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Original Article

Association between the *MARS* rs6782181 polymorphism and serum lipid levels

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Abstract: Little is known about the association between the muscle Ras (MRAS) gene rs6782181 polymorphism and serum lipid levels. The aim of the present study was to investigate the association between the *MRAS* rs6782181 polymorphism and serum lipid levels in the Mulao and Han populations. A total of 632 subjects of Han and 629 unrelated subjects of Mulao nationalities were randomly selected from our previous stratified randomized samples. Genotypes of the *MARS* rs6782181 polymorphism were determined via polymerase chain reaction and restriction fragment length polymorphism. The subjects with GG genotype had higher serum total cholesterol (TC), triglyceride (TG), low-density lipoprotein cholesterol (LDL-C), and apolipoprotein (Apo) B levels in Han, and higher serum TC and LDL-C levels in Mulao than the subjects with AA/AG genotypes ($P < 0.05$-0.01). Subgroup analyses showed that the subjects with GG genotype had higher TC, TG, high-density lipoprotein cholesterol (HDL-C), LDL-C, ApoAI and ApoB in Han males, lower ApoAI and the ratio of ApoAI to ApoB in Han females; and higher LDL-C levels in Mulao males but not in Mulao females than the subjects with AG/AA genotypes. The association of the *MARS* rs6782181 polymorphism and serum lipid levels is different between the Mulao and Han populations, or between males and females in the both ethnic groups. There may be an ethnic- and/or sex-specific association between the *MRAS* rs6782181 polymorphism and serum lipid levels in our study populations.

Keywords: Muscle Ras, MRAS, 3q22.3, single nucleotide polymorphism, serum lipid level, rs6782181

Introduction

Cardiovascular disease (CVD), which includes both coronary heart disease (CHD) and cerebrovascular disease (stroke), remains the one of the leading causes of death in western society, and is of growing concern in developing countries, despite the great advances have made in understanding its underlying pathophysiology [1-4]. It is generally accepted that hypercholesterolemia is a major health problem associated with an increased risk of CVD, especially low-density lipoprotein cholesterol (LDL-C) level elevation and conversely low level of high-density lipoprotein cholesterol (HDL-C) are mainly involved in disease development and progression [5-8], and serum triglyceride (TG) concentration plays a pivotal independent risk factor for atherosclerosis [9-11]. Dyslipidemia is a multi-factorial disease influenced by both environmental and genetic factors [12-14], accumulating evidences have shown that the heritability estimates of the interindividual variation give rise to a considerable genetic contribution [15-18].

Recently, a new susceptibility locus for dyslipidemia, rs6782181 single nucleotide polymorphism (SNP) was identified on chromosome 3q22.3 in Saudi individuals [19]. The muscle Ras (MRAS) gene resides on chromosome 3q22.3 and encodes a member of the membrane-associated Ras small GTPase proteins, which function as signal transducers in multiple processes including cell growth and differentiation [19, 20]. Several studies revealed a region

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

Parameter	Han	Mulao	t (χ^2)	P
Number	632	629		
Male/female	267/365	290/339	1.903	0.168
Age (years)	53.40 ± 14.26	53.09 ± 14.41	0.394	0.694
Height (cm)	154.18 ± 8.27	155.28 ± 8.17	-2.393	0.017
Weight (kg)	53.69 ± 9.09	52.67 ± 9.33	1.958	0.050
Body mass index (kg/m^2)	22.56 ± 3.36	21.78 ± 3.04	4.344	0.000
waist circumference (cm)	75.47 ± 7.83	74.97 ± 8.52	1.089	0.277
Cigarette smoking (n %)				
Nonsmoker	453 (71.7)	467 (74.2)		
≤ 20 cigarettes/day	160 (25.3)	133 (21.1)	4.777	0.092
> 20 cigarettes/day	19 (3.0)	29 (4.6)		
Alcohol consumption [n (%)]				
Nondrinker	498 (78.8)	464 (73.8)		
≤ 25 g/day	65 (10.3)	62 (9.9)	7.986	0.018
> 25 g/day	69 (10.9)	103 (16.4)		
Systolic blood pressure (mmHg)	130.52 ± 19.49	129.48 ± 21.63	0.896	0.370
Diastolic blood pressure (mmHg)	82.81 ± 11.46	80.87 ± 11.58	2.983	0.003
Pulse pressure (mmHg)	47.71 ± 15.02	48.61 ± 16.27	-1.017	0.309
Glucose (mmol/L)	6.17 ± 1.89	6.06 ± 1.68	1.214	0.225
Total cholesterol (mmol/L)	5.00 ± 0.87	5.04 ± 1.05	-0.598	0.550
Triglyceride (mmol/L)	1.10 (0.85)	1.08 (0.73)	0.841	0.478
HDL-C (mmol/L)	1.73 ± 0.53	1.78 ± 0.45	-1.738	0.082
LDL-C (mmol/L)	2.91 ± 0.78	2.98 ± 0.80	-1.473	0.141
Apolipoprotein (Apo) AI (g/L)	1.33 ± 0.24	1.33 ± 0.40	0.309	0.757
ApoB (g/L)	0.86 ± 0.18	0.99 ± 0.57	-5.587	0.000
ApoAI/Apo B	1.63 ± 0.49	1.59 ± 0.79	1.144	0.253

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

on 3q22.3, which encompasses the *MRAS*, rs9818870 SNP as a risk factor for CHD [21, 22]. In the present study, our aim was to investigate the association between the *MARS* rs6782181 SNP and serum lipid levels in the Han and Mulao populations.

