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Original Article

Association of the *SNX13* rs4142995 SNP and serum lipid levels in the Jing and Han populations

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Abstract: The single nucleotide polymorphism (SNP) of the sorting nexin 13 gene (*SNX13*) rs4142995 locus has been associated with high-density lipoprotein cholesterol (HDL-C) levels in a previous genome-wide association study (GWAS), but little is known about the association of the *SNX13* rs4142995 SNP and serum lipid profiles in the Chinese populations. The present study was to detect the association of the *SNX13* rs4142995 SNP and several environmental factors with serum lipid levels in the Jing and Han populations. Genotyping of the *SNX13* rs4142995 SNP was performed in 670 subjects of Jing and 670 subjects of Han peoples using polymerase chain reaction and restriction fragment length polymorphism, and then confirmed by direct sequencing. The G allele carriers in the Jing population had lower serum total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C) and apolipoprotein (Apo) B levels than the G allele non-carriers. Subgroup analyses showed that the G allele carriers had lower TC, LDL-C and ApoB levels in Jing females but not in Jing males. Serum lipid parameters were also correlated with several environmental factors in the Jing and Han populations, or in males and females in both ethnic groups. In the present study, we failed to find any association of the *SNX13* rs4142995 SNP and serum HDL-C levels in our study populations. Thus, there may be an ethnic- and/or sex-specific association of the *SNX13* rs4142995 SNP and serum lipid levels.

Keywords: Sorting nexin 13 gene, single nucleotide polymorphism, rs4142995, lipids

Introduction

Atherosclerotic cardiovascular disease (CVD) is a major disease burden worldwide and lipid modification plays an important role in the reduction of CVD risk [1-3]. Dislipidemia accounts for ~50% of the population attributable risk of developing CVD [4]. Dislipidemia 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 [5-7]. Dyslipidemia is proved to be caused by genetic and environmental factors and their interactions [8]. By family history and twin studies, Heller *et al*. illustrated that almost 40%-70% of the interindividual variation in plasma lipid phenotypes can be explained by genetic polymorphisms [9, 10]. It has been demonstrated that identifications of gene variants involved in hyperlipidemia can prove a way to identify targets for new therapies for cholesterol management and prevention of CVD [11, 12]. People have been trying to study the relationship between plasma lipid levels and genetic mutations [13]. In the past ten years, genome-wide association studies (GWASes) have implicated numerous common genetic variants and respective proteins in the determination of lipid and lipoprotein levels [14-16]. More than 157 loci associated with serum lipid levels had been identified in recent years [17]. We predict that more new loci involving in lipid metabolism will be found through GWASes.

Sorting nexin 13 gene (*SNX13*; http://www.ncbi.nlm.nih.gov/gene) is located on chromosome 7P21.1. This gene encodes a PHOX domain- and RGS domain-containing protein that belongs to the sorting nexin (SNX) family and the regulator of G protein signaling (RGS) family. The PHOX domain is a phosphoinositide binding domain, and the SNX family members are involved in intracellular trafficking. The RGS family members are regulatory molecules that act as GTPase activating proteins for G alpha

subunits of heterotrimeric G proteins. The RGS domain of this protein interacts with G alpha(s), accelerates its GTP hydrolysis, and attenuates G alpha(s)-mediated signaling. Overexpression of this protein delays lysosomal degradation of the epidermal growth factor receptor. Because of its bifunctional role, this protein may link heterotrimeric G protein signaling and vesicular trafficking (the above SNX13 gene function was provided by http://www.ncbi.nlm.nih.gov/gene). In 2007, Rao *et al*. used GWAS to identify a single nucleotide polymorphism (SNP) located in the SNX13, rs4721661, which was associated with albumin excretion [18]. Albumin excretion is a risk factor for adverse cardiovascular events, even in subjects without primary glomerular disease [19]. Indeed, even albumin excretion values less than those typically cited as constituting microalbuminuria or incipient nephropathy [18-21] may signal cardiovascular risk. A newly study has identified 157 loci associated with lipid levels at $P < 5 \times 10^{-8}$ which has mentioned that the *SNX13* rs4142995 SNP was associated with high-density lipoprotein cholesterol (HDL-C) levels [17]. However, the biological function of the *SNX13* rs4142995 SNP on serum lipid metabolism remains unclear. Importantly, the genetic variation has different effect in the different ethnic groups. Therefore, it would be necessary to characterize the relationship between the *SNX13* rs4142995 SNP and serum lipid levels in the Chinese populations.

China is a multiethnic country of 56 ethnic groups; the custom of every ethnic group is not identical. Han is the dominant ethnic group and Jing is a native minority existing 28199 people among the 55 minority groups according to the sixth national census statistics of China in 2010. Most of them live in the so called "Three Islands of Jing Nationality", Dongxing City, Guangxi Zhuang Autonomous Region, People's Republic of China [3]. The history of this minority can be traced back to the 1600s. Jing is mainly engaged in coastal fisheries. Jing is unique in Chinese ethnic minorities living in the nation of the sea; the way of life is single. It has a lot of differences between Jing and Han (as well as the other landlocked nationalities) nationality in diet custom and culture characteristics [3]. In a previous study, Lin *et al*. suggested that Jing ethnic population has an origin of Southeast Asia and is belonged to the southern group of Chinese populations [22]. These indicated that the genetic background of Jing population may be less heterogeneous within the population. To our knowledge, the association of *SNX13* SNPs and serum lipid profiles has not been previously reported in this population. Therefore, the aim of the present study was to detect the association of the *SNX13* rs4142995 SNP and several environmental factors with serum lipid profiles in the Jing and Han populations.

