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Original Article

Polymorphism of rs998584 near the vascular endothelial growth factor A gene and serum lipid levels in the Maonan and Han populations

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Abstract: The association of rs998584 single nucleotide polymorphism (SNP) near the vascular endothelial growth factor A gene (*VEGFA*) and serum lipid levels in the ethnic minorities of China is very little-known. In the Chinese Maonan ethnic group has not been reported previously. The present study aimed to detect the influence of the rs998584 SNP and several environmental factors on serum lipid profiles between the Maonan and Han populations. The genotypes of rs998584 SNP in 780 individuals of Maonan nationality and 749 participants of Han nationality were determined by polymerase chain reaction and restriction fragment length polymorphism. The genotype and allele frequencies were different between the Han and Maonan populations (TT, 31.78% *vs*. 37.44%; GT, 50.20% *vs*. 51.02%; GG, 18.02% *vs*. 11.54%, $P = 0.004$; T, 56.88% *vs*. 62.95%; G, 43.12% *vs*. 37.05%, $P = 0.001$) and between males and famales in the Maonan population (T, 66.07% *vs*. 60.74%; G, 33.93% *vs*. 39.26%, $P = 0.034$); respectively. The G allele carriers had lower levels of serum total cholesterol (TC), triglyceride, apolipoprotein (Apo) B and higher ApoA1/ApoB ratio than the G allele non-carriers in Maonan but not in Han populations ($P < 0.05$-0.01). Gender subgroup analyses showed that the G allele carriers had lower ApoB in Han famales but not in males ($P < 0.05$), lower TC, ApoB and higher ApoA1/ApoB ratio in Maonan males and lower ApoB in Maonan females than the G allele non-carriers ($P < 0.05$-0.01). Serum lipid traits were also associated with several environmental factors in the Han and Maonan populations, or in males and females in both ethnic groups. These fidnings suggested that the genetic variant of rs998584 near the *VEGFA* and blood lipid traits might have racial/ethnic- and/or sex-specific in our study populations.

Keywords: Vascular endothelial growth factor A, single nucleotide polymorphism, lipids, environmental factor

Introduction

With a rapidly aging population and un-healthy lifestyle habits, coronary heart disease (CHD) has become a major public helath problem and leading cause of morbidity and mortality all over the world [1]. In China, CHD contributes to 51.4% of the mortality in urban areas and 32.8% in rural areas [2]. As an important CHD risk factor, lipid disorder has been recognized to play a major role in the pathogenesis of arteriosclerosis. An enormous amount of laboratory and clinical evidences have demonstrated that high levels of total cholesterol (TC) [3], triglyceride (TG) [4], low-density lipoprotein cholesterol (LDL-C) [5] and ApoB [6], and low levels of high-density lipoprotein cholesterol (HDL-C) [7], ApoA1 [8] and the ApoA1/ApoB ratio [9] were closely associated with atherosclerosis and CHD, especially elevated TC and TG levels [10]. Previous researches have showed that dyslipidemia is a common result that is determined by age, gender, genetic, ethnicity, environmental factors and their interactions [11, 12]. Heritability studies based on twins suggested that dyslipidemia individual differences in heritability were estimated from the range of 0.48 to 0.87 [13]. Since 2006, genome-wide association stduy (GWAS) identified 95 loci associated with serum blood lipids and showed that variants with small effects can point to pathways and therapeutic targets that enable clinically-important changes in blood lipids [14]. Meanwhile, it has reported that the identified com-

mon variants associated with blood lipids, can account for ~10-12% of total trait variations [15]. In summary, understanding of the association of single nucleotide polymorphism (SNP) and serum lipid levels can identify targets for new therapies and prevention of CHD.

Previous study has indentified that several common variants on the vascular endothelial growth factor A gene (*VEGFA*: also known as: VPF; VEGF; MVCD1; Gene ID: 7422; HGNC ID: 12680; chromosomal location: 6p21.1) are highly associated with serum LDL-C and HDL-C levels [16]. A GWAS including 157 loci associated with lipid levels has reported genetic variant of rs998584 near the *VEGFA* as hyperlipidemic locus in European population [17]. The *VEGFA* is a main component of the VEGF family and also includes members such as VEGF-B, VEGF-C, VEGF-D and placental growth factor (PLGF), which plays decisive role in vasculogenesis, vascular repair, generation, remodelling during embryogenesis and in adult [16, 18]. Besides the angiogenic action, *VEGF* stimulates virtually all aspects of endothelial function, over suppression of VEGF action by its soluble receptor-1 (sVEGFR-1) can lead to endothelial dysfunction [19]. Likewise, VEGF gene transfer inhibits thickening of the media and promotes vascular remodeling [20]. Animal models and *in vitro* studies have indentified that *VEGFA* is expressed predominantly in aggregating macrophages in atherosclerotic lesions and the expression of *VEGFA* increase during atherogenesis, which causes the formation of atherosclerotic and unstable atherosclerotic plaques [21]. Furthermore, there has been some evidence that *VEGFA* could also be involved in the regulation of lipid metabolism. Increased levels of circulating *VEGFA* have been found in subjects with uncomplicated hyperlipidemia in a small-sample-size study [22], and a similar finding has been shown in a pilot study in patients with hypercholesterolemia [23]. Whereas in a supposedly healthy population in the SAPHIR study, *VEGFA* was negatively correlated with LDL-C, TC and ApoB only in women [24]. Although several previous studies have provided evidence for the genetic basis of plasma variability of *VEGFA*, the exact biological function of this gene variation in lipid metabolism is inadequately understood. As is known to all, the genetic variation has different magnitudes of effect in the different ethnicities, however, in Chinese, whether *VEGFA* rs998584 is associated with serum lipid levels or whether it shows ethnic/gender specific association as the previous reports remains elusive.

