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Original Article

BRCA2 rs9534275 polymorphism and serum lipid traits in the Maonan and Han populations

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Abstract: Maonan nationality is relatively conservative and isolated minority in China. Little is known about the association of breast susceptibility gene 2 (*BRCA2*) rs9534275 single nucleotide polymorphism (SNP) and serum lipid levels in this population. The aim of this study was to compare the effect of the *BRCA2* rs9534275 SNP and several environmental factors on serum lipid profiles between the Chinese Maonan and Han populations. Genotypes of the *BRCA2* rs9534275 SNP in 828 individuals of Maonan nationality and 795 participants of Han nationality were determined by polymerase chain reaction and restriction fragment length polymorphism. The frequencies of TT, GT and GG genotypes were 35.72%, 44.15% and 20.13% in Han, and 23.30%, 56.28% and 20.42% in Maonan populations ($P < 0.001$). The frequency of the G allele was 42.20% in Han and 48.55% in Maonan individuals ($P < 0.001$). The G allele carriers had higher low-density lipoprotein cholesterol (LDL-C) and the apolipoprotein (Apo) A1/ApoB ratio in Han; and higher total cholesterol (TC), LDL-C, ApoB and lower ApoA1/ApoB ratio in Maonan than the G allele non-carriers. Subgroup analyses indicated that the G allele carriers had higher LDL-C, ApoA1 levels and the ApoA1/ApoB ratio in Han females; and higher TC, LDL-C, ApoB levels and lower ApoA1/ApoB ratio in both Maonan males and females ($P < 0.05$-0.001). Serum lipid parameters in the two ethnic groups were also associated with several environmental factors. These findings revealed that there might be a racial/ethnic- and/or sex-specific association between the *BRCA2* rs9534275 SNP and serum lipid parameters in some populations.

Keywords: Breast susceptibility gene 2, single nucleotide polymorphism, lipids, environmental factor

Introduction

Over the past several decades, cardiovascular disease (CVD) has become the leading cause of mortality, morbidity, disability, functional decline and healthcare costs [1, 2]. The rates were 271.9 for white males, 352.4 for black males, 188.1 for white females, and 248.6 for black females and every 229.6 per 100,000 Americans were died of CVD in 2011 [3]. We had established risk status measurement of a standard lipid profile to evaluate risk severity, just as total cholesterol (TC) [4], triglyceride (TG) [5], low-density lipoprotein cholesterol (LDL-C) [6], apolipoprotein (Apo) B [7], high-density lipoproteins cholesterol (HDL-C) [8], ApoA1 [9] and the ratio of ApoA1 to ApoB [10] is recommended from an integral component of approaches to cardiovascular risk prediction. At the same time, the metabolic syndrome give rise to an ascended risk of cardiometabolic anomalies and CVD is greatly among the widespread popularity [11]. Previous research about CVD risk factors as everyone knew that they are usually differ between men and women [12], are also affected by age [13] and ethnicity [14], and are adjusted by behavioral choices [15], counting in poor diet [16] and without exercise lifestyle [17], environmental factors [18], and personal genetic profile [19, 20]. Even if it is well known and widely accepted that all these risk factors taken individually are characterized by a significant genetic component, there is still a lot of uncertainty in the true magnitude of risk factor clustering, as well as on the role of genetic factors in risk factor clustering in individuals.

Taking an example as twin and family research that studies have demonstrated that there is a

significant genetic component to human variability in CVD risk factors when considered individually [21, 22]. In the meantime, studies also have got a documentory proof that these risks that we previous spoke of elements are all exhibited as familial resemblance and significant heritability estimates [23]. The target of genome-wide association studies (GWAS) was to locate which part can identify common single nucleotide polymorphisms (SNPs) and calculate the numbers of the phenotypic variance is actually found out them [24].

Previously on a few GWAS have proved the association of several SNPs close to the breast cancer susceptibility gene 2 (*BRCA2*; Also knows as: FAD; FACD; FAD1; GLM3; BRCC2; FANCD; PNCA2; FANCD1; XRCC11; BROVCA2, Gene ID: 675, HGNC ID: 1101, synonyms: "BRCA1/BRCA2-containing complex, subunit 2", BRCC2, FAD, FAD1, XRCC11, locus type: gene with protein product, chromosomal location: 13q13.1) has been proved to its mutation can cause an increased risk for breast cancer [25]. Women carrying *BRCA* mutations have metabolic deregulations in their breast tissue that may be precursors to malignant transformation, and also lead to exhibit a reduction of 79% in metabolite level, while both lipid unsaturation and TG levels increased by 19%. Besides these, women carrying *BRCA2* mutations showed an increased lipid unsaturation of 21% and the metabolic changes in women carrying *BRCA1* mutations are different from those in women carrying *BRCA2* mutations, with a 47% increase in cholesterol level recorded in those with *BRCA2* mutations [26]. The mechanism was supposed to have a connection with lipid metabolism [27]. Previous GWAS on plasma lipid levels have identified the rs9534275 SNP near the *BRCA2* as hyperlipidemia loci in European. The association between the *BRCA2* rs9534275 SNP and TC and LDL-C might have ethnic- and/or sex-specificity [28, 29]. Whether *BRCA2* rs9534275 SNP is associated with serum lipid levels or whether it shows ethnic and/or sex specific association as the previously reported *BRCA2 rs9534275* SNP remains dubious.

Han nationality is the largest group among the 56 ethnic groups in China. Maonan nationality is a relatively conservative and isolated minority, and preserves their custom of intra-ethnic marriage as one of the minorities. Divertingly, they have their culture of consanguineous marriage to cousins of maternal side, suggesting that the genetic background of Maonan population may be less heterogeneous within the population. Local people widely utilize endemic species, and they have developed their own traditional medicinal knowledge [30]. Height, fat mass and fat distribution differs substantially between men and women, and these differences may, in part, explain the sex-specific susceptibilities to certain diseases such as coronary artery disease [31]. These considerable differences in anthropometry may reflect sex-specific differences in steroid hormone regulation, adipogenesis, lipid storage, muscle metabolism, composition, and contractile speed, skeletal growth and maturation, or lipolysis, and suggest a genetic underpinning [32]. Sexual dimorphism has been demonstrated as the potential of dyslipidemia and CVD risk factors. This study, therefore, was undertaken to detect the association of the *BRCA2 rs9534275* SNP and several environmental factors with serum lipid levels between males and females in the Maonan and Han populations.

