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Research Paper

Association of the rs7395662 SNP in the MADD-FOLH1 and Several Environmental Factors with Serum Lipid Levels in the Mulao and Han Populations

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Abstract

Background: The rs7395662 single nucleotide polymorphism (SNP) in the *MADD-FOLH1* has been associated with serum lipid traits, but the results are inconsistent in different populations. The present study was undertaken to investigate the association of rs7395662 SNP and several environmental factors with serum lipid levels in the Guangxi Mulao and Han populations.

Method: A total of 721 subjects of Mulao and 727 subjects of Han Chinese were randomly selected from our previous stratified randomized samples. Genotyping of the SNP was performed by polymerase chain reaction and restriction fragment length polymorphism combined with gel electrophoresis, and confirmed by direct sequencing.

Results: Serum apolipoprotein (Apo) B levels were higher in Mulao than in Han ($P < 0.01$). The allelic and genotypic frequencies in Han were different between males and females ($P < 0.05$ for each), but there was no difference between Mulao and Han or between Mulao males and females. The levels of low-density lipoprotein cholesterol (LDL-C) and ApoB in Mulao females were different among the genotypes ($P < 0.05$), the G allele carriers had higher LDL-C and ApoB levels than the G allele non-carriers. The levels of total cholesterol (TC), triglyceride (TG), LDL-C and ApoB in Han males and TC, TG and high-density lipoprotein cholesterol (HDL-C) in Han females were different among the genotypes ($P < 0.05$-0.01), the subjects with GG genotype in Han males had higher TC, TG, and ApoB and lower LDL-C levels than the subjects with AA or AG genotype, and the G allele carriers in Han females had lower TC and HDL-C levels than the G allele non-carriers. The levels of LDL-C and ApoB in Mulao females were correlated with the genotypes ($P < 0.05$ for each). The levels of HDL-C and ApoAI in Han males and HDL-C in Han females were correlated with genotypes ($P < 0.05$-0.001). Serum lipid parameters were also correlated with several environmental factors in both ethnic groups ($P < 0.05$-0.01).

Conclusion: The association of rs7395662 SNP and serum lipid levels is different between the Mulao and Han populations, and between males and females in both ethnic groups.

Key words: environmental factors, *MADD-FOLH1*, lipid profiles, single nucleotide polymorphism.

Introduction

It is well known that coronary artery disease (CAD) is a multifactorial chronic disease, whose morbidity and mortality remain high worldwide [1-3]. Dislipidemia has been shown to play a prominent role

in the process of CAD and it accounts for ~50% of the population attributable risk of developing CAD [4]. Based upon the results of recent studies, which had demonstrated that dislipidemia is a distinct complex trait resulted from multiple environmental and genetic factors as well as their interactions [5-7]. In twin and family studies, it was surveyed that plasma lipid levels were estimated to range from 0.28-0.78 with heritability [8]. However, the genetic architecture of serum lipid profiles is assumed to be intricate and continues to be poorly understood.

Numerous genetic variants associated with serum lipid levels have been identified in the genome-wide association studies (GWAS) in different populations [2, 5, 9-29]. On one hand, common variants at these loci collectively are estimated to explain only a small proportion of variation in each lipid trait [14, 15, 27] and 25%-30% of the genetic variability in plasma lipid phenotypes [5]; on the other hand, the heritability of lipid traits may also lead to large individual effects for other rare variants [27]. Previous GWAS have also discovered a large number of novel loci that impact phenotypes of serum lipid levels [5, 14, 30-32]. One of these newly identified single nucleotide polymorphisms (SNPs) is the rs7395662 SNP in the *MADD-FOLH1*.

The rs7395662 SNP is located on chromosome 11p11 in a flanking locus between *MADD* and *FOLH1*, which represents a gene desert close to the centromere with no known gene on the 500-kb flanking region [14]. The *MADD-FOLH1* stands for MAP-kinase activating death domain and folate hydrolase respectively and the roles of *MADD* and *FOLH1* in lipid metabolism are still poorly defined [14, 31]. In light of our best knowledge, *MADD* encodes MAP-kinase activating death domain containing protein. This protein can modulate the tumor necrosis factor-alpha (TNF-α) and propagate the apoptotic signal. For *FOLH1*, we learn that a mutation in *FOLH1* may be interacted with impaired absorption of dietary folates. In European population, the rs7395662 SNP in the *MADD-FOLH1* has been shown that it is significant associated with high-density lipoprotein cholesterol (HDL-C) concentration [14, 33]. Exactly as patterns of linkage-disequilibrium vary between populations, the results are complicated and inconsistent across different populations [34, 35]. In practical, there are still a large amount of variants have not been largely explored in various racial/ethnic groups, and more studies need to be carried on for further understanding about rs7395662 SNP in the *MADD-FOLH1*.