China is a multi-ethnic county composed of 56 different ethnic groups. The Han population accounts for the majority of the total population in our country. Maonan is a relatively consevative and isolated mountainous nation, and mainly lives in Huanjiang Maonan Autonomous County of Guangxi Zhuang Autonomous Region, with population of 107,166 according to the fifth national census statistics of China in 2000. Maonan population has various lifestyles and different eating habits which may result in the effect of hereditary variation to be further modified. Furthermore, they have their tradition of ethnic endogamy and consanguineous marriage to cousins of maternal side, suggesting that the genetic hereditary background of Maonan population may be less heterogeneous within the population.

Sexual dimorphism has been demonstrated as the potential risk factors of lipid metabolism disorder and cardiovescular disease. Differences in lipid profiles and metabolism between men and women have been well documented [25]. Males have more unfavorable plasma lipid profiles than premenopausal females, with higher levels of TC, TG and LDL-C, and lower of HDL-C levels [26]. It seems to be the result of a complex network of gene expression or sexual hormones in sexually dimorphic manner [27]. However, the reasons for these differences concerned with gender remain unclear. This study was undertaken to detect the association of the rs998584 SNP near the *VEGFA* and several environmental factors with serum lipid levels between males and females in the Maonan and Han populations.

Materials and methods

Subjects

A total of 749 unrelated participants of Han (315 males, 42.06% and 434 females, 57.94%) and 780 unrelated subjects of Maonan (305 males, 39.10% and 475 females, 60.89%) were randomly selected from our previous stratified randomized samples [28]. All participants were agricultural workers living in Huanjiang Maonan Autonomous County, Guangxi Zhuang Autonomous Region, People's Republic of China. The age of the participants ranged from

25 to 80 years, with a mean age of 56.22 ± 13.68 years in Han and 57.03 ± 14.96 years in Maonan ($P > 0.05$), respectively. All study subjects were essentially healthy with no history of cardiovascular disease such as CHD and stroke, diabetes, hyper- or hypo-thyroids, and chronic renal disease. We excluded the subjects who had a history of taking medications known to affect serum lipid levels (lipid-lowering drugs such as statins or fibrates, beta blockers, diuretics, or hormones) before the blood sample was drawn. The present study was approved by the Ethics Committee of the First Affiliated Hospital, Guangxi Medical University (No. Lunshen-2014-KY-Guoji-001, Mar. 7, 2014). Informed consent was taken from all participants.

Epidemiological survey

The survey was carried out using internationally standardized methods, following a common protocol [29]. 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-drinker), < 20 and ≥ 20. Several parameters such as blood pressure, height, weight, waist circumference, and body mass index (BMI) were measured or calculated. The methods of measuring above parameters were referred to previous studies [30, 31].

Biochemical measurements

At least 12 hours of fasting venous blood sample of 5 mL was drawn from the participants. A part of 2 mL was collected into glass tubes and used to determine serum lipid levels, and another part of 3 mL was shifted 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, CrumlinCo. Antrim, United Kingdom, BT29 4QY; Daiichi Pure Chemicals Co., Ltd., Tokyo, Japan). Serum ApoA1 and ApoB levels were measured 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 [32].

DNA amplification and genotyping

Genomic DNA of the samples was extracted from peripheral blood leucocytes according to the phenol-chloroform method [33]. The extracted DNA was stored at 4°C until analysis. Genotyping of the *VEGFA* rs998584 SNP was performed by polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP). PCR amplification was performed using 5'-TGGGACTTAGGTCTGG-3' and 5'-TTGGCAGATGAGAAGCAGGA-3' (Sangon, Shanghai, People's Republic of China) as the forward and reverse primer pairs, respectively. Each 25 μL PCR reaction mixture consisted of 2.0 μL genomic DNA, 1.0 μL each primer (10 μmol/L), 12.5 μL of 2 × *Taq* PCR Master mix (constituent: 0.1 U *Taq* polymerase/μL, 500 μM dNTP each and PCR buffer.), and 8.5 μL of ddH_2O (DNase/RNase-free). PCR was performed with an initialization step of 95°C for 5 min, followed by 30 s denaturing at 95°C, 30 s of annealing at 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 reation 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 *HpyF10*VI 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. Genotypes were scored by an experienced reader blinded to the epidemiological and serum lipid results. 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.