Materials and methods

Subjects

The study populations including 795 unrelated subjects (306 males, 38.49% and 489 females, 61.51%) of Han and 828 unrelated participants (332 males, 40.10% and 496 females, 59.90%) of Maonan were randomly selected from our previous stratified randomized samples [33]. The participants were all agricultural workers from Huanjiang Maonan Autonomous County, Guangxi Zhuang Autonomous Region, People's Republic of China. The participants' age ranged from 25 to 75 years with the mean age of 57.36±13.96 years in Han and 57.16±15.07 years in Maonan, respectively. The age distribution and gender ratio were matched between the two groups. All participants were essentially healthy with no history of CVD such as coronary artery disease, stroke, diabetes, hyper- or hypo-thyroids, and chronic renal disease. They were free from medications known to affect serum lipid levels. Informed consent was taken from all participants. The Ethics Committee of the First Affiliated Hospital, Guangxi Medical University,

approved the study design (No. Lunshen-2014-KY-Guoji-001, Mar. 7, 2014). Informed consent was taken from all participants.

Epidemiological survey

The epidemiological survey was carried out using internationally standardized methods, following a common protocol [34]. Information on demographics, socioeconomic status, and lifestyle factors was collected with standardized questionnaires. Alcohol consumption was categorized into groups of grams of alcohol per day: 0 (non-drinker), < 25 and ≥ 25. Smoking status was categorized into groups of cigarettes per day: 0 (non-smoker), < 20 and ≥ 20. Several parameters such as blood pressure, height, weight, waist circumference, and body mass index (BMI) were measured. The methods of measuring above parameters were referred to previous studies [35].

Biochemical measurements

A fasting venous blood sample of 5 ml was drawn from the participants. A part of the sample (2 mL) was collected into glass tubes and used to determine serum lipid levels. Another part of the sample (3 mL) was transferred to tubes with anticoagulants (4.80 g/L citric acid, 14.70 g/L glucose and 13.20 g/L tri-sodium citrate) and used to extract deoxyribonucleic acid (DNA). Measurements of serum TC, TG, HDL-C, and LDL-C levels in the samples were performed by enzymatic methods with commercially available kits (RANDOX Laboratories Ltd., Ardmore, Diamond Road, and Crumlin Co. Antrim, United Kingdom, BT29 4QY; Daiichi Pure Chemicals Co., Ltd., Tokyo, Japan). Serum ApoA1 and ApoB levels were detected by the immunoturbidimetric immunoassay using a commercial kit (RANDOX Laboratories Ltd.). All determinations were performed with an auto-analyzer (Type 7170A; Hitachi Ltd., Tokyo, Japan) in the Clinical Science Experiment Center of the First Affiliated Hospital, Guangxi Medical University [36, 37].

DNA amplification and genotyping

Genomic DNA of the samples was isolated from peripheral blood leucocytes according to the phenol-chloroform method [38, 39]. The extracted DNA was stored at 4°C until analysis. Genotyping of the *BRCA2* rs9534275 SNP was performed by polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP). PCR amplification was performed using 5'-TCTTGGCCCAGATGCTTACT-3' as the forward and 5'-TACCAACACTACCACCAGCA-3' as reversed primer pair (Sangon, Shanghai, People's Republic of China), respectively. Each 25 μL PCR reaction mixture consisted of 2.0 μL genomic DNA, 1.0 μL each primer (10 μmol/L), 12.5 μL of 2 × *Taq* PCR Master Mix (constituent: 0.1 U *Taq* polymerase/μL, 500 μM dNTP each and PCR buffer.), and 8.5 μL of ddH_2O (DNase/RNase-free). PCR was performed with an initialization step of 95°C for 5 min, followed by 30 s denaturing at 95°C, 30 s of annealing at 59°C and 35 s of elongation at 72°C for 33 cycles. The amplification was completed by a final extension at 72°C for 7 min. Following electrophoresis on a 2.0% agarose gel with 0.5 μg/mL ethidium bromide, the amplification products were visualized under ultraviolet light. Subsequently, each restriction enzyme reaction was performed with 5.0 μL amplified DNA, 8.8 μL nuclease-free water, 1.0 μL of 10 × buffer solution and 0.2 μL *Rsa*I restriction enzyme in a total volume of 15 μL 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 visualized with UV illumination. An experienced reader blinded to the epidemiological and serum lipid results scored genotypes. Six samples (TT, GT and GG genotypes in two; respectively) detected by the PCR-RFLP were also confirmed by direct sequencing with an ABI Prism 3100 (Applied Biosystems) in Shanghai Sangon Biological Engineering Technology & Services Co., Ltd., People's Republic of China.

Diagnostic criteria

The normal values of serum TC, TG, HDL-C, LDL-C, ApoA1, ApoB levels and the ApoA1/ApoB ratio in our Clinical Science Experiment Center were 3.10-5.17, 0.56-1.70, 1.16-1.42, 2.70-3.10 mmol/L, 1.20-1.60, 0.80-1.05 g/L and 1.00-2.50, respectively. The individuals with TC > 5.17 mmol/L and/or TG > 1.70 mmol/L were defined as hyperlipidaemic [38]. Hypertension was diagnosed according to the 1999 and 2003 criteria of the World Health Organization-International Society of Hypertension Guidelines for the management of hypertension [40].