Materials and methods

Study population

The study population included 670 unrelated subjects of Jing and 670 unrelated participants of Han Chinese. All participants were rural agricultural workers (Han) and/or fishermen (Jing) from Jiangping Down, Dongxing City, Guangxi Zhuang Autonomous Region, People's Republic of China. The Jing subjects comprised 270 (40.3%) males and 400 (59.7%) females. The participants of Han nationality comprised 271 (40.4%) males and 339 (59.6%) females. They were randomly selected from our previous stratified randomized samples. The ages of the participants ranged from 27 to 92 years. The mean age of Jing participants was 56.68± 12.58 years, whereas that of Han subjects was 56.43±13.38 years. All participants were healthy and had no evidence of diseases related to atherosclerosis, CAD and diabetes. None of them were using lipid-lowering medication. The present study was approved by the Ethics Committee of the First Affiliated Hospital, Guangxi Medical University. Informed consent was obtained from all participants.

Epidemiological survey

The survey was carried out using internationally standardized methods, following a common protocol [23]. Information on demographics, socioeconomic status, and lifestyle factors was collected with standardized questionnaires. The alcohol information included questions about the number of *liang* (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 ≤ 20 or > 20 cigarettes per day. In the physical examination,

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

Parameter	Han	Jing	t (x^2)	P
Number	670	670		
Male/female	271/399	270/400	0.003	0.956
Age (year)	56.43±13.38	56.68±12.58	0.359	0.719
Height (cm)	156.41±7.802	157.03±7.559	1.458	0.145
Weight (kg)	55.67±9.352	57.90±9.838	4.248	0.000
Body mass index (kg/m^2)	22.71±3.18	23.42±3.21	4.036	0.000
Waist circumference (cm)	77.23±8.91	79.92±9.06	5.471	0.000
Cigarette smoking [n (%)]				
Non-smoker	566 (84.5)	579 (86.4)		
< 20 cigarettes/day	27 (4.0)	24 (3.6)		
≥ 20 cigarettes/day	77 (11.5)	67 (10)	1.019	0.601
Alcohol consumption [n (%)]				
Non-drinker	558 (83.3)	598 (89.3)		
≤ 25 g/day	26 (3.9)	36 (5.4)		
> 25 g/day	86 (12.8)	36 (5.4)	23.489	0.000
Systolic BP (mmHg)	131.21±19.21	131.44±21.79	0.203	0.839
Diastolic BP (mmHg)	80.68±10.39	80.23±10.20	-0.795	0.427
Pulse pressure (mmHg)	50.53±15.46	51.21±17.48	0.749	0.454
Glucose (mmol/L)	6.61±1.05	6.80±1.40	2.788	0.006
Total cholesterol (mmol/L)	4.90±0.88	5.03±0.92	2.558	0.011
Triglyceride (mmol/L)	1.31 (0.63)	1.42 (0.72)	14.874	0.000
HDL-C (mmol/L)	1.79±0.50	1.78±0.45	-0.253	0.800
LDL-C (mmol/L)	2.85±0.44	2.78±0.44	-2.763	0.006
Apolipoprotein (Apo) A1 (g/L)	1.32±0.20	1.29±0.23	-2.340	0.019
ApoB (g/L)	1.04±0.25	1.00±0.24	-2.354	0.019
ApoA1/ApoB	1.35±0.38	1.30±0.38	-2.281	0.023

TC, total cholesterol; TG, triglyceride; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; ApoAI, apolipoprotein AI; 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 their 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.

several parameters such as blood pressure, height, weight, waist circumference were measured, and body mass index (BMI, kg/m^2) was calculated from the height and weight measurements. The methods of measuring above parameters were referred to a previous study [3].

Biochemical measurements

A fasting venous blood sample of 5 ml was drawn from the participants. The levels of total cholesterol (TC), triglyceride (TG), HDL-C, and low-density lipoprotein cholesterol (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 [3, 24, 25].

DNA amplification and genotyping

Genomic DNA was isolated from peripheral blood leukocytes using the phenol-chloroform method [3, 23-25]. The extracted DNA was stored at -20°C until analysis. Genotyping of the *SNX13* rs4142995 SNP was performed by polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP). PCR amplification was performed using 5'-TCCTCCTACCTCAGCCTTCT-3' and 5'-GTGTCAGTCCAGTGCGTTTT-3' (Sangon, Shanghai, People's Republic of China) as the forward and reverse primer pairs; respectively. Each amplification reaction was performed in a total volume of 25 μL, containing 10 × PCR buffer (1.8 mM $MgCl_2$) 2.5 μL, 1 U *Taq* polymerase, 2.5 mmol/L of each dNTP (Tiangen, Beijing, People's Republic of China) 2.0 μL, 20 pmol/L of each primer and 50 ng of genomic DNA, processing started with 95°C for 7 min and followed by 40 s of denaturing at 95°C, 45 s of annealing at 58°C and 1 min of elongation at 72°C for 33 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 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 epide-