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	749	780		
Male/female	315/434	305/475	1.383	0.240
Age (years)	56.22 ± 13.68	57.03 ± 14.96	-1.013	0.270
Height (cm)	154.47 ± 7.82	153.83 ± 8.04	1.580	0.114
Weight (kg)	52.97 ± 8.71	53.14 ± 10.64	-0.351	0.725
Body mass index (kg/m^2)	22.17 ± 3.17	22.35 ± 3.60	-0.983	0.326
Waist circumference (cm)	75.55 ± 8.33	76.56 ± 9.18	-2.264	0.024
Smoking status [n (%)]				
Non-smoker	619 (82.64)	615 (78.84)		
≤ 20 cigarettes/day	87 (11.62)	75 (9.61)		
> 20 cigarettes/day	43 (5.74)	90 (11.54)	17.897	0.000
Alcohol consumption [n (%)]				
Non-drinker	623 (83.18)	620 (79.48)		
≤ 25 g/day	50 (6.67)	60 (7.69)		
> 25 g/day	76 (10.15)	91 (11.67)	164.478	0.000
Systolic blood pressure (mmHg)	129.96 ± 20.14	135.47 ± 23.62	-4.913	0.000
Diastolic blood pressure (mmHg)	80.68 ± 12.11	82.99 ± 12.33	-3.703	0.000
Pulse pressure (mmHg)	49.29 ± 14.48	52.48 ± 17.35	-3.906	0.000
Glucose (mmol/L)	6.48 ± 1.76	6.17 ± 1.42	3.738	0.000
Total cholesterol (mmol/L)	5.05 ± 0.89	4.99 ± 1.04	1.095	0.274
Triglyceride (mmol/L)	1.20 (0.81)	1.29 (0.88)	-2.781	0.005
HDL-C (mmol/L)	1.71 ± 0.52	1.61 ± 0.41	3.979	0.000
LDL-C (mmol/L)	2.88 ± 0.68	2.86 ± 0.75	0.553	0.580
ApoA1 (g/L)	1.34 ± 0.24	1.38 ± 0.31	-2.942	0.003
ApoB (g/L)	0.88 ± 0.21	0.87 ± 0.20	0.178	0.858
ApoA1/ApoB	1.61 ± 0.48	1.66 ± 0.57	-1.997	0.046

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.

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 [34]. Hypertension diagnosis standard is according to the cirteria of 1999 and 2003 World Health Organization-International Society of Hypertension Guidelines for the management of hypertension [35]. 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.

Statistical analyses

The statistical analyses were performed with the statistical software package SPSS 22.0 (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 HardyWeinberg 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 charactistics between two ethnic groups were compared by the Student's unpaired *t*-test. The

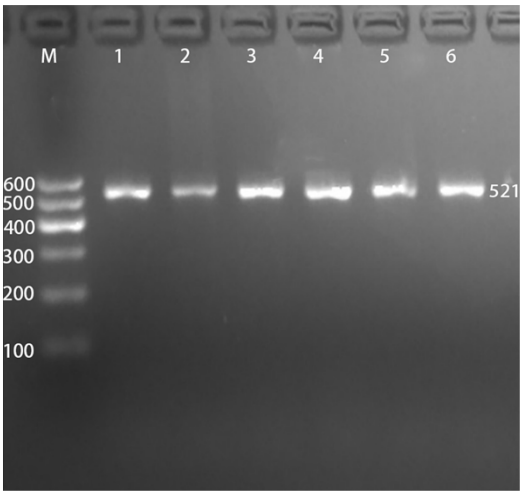

Figure 1. Electrophoresis of polymerase chain reaction products of the samples. Lane M is the 100 bp marker ladder; Lanes 1-6 are samples, the 521 bp bands are the target genes.

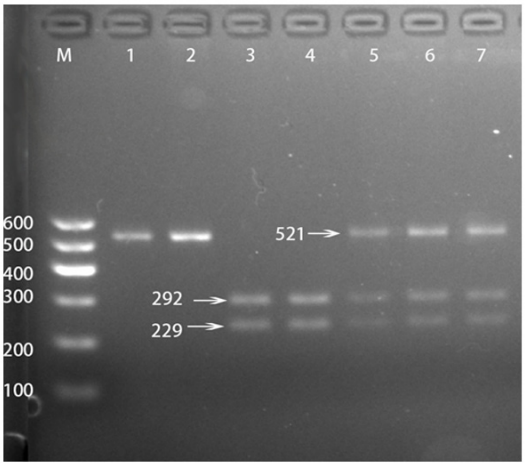

Figure 2. Genotyping of the *VEGFA* rs998584 SNP. Lane M, 100 bp marker ladder; lanes 1 and 2, TT genotype (521-bp); lanes 3 and 4, GG genotype (292- and 229-bp); lanes 5-7, GT genotype (521-, 292- and 229-bp).

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 and GG = 3) and several environmental factors with serum lipid levels in males and females of Han and Maonan populations. Two sideds P value < 0.05 was considered statistically significant.

Results

General characteristics and serum lipid levels

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

Results of electrophoresis and genotyping

After the genomic DNA of the samples was amplified using PCR and visualized with 2% agarose gel electrophoresis, the products of 521 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 229 bp and 292 bp), the GT genotype is heterozygous for the presence and absence of the site (bands at 521-, 229- and 292-bp) and the TT genotype is homozygous for the absence of the site (bands at 521 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 *VEGFA* rs998584 SNP are shown in **Table 2**. The genotype and allele frequencies were significant different between Han and Maonan populations (TT, 31.78% *vs*. 37.44%; GT, 50.02% *vs*. 51.02%; GG, 18.02% *vs*. 11.54%; $P = 0.004$; T, 56.88% *vs*. 62.95%; G, 43.12% *vs*. 37.05%; $P = 0.001$). Gender subgroup analysis showed that there were no differences in the genotypic frequencies between males and females in the two ethnic groups ($P > 0.05$ for

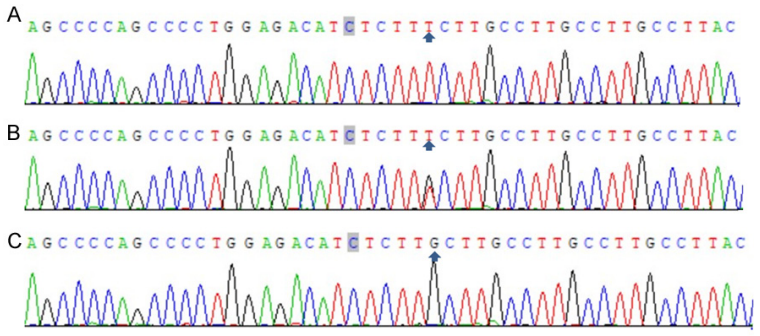

Figure 3. A part of the nucleotide sequence of the *VEGFA* rs998584 SNP. A: TT genotype; B: GT genotype; C: GG genotype.

each). The allele frequencies were different between males and famales in Maonan but not in Han populations (T, 66.07% *vs*. 60.74%; G, 33.93% *vs*. 39.26%; $P = 0.034$).