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

Parameter	Han	Maonan	t (x^2)	P
Number	795	828		
Male/female	306/489	332/496	0.439	0.508
Age (years)	57.36±13.96	57.16±15.07	0.244	0.621
Height (cm)	154.05±7.77	153.83±8.06	1.745	0.187
Weight (kg)	53.32±9.06	53.23±10.65	15.287	0.000
Body mass index (kg/m^2)	22.42±3.20	22.39±3.59	1.792	0.027
Waist circumference	75.23±7.87	76.78±9.19	14.167	0.000
Smoking status [*n* (%)]				
Non-smoker	593 (74.59)	648 (78.26)		
≤ 20 cigarettes/day	94 (11.83)	152 (18.36)		
> 20 cigarettes/day	108 (13.58)	28 (3.38)	74.338	0.000
Alcohol consumption [*n* (%)]				
Non-drinker	641 (80.63)	657 (79.35)		
≤ 25 g/day	115 (14.46)	136 (16.43)		
> 25 g/day	39 (4.91)	35 (4.22)	1.500	0.472
Systolic blood pressure (mmHg)	129.68±19.54	134.89±23.29	30.373	0.000
Diastolic blood pressure (mmHg)	81.73±11.20	82.32±12.14	1.838	0.175
Pulse pressure (mmHg)	47.95±15.20	52.57±18.33	27.370	0.000
Glucose (mmol/L)	6.18±1.92	6.21±1.42	14.966	0.000
Total cholesterol (mmol/L)	4.98±0.99	5.55±1.17	6.593	0.010
Triglyceride (mmol/L)	1.32 (0.62)	1.43 (0.70)	0.399	0.528
HDL-C (mmol/L)	1.74±0.45	1.61±0.40	0.823	0.364
LDL-C (mmol/L)	2.89±0.82	3.37±0.93	10.299	0.001
ApoA1 (g/L)	1.35±0.24	1.41±0.40	6.036	0.014
ApoB (g/L)	0.86±0.21	0.89±0.21	0.244	0.621
ApoA1/ApoB	1.65±0.50	1.66±0.56	2.363	0.067

HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; Apo, Apolipoprotein. The value of triglyceride was presented as median (interquartile range); the difference between the two ethnic groups was determined by the Wilcoxon-Mann-Whitney test.

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 [41].

Statistical analyses

The statistical analyses were performed with the statistical software package SPSS 21.0 (SPSS Inc., Chicago, Illinois). The quantitative variables were presented as mean ± standard deviation (serum TG levels were presented as medians and interquartile ranges). Allele frequency was determined via direct counting, and the Hardy-Weinberg equilibrium was verified with the standard goodness-of-fit test. The genotype distribution between the two groups was analyzed by the chi-square test. General characteristics between two ethnic groups were compared by the Student's unpaired *t*-test. The association between genotypes and serum lipid parameters was tested by covariance analysis (ANCOVA). Gender, age, BMI, blood pressure, alcohol consumption and cigarette smoking were adjusted for the statistical analysis. Multivariable linear regression analyses with stepwise modeling were used to determine the correlation between the genotypes (TT = 1, GT = 2, GG = 3) and several environmental factors with serum lipid levels in males and females of Han and Maonan populations. Two sided P value < 0.05 was considered statistically significant.

Results

General characteristics and serum lipid levels

The general characteristics and serum lipid levels between the Han and Maonan populations are summarized in **Table 1**. The percentages of cigarette smoking, the levels of weight were higher in Han than in Maonan (P < 0.001), whereas the levels of systolic blood pressure, pulse pressure, waist circumference, blood glucose, serum TC, LDL-C and ApoA1 were lower in Han than in Maonan (P < 0.05-0.001). There were no significant differences in the gender ratio, age structure, body height, BMI, percentage of alcohol consumption, diastolic blood pressure, serum TG, HDL-C, ApoB levels and the ApoA1/ApoB ratio between the two ethnic groups (P > 0.05 for all).

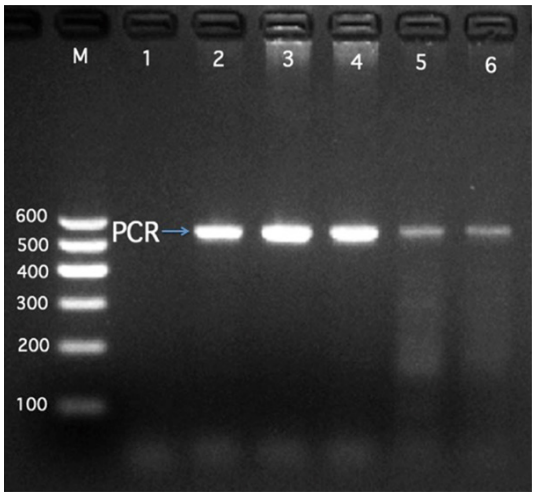

Figure 1. Electrophoresis of polymerase chain reaction products of the samples. Lane M is the 100 bp marker ladder; Lanes 1-6 are samples, the 550 bp bands are the target genes.

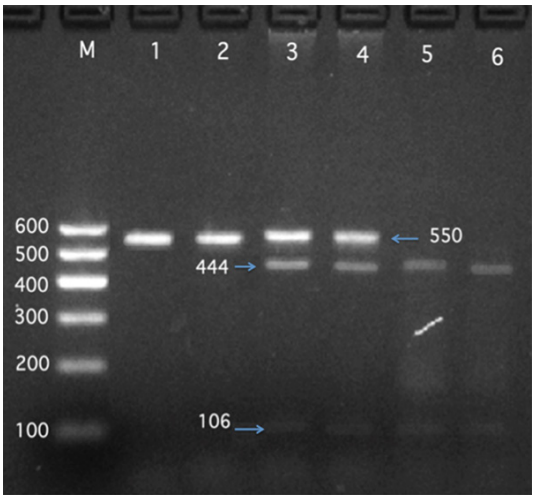

Figure 2. Genotyping of the *BRCA2* rs9534275 SNP. Lane M, 100 bp marker ladder; lanes 1 and 2, TT genotype (550 bp); lanes 3 and 4, GT genotype (444- and 106-bp); lanes 5 and 6, GG genotype (444- and 106-bp).

