)
x^2			6.989		5.442	
P			0.021		0.020	
Jing						
Male	458	406 (88.6)	51 (11.1)	1 (0.2)	863 (94.2)	53 (5.8)
Female	607	534 (88.0)	69 (11.4)	4 (0.7)	1137 (93.7)	77 (6.3)
x^2			0.953		0.282	
P			0.641		0.595	

hol consumption, and cigarette smoking were adjusted for the statistical analysis.

Relationships between serum lipid levels and genotypes and several envirnmental factors were assessed by multiple linear regression analysis with stepwise modeling. A two-tailed *P* value < 0.05 was considered statistically significant.

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, whereas the levels of ApoA1, LDL-C, the ratio of ApoA1/ApoB and the percentages of subjects consuming alcohol were lower in Jing than in Han (*P* < 0.05-0.001). The values of gender ratio, age, SBP, DBP, pulse pressure, HDL-C, 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 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 the cutting site indicates the T allele; while its presence indicates the A allele (can be cut). AA genotype is homozygous for the presence of the site (96- and 260-bp), AT genotype is heterozygous for the presence and absence of the site (96-, 260- and 356-bp), and TT genotype is homozygous for the absence of the site (356 bp; **Figure 2**).

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	892	4.90 ± 0.87	1.31 (0.62)	1.76 ± 0.51	2.86 ± 0.44	1.32 ± 0.20	1.03 ± 0.24	1.34 ± 0.37
AT/TT	169	4.91 ± 0.93	1.33 (0.69)	1.84 ± 0.39	2.86 ± 0.45	1.32 ± 0.20	1.04 ± 0.26	1.35 ± 0.42
F		0.051	-1.122	1.374	0.020	0.108	0.318	0.039
P		0.821	0.262	0.241	0.889	0.743	0.573	0.844
Jing								
AA	940	5.12 ± 0.93	1.44 (0.73)	1.78 ± 0.44	2.86 ± 0.44	1.29 ± 0.23	1.06 ± 0.24	1.29 ± 0.37
AT/TT	125	5.00 ± 0.86	1.54 (0.82)	1.61 ± 0.48	2.86 ± 0.46	1.26 ± 0.18	0.99 ± 0.20	1.29 ± 0.32
F		0.383	-2.352	20.764	1.188	5.406	4.882	0.651
P		0.536	0.019	0.000	0.276	0.020	0.027	0.420

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	394	4.80 ± 0.81	1.30 (0.71)	1.62 ± 0.54	2.84 ± 0.41	1.31 ± 0.20	1.04 ± 0.23	1.33 ± 0.38
AT/TT	57	4.94 ± 1.12	1.34 (0.43)	1.85 ± 0.45	2.94 ± 0.55	1.33 ± 0.21	1.10 ± 0.30	1.29 ± 0.47
F		0.695	0.000	10.183	1.941	0.315	2.601	0.034
P		0.405	0.996	0.002	0.164	0.575	0.107	0.853
Han/female								
AA	498	4.98 ± 0.89	1.31 (0.54)	1.87 ± 0.48	2.87 ± 0.45	1.32 ± 0.20	1.03 ± 0.25	1.35 ± 0.36
AT/TT	112	4.89 ± 0.82	1.25 (0.80)	1.83 ± 0.36	2.82 ± 0.39	1.33 ± 0.19	1.01 ± 0.24	1.30 ± 0.47
F		0.784	3.530	2.505	1.290	0.009	0.039	0.000
P		0.376	0.061	0.114	0.256	0.924	0.844	0.995
Jing/male								
AA	406	5.12 ± 0.86	1.51 (0.87)	1.70 ± 0.44	2.83 ± 0.39	1.25 ± 0.21	1.08 ± 0.23	1.24 ± 0.38
AT/TT	52	5.05 ± 0.83	1.60 (0.88)	1.65 ± 0.52	2.79 ± 0.36	1.30 ± 0.22	1.01 ± 0.19	1.32 ± 0.37
F		0.193	0.022	0.651	0.003	0.911	4.041	1.261
P		0.660	0.882	0.420	0.960	0.340	0.045	0.262
Jing/female								
AA	534	5.12 ± 0.98	1.40 (0.63)	1.84 ± 0.43	2.83 ± 0.49	1.33 ± 0.25	1.04 ± 0.25	1.34 ± 0.36
AT/TT	73	4.99 ± 0.89	1.54 (0.74)	1.59 ± 0.45	2.74 ± 0.43	1.23 ± 0.16	1.00 ± 0.20	1.27 ± 0.29
F		0.753	9.766	27.984	1.061	10.879	0.869	3.411
P		0.386	0.002	0.000	0.303	0.001	0.352	0.065

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.