Genotypes and serum lipid levels

Tables 3 and **4** describe the association between genotypes and serum lipid levels. The serum levels of TC, TG, ApoB and the ratio of ApoA1 to ApoB were different among the three genotypes in Maonan but not in Han ($P < 0.05$-0.01), the G allele carriers had lower levels of TC, TG and ApoB, and higher ApoA1/ApoB ratio than the G allel non-carriers ($P < 0.05$). Subgroup analyses showed that the G allele carriers in Han famales but not in males had lower ApoB levels ($P < 0.05$), lower TC, ApoB levels and higher ApoA1/ApoB ratio in Maonan males; and lower ApoB levels in Maonan famels than the G allele non-carriers ($P < 0.05$).

Relative factors for serum lipid parameters

The risk factors for serum lipid parameters in Maonan and Han are shown in **Tables 5** and **6**. Multiple linear regression analysis showed that serum ApoB levels and ApoA1/ApoB ratio in the combined population of Han and Maonan; and TC, ApoB levels and ApoA1/ApoB ratio in Maonan were correlated with the genotypes of the *VEGFA* rs998584 SNP ($P < 0.05$ for all; **Table 5**). When the correlation of serum lipid parameters and the genotypes was analyzed according to gender, we showed that the ApoB levels were assocated with genotypes in Han females but not in males. Serum TC, ApoB levels and the ApoA1/ApoB ratio in Maonan males and ApoB in Maonan females were correlated with the genotypes ($P < 0.05$ for all; **Table 6**). Several environmental factors such as age, gender, weight, waist circumference, alcohol consumtion, and cigarette smoking and traditional cardiovascular risk factors such as BMI, fasting blood glucose and blood pressure levels were also correlated with serum lipid phenotypes in both ethnic groups or in males and females ($P < 0.05$-0.001; **Tables 5** and **6**).

Discussion

In the present study, we observed that the levels of serum TG, ApoB and ApoA1/ApoB ratio were lower in Han than in Maonan ($P < 0.05$). There were no significant differences in the levels of serum HDL-C, LDL-C and ApoA1 levels between the two ethnic groups. It is widely accepted that variation of plasma lipid and apolipoprotein levels between individuals is considered to the result of the multipale environmetal factors such as diet, lifestyle, alcohol consumption, cigarette smoking, hypertension, overweight and genetic inheritance. Maonan nationality is peculiar minority mainly living in the northern of Guangxi. They have been abided with special customs and eating habits. They favor of acid food, corn and rice are the staple food for them. In addition, sweet potatoes, pumpkin and sorghum is another important complement. Maonan preference of ethnic intermarriage and their marriages arranged by parents were coomon, but intermarriage with Han or Zhuang people is seldom happened. Therefore, we can speculate that the hereditary characteristics of lipid metabolism-related genes in Maonan population may be different from those in Han Chinese.

The genotypic and allelic frequencies of the rs998584 SNP in different ethnic populations are pooly understood. According to the International HapMap data, the frequency of rs998584 G allele was 39.53% in Han Chinese from Beijing, 47.09% in Japanese, 52.49% in European and 88.93% in Yoruba. The minor allele frequency was lower in Asian than the Western populations. In the present study, we identified that the rs998584 G allele frequency was higher in Han than in Maonan populations (43.12% *vs*. 37.05%; $P < 0.01$), which were in close prox-

Table 2. Comparison of the genotype and allele frequencies of *VEGFA* rs998584 SNP in the Han and Maonan populations [*n* (%)]

Group	*n*	Genotype			Allele	
		TT	GT	GG	T	G
Han	749	238 (31.78)	376 (50.20)	135 (18.02)	852 (56.88)	646 (43.12)
Maonan	780	292 (37.44)	398 (51.02)	90 (11.54)	982 (62.95)	578 (37.05)
x^2			11.034		11.740	
P			0.004		0.001	
Han						
Male	315	100 (31.75)	152 (48.25)	63 (20.10)	352 (55.87)	278 (44.13)
Female	434	138 (31.79)	224 (51.61)	72 (16.59)	500 (57.60)	368 (42.40)
x^2			1.060		0.446	
P			0.588		0.504	
Maonan						
Male	305	128 (41.97)	147 (48.20)	30 (9.84)	403 (66.07)	207 (33.93)
Female	475	163 (34.32)	251 (52.84)	61 (12.84)	577 (60.74)	373 (39.26)
x^2			4.710		4.516	
P			0.095		0.034	