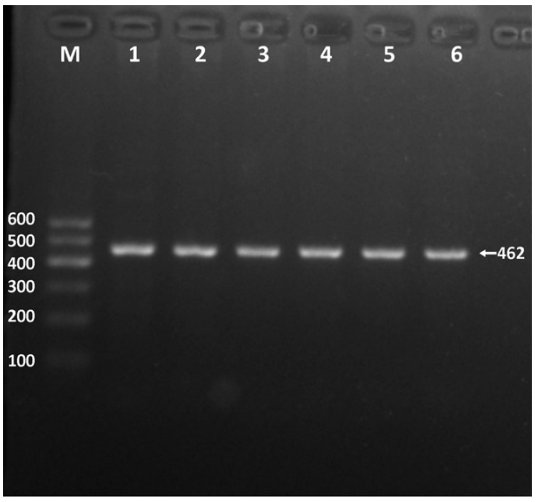

Figure 1. Electrophoresis of PCR products of the samples. Lane M, 100 bp marker ladder; lanes 1-6, samples. The 462 bp bands are the target genes.

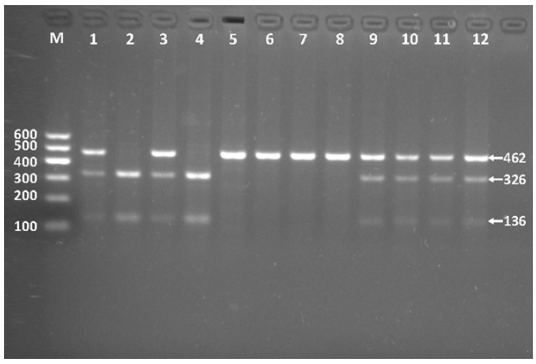

Figure 2. Genotyping of the *SNX13* rs4142995 SNP. Lane M is the 100 bp Marker Ladder; lanes 1, 3 and 9-12, GT genotype (462-, 326- and 136-bp); and lanes 2 and 4, GG genotype (326- and 136-bp); lanes 5-8, TT genotype (462-bp).

miological and lipid results. Six samples (TT, GT 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 [3, 25, 26].

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 hyperlipidemic [27]. Hypertension was assessed according to the criteria outlined by the 1999 World Health Organization-International Society of Hypertension Guidelines for the management of hypertension [28, 29]. 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 [3, 30].

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 are 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 (TT = 1, GT = 2 and GG = 3) 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 of genotypes (TT = 1, GT = 2 and GG = 3) and serum lipid parameters was tested by analysis of covariance (ANCOVA). In order to evaluate the association of serum lipid levels with genotypes and several environmental factors, multiple linear regression analysis with stepwise modeling was also performed in the combined population of Jing and Han, Jing, Han, males and females; respectively. A *P* value of less than 0.05 was considered statistically significant. The methods of statistical analyses were referred to our previous studies [3, 24, 31].

Results

General and biochemical characteristics of the subjects

Table 1 shows the general characteristics and serum lipid levels between the Jing and Han

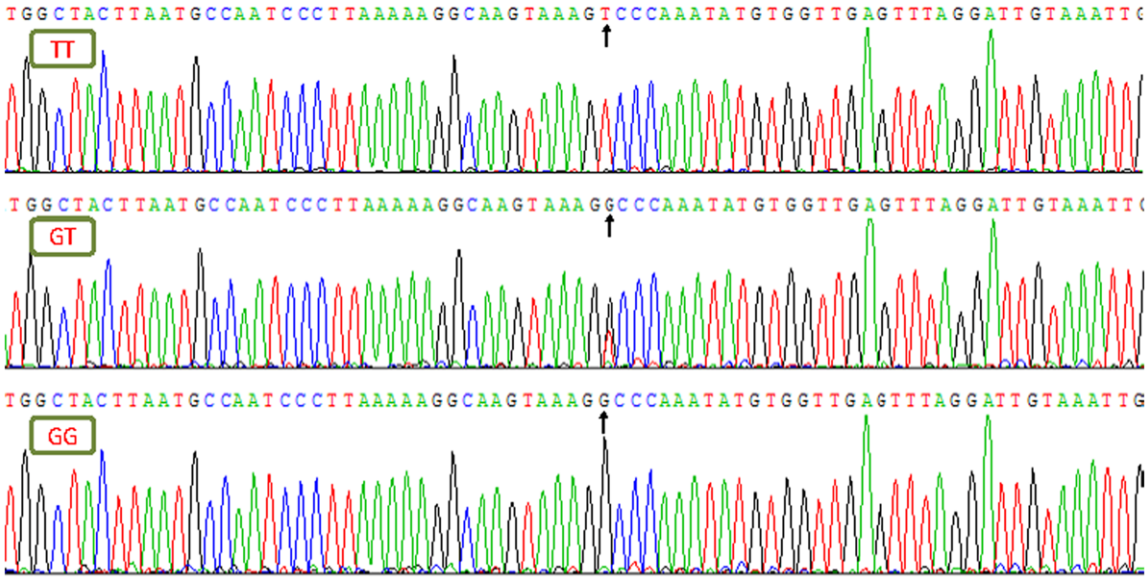

Figure 3. A part of the *SNX13* rs4142995 SNP sequence. TT: TT genotype; GT: GT genotype; GG: GG genotype.