Results of electrophoresis and genotyping

After the genomic DNA of the samples was amplified using PCR and visualized with 2% agarose gel electrophoresis, the products of 550 bp nucleotide sequences were observed in all samples (**Figure 1**). The genotypes identified were termed according to the presence (G allele) or absence (T allele) of the enzyme restriction sites. Thus, the GG genotype is homozygous for the presence of the site (bands at 444 bp and 106 bp), the GT genotype is heterozygous for the presence and absence of the site (bands at 550-, 444- and 106-bp) and the TT genotype is homozygous for the absence of the site (bands at 550 bp; **Figure 2**). The TT, GT and GG genotypes detected by PCR-RFLP were also confirmed by direct sequencing (**Figure 3**), respectively.

Genotypic and allelic frequencies

The genotypic and allelic frequencies of the *BRCA2* rs9534275 SNP are shown in **Table 2**. The frequencies of T and G alleles were 57.80% and 42.20% in Han, and 51.45% and 48.55% in Maonan populations ($P < 0.001$), respectively. The frequencies of TT, GT and GG genotypes were 35.72%, 44.15% and 20.13% in the Han population, and 23.30%, 56.28% and 20.42% in the Maonan population ($P < 0.005$), respectively. No difference in the genotypic and allelic frequencies was found between males and females in the two ethnic groups ($P > 0.05$ for each).

Genotypes and serum lipid levels

Tables 3 and **4** describe the association between genotypes and serum lipid levels. The levels of LDL-C and the ratio of ApoA1 to ApoB in Han were different between the genotypes ($P < 0.05$ for each); the G allele carriers had higher LDL-C levels and the ApoA1/ApoB ratio than the G allele non-carriers. The levels of TC, LDL-C, ApoB and the ratio of ApoA1 to ApoB in Maonan were different between the genotypes ($P < 0.05$); the G allele carriers had higher TC, LDL-C and ApoB levels and lower ApoA1/ApoB ratio than the G allele non-carriers. Subgroup analyses showed that the G allele carriers had higher LDL-C, ApoA1 levels and the ApoA1/ApoB ratio in Han females; and higher TC, LDL-C, ApoB levels and lower ApoA1/ApoB ratio in both Maonan males and females ($P < 0.05$-0.001).

Relative factors for serum lipid parameters

Multiple linear regression analysis showed that serum TC, LDL-C and ApoB levels in the combined population of Han and Maonan; and TC, LDL-C, ApoB levels and the ApoA1/ApoB ratio in Maonan were correlated with the genotypes of the *BRCA2* rs9532475 SNP ($P < 0.05$ for all; **Table 5**). When the correlation of serum lipid

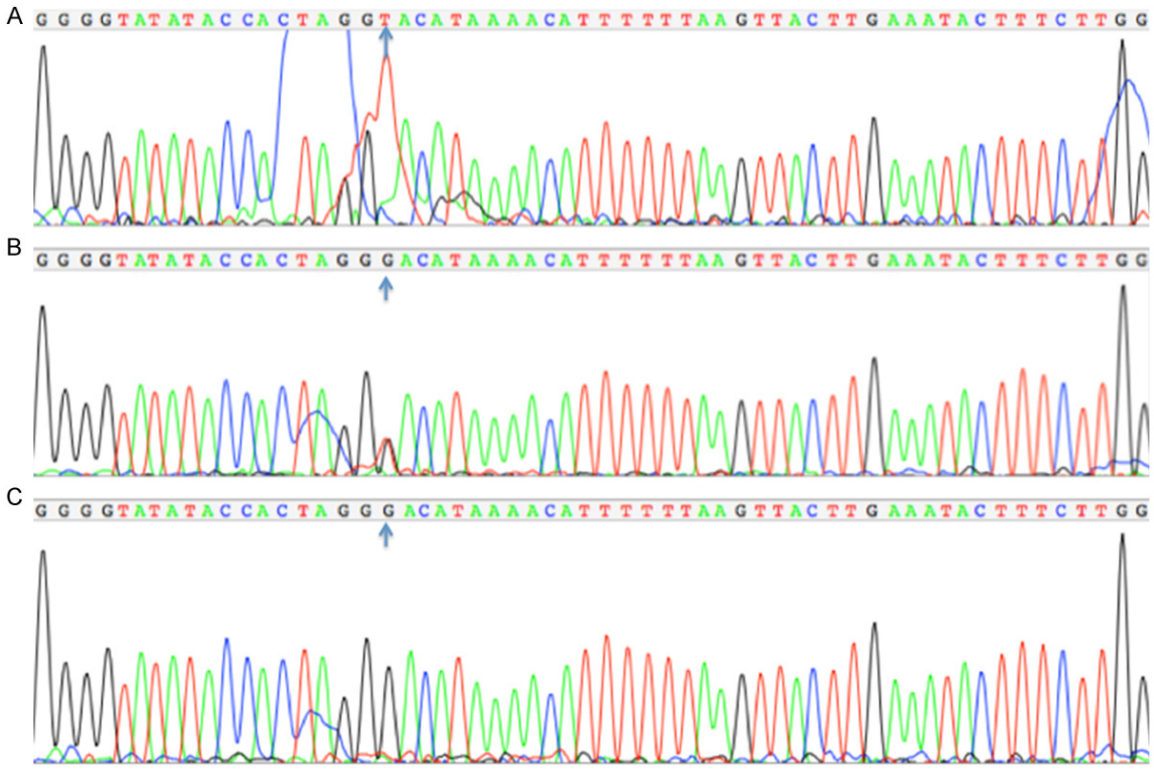

Figure 3. A part of the nucleotide sequence of the *BRCA2* rs9534275 SNP. A: TT genotype; B: GT genotype; C: GG genotype.