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	238	5.10 ± 0.98	1.22 (0.94)	1.73 ± 0.38	2.93 ± 0.75	1.36 ± 0.23	0.89 ± 0.20	1.58 ± 0.43
GT	376	5.04 ± 0.94	1.09 (0.89)	1.71 ± 0.58	2.89 ± 0.73	1.34 ± 0.26	0.86 ± 0.21	1.65 ± 0.54
GG	135	4.92 ± 0.89	1.07 (0.61)	1.69 ± 0.39	2.82 ± 0.65	1.34 ± 0.24	0.87 ± 0.18	1.59 ± 0.40
F		1.022	1.987	0.130	0.685	0.484	1.600	1.198
P		0.361	0.370	0.878	0.505	0.616	0.203	0.303
Maonan								
TT	292	5.12 ± 1.03	1.37 (0.84)	1.63 ± 0.42	2.92 ± 0.83	1.39 ± 0.25	0.91 ± 0.21	1.60 ± 0.47
GT	398	4.92 ± 1.06	1.21 (0.91)	1.60 ± 0.40	2.88 ± 0.71	1.39 ± 0.37	0.85 ± 0.19	1.72 ± 0.66
GG	90	4.87 ± 1.07	1.21 (0.68)	1.61 ± 0.39	2.75 ± 0.77	1.36 ± 0.20	0.88 ± 0.22	1.64 ± 0.46
F		3.379	6.905	0.319	1.686	0.293	5.843	3.293
P		0.035	0.032	0.727	0.186	0.746	0.003	0.038

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

imity to those of Han Chinese Bejing (39.53%); the distribution of GG and GT genotypes was different in two ethnic groups ($P < 0.05$); the frequencies of GG genotype were higher in Han than in Maonan. Gender subgroup analysis showed that the G allele frequency between male and females were different in Maonan but not in Han. These findings indicate that the *VEGFA* rs998584 SNP is inconsistent among diverse ethnic groups.

In addition to the crucial role in vascular maintence and neovascularization, the *VEGFA* also showed a significant correlation to lipid metabolism. Zhou and colleagues have reported that the *VEGFA* was an activators of sterol regulatory element binding proteins (SREBPs) in human microvascular endothelial cells [36]. Kivelä et al. further indentified that *VEGFA* inhibits endothelial lipase (EL) expression and increase serum HDL-C level both *in vitro* and *in vivo* [37].

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	100	5.20 ± 0.89	1.25 (1.02)	1.72 ± 0.38	2.97 ± 0.72	1.36 ± 0.26	0.90 ± 0.22	1.58 ± 0.43
GT	152	5.23 ± 1.07	1.19 (0.93)	1.68 ± 0.43	3.02 ± 0.69	1.38 ± 0.28	0.91 ± 0.20	1.59 ± 0.49
GG	63	4.97 ± 0.96	1.09 (0.78)	1.60 ± 0.44	2.91 ± 0.64	1.31 ± 0.28	0.91 ± 0.16	1.47 ± 0.34
F		1.024	1.077	1.134	0.355	0.767	0.058	1.184
P		0.361	0.584	0.324	0.701	0.466	0.944	0.308
Han/female								
TT	138	5.02 ± 1.03	1.18 (0.86)	1.73 ± 0.38	2.90 ± 0.78	1.36 ± 0.21	0.89 ± 0.19	1.58 ± 0.42
GT	224	4.91 ± 0.83	1.01 (0.76)	1.72 ± 0.65	2.81 ± 0.76	1.32 ± 0.24	0.83 ± 0.21	1.70 ± 0.56
GG	72	4.88 ± 0.84	1.09 (0.44)	1.70 ± 0.33	2.74 ± 0.65	1.35 ± 0.19	0.84 ± 0.18	1.69 ± 0.42
F		0.627	1.407	0.135	0.802	1.231	3.401	1.565
P		0.535	0.495	0.874	0.449	0.293	0.035	0.211
Maonan/male								
TT	128	5.17 ± 1.06	1.50 (0.86)	1.59 ± 0.47	2.89 ± 0.88	1.39 ± 0.30	0.93 ± 0.21	1.56 ± 0.50
GT	147	4.86 ± 0.85	1.32 (1.19)	1.57 ± 0.43	2.78 ± 0.73	1.43 ± 0.53	0.86 ± 0.19	1.78 ± 0.91
GG	30	4.55 ± 0.95	1.09 (0.59)	1.52 ± 0.40	2.52 ± 0.71	1.32 ± 0.23	0.82 ± 0.18	1.67 ± 0.44
F		6.063	4.436	0.307	2.590	0.843	4.842	2.738
P		0.003	0.109	0.736	0.077	0.431	0.009	0.066
Maonan/female								
TT	163	5.09 ± 1.00	1.33 (0.69)	1.65 ± 0.39	2.95 ± 0.79	1.40 ± 0.22	0.91 ± 0.23	1.64 ± 0.45
GT	251	5.04 ± 1.18	1.16 (0.83)	1.62 ± 0.38	2.92 ± 0.69	1.37 ± 0.24	0.85 ± 0.19	1.69 ± 0.45
GG	61	4.96 ± 1.09	1.22 (0.88)	1.65 ± 0.38	2.87 ± 0.77	1.38 ± 0.18	0.90 ± 0.20	1.62 ± 0.48
F		0.621	3.335	0.428	0.238	0.703	3.479	0.799
P		0.538	0.189	0.652	0.788	0.495	0.032	0.451