Nucleotide sequences

The results were separated into AA, AT and TT genotypes of the rs2929282 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 rs2929282SNP in the both ethnic groups are shown in **Table 2**. The genotypic distribution was followed Hardy-Weinberg equilibrium

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	0.038	0.004	0.405	9.840	0.000
	Cigarette smoking	0.271	0.028	0.209	9.601	0.000
	Glucose	0.095	0.016	0.121	5.974	0.000
	Height	-0.045	0.012	-0.418	-3.752	0.000
	Diastolic blood pressure	0.006	0.002	0.073	3.566	0.000
	Body mass index	-0.121	0.038	-0.469	-3.171	0.002
	Genotype	-0.121	0.050	-0.049	-2.425	0.015
	Weight	0.038	0.016	0.433	2.348	0.019
TC	Glucose	0.141	0.018	0.168	7.951	0.000
	Age	0.008	0.002	0.115	5.255	0.000
	Height	-0.008	0.002	-0.072	-3.340	0.001
	Diastolic blood pressure	0.005	0.002	0.058	2.749	0.006
HDL-C	Waist circumference	-0.014	0.001	-0.282	-13.269	0.000
	Gender	0.127	0.024	0.132	5.373	0.000
	Alcohol consumption	0.120	0.018	0.159	6.673	0.000
	Cigarette smoking	-0.059	0.017	-0.082	-3.404	0.001
	Diastolic blood pressure	0.003	0.001	0.059	2.815	0.005
	Genotype	0.076	0.028	0.056	2.698	0.007
LDL-C	Age	0.003	0.001	0.090	4.061	0.000
	Glucose	0.025	0.009	0.063	2.903	0.004
	Diastolic blood pressure	0.004	0.001	0.088	4.067	0.000
	Height	-0.003	0.001	-0.055	-2.494	0.013
ApoA1	Weight	-0.013	0.004	-0.614	-3.236	0.001
	Alcohol consumption	0.066	0.008	0.190	8.124	0.000
	Glucose	-0.016	0.004	-0.080	-3.804	0.000
	Gender	0.058	0.012	0.133	4.741	0.000
	Height	0.009	0.003	0.332	2.842	0.005
	Body mass index	0.022	0.010	0.337	2.177	0.030
ApoB	Waist circumference	0.005	0.001	0.191	8.840	0.000
	Age	0.002	0.000	0.092	4.022	0.000
	Systolic blood pressure	0.001	0.000	0.051	2.191	0.029
ApoA1/ApoB	Waist circumference	-0.008	0.002	-0.185	-4.324	0.000
	Alcohol consumption	0.076	0.014	0.126	5.462	0.000
	Gender	0.102	0.023	0.134	4.499	0.000
	Height	0.028	0.005	0.597	5.166	0.000
	Glucose	-0.015	0.007	-0.045	-2.137	0.033
	Weight	-0.034	0.007	-0.895	-4.726	0.000
	Body mass index	0.071	0.017	0.632	4.149	0.000
	Age	-0.002	0.001	-0.055	-2.379	0.017
Han						
TG	Waist circumference	0.034	0.005	0.384	6.702	0.000
	Cigarette smoking	0.207	0.038	0.175	5.512	0.000
	Height	-0.014	0.003	-0.141	-3.950	0.000
	Body mass index	-0.031	0.013	-0.130	-2.289	0.022
	Diastolic blood pressure	0.007	0.002	0.085	2.833	0.005
TC	Glucose	-0.322	0.085	-0.115	-3.799	0.000
	Cigarette smoking	-0.111	0.039	-0.087	-2.869	0.004