Table 2. Comparison of the genotype and allele frequencies of BRCA2 rs9534275 SNP in the Han and Maonan populations [*n* (%)]

Group	*n*	Genotype			Allele	
		TT	GT	GG	T	G
Han	795	284 (35.72)	351 (44.15)	160 (20.13)	919 (57.80)	671 (42.20)
Maonan	828	193 (23.30)	466 (56.28)	169 (20.42)	852 (51.45)	804 (48.55)
x^2			33.137		13.191	
P			0.000		0.000	
Han						
Male	306	114 (37.25)	133 (43.46)	59 (19.29)	361 (58.99)	251 (41.01)
Female	489	170 (34.76)	218 (44.58)	101 (20.66)	558 (57.05)	420 (42.95)
x^2			0.556		0.575	
P			0.757		0.448	
Maonan						
Male	332	69 (20.78)	188 (56.63)	75 (22.59)	326 (49.10)	338 (50.90)
Female	496	124 (25.00)	278 (56.05)	94 (18.95)	526 (54.12)	466 (45.88)
x^2			2.819		2.457	
P			0.244		0.117	

parameters and the genotypes was analyzed according to sex, we showed that TC, LDL-C, ApoB levels and the ApoA1/ApoB ratio in Maonan males; serum TC, LDL-C and ApoB levels in Maonan females were correlated with the genotypes ($P < 0.05$ for all; **Table 6**). Serum lipid parameters were also associated with age, gender, BMI, waist circumference, systolic and diastolic blood pressure, pulse pressure, fasting blood glucose levels, cigarette smoking

Table 3. Comparison of the genotypes and serum lipid levels in the Han and Maonan 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	284	4.91±1.02	1.32 (0.60)	1.74±0.41	2.81±0.86	1.35±0.26	0.86±0.21	1.67±0.52
GT	351	5.06±0.97	1.32 (0.60)	1.74±0.51	2.98±0.82	1.33±0.22	0.87±0.19	1.60±0.45
GG	160	4.94±0.96	1.32 (0.72)	1.77±0.41	2.89±0.82	1.38±0.25	0.85±0.24	1.72±0.53
F		2.035	0.062	0.339	3.585	1.904	0.876	3.609
P		0.131	0.951	0.671	0.028	0.150	0.417	0.028
Maonan								
TT	193	5.26±1.09	1.43 (0.73)	1.58±0.41	3.19±0.83	1.40±0.48	0.85±0.19	1.71±0.70
GT	466	5.47±1.13	1.43 (0.73)	1.62±0.41	3.28±0.88	1.41±0.37	0.88±0.20	1.69±0.53
GG	169	6.08±1.19	1.42 (0.67)	1.60±0.34	3.80±1.03	1.43±0.38	0.99±0.22	1.50±0.40
F		26.142	0.177	0.713	24.662	0.221	22.957	9.015
P		0.000	0.859	0.490	0.000	0.810	0.000	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. 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 Maonan 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	114	5.03±1.58	1.30 (0.66)	1.67±0.57	2.90±1.39	1.34±0.42	0.91±0.32	1.56±0.67
GT	133	5.16±1.07	1.30 (0.66)	1.70±0.57	2.92±0.97	1.36±0.36	0.91±0.27	1.55±0.68
GG	59	5.10±1.49	1.37 (0.85)	1.67±0.58	2.95±1.10	1.34±0.34	0.88±0.27	1.59±0.64
F		0.511	0.604	0.143	0.060	0.286	0.508	0.101
P		0.600	0.546	0.866	0.942	0.752	0.602	0.904
Han/female								
TT	170	4.82±1.34	1.35 (0.59)	1.73±0.57	2.76±1.09	1.36±0.33	0.82±0.26	1.75±0.77
GT	218	5.00±1.53	1.30 (0.66)	1.76±0.78	3.01±1.25	1.32±0.27	0.85±0.27	1.63±0.62
GG	101	4.85±1.27	1.31 (0.64)	1.83±0.57	2.77±1.05	1.40±0.35	0.83±0.38	1.80±0.78
F		1.728	0.566	0.784	5.562	5.309	0.678	4.990
P		0.179	0.578	0.457	0.004	0.005	0.508	0.007
Maonan/male								
TT	69	5.12±0.98	1.52 (0.92)	1.49±0.39	3.07±0.73	1.43±0.76	0.84±0.20	1.78±1.02
GT	188	5.43±0.98	1.30 (0.66)	1.60±0.46	3.16±0.81	1.45±0.48	0.89±0.20	1.70±0.62
GG	75	6.10±1.18	1.39 (0.72)	1.50±0.35	3.72±1.12	1.40±0.41	1.01±0.22	1.43±0.44
F		17.883	0.411	2.651	12.942	0.199	14.323	5.371
P		0.000	0.681	0.072	0.000	0.819	0.000	0.005
Maonan/female								
TT	124	5.34±1.15	1.40 (0.72)	1.63±0.42	3.26±0.87	1.38±0.22	0.86±0.19	1.68±0.45
GT	278	5.49±1.22	1.30 (0.66)	1.63±0.38	3.36±0.92	1.39±0.28	0.87±0.20	1.68±0.46
GG	94	6.07±1.20	1.43 (0.64)	1.67±0.33	3.86±0.96	1.45±0.36	0.97±0.21	1.54±0.36
F		10.937	0.047	0.528	13.161	1.654	9.195	3.691
P		0.000	0.963	0.590	0.000	0.192	0.000	0.026