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

In a previous study, Alber et al. [22] reported that the subjects with uncomplicated hyperlipidemia had higher level of VEGFA than the healthy controls. Another study found significant associations between HDL-C and VEGFA levels in a healthy population from Japan [38]. These findings show that the *VEGFA* has an important effect on cardiac diseases and on the variation of related risk factors such as lipid parameters. Recently, a new GWAS including 157 lipid-related loci has confirmed that 62 new loci not previously associated with lipid levels in humans, which has mentioned polymorphsim rs998584 SNP associated with TG and HDL-C levels [17]. Besides this, there were hardly any previous studies presented the direct influence. In the current study, we found that the TC, TG, ApoB levels and the ApoA1/ApoB ratio were different among the three genotypes in Maonan but not in Han ($P < 0.05$-0.01); the G allele carriers had lower TC, TG, ApoB levels and higher ApoA1/ApoB ratio than the G allele non-carriers ($P < 0.05$). Subgroup analysis according to sex showed that the G allele carriers had lower ApoB levels in Han females ($P < 0.05$); lower TC, ApoB levels and higher ApoA1/ApoB ratio in Maonan males; and lower ApoB levels in Maonan females than the G allele non-carriers ($P < 0.05$). These experimental results suggest that there may be an ethnic or geneder specific-association of the *VEGFA* rs998584 SNP and serum lipid levels.

Previous study reported that serum androgen levels declined with age in healthy men, which is related to the risk increase of male CHD, aortic or carotid atherosclerosis [39]. Meanwhile, compared to perimenopausal women, the aged postmenopausal women have lower serum level of estradiol, and are more likely to have abnormal lipid metabolism and higher prevalence of CHD [40, 41]. The influence of sex on the development of lipid disorder is well estalished. There are differences between the males and famales in the serum lipid profile, for example, LDL-C, TC and TG levels are generally lower in women than in men until menopause. Previous study of cardiovascular disease in wo-

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	Age	0.007	0.002	0.107	3.142	0.002
	Alocohol consumption	0.102	0.043	0.079	2.390	0.017
	Waist circumference	0.014	0.005	0.126	2.714	0.007
TG	Alocohol consumption	0.278	0.075	0.119	3.712	0.000
	Glucose	0.084	0.031	0.077	2.688	0.007
	Height	-0.070	0.030	-0.309	-2.339	0.020
	Weight	0.101	0.041	0.550	2.477	0.013
	Body mass index	-0.207	0.088	-0.396	-2.360	0.018
	Waist circumference	0.044	0.009	-0.215	4.760	0.000
HDL-C	Ethnic group	-0.81	0.026	-0.090	-3.170	0.002
	Gender	0.092	0.035	0.101	2.592	0.010
	Alcohol consumption	0.101	0.018	0.176	5.479	0.000
	Waist circumference	-0.009	0.002	-0.180	-3.963	0.000
	Glucose	-0.017	0.008	-0.065	-2.234	0.026
LDL-C	Waist circumference	0.009	0.004	0.110	2.357	0.019
	Age	0.006	0.002	0.119	3.471	0.001
ApoA1	Alcohol consumption	0.077	0.012	0.207	6.276	0.000
	Ethnic group	0.047	0.017	0.079	2.733	0.006
ApoB	Waist circumference	0.006	0.001	0.265	5.991	0.000
	Genotype	-0.018	0.006	-0.083	-3.056	0.002
	Age	0.002	0.000	0.131	4.033	0.000
	Gender	-0.028	0.012	-0.067	-2.237	0.020
	Glucose	0.008	0.003	0.064	2.273	0.023
ApoA1/ApoB	Ethnic group	0.080	0.031	0.073	2.604	0.009
	Genotype	0.035	0.016	0.058	2.105	0.036
	Age	-0.004	0.001	-0.113	-3.415	0.001
	Alcohol consumption	0.076	0.022	0.110	3.455	0.001
	Waist circumference	-0.014	0.003	-0.225	-4.991	0.000
	Glucose	-0.021	0.009	-0.065	-2.275	0.023
Han						
TC	Alcohol consumption	0.151	0.052	0.147	2.910	0004
	Glucose	0.051	0.022	0.106	2.275	0.023
TG	Cigarette smoking	0.709	0.180	0.195	3.942	0.000
	Waist circumference	0.072	0.018	0.306	4.053	0.000
	Glucose	0.115	0.045	0.117	2.557	0.011
HDL-C	Alcohol consumption	0.092	0.027	0.173	3.349	0.001
	Height	-0.006	0.002	-0.084	-2.254	0.025
	Weight	-0.008	0.002	-0.129	-3.480	0.001
	Diastolic blood pressure	-0.004	0.002	-0.089	-2.389	0.017
LDL-C	Age	0.007	0.002	0.128	2.847	0.005
	Glucose	0.036	0.017	0.096	2.033	0.043
ApoA1	Alcohol consumption	0.071	0.013	0.264	5.260	0.000
	Weight	-0.019	0.009	-0.672	-2.180	0.030
	Waist circumference	0.006	0.002	0.198	2.607	0.009
ApoB	Age	0.001	0.001	0.102	2.068	0.039