	Systolic blood pressure	0.006	0.001	0.128	4.246	0.000
HDL-C	Waist circumference	-0.012	0.002	-0.223	-7.375	0.000
	Alcohol consumption	0.117	0.025	0.167	4.613	0.000
	Gender	0.110	0.036	0.110	3.052	0.002
	Glucose	-0.169	0.047	-0.107	-3.641	0.000
	Diastolic blood pressure	0.004	0.001	0.084	2.808	0.005
	Cigarette smoking	-0.113	0.026	-0.156	-4.369	0.000
LDL-C	Glucose	-0.128	0.042	-0.091	-3.020	0.003
	Systolic blood pressure	0.004	0.001	0.159	5.242	0.000
ApoA1	Weight	-0.016	0.005	-0.784	-3.513	0.000
	Alcohol consumption	0.074	0.010	0.263	7.619	0.000
	Gender	0.065	0.017	0.160	3.881	0.000
	Height	0.012	0.004	0.486	3.315	0.001
	Body mass index	0.030	0.011	0.507	2.714	0.007
ApoB	Waist circumference	0.006	0.001	0.212	6.915	0.000
	Systolic blood pressure	0.001	0.000	0.114	3.728	0.000
	Glucose	-0.059	0.023	-0.075	-2.522	0.012
	Height	-0.004	0.001	-0.135	-3.524	0.000
	Gender	-0.053	0.019	-0.105	-2.804	0.005
ApoA1/ApoB	Waist circumference	-0.007	0.002	-0.165	-2.792	0.005
	Alcohol consumption	0.081	0.018	0.152	4.483	0.000
	Gender	0.148	0.031	0.194	4.777	0.000
	Height	0.036	0.007	0.798	5.541	0.000
	Systolic blood pressure	-0.002	0.001	-0.095	-3.163	0.002
	Weight	-0.041	0.009	-1.061	-4.740	0.000
	Body mass index	0.087	0.020	0.789	4.328	0.000
Jing						
TG	Waist circumference	0.046	0.006	0.467	8.081	0.000
	Cigarette smoking	0.298	0.045	0.213	6.653	0.000
	Glucose	0.094	0.017	0.155	5.450	0.000
	Height	-0.033	0.005	-0.290	-6.704	0.000
	Gender	-0.251	0.074	-0.139	-3.382	0.001
	Age	-0.006	0.002	-0.093	-2.880	0.004
	Body mass index	-0.045	0.015	-0.162	-2.936	0.003
	Diastolic blood pressure	0.007	0.003	0.074	2.578	0.010
TC	Glucose	0.146	0.018	0.234	8.072	0.000
	Age	0.019	0.002	0.274	8.432	0.000
	Pulse pressure	-0.008	0.002	-0.150	-4.688	0.000
	Cigarette smoking	0.209	0.047	0.145	4.419	0.000
	Gender	0.230	0.062	0.124	3.733	0.000
	Diastolic blood pressure	0.007	0.003	0.082	2.815	0.005
HDL-C	Waist circumference	-0.023	0.003	-0.456	-8.251	0.000
	Genotype	0.183	0.039	0.131	4.650	0.000
	Alcohol consumption	0.163	0.027	0.186	6.130	0.000
	Gender	0.192	0.033	0.211	5.876	0.000
	Age	0.003	0.001	0.087	2.826	0.005
	Weight	0.007	0.003	0.149	2.477	0.013
LDL-C	Age	0.005	0.001	0.145	4.613	0.000
	Glucose	0.034	0.009	0.114	3.773	0.000
	Cigarette smoking	0.074	0.024	0.108	3.137	0.002

	Diastolic blood pressure	0.003	0.001	0.074	2.452	0.014
	Gender	0.063	0.031	0.071	2.043	0.041
ApoA1	Glucose	-0.016	0.005	-0.101	-3.321	0.001
	Alcohol consumption	0.051	0.014	0.114	3.565	0.000
	Gender	0.060	0.015	0.130	4.085	0.000
	Genotype	0.050	0.021	0.070	2.368	0.018
	Waist circumference	-0.005	0.001	-0.187	-6.120	0.000
	Pulse pressure	0.001	0.000	0.069	2.287	0.022
ApoB	Waist circumference	0.002	0.001	0.091	1.698	0.090
	Age	0.003	0.001	0.160	5.169	0.000
	Genotype	0.046	0.022	0.063	2.097	0.036
	Weight	0.003	0.001	0.108	1.987	0.047
ApoA1/ApoB	Waist circumference	-0.010	0.001	-0.256	-8.544	0.000
	Age	-0.002	0.001	-0.078	-2.605	0.009
	Alcohol consumption	0.078	0.023	0.109	3.436	0.001
	Gender	0.072	0.024	0.096	3.008	0.003
	Glucose	-0.019	0.008	-0.075	-2.505	0.012

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.