As a multiethnic country, China possesses 56 ethnic groups and Han nationality is the largest member in the 56 ethnic groups. Mulao nationality is one of the 55 minorities with population of 207,352 according to the fifth national census statistics of China in 2000. Ninety percent of them live in the Luocheng Mulao Autonomous Country, Guangxi Zhuang Autonomous Region, People's Republic of China. Dating back to the Jin Dynasty (AD265-420), this minority had been written down into gorgeous history in China. It is established that the Mulao population are the descendants of the ancient Baiyue tribe in south China. Besides, they are related ethnically to the neighboring ethnic groups. In a previous study, Xu et al. [36] showed that the genetic relationship between Mulao nationality and other minorities in Guangxi was much closer than that between Mulao and Han or Uighur nationality. Owing to a variety of lifestyles and environments in our population resident in Guangxi, the effect of genetic variation may be further modified. Since Mulao population has different lifestyles and customs, it tends to be more genetically isolated. However, whether rs7395662 SNP confer significant association with serum lipid levels remains unexplored. Thus, the aim of the present study was to detect and get insight into the association of rs7395662 SNP in the *MADD-FOLH1* and several environmental factors with serum lipid traits in the Guangxi Mulao and Han populations.

Materials and methods

Participants

Participants in the present study included 721 individuals of Mulao nationality living in Luocheng Mulao Autonomous County, Guangxi Zhuang Autonomous Region, People's Republic of China. They were randomly selected from our previous stratified randomized samples [37]. The ages of the participants ranged from 15 to 86 years, with an average age of 52.31 ± 14.50 years. There were 336 males (46.6%) and 385 females (53.4%). All participants were rural agricultural workers. Meanwhile, a total of 727 Han nationality who reside in the same villages were also randomly selected from our previous stratified randomized samples. The average age of the subjects was 51.94 ± 12.92 years, which ranged from 15 to 84 years. There were 346 men (47.6%) and 381 women (52.4%). All of them were also rural agricultural workers. All study subjects were essentially healthy and had no evidence of any chronic illness, including hepatic, renal, or thyroid. The participants with a history of heart attack of myocardial infarction, stroke, congestive heart failure, diabetes or fasting blood glucose ≥ 7.0 mmol/L determined by glucose meter were excluded from the analyses. The participants were not taking medications known to affect serum lipid levels (lipid-lowering drugs such as statins or fibrates, beta-blockers, diuretics, or hormones). The experimental

design was approved by the Ethics Committee of the First Affiliated Hospital Guangxi Medical University. All participants in this study provided written informed consent.

Epidemiological survey

The survey was carried out using internationally standardized methods [38]. All participants underwent a complete history, physical examination, and laboratory assessment of cardiovascular risk factors, including cigarette smoking, family history of myocardial infarction, blood pressure, presence of diabetes mellitus. Information on demographics, socioeconomic status, and lifestyle factors was collected with standardized questionnaires. The alcohol information included questions about the number of liangs (about 50g) of rice wine, corn wine, rum, beer, or liquor consumed during the preceding 12 months. Alcohol consumption was categorized into groups of grams of alcohol per day: ≤ 25 and > 25. Smoking status was categorized into groups of cigarettes per day: ≤ 20 and > 20. At the physical examination, several parameters were measured. Sitting blood pressure was measured three times with the use of a mercury sphygmomanometer after the subjects had a 5-minute rest, and the average of the three measurements was used for the level of blood pressure. Systolic blood pressure was determined by the first Korotkoff sound, and diastolic blood pressure was determined by the fifth Korotkoff sound. Body weight, to the nearest 50 grams, was measured by using a portable balance scale. Subjects were weighed without shoes and minimum of clothing. Height was measured, to the nearest 0.5 ㎝, using a portable measuring device. From these two measurements body mass index (BMI, kg/m²) was calculated.

Determination of serum lipid levels

Venous blood samples were collected after an overnight (at least 12 hours) fast. A part of the sample (2 mL) was collected into glass tubes and allowed to clot at room temperature, and used to determine serum lipid levels. Another part of the sample (3 mL) was transferred to tubes with anticoagulate solution (4.80 g/L citric acid, 14.70 g/L glucose, and 13.20 g/L tri-sodium citrate) and used to extract DNA. Serum total cholesterol (TC), triglyceride (TG), HDL-C, and low-density lipoprotein cholesterol (LDL-C) levels in the samples were measured according to standard enzymatic methods. Serum apolipoprotein (Apo) AI and ApoB levels were determined by the immunoturbidimetric immunoassay. All determinations were performed with an autoanalyzer (Type 7170A; Hitachi Ltd, Tokyo, Japan) in the Clinical Science Experiment Center of the First Affiliated Hospital, Guangxi Medical University [6, 7].