	Alcohol consumption	0.026	0.011	0.120	2.450	0.015
	Waist circumference	0.005	0.002	0.217	2.928	0.005
	Glucose	0.014	0.005	0.133	2.967	0.003
ApoA1/ApoB	Age	-0.005	0.002	-0.131	-2.593	0.010
	Glucose	-0.025	0.011	-0.103	-2.257	0.024
Maonan						
TC	Genotype	-0.086	0.042	-0.075	-2.034	0.042
	Age	0.010	0.003	0.145	3.195	0.001
	Height	-0.056	0.024	-0.427	-2.359	0.019
	Weight	0.081	0.032	0.815	2.504	0.013
	Body mass index	-0.161	0.069	-0.554	-2.322	0.021
	Waist circumference	0.015	0.007	0.130	2.214	0.027
TG	Alcohol consumption	0.490	0.111	0.185	4.403	0.000
	Height	-0.136	0.038	-0.623	-3.604	0.000
	Weight	0.201	0.051	1.217	3.911	0.000
	Body mass index	-0.388	0.111	-0.797	-3.495	0.001
	Waist circumference	0.031	0.011	0.163	2.896	0.004
HDL-C	Gender	0.117	0.044	0.141	2.672	0.008
	Alcohol consumption	0.115	0.026	0.188	4.437	0.000
	Waist circumference	-0.011	0.003	0.255	-4.498	0.000
	Pulse pressure	-0.003	0.001	-0.114	-2.789	0.005
LDL-C	Age	0.008	0.002	0.161	3.522	0.000
	Alcohol consumption	-0.123	0.050	-0.108	-2.442	0.015
ApoA1	Waist circumference	-0.005	0.002	-0.145	-2.435	0.015
	Alcohol consumption	0.090	0.021	0.189	4.275	0.000
	Pulse pressure	-0.002	0.001	-0.088	-2.064	0.039
ApoB	Genotype	-0.023	0.008	-0.104	-2.977	0.003
	Age	0.002	0.001	0.157	3.648	0.000
	Waist circumference	0.007	0.001	0.301	5.394	0.000
ApoA1/ApoB	Genotype	0.044	0.022	0.071	1.995	0.046
	Age	-0.004	0.002	-0.102	-2.331	0.020
	Alcohol consumption	0.155	0.037	0.179	4.256	0.000
	Waist circumference	-0.018	0.004	-0.295	-5.230	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; B, unstandardized coefficient; Beta, standardized coefficient.

men showed that high TG contrations may be an independent and better predictor of heart disease risk than TC or LDL-C in females [25, 42]. The effects of progestogens and androgens can explain only a small part of the differences and it is likely that an underlying mechanism is differential gene regulation or genotype-sex interaction in males and females [27]. Thus, sex-specific genetic associations between SNPs and serum lipid levels may provide valuable insight into prevention and treatment strategy for dyslipidemia. In our reaserch, gender subgroup analysis showed that the G allele frequency was higher in females than males just in Maonan population. We also found that the G allele carriers had lower ApoB levels in both Han and Maonan females, whereas the G alleles carrers had lower TC, ApoB levels and higher ApoA1/ApoB ratio in Maonan males than the G allele non-carriers. However, this is the first study to report the gender specific association of *VEGFA* rs998584 SNP and serum lipid parameters, further studies with larger sample size should be added to confirm this association.

It is well known that environmental factors such as dietary patterns, lifestyle, obesity, hyperten-

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	Glucose	0.064	0.030	0.125	2.165	0.031
TG	Weight	0.044	0.014	0.173	3.028	0.003
	Body mass index	0.074	0.037	0.115	1.989	0.048
	Waist circumference	0.057	0.015	0.211	3.913	0.000
HDL-C	Height	-0.012	0.003	-0.196	-3.452	0.001
	Body mass index	-0.018	0.008	-0.137	-2.388	0.018
	Waist circumference	-0.010	0.003	-0.183	-3.217	0.001
LDL-C	Glucose	-0.035	0.015	-0.136	-2.373	0.018
ApoA1	Weight	-0.005	0.002	-0.180	-3.152	0.002
	Waist circumference	-0.005	0.002	-0.150	-2.615	0.009
	Body mass index	-0.009	0.004	-0.120	-2.087	0.038
ApoB	Glucose	0.016	0.007	0.137	2.390	0.017
	Body mass index	0.011	0.003	0.199	3.504	0.001
	Waist circumference	0.006	0.001	0.241	4.276	0.000
	Weight	0.004	0.001	0.163	2.846	0.005
ApoA1/ApoB	Glucose	-0.036	0.015	-0.141	-2.453	0.015
Han/female						
TC	Age	0.012	0.003	0.185	3.817	0.000
	Waist circumference	0.019	0.009	0.171	2.141	0.033
	Glucose	0.065	0.024	0.135	2.772	0.006
TG	Waist circumference	0.035	0.011	0.258	3.296	0.001
	Glucose	0.101	0.029	0.168	3.462	0.001
HDL-C	Waist circumference	-0.007	0.003	-0.101	-2.066	0.039
	Diastolic blood pressure	-0.005	0.002	-0.102	-2.088	0.037
LDL-C	Age	0.010	0.003	0.196	4.053	0.000
	Waist circumference	0.018	0.007	0.126	2.591	0.010
	Glucose	0.042	0.019	0.108	2.204	0.028
ApoA1	Diastolic blood pressure	-0.002	0.001	-0.097	-1.981	0.048
ApoB	Genotype	-0.033	0.013	-0.147	-2.508	0.013
	Age	0.003	0.001	0.189	3.901	0.000
	Waist circumference	0.008	0.002	0.315	4.020	0.000
ApoA1/ApoB	Age	-0.007	0.002	-0.176	-3.622	0.000
	Waist circumference	-0.013	0.005	-0.211	-2.669	0.008
Maonan/male						
TC	Genotype	-0.152	0.061	-0.148	-2.471	0.014
	Height	-0.141	0.067	-0.969	-2.106	0.036
	Glucose	0.082	0.039	0.123	2.120	0.035
TG	Alcohol consumption	0.020	0.006	0.193	3.300	0.001
	Height	0.060	0.022	0.159	2.765	0.006
	Weight	0.066	0.028	0.266	2.332	0.020
	Glucose	0.204	0.103	0.114	1.981	0.049
HDL-C	Waist circumference	-0.022	0.005	-0.455	-4.423	0.000
	Alcohol consumption	0.004	0.001	0.201	3.469	0.001
LDL-C	Age	0.009	0.004	0.177	2.287	0.023
	Alcohol consumption	-0.005	0.002	-0.163	-2.635	0.009