(HWE). The allelic and genotypic frequencies of the *FRMD5* rs2929282 SNP were different between Jing and Han ($P < 0.05$). The frequency of minor T allele was lower in Jing than in Han. The allelic and genotypic frequencies of the *FRMD5* rs2929282 SNP were different between males and females in Han but not in Jing ($P < 0.05$). The frequency of minor T allele was lower in Han males than in Han females.

Genotypes and serum lipid levels

As shown in **Tables 3** and **4**, the *FRMD5* rs29-29282 T allele carriers had lower serum HDL-C, ApoA1 and ApoB levels, and higher TG levels in Jing but not in Han. Subgroup analysis according to sex showed that the T allele carriers had higher serum TG levels in Jing females but not in males than the T allele non-carriers ($P < 0.05$). The T allele carriers had higher HDL-C levels in Han males but not in Han females, and lower HDL-C levels in Jing females but not in Jing males than the T allele non-carriers ($P < 0.05$). The T allele carriers had lower ApoA1 levels in Jing females but not in Jing males, and lower ApoB levels in Jing males but not in Jing females than the T allele non-carriers ($P < 0.05$).

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 TG and HDL-C levels in Jing and Han and HDL-C, ApoA1, ApoB levels in Jing were correlated with genotypes ($P < 0.05$), respectively. When serum lipid data were analyzed according to gender, HDL-C, ApoA1, and TG levels in Jing females, TG levels in Han females and HDL-C levels in Jing males were associated with genotypes ($P < 0.05$). Serum lipid parameters were also associated with environmental factors such as age, gender, BMI, waist circumference, blood pressure, blood glucose, cigarette smoking, and alcohol consumption in both ethnic groups ($P < 0.05$, **Tables 5** and **6**).

Discussion

Recently, large numbers of new candidate genes were claimed to be related to dyslipidemia by GWASes, mainly in a European population, and replicating these results in independent populations has generally been necessary. To the best of our knowledge, this is the first study to detect the association of the *FRMD5* rs2929282 polymorphism and serum lipid levels in the Chinese populations. It is well known that dyslipidemia is a multifactorial and complicated origin which combined by genetic and environmental factors. Given that genetic factors and interactions with environmental factors are important in common forms of serum lipid levels, prediction of the risk for dyslipidemia on the basis of genetic variants would