DNA preparation and genotyping

Genomic DNA was isolated from peripheral blood leukocytes using the phenol-chloroform method [39, 40]. Genotyping of the rs7395662 SNP was performed by polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP). PCR amplification was performed using 5'-ACCCCTATGTTGAACCCT-3' and 5'-AGCATGCAGGGAAAATCATTTATTA-3' (Sangon, Shanghai, People's Republic of China) as the forward and reverse primer pairs; respectively. Each amplification reaction was performed using 100 ng (2 μL) of genomic DNA in 25 μL of reaction mixture consisting of 1.0 μL of each primer (10 μmol/L), 12.5 μL 2 × *Taq* PCRMasterMix (constituent: 0.1 U Taq polymerase/μL, 500 μM dNTP each, 20 mM Tris-HCl, pH 8.3, 100 mM KCL, 3 mM $MgCl_2$, and stabilizers), and nuclease-free water 8.5 μL. After initial denaturizing at 95 °C for 5 min, the reaction mixture was subjected to 35 cycles of denaturation at 95 °C at 45 s, annealing at 59 °C for 45 s and extension 45 s at 72 °C, followed by a final 5 min extension at 72 °C. After electrophoresis on a 2% agarose gel with 0.5 μg/mL ethidium-bromide, the amplification products were visualized under ultraviolet light. Then 2.5 U of *Hin1*II restriction enzyme, 9 μL nuclease-free water and 1 μL of 10 × buffer solution were added directly to the PCR products (5 μL) and digested at 37 °C overnight. After restriction enzyme digestion of the amplified DNA, genotypes were identified by electrophoresis on 2.5% agarose gel and visualized with ethidium-bromide staining ultraviolet illumination. Genotypes were scored by an experienced reader blinded to epidemiological data and serum lipid levels.

DNA sequencing

Six samples (AA, AG and GG genotypes in two; respectively) detected by PCR-RFLP were also confirmed by direct sequencing. The PCR products were purified by low melting point gel electrophoresis and phenol extraction, and then the DNA sequences were analyzed in Shanghai Sangon Biological Engineering Technology & Services Co., Ltd., People's Republic of China.

Diagnostic criteria

The normal values of serum TC, TG, HDL-C, LDL-C, ApoAI, ApoB levels and the ratio of ApoAI 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 [41]. The individuals with TC > 5.17 mmol/L, and/or TG > 1.70 mmol/L were defined as hyperlipidemic [6,

7, 37]. Hypertension was diagnosed according to the criteria of 1999 World Health Organization-International Society of Hypertension Guidelines for the management of hypertension. Hypertension was defined as an average systolic blood pressure (SBP) ≥ 140 mm Hg, an average diastolic blood pressure (DBP) ≥ 90 mmHg, and/or self-reported current treatment for hypertension with antihypertensive medication [39, 40]. 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, > 28 kg/m^2; respectively [41].

Statistical analysis

The statistical analyses were done with the statistical software package SPSS16.0 (SPSS Inc., Chicago, Illinois). Quantitative variables were expressed as mean ± standard deviation (serum TG levels were presented as medians and interquartile ranges). Qualitative variables were expressed as percentages. Allele frequency was determined via direct counting, and the standard goodness-of-fit test was used to test the Handy-Weinberg equilibrium. Difference in genotype distribution between the groups was obtained using the chi-square test. The difference in general characteristics between two ethnic groups was tested by the Student's unpaired *t*-test. The association of genotypes and serum lipid parameters was tested by analysis of covariance (ANOVA). Age, sex, BMI, blood pressure, alcohol consumption, and cigarette smoking were included in the statistical models as covariates. Multiple linear regression analyses adjusted for age, sex, BMI, blood pressure, alcohol consumption, and cigarette smoking were also performed to assess the association of serum lipid levels with genotypes (AA = 1, AG = 2, GG = 3; or AA = 1, AG/GG = 2) and several environment factors. A *P* value of less than 0.05 was considered statistically significant.

Results

General characteristics and serum lipid levels

Table 1 shows the general characteristics and serum lipid levels of the study populations. The levels of ApoB and the percentage of subjects who consumed alcohol were higher but the levels of body weight, BMI, and diastolic blood pressure were lower in Mulao than in Han (P < 0.05-0.001). There were no significant differences in the levels of age, systolic blood pressure, pulse pressure, blood glucose, TC, TG, HDL-C, LDL-C, ApoAI; and the ratio of ApoAI to ApoB and male to female; the percentage of subjects who smoked cigarette between the two ethnic groups (P > 0.05 for all).