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

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

Lipid	Risk factor	B	Std.error	Beta	*t*	*P*
Han and Maonan						
TC	Waist circumference	0.017	0.005	0.127	3.303	0.001
	Age	0.009	0.002	0.113	3.958	0.000
	Ethnic group	-0.497	0.053	-0.222	-9.323	0.000
	Diastolic blood pressure	0.007	0.002	0.070	2.881	0.005
	Genotype	0.169	0.038	0.106	4.500	0.000
TG	Waist circumference	0.043	0.008	0.211	5.411	0.000
	Alcohol consumption	0.388	0.126	0.089	3.068	0.002
	Glucose	0.079	0.025	0.076	3.128	0.002
	Diastolic blood pressure	0.011	0.004	0.071	2.813	0.005
HDL-C	Waist circumference	-0.010	0.002	-0.206	-5.289	0.000
	Gender	0.148	0.034	0.167	4.403	0.000
	Cigarette smoking	0.085	0.032	0.083	2.655	0.008
	Alcohol consumption	0.146	0.031	0.135	4.651	0.000
	Ethnic group	0.115	0.021	0.133	5.545	0.000
LDL-C	Ethnic group	-0.420	0.043	-0.230	-9.749	0.000
	Age	0.009	0.002	0.137	4.816	0.000
	Cigarette smoking	-0.160	0.066	-0.750	-2.429	0.015
	Waist circumference	0.016	0.004	0.151	3.943	0.000
	Genotype	0.123	0.031	0.095	4.069	0.000
ApoA1	Cigarette smoking	0.098	0.026	0.124	3.827	0.000
	Alcohol consumption	0.112	0.025	0.145	4.826	0.000
	Gender	0.089	0.027	0.129	3.287	0.001
	Ethnic group	-0.073	0.017	-0.109	-4.360	0.000
ApoB	Waist circumference	0.005	0.001	0.213	5.555	0.000
	Diastolic blood pressure	0.002	0.000	0.087	3.510	0.000
	Age	0.002	0.000	0.122	4.266	0.000
	Genotype	0.018	0.007	0.061	2.624	0.009
ApoA1/ApoB	Waist circumference	-0.014	0.002	-0.221	-5.708	0.000
	Age	-0.040	0.001	-0.980	-3.413	0.001
	Gender	0.172	0.041	0.159	4.215	0.000
	Cigarette smoking	0.143	0.039	0.115	3.679	0.002
	Alcohol consumption	0.155	0.038	0.118	4.069	0.000
Han						
TC	Waist circumference	0.019	0.007	0.152	2.611	0.009
	Diastolic blood pressure	0.010	0.003	0.117	3.142	0.002
TG	Waist circumference	0.061	0.013	0.273	4.743	0.006
	Glucose	0.098	0.032	0.107	3.027	0.003
	Cigarette smoking	0.483	0.183	0.122	2.671	0.008
	Diastolic blood pressure	0.022	0.006	0.144	3.917	0.003
	Age	-0.013	0.005	-0.108	-2.556	0.011
HDL-C	Gender	0.141	0.042	0.152	2.718	0.007
	Waist circumference	-0.007	0.003	-0.120	-2.040	0.042
	Alcohol consumption	0.166	0.051	0.145	3.241	0.001
LDL-C	Gender	-0.218	0.094	-0.128	-2.320	0.021
	Age	0.009	0.003	0.146	3.402	0.001

	Cigarette smoking	-0.307	0.088	-0.162	-3.494	0.001
ApoA1	Gender	0.081	0.027	0.165	2.988	0.003
	Alcohol consumption	0.163	0.027	0.268	6.067	0.000
	Cigarette smoking	0.080	0.026	0.146	3.152	0.002
ApoB	Glucose	0.009	0.004	0.085	2.466	0.015
	Systolic blood pressure	0.001	0.001	0.196	5.436	0.000
	Age	0.001	0.001	0.084	2.033	0.042
	Gender	-0.066	0.023	-0.154	-2.871	0.004
ApoA1/ApoB	Cigarette smoking	0.161	0.051	0.141	3.122	0.002
	Gender	0.254	0.055	0.249	4.616	0.000
	Alcohol consumption	0.132	0.054	0.105	2.435	0.015
Maonan						
TC	Waist circumference	0.014	0.007	0.110	2.011	0.045
	Age	0.012	0.003	0.150	3.653	0.000
	Genotype	0.366	0.059	0.207	6.148	0.000
TG	Waist circumference	0.028	0.010	0.147	2.731	0.006
	Alcohol consumption	0.540	0.170	0.127	3.177	0.002
	Height	-0.103	0.035	-0.479	-2.936	0.003
	Weight	0.156	0.048	0.959	3.237	0.001
	Body mass index	-0.299	0.105	-0.620	-2.854	0.004
HDL-C	Waist circumference	-0.012	0.002	-0.273	-5.069	0.000
	Gender	0.157	0.044	0.192	3.601	0.000
	Alcohol consumption	0.138	0.039	0.140	3.510	0.000
	Cigarette smoking	0.087	0.042	0.090	2.069	0.039
LDL-C	Age	0.009	0.002	0.151	3.730	0.001
	Alcohol consumption	-0.211	0.091	-0.092	-2.307	0.021
	Waist circumference	0.021	0.005	0.205	3.801	0.000
	Genotype	0.278	0.047	0.197	5.939	0.000
ApoA1	Alcohol consumption	0.098	0.042	0.099	2.361	0.018
	Gender	0.094	0.046	0.114	2.036	0.046
	Cigarette smoking	0.117	0.044	0.099	2.362	0.018
ApoB	Waist circumference	0.006	0.001	0.281	5.366	0.000
	Genotype	0.052	0.010	0.165	5.101	0.000
	Age	0.002	0.001	0.161	4.099	0.001
ApoA1/ApoB	Age	-0.004	0.001	-0.097	-2.440	0.015
	Waist circumference	-0.019	0.003	-0.318	-6.019	0.000
	Alcohol consumption	0.198	0.054	0.144	3.685	0.000
	Cigarette smoking	0.122	0.058	0.090	2.118	0.035
	Genotype	-0.079	0.028	-0.094	-2.874	0.004

TC, total cholesterol; TG, triglyceride; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; ApoA1, apolipoprotein A1; ApoB, apolipoprotein B; ApoA1/ApoB, the ratio of apolipoprotein A1 to apolipoprotein B; B, unstandardized coefficient; Beta, standardized coefficient.

and alcohol consumption in both ethnic groups or in males and females ($P < 0.05$-0.001; **Tables 2** and **3**).