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						
TG	Waist circumference	0.040	0.005	0.383	8.390	0.000
	Cigarette smoking	0.285	0.050	0.243	5.657	0.000
	Glucose	0.118	0.028	0.183	4.275	0.000
	Height	-0.025	0.007	-0.160	-3.556	0.000
TC	Glucose	0.102	0.025	0.188	4.168	0.000
	Age	0.017	0.003	0.286	5.723	0.000
	Pulse pressure	-0.020	0.005	-0.382	-4.366	0.000
	Cigarette smoking	0.137	0.047	0.139	2.916	0.004
	Body mass index	0.102	0.024	0.371	4.220	0.000
	Waist circumference	-0.028	0.008	-0.319	-3.527	0.000
	Alcohol consumption	0.142	0.054	0.118	2.646	0.008
	Systolic blood pressure	0.008	0.004	0.179	2.074	0.039
LDL-C	Body mass index	0.044	0.011	0.356	3.904	0.000
	Waist circumference	-0.011	0.004	-0.276	-3.000	0.003
	Pulse pressure	-0.003	0.001	-0.120	-2.578	0.010
	Glucose	0.026	0.012	0.109	2.300	0.022
HDL-C	Waist circumference	-0.024	0.004	-0.528	-6.097	0.000
	Alcohol consumption	0.166	0.027	0.262	6.034	0.000
	Glucose	0.028	0.013	0.097	2.204	0.028
	Body mass index	0.025	0.012	0.172	2.030	0.043
ApoA1	Waist circumference	-0.006	0.001	-0.282	-6.277	0.000
	Alcohol consumption	0.053	0.013	0.181	4.020	0.000
ApoB	Weight	0.006	0.001	0.284	6.097	0.000
	Age	0.002	0.001	0.109	2.333	0.020
ApoA1/ApoB	Waist circumference	-0.010	0.002	-0.223	-5.576	0.000
	Height	0.009	0.002	0.146	3.663	0.000
	Glucose	-0.034	0.010	-0.130	-3.293	0.001
Jing/female						
TG	Waist circumference	0.026	0.004	0.274	7.171	0.000
	Cigarette smoking	1.801	0.293	0.228	6.149	0.000
	Glucose	0.051	0.021	0.091	2.425	0.016
	Height	-0.022	0.006	-0.163	-3.824	0.000
	Age	0.006	0.003	0.089	2.138	0.033
	Genotype	-0.282	0.089	-0.117	-3.162	0.002
TC	Glucose	0.197	0.026	0.285	7.468	0.000
	Age	0.022	0.003	0.278	6.689	0.000
	Pulse pressure	-0.006	0.002	-0.126	-3.011	0.003
LDL-C	Body mass index	-0.012	0.006	-0.078	-1.982	0.048
	Glucose	0.055	0.014	0.161	4.069	0.000
	Age	0.008	0.002	0.216	5.507	0.000
HDL-C	Waist circumference	-0.018	0.002	-0.340	-8.843	0.000
	Genotype	0.276	0.051	0.202	5.380	0.000
	Diastolic blood pressure	0.004	0.002	0.088	2.281	0.023
	Cigarette smoking	-0.381	0.168	-0.085	-2.268	0.024
ApoA1	Body mass index	-0.011	0.003	-0.144	-3.591	0.000
	Genotype	0.101	0.029	0.137	3.455	0.001
	Glucose	-0.016	0.007	-0.094	-2.339	0.020

ApoB	Age	0.004	0.001	0.206	5.214	0.000
	Waist circumference	0.004	0.001	0.136	3.449	0.001
ApoA1/ApoB	Waist circumference	-0.010	0.002	-0.223	-5.576	0.000
	Height	0.009	0.002	0.146	3.663	0.000
	Glucose	-0.034	0.010	-0.130	-3.293	0.001
Han/male						
TG	Waist circumference	0.026	0.005	0.242	5.342	0.000
	Cigarette smoking	0.214	0.044	0.218	4.908	0.000
	Diastolic blood pressure	0.011	0.004	0.126	2.798	0.005
TC	Glucose	-0.427	0.107	-0.183	-3.970	0.000
	Systolic blood pressure	0.007	0.002	0.150	3.271	0.001
LDL-C	Waist circumference	0.006	0.002	0.107	2.294	0.022
	Glucose	-0.187	0.055	-0.158	-3.424	0.001
	Systolic blood pressure	0.003	0.001	0.129	2.755	0.006
HDL-C	Waist circumference	-0.018	0.003	-0.279	-6.126	0.000
	Alcohol consumption	0.116	0.027	0.201	4.311	0.000
	Glucose	-0.278	0.063	-0.192	-4.446	0.000
	Genotype	-0.210	0.070	-0.132	-3.018	0.003
	Diastolic blood pressure	0.006	0.002	0.122	2.716	0.007
	Cigarette smoking	-0.100	0.028	-0.170	-3.505	0.001
	Age	0.006	0.002	0.132	2.776	0.006
	Height	0.009	0.004	0.104	2.197	0.029
ApoA1	Waist circumference	-0.005	0.001	-0.217	-4.934	0.000
	Alcohol consumption	0.078	0.010	0.350	7.965	0.000
	Glucose	-0.060	0.024	-0.108	-2.483	0.013
ApoB	Waist circumference	0.007	0.001	0.248	5.501	0.000
	Glucose	-0.102	0.029	-0.156	-3.507	0.000
	Systolic blood pressure	0.002	0.001	0.149	3.299	0.001
ApoA1/ApoB	Alcohol consumption	0.095	0.019	0.223	4.972	0.000
	Age	0.004	0.001	0.132	2.863	0.004
	Waist circumference	-0.014	0.002	-0.295	-6.565	0.000
	Systolic blood pressure	-0.004	0.001	-0.158	-3.374	0.001
Han/female						
TG	Waist circumference	0.023	0.003	0.300	7.790	0.000
	Age	0.006	0.002	0.106	2.758	0.006
	Genotype	-0.152	0.074	-0.079	-2.053	0.041
TC	Pulse pressure	0.006	0.002	0.116	2.885	0.004
	Cigarette smoking	-0.838	0.359	-0.094	-2.336	0.020
LDL-C	Systolic blood pressure	0.003	0.001	0.147	3.653	0.000
	Height	-0.006	0.003	-0.095	-2.352	0.019
	Cigarette smoking	-0.377	0.177	-0.085	-2.127	0.034
HDL-C	Body mass index	-0.023	0.005	-0.186	-4.669	0.000
ApoA1	Body mass index	-0.006	0.002	-0.115	-2.850	0.005
ApoB	Age	0.002	0.001	0.112	2.694	0.007
	Waist circumference	0.005	0.001	0.204	5.190	0.000
	Height	-0.006	0.002	-0.152	-3.628	0.000
ApoA1/ApoB	Weight	-0.057	0.011	-1.408	-5.386	0.000
	Age	-0.004	0.001	-0.128	-3.178	0.002
	Height	0.049	0.008	0.893	5.952	0.000
	Body mass index	0.104	0.023	1.075	4.430	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.