Table 2. Comparison of the genotype and allele frequencies of *SNX13* rs4142995 SNP in the Han and Jing populations [n (%)]

Group	N	Genotype			Allele	
		TT	GT	GG	T	G
Han	670	194 (29.0)	355 (53.0)	121 (18.1)	743 (55.45)	597 (44.55)
Jing	670	202 (30.1)	332 (49.6)	136 (20.3)	736 (54.93)	604 (45.07)
x^2			1.807		0.074	
P			0.405		0.786	
Han						
Male	271	72 (26.6)	148 (54.6)	51 (18.8)	292 (53.87)	250 (46.13
Female	399	122 (30.6)	207 (51.9)	70 (17.5)	451 (56.52)	347 (43.48)
x^2			1.268		0.912	
P			0.530		0.340	
Jing						
Male	270	71 (26.3)	151 (55.9)	48 (17.8)	293 (54.26)	247 (45.74)
Female	400	131 (32.8)	181 (45.3)	88 (22.0)	443 (55.38)	357 (44.62)
x^2			7.350		0.162	
P			0.025		0.687	

populations. The levels of weight, waist circumference, BMI, TC, TG, glucose were higher in Jing than in Han but the levels of ApoA1, ApoB, ApoA1/ApoB ratio, LDL-C 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, height, systolic blood pressure, diastolic blood pressure, pulse pressure, HDL-C and the percentages of smoking were no difference ($P > 0.05$ 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 462-bp nucleotide sequences were seen in all samples (**Figure 1**). The genotypes identified were named according to the presence or absence of the enzyme restriction sites. The absence of the cutting site indicates the T allele; while its presence indicates the G allele (can be cut). GG genotype is homozygote for the presence of the site (136- and 326-bp), GT genotype is heterozygote for the presence and absence of the site (136-, 326- and 462-bp), and TT genotype is homozygote for the absence of the site (462 bp; **Figure 2**).

Nucleotide sequences

The results were shown as TT, GT and GG genotypes by PCR-RFLP, the TT, GT and GG genotypes were also confirmed by direct sequencing (**Figure 3**); respectively.

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								
TT	194	4.83±0.81	1.27 (0.66)	1.75±0.50	2.81±0.41	1.33±0.21	1.02±0.23	1.37±0.38
GT	355	4.95±0.91	1.35 (0.68)	1.80±0.51	2.88±0.46	1.31±0.19	1.05±0.26	1.33±0.37
GG	121	4.89±0.89	1.27 (0.48)	1.82±0.49	2.69±0.50	1.33±0.21	1.03±0.24	1.36±0.41
F		1.626	1.836	0.389	1.856	0.786	0.881	1.122
P		0.198	0.399	0.678	0.157	0.456	0.415	0.326
Jing								
TT	202	5.10±0.87	1.41 (0.59)	1.80±0.41	2.82±0.40	1.29±0.21	1.03±0.22	1.31±0.33
GT	332	5.08±0.93	1.45 (0.78)	1.79±0.46	2.82±0.43	1.29±0.25	1.02±0.26	1.27±0.38
GG	136	4.79±0.93	1.43 (0.72)	1.76±0.47	2.65±0.50	1.28±0.21	0.93±0.22	1.35±0.43
F		5.690	0.650	0.971	7.888	0.475	6.810	1.540
P		0.04	0.723	0.379	0.000	0.622	0.001	0.215

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 among the genotypes was determined by the Kruskal-Wallis 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								
TT	72	4.83±0.83	1.31 (0.69)	1.67±0.56	2.85±0.42	1.32±0.23	1.05±0.22	1.33±0.40
GT	148	4.87±0.91	1.37 (0.75)	1.70±0.53	2.86±0.45	1.30±0.19	1.06±0.24	1.30±0.34
GG	51	4.70±0.78	1.25 (0.57)	1.79±0.56	2.80±0.43	1.34±0.21	1.02±0.26	1.41±0.49
F		0.945	2.852	0.746	0.394	1.564	0.498	2.667
P		0.390	0.240	0.475	0.675	0.211	0.608	0.071
Han/female								
TT	122	4.82±0.80	1.27 (0.63)	1.80±0.45	2.79±0.40	1.33±0.20	1.00±0.24	1.40±0.36
GT	207	5.00±0.92	1.32 (0.62)	1.88±0.49	2.89±0.47	1.32±0.19	1.04±0.28	1.36±0.39
GG	70	5.03±0.94	1.34 (0.48)	1.84±0.43	2.86±0.44	1.32±0.22	1.04±0.22	1.32±0.34
F		1.816	0.851	0.820	2.07	0.106	0.810	0.550
P		0.164	0.653	0.441	0.128	0.899	0.446	0.577
Jing/male								
TT	71	5.17±0.75	1.34 (0.68)	1.81±0.43	2.82±0.32	1.29±0.25	1.01±0.23	1.33±0.35
GT	151	5.11±0.81	1.63 (0.89)	1.70±0.47	2.86±0.37	1.27±0.23	1.09±0.22	1.22±0.38
GG	48	4.91±0.96	1.32 (0.94)	1.69±0.43	2.72±0.43	1.28±0.20	1.04±0.22	1.30±0.44
F		1.151	5.041	0.674	2.534	0.080	1.771	0.960
P		0.318	0.080	0.511	0.081	0.923	0.172	0.384
Jing/female								
TT	131	5.06±0.93	1.42 (0.56)	1.79±0.40	2.82±0.44	1.30±0.20	1.04±0.21	1.30±0.31
GT	181	5.05±1.02	1.34 (0.64)	1.87±0.44	2.78±0.47	1.32±0.26	0.96±0.28	1.32±0.38
GG	88	4.71±0.92	1.45 (0.64)	1.79±0.48	2.60±0.54	1.28±0.22	0.88±0.20	1.37±0.42
F		5.164	1.559	1.543	5.927	0.864	10.958	1.284
P		0.006	0.459	0.215	0.003	0.422	0.000	0.278

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. The values of TG were presented as median (interquartile range), the difference among the genotypes was determined by the Kruskal-Wallis test.