Table 1. Comparison of demographics, lifestyle and serum lipid levels between the Mulao and Han populations

Parameter	Mulao	Han	t (χ^2)	P
Number	721	727	–	–
Male/female	336/385	346/381	0.143	0.706
Age (years)	52.31±14.50	51.94±12.52	0.514	0.608
Height (cm)	155.58±7.97	154.99±7.65	1.428	0.154
Weight (kg)	53.04±9.33	54.45±8.96	-2.930	0.003
Body mass index (kg/m^2)	21.85±3.07	22.64±3.22	-4.736	<0.001
Waist circumference	75.13±8.87	75.41±7.90	-0.647	0.518
Cigarette smoking [*n* (%)]				
Nonsmoker	537 (74.5)	530 (72.9)		
≤ 20 cigarettes/day	154 (21.4)	150(20.6)		
> 20 cigarettes/day	30 (4.1)	47 (6.5)	3.827	0.148
Alcohol consumption [*n* (%)]				
Nondrinker	537 (74.5)	538 (78.5)		
≤ 25g/day	61 (8.5)	74 (9.2)		
> 25g/day	123 (17.1)	115 (12.3)	6.753	0.034
Systolic blood pressure (mmHg)	129.40±21.69	130.31±18.52	-0.860	0.390
Diastolic blood pressure (mmHg)	81.23±11.67	82.86±10.94	-2.743	0.006
Pulse pressure (mmHg)	48.17±16.09	47.45±13.77	0.914	0.361
Blood glucose (mmol/L)	5.99±1.61	6.15±1.81	-1.877	0.061
Total cholesterol (mmol/L)	5.03±1.35	5.10±1.16	-1.027	0.305
Triglyceride (mmol/L)	1.07(0.82)	1.09(0.89)	-1.333	0.182
HDL-C (mmol/L)	1.75±0.46	1.75±0.58	0.024	0.981
LDL-C (mmol/L)	2.92±0.90	2.91±0.87	0.079	0.937
Apolipoprotein (Apo) AI (g/L)	1.32±0.41	1.35±0.26	-1.666	0.096
ApoB (g/L)	0.98±0.57	0.87±0.21	4.792	<0.001
ApoAI/ApoB	1.62±1.01	1.64±0.51	-0.432	0.666

HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol. 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.

Electrophoresis and genotypes

After the genomic DNA of the samples was amplified by PCR and imaged by 2.0% agarose gel electrophoresis, the PCR products of 225 bp nucleotide sequences could be seen in the samples (Supplementary Material: Figure S1). The genotypes identified were named according to the presence (A allele) or absence (G allele) of the enzyme restriction sites. Thus, AA genotype is heterozygote for the presence of the site (bands at 176-, 30- and 19-bp), AG genotype is heterozygote for the absence and presence of the site

(bands at 206-, 176-, 30- and 19-bp), and the GG genotype is homozygote for the absence of the site (bands at 206- and 19-bp; Supplementary Material: Figure S2). The 30-bp and 19-bp fragments were invisible in the gel owing to their fast migration speed. The genotypes of the rs7395662 SNP were followed by the Hardy-Weinberg equilibrium.

Nucleotide sequences

The results were shown as AA, AG and GG genotypes of the rs7395662 SNP by PCR-RFLP, the genotypes were also confirmed by sequencing (Supplementary Material: Figure S3); respectively.

Genotypic and allelic frequencies

The genotypic and allelic frequencies of the rs7395662 SNP in the both ethnic groups are shown in Table 2. For the Han population, the allelic frequencies of A and G were 59.8% and 40.2% in males, and 53.1% and 46.9% in females ($P < 0.05$); respectively. The frequencies of AA, AG and GG genotypes were 36.4%, 46.8% and 16.8% in males, and 30.2%, 45.9% and 23.9% in females ($P < 0.05$); respectively. They are both in Hardy Weinberg Equilibrium ($P = 0.780466$ for Mulao, $P = 0.119036$ for Han). There were no significant differences in the allelic and genotypic frequencies between Mulao and Han, or between Mulao males and females.

Genotypes and serum lipid levels

As shown in Table 3, the levels of LDL-C and ApoB in Mulao females but not males were different among the genotypes ($P < 0.05$), the G allele carriers had higher LDL-C and ApoB levels than the G allele non-carriers.

The levels of TC, TG, LDL-C and ApoB in Han males and TC, TG and HDL-C in Han females were different among the genotypes ($P < 0.05$-0.01), the subjects with GG genotype in Han males had higher TC, TG, and ApoB and lower LDL-C levels than the subjects with AA or AG genotype, and the G allele carriers in Han females had lower TC and HDL-C levels than the G allele non-carriers. The subjects with GG genotype in Han females also had higher TG levels that the subjects with AG genotype.

Risk factors for lipid parameters

The correlation between the genotypes of rs7395662 SNP and serum lipid parameters in Mulao and Han is shown in Table 4. The levels of LDL-C and ApoB in Mulao females were correlated with the genotypes ($P < 0.05$ for each). The levels of HDL-C and ApoAI in Han males and HDL-C in Han females were correlated with genotypes ($P < 0.05$-0.001).