Discussion

The results of the present study showed that the levels of serum TC, LDL-C, ApoB and ApoA1/ApoB ratio were lower in Han than in Maonan. There were no significant differences in the levels of serum TG, HDL-C and ApoA1 levels between the two ethnic groups. It was widely realized that dyslipidemia as a serious risk factor for CVD is caused by various elements, mainly including genetic and environmental factors and their interaction [42, 43]. Maonan

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

Lipid	Risk factor	B	Std.error	Beta	*t*	*P*
Han/male						
TC	Diastolic blood pressure	0.014	0.005	0.165	2.716	0.007
TG	Glucose	0.185	0.079	0.140	2.324	0.021
	Diastolic blood pressure	0.029	0.012	0.144	2.376	0.018
HDL-C	Alcohol consumption	0.158	0.049	0.195	3.240	0.001
LDL-C	Cigarette smoking	-0.297	0.097	-0.177	-3.054	0.002
ApoA1	Cigarette smoking	0.079	0.030	0.144	2.614	0.009
	Alcohol consumption	0.171	0.031	0.320	5.421	0.000
ApoB	Systolic blood pressure	0.003	0.001	0.161	2.764	0.006
	Glucose	0.022	0.007	0.187	3.233	0.001
ApoA1/ApoB	Weight	-0.041	0.017	-0.789	-2.448	0.015
	Alcohol consumption	0.247	0.075	0.178	3.301	0.001
	Glucose	-0.030	0.015	-0.114	-2.011	0.045
Han/female						
TC	Age	0.018	0.004	0.243	4.480	0.000
TG	Diastolic blood pressure	0.022	0.005	0.201	4.118	0.000
	Glucose	0.082	0.027	0.138	3.052	0.002
LDL-C	Body mass index	0.316	0.141	1.131	2.249	0.025
	Age	0.021	0.003	0.336	6.305	0.000
ApoA1	Height	-0.034	0.012	-0.904	-2.938	0.005
	Body mass index	-0.127	0.040	-1.691	-3.189	0.000
	Weight	0.510	0.180	1.634	2.867	0.004
ApoB	Cigarette smoking	-0.132	0.057	-0.127	-2.677	0.008
	Diastolic blood pressure	0.004	0.001	0.218	4.646	0.000
	Age	0.003	0.001	0.185	3.465	0.001
ApoA1/ApoB	Age	-0.008	0.002	-0.203	-3.752	0.000
	Diastolic blood pressure	-0.005	0.002	-0.114	-2.393	0.017
	Cigarette smoking	0.521	0.140	0.179	3.723	0.000
Maonan/male						
TC	Genotype	0.415	0.086	0.253	4.802	0.000
	Age	0.010	0.005	0.136	2.120	0.035
TG	Cigarette smoking	0.635	0.271	0.128	2.343	0.020
HDL-C	Cigarette smoking	0.090	0.045	0.106	2.004	0.046
	Alcohol consumption	0.173	0.046	0.204	3.745	0.000
LDL-C	Genotype	0.297	0.073	0.216	4.409	0.000
	Alcohol consumption	-0.352	0.098	-0.194	-3.582	0.000
ApoA1	Cigarette smoking	0.079	0.030	0.144	2.614	0.009
ApoB	Age	0.003	0.001	0.199	3.193	0.002
	Genotype	0.067	0.017	0.207	4.028	0.000
	Glucose	0.016	0.008	0.107	2.106	0.036
ApoA1/ApoB	Age	-0.006	0.003	-0.127	-1.977	0.049
	Alcohol consumption	0.247	0.075	0.178	3.301	0.001
	Genotype	-0.130	0.056	-0.123	-2.332	0.002
Maonan/female						
TC	Genotype	0.332	0.081	0.180	4.119	0.000
	Age	0.014	0.004	0.171	3.277	0.001

TG	Pulse pressure	0.008	0.002	0.156	3.278	0.001
HDL-C	Weight	-0.031	0.015	-0.743	-2.105	0.000
	Height	0.024	0.011	0.379	2.301	0.022
	Pulse pressure	-0.003	0.001	-0.144	-2.992	0.003
	Glucose	-0.024	0.012	-0.089	-1.984	0.048
LDL-C	Genotype	0.272	0.061	0.191	4.439	0.000
	Age	0.013	0.003	0.209	4.056	0.000
ApoB	Age	0.003	0.001	0.186	3.650	0.000
	Genotype	0.044	0.013	0.142	3.342	0.001
	Pulse pressure	0.001	0.001	0.118	2.517	0.012
ApoA1/ApoB	Pulse pressure	-0.003	0.001	-0.138	-2.922	0.004
	Age	-0.004	0.002	-0.143	-2.778	0.006

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 toapolipoprotein B; B, unstandardized coefficient.

belongs to inland nationality and is mainly occupied with cereal and miscellaneous grain crops. The history of Maonan can be traced back to the 11th century. According to the statistics in 2000, the numbers of Maonan population were 107166, mainly engaged in agriculture and were good at raising beef cattle and prepare the bamboo hat. The main food for them was rice, besides this, corn, sorghum, millet, sweet potatoes and pumpkin is another important complement. Therefore, they were enjoyed a very special lifestyle and dietary habits compared with the other nationalities. Maonan people were keening on spicy and acid food. Parents mainly arrange their marriages. Maonan stays endogamy; intermarriage with Han or Zhuang people is seldom happened. Therefore, it is considered that the hereditary characteristics and genotypes of certain lipid metabolism-related genes in this population might be different from those in the Han Chinese.