Genotypic and allelic frequencies

The genotypic and allelic distribution of the *SNX13* rs4142995 SNP is shown in **Table 2**. The genotypic distribution was followed Hardy-Weinberg equilibrium (HWE). The genotype and allele frequencies of the *SNX13* rs4142995 SNP have no differences between the Jing and Han populations ($P > 0.05$ for each). The genotypic frequencies but not allele frequencies of the *SNX13* rs4142995 SNP were different between males and females in Jing but not in Han ($P < 0.05$).

Genotypes and serum lipid levels

As shown in **Tables 3** and **4**, the G allele carriers in Jing had lower serum TC, LDL-C, and ApoB levels than the G allele non-carriers ($P < 0.05$ for all). When serum lipid parameters were analysed according to the sex subgroups, the G allele carriers in Jing females had lower TC, LDL-C, and ApoB levels than the G allele non-carriers ($P < 0.05$ for all). There was no significant difference in the remaining serum lipid parameters among the three genotypes in Jing, Han, males, or females ($P > 0.05$ for all).

Risk factors for serum lipid parameters

As described in **Tables 5** and **6**, multiple linear regression analyses showed that the levels of TC, ApoB and LDL-C in the combined population (Jing plus Han) were correlated with genotypes ($P < 0.05$-0.01). Serum TC, LDL-C and ApoB levels were correlated with genotypes in Jing ($P < 0.05$ for all) but not in Han. When the association of rs4142955 genotypes and serum lipid levels were analyzed according to sex subgroups, we showed that the levels of TC, LDL-C and ApoB in Jing females ($P < 0.05$-0.01). Serum LDL-C levels were also correlated with genotypes in Jing males ($P < 0.05$). Serum ApoA1/ApoB ratio was correlated with genotypes only in Han males ($P < 0.05$). We also found that several environmental factors such as age, gender, height, weight, waist circumference, alcohol consumption and cigarette smoking, and traditional cardiovascular risk factors such as BMI, fasting blood glucose and blood pressure levels were correlated with serum lipid parameters in the merged populations (Jing plus Han) and in males and females of both ethnic groups ($P < 0.05$-0.01).

Discussion

Dyslipidemia is one of the important CVD risk factors [32, 33], whereas CVD is a leading cause of death [34]. The mortality and morbidity of CVD can reduce by controlling the conventional risk factors for CVD [35, 36]. Human genetic studies of lipid levels can provide new therapies to discover lipid-lowering medicines target which is a necessary for comprehensive prevention of CVD [37]. Recently, GWASes have found large numbers of candidate genes related to dyslipidemia. But most of subjects in these studies were Europeans; these findings may be limited to other ethnic groups. It is necessary to replicate these results in different populations. It is well known that dyslipidemia is a multifactorial origin, including hereditary and acquired risk factors. The Jing ethnic group is a conservative and isolated minority. Their marriages were arranged by their parents in the past. They find their life partners by singing song to each other. 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. While the formal engagement ceremony and wedding they need pork, cake, tea, wine, glutinous rice as gifts. Intermarriage is not accepted among the Jing and they seldom marry with someone with the same last name. All marriages were monogamous among Jing and cross-cousin marriage is also strictly forbidden. From the facts, 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 distribution of the *SNX13* rs4142995 SNP varies among different ethnic groups. According to the 1000 genomes project data, the frequency of G allele of rs4142995 SNP was 48.06% in Han Chinese from Beijing, 49.52% in Southern Han Chinese, 54.81% in Japanese, 62.21% in bengali from Bangladesh, 60.44% in British in England and Scotland, 74.75% in Finnish in Finland. However, it was lower in African Ancestry, 30.33% in Americans of African Ancestry in SW USA, 21.71% of Esan in Nigeria, 13.27% in Gambian in Western Divisions in the Gambia. In the present study, we showed that the G allele frequency of the rs4142995 SNP was 44.07% in Jing