Serum lipid parameters were also correlated with several environmental factors such as age, gender, height, weight, BMI, waist circumference, alcohol consumption, cigarette smoking, blood pressure and blood glucose in both ethnic groups ($P < 0.05$-0.001; Table 5).

Table 2. Comparison of the genotypic and allelic frequencies of *FOLH1* rs7395662 SBNP between the Mulao and Han populations [*n* (%)]

Group	*n*	Genotype			Allele	
		AA	AG	GG	A	G
Mulao	721	221 (30.6)	353 (49.0)	147 (20.4)	795 (55.1)	647 (44.9)
Han	727	241 (33.1)	337 (46.4)	149 (20.5)	819 (56.3)	635 (43.7)
χ^2	–	1.225			0.419	
P	–	0.542			0.517	
Mulao						
Male	336	104 (31.0)	169 (50.3)	63 (18.8)	377 (56.1)	295 (43.9)
Female	385	117 (30.4)	184 (47.8)	84 (21.8)	418 (54.3)	352 (45.7)
χ^2	–	1.077			0.478	
P	–	0.584			0.489	
Han						
Male	346	126 (36.4)	162 (46.8)	58 (16.8)	414 (59.8)	278 (40.2)
Female	381	115 (30.2)	175 (45.9)	91 (23.9)	405 (53.1)	357 (46.9)
χ^2	–	6.643			6.572	
P	–	0.036			0.010	

Table 3. The genotypes of rs7395662 SNP and serum lipid levels between the Mulao and Han populations

Genotype	*n*	TC (mmol/L)	TG (mmol/L)	HDL-C (mmol/L)	LDL-C (mmol/L)	ApoAI (g/L)	ApoB (g/L)	ApoAI/ ApoB
Mulao								
AA	221	4.96±1.11	1.01(0.73)	1.80±0.51	2.86±0.85	1.33±0.41	0.92±0.52	1.69±0.75
AG	353	5.01±1.26	1.10(0.86)	1.72±0.44	2.89±0.88	1.31±0.41	0.99±0.60	1.64±1.05
GG	147	5.16±1.79	1.17(0.75)	1.72±0.43	3.07±1.02	1.33±0.38	1.04±0.57	1.49±0.64
F	–	1.608	5.061	2.079	2.653	0.199	2.104	1.664
P	–	0.344	0.080	0.126	0.071	0.820	0.123	0.190
Han								
AA	241	5.21±1.16	1.14 (1.03)	1.82±0.80	3.05±0.90	1.39±0.27	0.90±0.23	1.67±0.95
AG	337	4.96±1.01	0.97 (0.79)	1.72±0.42	2.86±0.84	1.33±0.26	0.84±0.19	1.59±0.61
GG	149	5.25±1.40	1.28(0.72)	1.68±0.42	2.82±0.90	1.35±0.25	0.89±0.21	1.59±0.48
F	–	5.013	15.918	3.283	4.577	2.945	6.654	1.123
P	–	0.007	<0.001	0.038	0.011	0.063	0.001	0.326
Mulao/male								
AA	104	5.08±1.48	0.99(0.90)	1.80±0.58	2.90±0.82	1.33±0.45	0.98±0.59	1.60±0.80
AG	169	5.13±1.38	1.29(0.97)	1.72±0.46	2.85±0.87	1.33±0.43	1.06±0.43	1.49±0.65
GG	63	5.21±2.17	1.33(0.96)	1.70±0.45	2.91±0.95	1.36±0.43	1.04±0.57	1.51±0.65
F	–	0.162	5.141	1.143	0.130	0.126	0.509	0.091
P	–	0.850	0.077	0.320	0.878	0.881	0.601	0.407
Mulao/female								
AA	117	4.86±1.12	1.03 (0.64)	1.80±0.41	2.83±0.87	1.34±0.36	0.86±0.44	1.76±0.70
AG	184	4.91±1.13	1.00(0.69)	1.73±0.43	2.92±0.88	1.30±0.40	0.92±0.50	1.78±1.10
GG	84	5.13±1.46	1.06(0.67)	1.74±0.41	3.18±1.06	1.31±0.34	1.05±0.57	1.49±0.64
F	–	1.374	1.368	0.986	3.749	0.356	3.340	1.845
P	–	0.254	0.505	0.374	0.024	0.701	0.036	0.159
Han/male								
AA	126	5.27±1.32	1.22(1.10)	1.72±0.42	3.11±0.96	1.43±0.31	0.95±0.25	1.61±0.57
AG	162	5.16±0.70	1.15(0.95)	1.70±0.42	2.90±0.70	1.37±0.26	0.88±0.15	1.60±0.45
GG	58	5.83±1.86	1.48 (1.00)	1.62±0.44	2.78±1.01	1.35±0.24	0.99±0.23	1.44±0.43
F	–	6.601	6.295	1.090	3.333	2.251	7.326	2.713
P	–	0.002	0.043	0.337	0.037	0.107	0.001	0.067
Han/female								
AA	115	5.13±0.95	1.07(0.88)	1.96±1.06	2.99±0.82	1.34±0.20	0.85±0.21	1.69±0.50
AG	175	4.76±1.20	0.87(0.59)	1.74±0.43	2.82±0.94	1.30±0.26	0.80±0.21	1.73±0.53
GG	91	4.89±0.83	1.11 (0.82)	1.73±0.41	2.85±0.76	1.35±0.25	0.82±0.16	1.69±0.49
F	–	4.831	13.283	3.578	1.469	1.318	2.143	0.252
P	–	0.013	0.001	0.029	0.231	0.269	0.119	0.778