be beneficial for personalized prevention of this condition [25, 26]. Several previous studies have showed that the associations of variants in several lipid-related genes and serum lipid profiles were significantly different between the Jing and Han populations and their gender subgroups [23, 27]. Therefore, it is compelling us to believe that some hereditary characteristics and genotypes of lipid metabolism-related genes in this population may differ from Han nationality.

In the present study, we found that the levels of height, weight, waist circumference, BMI, TC, TG, glucose were higher in Jing than in Han, whereas the levels of ApoA1, LDL-C, the ratio of ApoA1/ApoB and the percentages of subjects consuming alcohol were lower in Jing than in Han ($P < 0.05$-0.001). It is well known that dyslipidemia is a complex trait caused by environmental, genetic factors and their interactions. Jing nationality is a relatively conservative and isolated minority in China that retains its regional and special customs. 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. Jing says that endogamy, intermarriage with Han or Zhuang people seldom happens. Jing people don't get married with someone sharing the same last name, also cross-cousin marriage is strictly forbidden. They find their life partners by singing songs to each other. After antiphonal singing, if the boy is into the girl he would kick sand toward her while approaching her. If the girl feels the same she would kick back, which means engagement. While the formal engagement ceremony and wedding they need pork, cake, tea, wine, glutinous rice as gifts (https://www.chinatravel.com/facts/jing-ethnic-minority.htm). Therefore, we believe that the genetic background and some lipid-associated genetic variants in this population may be different from those in Han nationality.

The genotypic and allelic frequencies of rs2929282 SNP in the *FRMD5* in diverse racial/ethnic groups are inconsistent. According to the 1000 genomes project data, the frequency of the rs2929282 T allele was 5.83% in Han Chinese from Beijing, 6.67% in Southern Han Chinese, 2.40% in Japanese from Tokyo, 6.04% in British in England and Scotland, 2.53% in Finnish in Finland, 5.14% in Iberian population in Spain. However, in African ancestry, the frequency of the rs2929282 T allele was 31.15% in Americans of African ancestry in SW USA, 41.92% of Esan in Nigeria, 33.63% in Gambian in Western Divisions in the Gambia. Apparently, the minor T allele frequency was lower in the Western than African ancestry populations. In the present study, The frequency of the T allele was higher in Han than in Jing (8.2% *vs.* 6.1%). The genotypic and allelic frequencies of the *FRMD5* rs2929282 SNP were significantly different between Han males and females ($P < 0.05$ for each), but not between Jing males and females. The frequency of the T allele was higher in Han females than in Han males (9.3% *vs.* 6.5%). These results indicated that the prevalence of the *FRMD5* rs2929282 T allele may have racial/ethnic as well as gender specificity.