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						
TC	Glucose	0.185	0.019	0.255	9.594	0.000
	Age	0.007	0.002	0.105	3.957	0.000
	Genotype	-0.161	0.060	-0.070	-2.694	0.007
TG	Waist circumference	0.039	0.005	0.411	8.015	0.000
	Cigarette smoking	0.332	0.037	0.246	9.095	0.000
	Glucose	0.113	0.018	0.164	6.403	0.000
	Height	-0.020	0.003	-0.179	-5.863	0.000
	Diastolic blood pressure	0.006	0.002	0.071	2.732	0.006
	Body mass index	-0.033	0.013	-0.124	-2.509	0.012
	Age	-0.004	0.002	-0.065	-2.477	0.013
HDL-C	Waist circumference	-0.016	0.001	-0.3000	-11.130	0.000
	Gender	0.198	0.038	0.204	5.199	0.000
	Alcohol consumption	0.120	0.024	0.151	5.091	0.000
	Cigarette smoking	-0.070	0.023	-0.093	-3.030	0.002
	Height	0.007	0.002	0.116	3.196	0.001
	Age	0.002	0.001	0.060	2.075	0.038
LDL-C	Glucose	0.051	0.010	0.144	5.297	0.000
	Age	0.004	0.001	0.104	3.826	0.000
	Genotype	-0.102	0.030	-0.090	-3.397	0.001
	Diastolic blood pressure	0.003	0.001	0.071	2.648	0.008
ApoAI	Weight	-0.005	0.001	-0.208	-7.650	0.000
	Alcohol consumption	0.054	0.010	0.149	5.497	0.000
ApoB	Waist circumference	0.006	0.001	0.212	7.651	0.000
	Age	0.001	0.001	0.063	2.042	0.041
	Genotype	-0.050	0.016	-0.080	-3.056	0.002
	Gender	-0.076	0.018	-0.152	-4.215	0.000
	Height	-0.004	0.001	-0.115	-3.116	0.002
	Systolic blood pressure	0.001	0.000	0.069	2.360	0.018
ApoAI/ApoB	Waist circumference	-0.007	0.002	-0.163	-3.379	0.001
	Age	-0.003	0.001	-0.091	-3.442	0.001
	Body mass index	-0.017	0.006	-0.140	-2.931	0.003
	Alcohol consumption	0.061	0.018	0.097	3.318	0.001
	Gender	0.059	0.023	0.076	2.566	0.010
Jing						
TC	Glucose	0.149	0.024	0.227	6.202	0.000
	Age	0.016	0.003	0.214	5.356	0.000
	Genotype	-0.278	0.083	-0.122	-3.354	0.001
	Cigarette smoking	0.191	0.057	0.128	3.323	0.001
	Pulse pressure	-0.006	0.002	-0.120	-3.000	0.003
	Body mass index	0.025	0.011	0.087	2.373	0.018
	Height	-0.010	0.005	-0.084	-2.122	0.034
TG	Waist circumference	0.034	0.004	0.353	9.511	0.000
	Cigarette smoking	0.377	0.053	0.267	7.114	0.000
	Glucose	0.098	0.022	0.157	4.458	0.000
	Height	-0.019	0.004	-0.164	-4.220	0.000
HDL-C	Waist circumference	-0.017	0.002	-0.351	-9.749	0.000
	Alcohol consumption	0.167	0.035	0.183	4.775	0.000

	Gender	0.130	0.035	0.143	3.729	0.000
LDL-C	Age	0.005	0.001	0.151	3.998	0.000
	Genotype	-0.164	0.041	-0.149	-3.989	0.000
	Glucose	0.036	0.012	0.115	3.069	0.002
	Cigarette smoking	0.074	0.027	0.104	2.769	0.006
ApoAI	Weight	-0.004	0.001	-0.180	-4.737	0.000
ApoB	Waist circumference	0.005	0.001	0.199	5.381	0.000
	Gender	-0.070	0.018	-0.141	-3.767	0.000
	Genotype	-0.074	0.022	-0.124	-3.366	0.001
	Age	0.002	0.001	0.119	3.180	0.002
ApoAI/ApoB	Waist circumference	-0.007	0.003	-0.161	-2.480	0.013
	Age	-0.003	0.001	-0.114	-2.961	0.003
	Weight	-0.005	0.003	-0.132	-2.010	0.045
Han						
TC	Glucose	0.254	0.031	0.303	8.214	0.000
	Gender	0.152	0.066	0.085	2.297	0.022
TG	Waist circumference	0.026	0.004	0.272	7.335	0.000
	Cigarette smoking	0.278	0.051	0.216	5.499	0.000
	Glucose	0.120	0.029	0.149	4.119	0.000
	Height	-0.013	0.004	-0.117	-2.931	0.003
HDL-C	Waist circumference	-0.013	0.002	-0.238	-6.260	0.000
	Alcohol consumption	0.117	0.034	0.159	3.473	0.001
	Gender	0.207	0.054	0.202	3.799	0.000
	Cigarette smoking	-0.117	0.035	-0.152	-3.356	0.001
	Height	0.008	0.003	0.121	2.493	0.013
LDL-C	Glucose	0.085	0.016	0.203	5.262	0.000
	Systolic blood pressure	0.003	0.001	0.122	3.156	0.002
ApoA1	Body mass index	-0.012	0.002	-0.188	-5.047	0.000
	Alcohol consumption	0.071	0.013	0.241	5.543	0.000
	Gender	0.060	0.018	0.146	3.367	0.001
ApoB	Waist circumference	0.007	0.001	0.234	6.068	0.000
	Systolic blood pressure	0.002	0.000	0.122	3.228	0.001
	Height	-0.005	0.002	-0.150	-3.077	0.002
	Gender	-0.057	0.024	-0.111	-2.341	0.020
ApoA1/ApoB	Waist circumference	-0.013	0.002	-0.296	-7.876	0.000
	Glucose	-0.038	0.014	-0.106	-2.823	0.005
	Pulse pressure	-0.002	0.001	-0.065	-1.714	0.087
	Alcohol consumption	0.069	0.024	0.124	2.917	0.004
	Gender	0.131	0.040	0.169	3.310	0.001
	Height	0.007	0.002	0.137	2.845	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.