TC, total cholesterol; TG, triglyceride; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; ApoAI, apolipoprotein AI; ApoB, apolipoprotein B; ApoAI/ApoB, the ratio of apolipoprotein AI 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.

Table 4. Correlation between the genotypes of rs7395662 SNP and serum lipid parameters in the Mulao and Han populations

Lipid parameter	Risk factor	Unstandardized coefficient	Std. error	Standardized coefficient	*t*	*P*
Mulao and Han						
HDL-C	Genotype	0.063	0.019	0.086	3.371	0.001
ApoAI/ApoB	Genotype	0.073	0.029	0.066	2.568	0.010
Mulao						
ApoB	Genotype	-0.063	0.030	-0.078	-2.129	0.034
Han Chinese						
HDL-C	Genotype	0.103	0.030	0.126	3.392	0.001
ApoAI	Genotype	0.034	0.013	0.094	2.720	0.007
ApoAI/ApoB	Genotype	0.079	0.023	0.116	3.424	0.001
Mulao/Female						
LDL-C	Genotype	-0.146	0.064	-0.113	-2.265	0.024
ApoB	Genotype	-0.087	0.035	-0.125	-2.524	0.012
Han/Male						
HDL-C	Genotype	0.078	0.032	0.126	2.451	0.015

ApoAI	Genotype	0.071	0.020	0.174	3.613	<0.001
Han/Female						
HDL-C	Genotype	0.129	0.050	0.135	2.591	0.010

HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; ApoAI, Apolipoprotein AI; ApoB, Apolipoprotein B; ApoAI/ApoB, the ratio of Apolipoprotein AI to Apolipoprotein B. The results are limited to associations with $P < 0.05$ and corrected by bonferroni correction; the results with $P > 0.05$ are not shown in the table.

Table 5. Correlation between environmental factors and serum lipid parameters in the Mulao and Han populations

Lipid parameter	Risk factor	Unstandardized coefficient	Std. error	Standardized coefficient	*t*	*P*
Mulao and Han						
TC	Age	0.011	0.002	0.118	4.512	<0.001
	Diastolic blood pressure	0.010	0.003	0.090	3.314	0.001
	Cigarette smoking	0.184	0.064	0.082	2.857	0.004
	Alcohol consumption	0.142	0.048	0.086	2.968	0.003
	BMI	0.045	0.010	0.114	4.372	<0.001
TG	Diastolic blood pressure	0.019	0.006	0.087	3.360	0.001
	Cigarette smoking	0.854	0.116	0.191	7.501	<0.001
	Weight	0.034	0.007	0.123	4.605	<0.001
	Blood glucose	0.147	0.036	0.103	4.110	<0.001
HDL-C	Weight	-0.013	0.002	-0.230	-8.696	<0.001
	Cigarette smoking	0.099	0.025	0.106	4.018	0.001
LDL-C	Gender	-0.120	0.057	-0.067	-2.108	0.035
	Age	0.010	0.002	0.159	6.250	<0.001
	Cigarette smoking	-0.232	0.050	-0.146	-4.615	<0.001
	BMI	0.052	0.007	0.185	7.251	<0.001
ApoAI	Gender	0.057	0.025	0.083	2.242	0.025
	Cigarette smoking	0.127	0.020	0.210	6.429	<0.001
	Alcohol consumption	0.048	0.014	0.108	3.412	<0.001
ApoB	Weight	0.007	0.001	0.140	5.161	<0.001
	Systolic blood pressure	0.001	0.001	0.067	2.532	0.011
	Blood glucose	0.022	0.006	0.091	3.486	0.001
ApoAI/ApoB	Gender	0.242	0.051	0.151	4.706	<0.001
	Age	-0.005	0.002	-0.080	-3.154	0.002
	Cigarette smoking	0.173	0.045	0.122	3.814	<0.001
	BMI	-0.049	0.006	-0.194	-7.568	<0.001
Mulao						
TC	BMI	0.062	0.016	0.142	3.837	<0.001
	Cigarette smoking	0.250	0.093	0.100	2.703	0.007
TG	Weight	0.043	0.009	0.180	4.785	<0.001
	Cigarette smoking	0.535	0.156	0.129	3.428	0.001
HDL-C	Height	-0.005	0.002	-0.095	-2.433	0.015
	Cigarette smoking	0.135	0.034	0.156	4.013	<0.001
	BMI	-0.040	0.005	-0.263	-7.319	<0.001
LDL-C	Height	-0.009	0.004	-0.078	-2.120	0.034
	BMI	0.049	0.011	0.168	4.553	<0.001
ApoAI	Gender	0.076	0.036	0.094	2.109	0.035
	Cigarette smoking	0.161	0.034	0.214	4.794	<0.001
ApoB	Weight	0.009	0.002	0.152	4.154	<0.001
	Blood glucose	0.039	0.013	0.111	3.015	0.003
ApoAI/ApoB	Gender	0.155	0.075	0.076	2.055	0.040
	BMI	-0.043	0.012	-0.131	-3.546	<0.001
	Blood glucose	-0.043	0.012	-0.085	-2.308	0.021
Han Chinese						
TC	Diastolic blood pressure	0.022	0.004	0.211	5.829	<0.001
	Gender	-0.275	0.126	-0.118	-2.190	0.029
	Waist circumference	0.045	0.009	0.310	5.178	<0.001
	BMI	0.061	0.022	0.171	2.793	0.005
	Alcohol consumption	0.206	0.068	0.130	3.049	0.002
TG	Waist circumference	0.117	0.020	0.333	5.711	<0.001