There were hardly any previous studies presented the direct relationship between the *FRMD5* rs2929282 SNP and serum lipid levels in humans except a large-scale association study which showed that the *FRMD5* rs2929282 T allele carriers had lower TG levels ($n = 83,616$, $P = 2 \times 10^{-9}$) and the risk of CVD ($n = 81,446$, $P = 2.8 \times 10^{-3}$) than the rs2929282 T allele non-carriers in the population of European descent [11]. In the present study, we found that the association of the *FRMD5* rs2929282 SNP and serum lipid levels was different between the Jing and Han populations. The *FRMD5* rs2929282 T allele carriers had lower serum HDL-C, ApoA1 and ApoB levels, and higher TG levels in Jing but not in Han. Subgroup analysis according to sex showed that the T allele carriers had higher serum TG levels in Jing females but not in males than the T allele non-carriers ($P < 0.05$). The T allele carriers had higher HDL-C levels in Han males but not in Han females, and lower HDL-C levels in Jing females but not in Jing males than the T allele non-carriers ($P < 0.05$). The T allele carriers had lower ApoA1 levels in Jing females but not in Jing males and lower ApoB levels in Jing males but not in Jing females than the T allele non-carriers ($P < 0.05$). These findings indicated that the association of the *FRMD5* rs2929282 SNP and serum lipid levels may have racial/ethnic and/or sex specificity. As far as we know, our study is the first replication of GWAS signals (studied in European) about the association of rs2929282

SNP and serum lipid levels in the Chinese populations. However the reason for these findings is unclear, probably because of different study designs, sample size, sex and age structure, experimental technique and multiethnic background trait, as well as different environmental and genetic factors and gene-environmental interactions. Therefore, further studies with larger sample size are still needed to confirm this association.

We also noted that serum lipid parameters were correlated to age, sex, waist circumference, BMI, blood pressure, alcohol consumption, and cigarette smoking in both ethnic groups. These data suggested that the environmental factors also played important roles in determining serum lipid levels. Life-style modification has been recommended by the National Cholesterol Education Program as the first approach to reduce serum lipid values and the risk for CAD. The dietary habits are different between the Jing and Han populations. Although Jing and local Han share same living environment, there is a significant difference in their diet and lifestyle. Rice and corn are Jing people's staple food. They prefer glutinous rice and seafood like fish, shrimp, crabs, shellfish and sandworms. The daily dishes are mainly fish and shrimp, and this is an inevitable seasoning ingredient at each meal. The typical food of the Jing people is fish based, which is also called silurid soluble. It is the traditional seasoning among the Jing people and is made from different kinds of small fishes which are first pickled before made into the sauce [27]. Jing people like to eat a kind of fish sauce called nuoc-mam which contains 17 amino acids (8 essential amino acids included of course) [28]. Jing nationality, as the only Chinese minority for coastal fisheries, has a diet pattern very similar with the Mediterranean diet. High intake of sea fish and other seafood indicates low content of saturated fatty acids and high content of composite carbohydrates and dietary fiber [28, 29]. Also, a Mediterranean diet may be related with favorable blood lipid profiles, but the association between the consumption of seafood and its benefits on blood lipid profiles and its benefits on CVD risk can be challenged by overconsumption of any form of dietary energy may increases lipid and lipoprotein levels [30].

In addition, we also found that the levels of weight and BMI were higher in Jing than in Han ($P < 0.05$ for each). Mandai et al. also showed that high BMI (> 26) was associated with higher 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 DBP, LDL-C, TG and FBG and decreased HDL-C. In contrast, decreased BMI was associated with decreased BP and LDL-C and increased HDL-C in both sexes, and decreased TG in men and FBG in women [31]. Consequently, the joint effects of different dietary habits, lifestyles, and environmental factors probably further modify the association of genetic variation and serum lipid levels in our study populations.

Conclusions

In conclusion, this study showed that the association of the *FRMD5* rs2929282 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 *FRMD5* rs2929282 SNP and serum lipid levels in our study populations.

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Informed consent including consent to publish was obtained from all participants by signature or by fingerprint (to express consent), as approved by the ethical review committee.

Disclosure of conflict of interest

None.

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