and 44.55% in Han ($P > 0.05$), which was lower than that in the Beijing Chinese (Beijing in the north of China) and Southern Han Chinese samples. This difference may be caused by different sample sizes and regions (Beijing *vs.* Guangxi). The prevalence of the rs4142995 G allele is higher in European than in Chinese and lower in people of African ancestry. The genotype frequencies were also different between Jing males and females ($P < 0.05$), but not between Han males and females. These results indicated that the genotype frequencies of the *SNX13* rs4142995 SNP may have gender specificity in the Jing population. However, these

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	Glucose	0.114	0.032	0.205	3.532	0.000
	Cigarette smoking	0.144	0.058	0.149	2.493	0.013
	Age	0.013	0.004	0.192	2.883	0.004
	Pulse pressure	-0.012	0.003	-0.227	-3.574	0.000
	Body mass index	0.106	0.034	0.392	3.126	0.002
	Weight	-0.025	0.011	-0.292	-2.281	0.023
	Alcohol consumption	0.150	0.067	0.128	2.226	0.027
TG	Waist circumference	0.044	0.006	0.426	7.272	0.000
	Cigarette smoking	0.322	0.066	0.270	4.842	0.000
	Glucose	0.135	0.037	0.198	3.650	0.000
	Height	-0.034	0.009	-0.211	-3.565	0.000
	Age	-0.013	0.005	-0.156	-2.672	0.008
HDL-C	Waist circumference	-0.018	0.003	-0.392	-7.115	0.000
	Alcohol consumption	0.175	0.036	0.272	4.932	0.000
LDL-C	Genotype	-0.131	0.059	-0.135	-2.224	0.027
	Cigarette smoking	0.056	0.026	0.128	2.112	0.036
ApoA1	Weight	-0.005	0.001	-0.223	-3.750	0.000
	Alcohol consumption	0.043	0.019	0.133	2.233	0.026
ApoB	Body mass index	0.022	0.004	0.304	5.215	0.000
ApoA1/ApoB	Weight	-0.014	0.002	-0.346	-6.046	0.000
Jing/female						
TC	Glucose	0.186	0.034	0.254	5.434	0.000
	Age	0.017	0.004	0.217	4.640	0.000
	Genotype	-0.323	0.110	-0.137	-2.946	0.003
TG	Waist circumference	0.024	0.004	0.271	5.663	0.000
	Cigarette smoking	1.129	0.315	0.169	3.581	0.000
	Glucose	0.067	0.026	0.119	2.535	0.012
	Height	-0.024	0.006	-0.186	-3.868	0.000
HDL-C	Waist circumference	-0.018	0.003	-0.342	-7.023	0.000
	Diastolic blood pressure	0.005	0.002	0.103	2.126	0.034
LDL-C	Genotype	-0.192	0.055	-0.166	-3.472	0.001
	Age	0.008	0.002	0.206	4.301	0.000
	Glucose	0.050	0.017	0.139	2.888	0.004
ApoA1	Body mass index	-0.011	0.003	-0.153	-3.097	0.002
ApoB	Body mass index	0.012	0.004	0.156	3.268	0.001
	Genotype	-0.119	0.028	-0.201	-4.202	0.000
	Age	0.004	0.001	0.194	4.058	0.000
ApoA1/ApoB	Body mass index	-0.023	0.005	-0.212	-4.349	0.000
	Age	-0.004	0.001	-0.137	-2.823	0.005
Han/male						
TC	Glucose	0.238	0.043	0.315	5.510	0.000
	Diastolic blood pressure	0.013	0.005	0.152	2.663	0.008
TG	Waist circumference	0.054	0.013	0.450	4.298	0.000
	Cigarette smoking	0.233	0.066	0.207	3.552	0.000
	Glucose	0.167	0.050	0.192	3.317	0.001
	Age	-0.018	0.005	-0.233	-3.681	0.000

	Weight	-0.036	0.013	-0.310	-2.846	0.005
	Diastolic blood pressure	0.013	0.005	0.135	2.378	0.018
HDL-C	Waist circumference	-0.016	0.004	-0.246	-4.081	0.000
	Alcohol consumption	0.106	0.037	0.180	2.839	0.005
	Diastolic blood pressure	0.007	0.003	0.138	2.316	0.021
	Cigarette smoking	-0.110	0.038	-0.181	-2.923	0.004
LDL-C	Glucose	0.100	0.022	0.264	4.544	0.000
	Diastolic blood pressure	0.006	0.002	0.149	2.566	0.011
ApoA1	Alcohol consumption	0.076	0.013	0.339	5.923	0.000
	Waist circumference	-0.006	0.001	-0.231	-4.038	0.000
ApoB	Waist circumference	0.007	0.002	0.229	3.931	0.000
	Systolic blood pressure	0.002	0.001	0.161	2.708	0.007
	Glucose	0.025	0.012	0.119	2.020	0.044
ApoA1/ApoB	Waist circumference	-0.013	0.003	-0.281	-4.855	0.000
	Alcohol consumption	0.089	0.025	0.207	3.585	0.000
	Systolic blood pressure	-0.004	0.001	-0.176	-3.050	0.003
	Genotype	0.113	0.057	0.113	1.984	0.048
Han/female						
TC	Glucose	0.269	0.044	0.295	6.162	0.000
TG	Waist circumference	0.022	0.004	0.285	6.008	0.000
	Glucose	0.118	0.035	0.160	3.381	0.001
HDL-C	Body mass index	-0.034	0.007	-0.244	-5.023	0.000
LDL-C	Glucose	0.080	0.023	0.175	3.457	0.001
	Systolic blood pressure	0.003	0.001	0.118	2.320	0.021
	Height	-0.008	0.003	-0.107	-2.197	0.029
ApoA1	Body mass index	-0.011	0.003	-0.193	-3.911	0.000
ApoB	Age	0.003	0.001	0.144	2.846	0.005
	Waist circumference	0.007	0.001	0.245	5.054	0.000
	Height	-0.006	0.002	-0.159	-3.083	0.002
ApoA1/ApoB	Weight	-0.082	0.025	-1.914	-3.258	0.001
	Body mass index	0.158	0.058	1.430	2.716	0.007
	Age	-0.003	0.001	-0.115	-2.259	0.024
	Height	0.064	0.018	1.077	3.592	0.000
	Glucose	-0.042	0.018	-0.111	-2.292	0.022