	Diastolic blood pressure	0.032	0.009	0.127	3.572	<0.001
	Cigarette smoking	1.299	0.170	0.271	7.624	<0.001
	Blood glucose	0.296	0.061	0.169	4.829	<0.001
HDL-C	Weight	-0.014	0.003	-0.216	-5.552	<0.001
	Alcohol consumption	0.062	0.031	0.078	2.022	0.044
	Blood glucose	-0.032	0.014	-0.088	-2.369	0.018
LDL-C	Gender	-0.455	0.094	-0.258	-4.834	<0.001
	Waist circumference	0.023	0.004	0.210	5.571	<0.001
	Cigarette smoking	-0.347	0.071	-0.230	-4.886	<0.001
ApoAI	Weight	-0.008	0.001	-0.281	-7.695	<0.001
	Cigarette smoking	0.067	0.018	0.150	3.786	<0.001
	Alcohol consumption	0.111	0.014	0.316	7.778	<0.001
ApoB	Gender	-0.068	0.019	-0.164	-3.654	<0.001
	Waist circumference	0.009	0.001	0.351	10.019	<0.001
	Diastolic blood pressure	0.003	0.001	0.146	4.332	<0.001
	Alcohol consumption	0.036	0.011	0.128	3.321	0.001
	Blood glucose	0.020	0.004	0.151	4.607	<0.001
ApoAI/ApoB	Gender	0.027	0.054	0.234	4.205	<0.001
	Waist circunference	-0.011	0.003	-0.186	-3.673	<0.001
	BMI	-0.038	0.007	-0.252	-5.123	<0.001
	Cigarette smoking	0.114	0.038	0.137	3.020	0.003
	Alcohol consumption	0.092	0.027	0.140	3.384	0.001
	Blood glucose	-0.023	0.010	-0.075	-2.167	0.030

TC, total cholesterol; TG, triglyceride; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; ApoAI, apolipoprotein AI; ApoB, apolipoprotein B; ApoAI/ApoB, the ratio of apolipoprotein AI to apolipoprotein B. The results are limited to associations with $P < 0.05$ and corrected by bonferroni correction; the results with $P > 0.05$ are not shown in the table.

Discussion

In the current study, we showed that serum ApoB levels were significantly higher in Mulao than in Han. Significant differences in the levels of TC, TG, HDL-C, LDL-C, ApoAI and the ratio of ApoAI to ApoB were not observed between the two ethnic groups. Proverbially, dislipidemia is one of the intermediate risk factors for CAD and it is a multifactorial and complicated origin which combined by genetic factors as well as environmental factors [6, 7]. Numerous studies have shown that serum lipid levels such as TG, HDL-C and LDL-C are intensively influenced by the genetic component of each individual. Hence, it may account for additional analytically challenges when studying non-European populations since most GWAS have been focused on populations of European descent exclusively.

Mulao nationality is one of the 11 minorities in Guangxi Zhuang Autonomous Region, which is a genetic feature distinctive nationality. Therefore, we surmised that the hereditary characteristic and some lipid-related gene polymorphisms in the Mulao population may be different from those in Han nationality.