ApoA1	Waist circumference	-0.016	0.005	-0.357	-3.373	0.001
	Alcohol consumption	0.003	0.001	0.204	3.459	0.001
ApoB	Glucose	0.017	0.008	0.119	2.154	0.032
	Age	0.002	0.001	0.180	2.557	0.011
	Genotype	-0.032	0.013	-0.152	-2.541	0.012
ApoA1/ApoB	Waist circumference	-0.022	0.008	-0.278	-3.671	0.008
	Alcohol consumption	0.006	0.002	0.201	3.442	0.001
	Genotype	0.108	0.046	0.141	2.344	0.020
Maonan/female						
TC	Age	0.013	0.004	0.167	2.915	0.004
	Waist circumference	0.025	0.009	0.193	2.821	0.005
TG	Waist circumference	0.030	0.006	0.332	5.185	0.000
HDL-C	Waist circumference	-0.007	0.003	-0.151	-2.249	0.025
	Diastolic blood pressure	0.005	0.002	0.147	2.169	0.031
	Systolic blood pressure	-0.003	0.001	-0.183	-2.542	0.011
LDL-C	Age	0.008	0.003	0.171	2.976	0.003
	Waist circumference	0.015	0.006	0.173	2.539	0.011
ApoA1	Glucose	-0.017	0.007	-0.107	-2.289	0.023
	Systolic blood pressure	-0.002	0.001	-0.166	-2.233	0.026
	Pulse pressure	-0.002	0.001	-0.120	-2.480	0.013
ApoB	Genotype	-0.024	0.010	-0.110	-2.450	0.015
	Age	0.002	0.001	0.163	3.035	0.003
	Waist circumference	0.009	0.001	0.381	5.950	0.000
ApoA1/ApoB	Waist circumference	-0.018	0.003	-0.345	-5.328	0.000
	Systolic blood pressure	-0.004	0.001	-0.208	-2.983	0.003

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.

sion and physical inactivity are all strongly related with serum lipid levels [43]. In the present study, multivariate linear regression analysis also showed that serum lipid parameters were correlated to age, sex, weight, waist circumference, BMI, blood pressure, fasting blood glucose levels, alcohol consumption, and cigarette smoking in both ethnic groups. These findings suggested that the environmental factors also play a critical role in determining serum lipid levels in our study populations. The people of Maonan live in an isolated environment all the year round with unique histories, cultural practices and dietary customs. Compared with the Han nationality, they prefer to eat cold foods and acidic and spicy dishes with lots of oil and salt. Rice is the Maonan people's staple food supplemented with corn, sweet potato, taro and other grains. Numerous studies have also stated that daily eating habits have definite affect on serum levels of TC, TG, LDL-C, ApoB, ApoA1 and their ratio, and which in turn cause an increased risk of CHD [44]. In our present sduty, the levels of serum TG, ApoA1 and the ratio of ApoA1 to ApoB were higher and the serum HDL-C levels were lower in Maonan than in the Han individuals. This discrepancy may be partly attributed to the difference in daily diet between the Maonan and Han populations.

In addition, we also noticed that the levels of waist circumference, blood pressure, the percentages of subjects who consumed alcohol and smoked cigarettes were higher in Maonan than in Han; the males' percentages of subjects who consumed alcohol and smoked cigarettes were significantly higher than the females' in both ethnic groups. Alcohol intake has a significant influence on the human serum lipid metabolism. Intake of alcohol only in moderation has been showed to protect against CHD death, which has been ascribed to the increase the concentration of serum HDL-C, TG and ApoA1 levels [45]. But consumption of

large amounts of ethanol would have the opposite result. An Italian Longitudinal Study on Aging showed that alcohol consumption increased serum LDL-C levels in older Italian males (65-84 years) [46]. Onat et al. [47] 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. Furthermore, consuming large amounts of alcohol was also asociatied with higher values for blood pressure [48] and waist circumference [49]. The relationship between cigarette smoking and serum lipid disorder has been taken more and more attention. A recent study in smoking males (ages from 25 to 35 years old) has found that a significant increase in TC and LDL-C in tobacco users [50]. Another meta-analysis also reported that smoking increased TG by 0.15 mmol/L, and decreased HDL-C by 0.09 mmol/L with every 20 cigarettes smoked [51]. Consequently, the results of exposure to different dietary habits, lifestyle and environmental factors may 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, although we observe significant association of the *VEGFA* rs998584 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-enviroment, and environment-environment on serum lipid levels are still to be determined. Third, this is the first study to report the sex-specific association of the rs998584 SNP and serum lipid levels, no previous evidence to support our findings and the number of subjects in our study is moderate, the statistical power is relatively reliable. Thus, further studies with larger samples are needed to replicate our findings in other populations.

Conclusions

The present study showed that the genotypic and allelic frequencies of the *VEGFA* rs998584 SNP were different between the Han and Maonan populations. The associations of the *VEGFA* rs998584 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 *VEGFA* rs998584 SNP and serum lipid levels.

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Disclosure of conflict of interest

None.

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