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.

findings still need to be confirmed in more populations with larger sample sizes.

Recently, a newly study identify and annotate 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 *SNX13* rs4142995 SNP and HDL-C levels [17]. Besides this, there were no GWAS explored the relation between the rs4142995 SNP and serum lipid level. 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 [17]. In our cuurent study, we found that the TC, ApoB and LDL-C levels were different among the TT, GT and GG genotypes of the rs4142995 SNP in Jing but not in Han, the G allele carriers in Jing had lower serum TC, LDL-C and ApoB levels than the G allele non-carriers. Stratified analyses by gender showed that the levels of TC, ApoB and LDL-C in Jing were different among the three genotypes only in females; the TC, LDL-C and ApoB levels in Jing females were

lower in the G allele carriers than in the G allele non-carriers. There was no significant difference in the remaining serum lipid parameters among the three 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 *SNX13* rs4142995 SNP and serum lipid parameters. But the reason for these results is still unclear, and we deduced that natural selective processes may be the reason of some of the population differences detected for specific genetic variants.

Many pursuers have proved that there are gender differences in lipid metabolism [38, 39]. Premenopausal women were proved to have more favorable plasma lipid profiles than males, with lower levels of TG, TC and LDL-C, and higher HDL-C levels than age-matched men [40-43]. Some researchers also reported that lipoprotein particle concentration, subclass distribution, and sizes were different between male and female [42]. But Sex differences in lipid metabolism and lipoprotein kinetics remain to be elucidated. Sex hormones, body size and compositioncould not completely explain the differences [42]. More work is necessary to fully understand the control of lipid metabolism by a person's sex. In 2010, a GWAS has identified more than 12 loci exhibiting sex heterogeneity [12], since then more gender specific loci were found and replicated in other studies [26, 44-47]. The sex-specific association of the *SNX13* rs4142995 SNP was firstly reported in the present study. Sex-specific genetic associations may help to understand the sexual dimorphism in the plasma lipid profile [47] and may also be useful for the prevention and treatment of CVD.

In the cuurent study, we also detected that serum lipid parameters were affected by several environmental factors such as age, weight, waist circumference, BMI, blood pressure, alcohol consumption, cigarette smoking, and blood glucose in both Jing and Han, or males and females in both ethnic groups. In the present study, the Jing population has higher weight, BMI, waist circumference, glucose, TC, TG levels than the Han population, but lower ApoA1/ApoB ratio, LDL-C and ApoA1 levels. These results suggest that the environmental factors also play crucial role in determining the plasma lipid profile in our populations. Although Jing and local Han share same living environment, there was 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 sandworm. Jing people like to eat sweet food such as sweet glutinous rice porridge, mung bean syrup, because they believe sweet food is a symbol for happiness and they also prefer a kind of fish sauce called nuoc-mam which contains 17 amino acids (8 essential amino acids included of course) [3]. It has been reported that all saturated fatty acids, except stearic acid, raise LDL-C levels, and the omega-3 fatty acids from fish lower TG levels [48]. Many studies have proved that fiber consumption facilitates weight loss and improves lipid profiles, and dietary fiber intake can reduce the risk of CVD and and type 2 diabetes mellitus [48-50]. Although dietary composition remains an important, modifiable predictor of dyslipidemia, over consumption of any form of dietary energy may increases lipid and lipoprotein levels [48]. It is important that developing healthy eating habits is a key strategy for prevention and regression of CVD. In the present study, we also found that the percentages of alcohol consumption were lower in Jing than in Han population ($P < 0.001$). Alcohol intake has a significant influence on the human serum lipid metabolism [51]. Many studies have shown that modest alcohol consumption is associated with the increasing of serum HDL-C levels [51-53]. It was reported that 50% the beneficial effects of moderate alcohol consumption on CVD can be attributed to increased HDL-C [54]. But many evidences show that heavy alcohol consumption can raise serum TG levels, blood pressure, liver damage [54, 55]. A Japan collaborative cohort study showed than heavy alcohol consumption is associated with increased mortality from total stroke, and total CVD for men, and from coronary heart disease for women [56]. These results suggest that serum lipid levels are also modifiable by the environmental factors, genetic factors and their interaction in the present study.

In conclusion, this study showed that the association of the *SNX13* rs4142995 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 *SNX13* rs4142995 SNP and serum lipid levels in different populations.

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Disclosure of conflict of interest

None.

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