The genotypic and allelic frequencies of the rs7395662 SNP have not been reported previously in Mulao and Han nationalities. The genotypic and allelic frequencies in previous GWAS are also inconsistent in different racial/ethnic groups. In the present study, we showed that the genotypic and allelic frequencies of the rs7395662 SNP in Han were different between males and females ($P < 0.05$ for each), but there were no significant differences in the allelic and genotypic frequencies between Mulao and Han, or between Mulao males and females. The data in the International HapMap Project's data-base have suggested that the frequency of G allele was 62.7% in European, 58.9% in Han Chinese in Beijing, 48.9% in Japanese and 44.2% in Sub-Saharan African. As compared with other populations, we found that the frequency of G allele in our study populations was lower than that in Han Chinese from Beijing, which may be caused by different sample sizes and Han Chinese from Beijing and Guangxi are different parts of Han. The results of the present study suggested that there may be a sex-specific difference in the rs7395662 SNP in our study populations.

The potential association of the rs7395662 SNP and serum/plasma lipid levels in humans has been investigated in several previous GWAS, but the results are inconsistent. Aulchenko et al. [14] showed that the rs7395662 SNP was significant association with HDL-C concentration ($P = 6 \times 10^{-11}$) in population of European descent. Chasman et al. [33] also found that this locus was associated with lipoprotein size measures and HDL-C. Furthermore, a study of copy number variants (CNVs) well-tagged by SNPs by Gamazon et al. [30] delivered that, the rs7395662 SNP was a replicated SNP associated with HDL-C which is tagging nearby CNV. As one of the newly identified lipid-associated loci, although the rs7395662

SNP had been shown consistent evidence for association across European cohorts just as other loci, its role in lipid metabolism was less obvious [14, 31]. Aulchenko et al. [14] at the same time reported that the genetic effect is modest for the rs7395662 SNP in the variance of HDL-C. Unfortunately, these several previously cited studies all failed to expressly confirm the function of the *MADD-FOLH1* and discover the direct evidence for a role in lipid metabolism of this SNP. Coincidentally, the Carotid Lesion Epidemiology And Risk (CLEAR) study [34] and a study of biochemical traits in Korčula Island [19] neither observed the significant association between this SNP with carotid artery disease and blood lipid profiles separately. Likewise, Weissglas-Volkov et al. [21] did not access the involvement in the increased susceptibility to dyslipidemia for the *MADD-FOLH1* in Mexicans. In the present study, we showed that the levels of LDL-C and ApoB in Mulao females but not males were different among the genotypes, the G allele carriers had higher LDL-C and ApoB levels than the G allele non-carriers. The levels of TC, TG, LDL-C and ApoB in Han males and TC, TG and HDL-C in Han females were different among the genotypes, the subjects with GG genotype in Han males had higher TC, TG, and ApoB and lower LDL-C levels than the subjects with AA or AG genotype, and the G allele carriers in Han females had lower TC and HDL-C levels than the G allele non-carriers. The subjects with GG genotype in Han females also had higher TG levels than the subjects with AG genotype. These findings suggested that there may be a sex-specific association of the rs7395662 SNP and serum lipid concentrations in our study populations.

Serum lipid levels have been demonstrated to be influenced by genetic factors and non-genetic factors, and the latter ones include environmental factors which are strongly related with serum lipid levels such as dietary patterns, lifestyle, obesity, physical inactivity, and hypertension [6,7]. Hence, different environmental modifiers that interact with genes influence serum lipid levels may become the major reason for inconsistency among studies conducted with lipid profiles [42, 43]. Moreover, owing to a variety of lifestyle and environments in our study populations resident in Guangxi, the effect of genetic variation may be further modified. In the current study, we simultaneously showed that serum lipid parameters were correlated with age, sex, height, weight, BMI, waist circumference, alcohol consumption, cigarette smoking, blood pressure and blood glucose in the both ethnic groups. These findings highlight the importance of the environmental factors in affecting serum lipid levels in our study populations. Although rice and corn are the staple food in the both ethnic groups, the people of Mulao nationality like to eat cold foods along with acidic and spicy dishes, therefore bean soy sauce and pickle vegetables become members of their most popular dishes. Meanwhile, they are fond of eating animal offals which contain abundant saturated fatty acid. It has been widely accepted that high-fat diets, particularly those contain rich saturated fatty acids, can raise the serum cholesterol concentrations as well as the risk of suffering cardiovascular disease [44].

Conclusion

We conclude from the present study that the genotypic and allelic frequencies of rs7395662 SNP in the *MADD-FOLH1* are different between Han males and females. The association of the rs7395662 SNP and serum lipid levels is also different between Mulao and Han, or between males and females in the both ethnic groups. There may be a sex-specific association of the rs7395662 SNP and serum lipid levels in our study populations.

Supplementary Material

Figures S1-S3.
http://www.medsci.org/v10p1537s1.pdf

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Competing Interests

The authors have declared that no competing interest exists.

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