To the best of our knowledge, the genotypic and allelic frequencies of the *BRCA2* rs9534275 SNP have not been reported previously in different ethnic groups. In the present study, we firstly showed that the G allele frequency of the *BRCA2* rs9534275 SNP was lower in Han than in Maonan populations (42.20% *vs*. 48.55%; $P < 0.001$). The distribution of the GT and GG genotypes was also different between the two ethnic groups ($P < 0.001$); the frequencies of GT and GG genotypes were lower in Han than in Maonan ethnic groups, respectively. No significant differences were observed in the genotypic and allelic frequencies between males and females in the two ethnic groups. These results indicate that the prevalence of *BRCA2* rs9534275 SNP may have racial/ethnic specificity.

The potential association of the *BRCA2* rs9534275 SNP and serum lipid levels has not been previously reported in different racial/ethnic groups. In a previous association study that *BRCA* mutations contribute to about 20% of all hereditary breast cancers and women carrying *BRCA1* and *BRCA2* mutation were easily caught up with breast cancer [44]. When we compared the *BRCA1* cohort with *BRCA2*, an increase in the glycerol backbone of TG was accompanied by an increase in unsaturation of the fatty acid chains. The increase in cholesterol level in the *BRCA2* cohort indicates lipid pathways are affected differently in the two different gene mutations. It has been documented elsewhere that women with a *BRCA2* mutation survive longer than women with a *BRCA1* mutation. These results suggest that there are biochemical differences that may explain this difference [26]. In the current study, we firstly showed that the G allele carriers in Han had higher serum LDL-C levels and the ApoA1/ApoB ratio than the G allele non-carriers. The G allele carriers in Maonan had higher serum TC, LDL-C and ApoB levels and lower ApoA1/ApoB ratio than the G allele non-carriers. Subgroup analyses showed that the G allele carriers in Han females had higher LDL-C, ApoA1 levels and the ApoA1/ApoB ratio than the G allele non-carriers; the G allele carriers in Maonan males and females had higher TC, LDL-C and ApoB levels and lower ApoA1/ApoB ratio than the G

allele non-carriers. These findings suggest that there may be an ethnic- and gender-specific association of the *BRCA2* rs9534275 SNP and serum lipid levels.

As we all known that environmental factors such as dietary patterns, lifestyle and physical inactivity are all strongly related with serum lipid levels [45]. In the present study, multivariate linear regression analysis also showed that serum lipid parameters were correlated to age, sex, waist circumference, BMI, blood pressure, blood glucose, alcohol consumption, and cigarette smoking in both ethnic groups. These findings suggest that the environmental factors also play a key role in determining serum lipid levels in our study populations. The dietary habits are different between the Han and Maonan populations. Rice is the Maonan people's staple food supplemented with corn, sweet potato and other grains. Maonan people prefer to eat spicy and acid food with lots of oil and salt. This preference of high in carbohydrates may be related to the higher blood glucose levels, weight, BMI and waist circumference in Maonan than in Han people. In the meantime, rich oil and salt can give rise to higher blood pressure, serum TC, LDL-C and ApoB levels in Maonan than in Han people. In many past studies proved that diet alone could account for the variability on serum lipid levels [46-48].

In addition, multiple linear regression analysis also showed that serum HDL-C and ApoA1 levels in Han males and serum LDL-C, HDL-C, ApoA1 levels and ApoA1/ApoB ratio were correlated with the alcohol consumption ($P < 0.001$). Compared with alcohol consumption, cigarette smoking was correlated with serum LDL-C, ApoA1 levels and ApoA1/ApoB ratio in Han males, serum ApoB levels and ApoA1/ApoB ratio in Han females, serum TG, HDL-C, LDL-C and ApoA1 levels in Maonan males. Several case-control and cohort studies have described a J- or U-shaped association between alcohol intake and atherogenesis [49]. A moderate intake of alcohol when taken on a regular basis has been showed to protect against CVD death, which has been ascribed to the changes in serum HDL-C, TG and ApoA1 levels [50]. However, alcohol consumption was also associated with worse hematological values of TC and LDL-C levels. Results from the Italian Longitudinal Study on Aging showed that in elderly men (65-84 years) alcohol consumption increases serum LDL-C levels [50]. Onat *et al*. [51] also showed that alcohol consumption is positively associated with TG, LDL-C, and ApoB levels in males and negatively correlated with TG and/or not correlated with LDL-C and ApoB levels in females. At the same time, an increase in HDL-C through lifestyle changes just as smoking cessation and physical exercise has positive effects [52]. Nevertheless, another research indicated that the effects of alcohol consumption on LDL-C appear to vary by specific patient types or patterns of alcohol intake, and sex as well as genetic variants [53]. Therefore, the results of exposure to different lifestyle and environmental factors probably further modify the association of genetic variations and serum lipid levels in our study populations.

Limitations

There are several potential limitations in our study. First, we were not able to alleviate the effect of diet and several environmental factors during the statistical analysis. Second, we could not completely exclude asymptomatic disorders such as atherosclerosis that may create a potentially significant bias due to poor field study condition. Third, although we observe significant association of the *BRCA2* rs9534275 SNP and serum lipid levels, there are still many unmeasured environmental and genetic factors that needed to be considered. The interactions of gene-gene, gene-environment, and environment-environment on serum lipid levels are remained to be determined. What's more, the relevance of this finding has to be defined in further high caliber of studies including incorporating the genetic information of the *BRCA2* rs9534275 SNP and *in vitro* functional studies to confirm the impact of a variant on a molecular level.

Conclusions

The present study showed that the genotypic and allelic frequencies of the *BRCA2* rs9534275 SNP were different between the Han and Maonan populations. The associations of the *BRCA2* rs9534275 SNP and serum lipid levels were also different between the both ethnic groups and between males and females in the Maonan population. There may be a racial/ethnic- and/or sex-specific association of the *BRCA2* rs9534275 SNP and serum lipid levels.

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Disclosure of conflict of interest

None.

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