Materials and methods

Study subjects

There were 632 subjects of Han population and 629 unrelated subjects of Mulao population who were randomly selected from our previous stratified randomized samples [23]. All subjects were rural agricultural workers reside in Luocheng Mulao Autonomous County, Guangxi Zhuang Autonomous Region, People's Republic of China. The participants of Han consisted of 267 (42.24%) males and 365 (57.76%) females, with a mean age of 53.40 ± 14.26 years. The subjects of Mulao consisted of 290 (46.10%) males and 339 (53.90%) females, with a mean age of 53.09 ± 14.41 years. Subjects with diseases related to atherosclerosis, CHD, diabetes or those who were using lipid-lowering medication (such as statins or fibrates, beta-blockers, diuretics, or hormones) were excluded from the study before the blood sample was taken. The study design was approved by the Ethics Committee of the First Affiliated Hospital, Guangxi Medical University. Written informed consent was obtained from all subjects before participation.

Epidemiological survey

The survey was carried out using internationally standardized methods, following a common protocol [24]. Information on demographics,

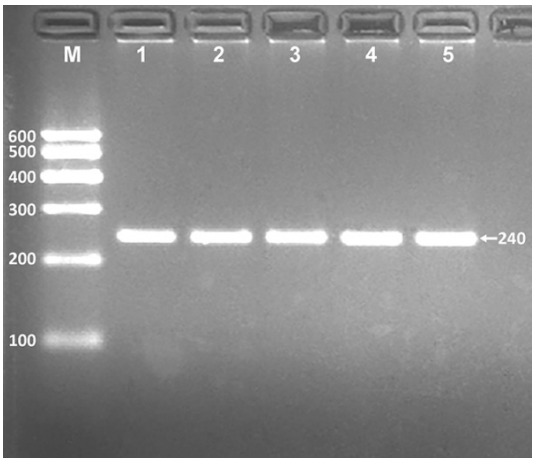

Figure 1. Electrophoresis of PCR products and genotyping of the *MARS* rs6782181 polymorphism. Lane M, 100 bp marker ladder; lanes 1-5, samples. The 240 bp bands are the PCR products.

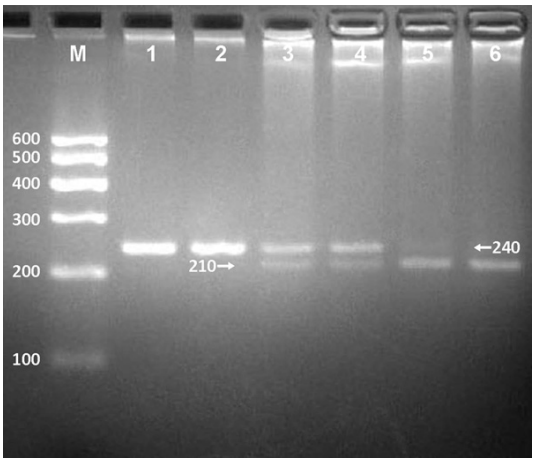

Figure 2. Genotyping of the *MARS* rs6782181 SNP. Lane M, 100 bp marker ladder; lanes 1 and 2, AA genotype (240 bp); lanes 3 and 4, AG genotype (240-, 210- and 30-bp); lanes 5 and 6, GG genotype (210- and 30-bp). The 30 bp fragments were invisible in the gel owing to their fast migration speed.

socioeconomic status, and lifestyle factors was collected with standardized questionnaires. The intake of alcohol was quantified as the number of *liang* (about 50 g) of rice wine, corn wine, rum, beer, or liquor consumed during the preceding 12 months. Alcohol consumption was categorized into groups of grams of alcohol per day: ≤ 25 and > 25. Smoking status was categorized into groups of cigarettes per day: ≤ 20 and > 20. In the physical examination, several parameters such as height, weight, and waist circumference were measured. Sitting blood pressure was measured three times with the use of a mercury sphygmomanometer after a 5-minute of rest, and the average of the three measurements was recorded. Systolic blood pressure was determined by the first Korotkoff sound, and diastolic blood pressure by the fifth Korotkoff sound. Body weight, to the nearest 50 grams, was measured using a portable balance scale. Subjects were weighed in a minimum of clothing with shoes off. Height was measured, to the nearest 0.5 cm, using a stadiometer. From these two measurements BMI was calculated.

Biochemical measurements

Blood samples were obtained in the fasting state. Biochemical parameters including total cholesterol (TC), TG, HDL-C, LDL-C were measured by enzymatic methods with commercially available kits. Serum apolipoprotein (Apo) AI and ApoB concentrations were quantified by the immunoturbidimetric immunoassay using a commercial kit. Fasting blood glucose was determined by glucose meter.

DNA amplification and genotyping

Genomic DNA was isolated from peripheral blood leukocytes using the phenol-chloroform method. The extracted DNA was stored at 4°C until analysis. Genotyping of the *MARS* rs6782181 SNP was performed by polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP). PCR amplification was performed using 5'-CTGTAATCACTGCCAACTC-3' and 5'-CAGCACGAACCTGTAAAA-3' (Sangon, Shanghai, People's Republic of China) as the forward and reverse primer pairs, respectively. Each amplification reaction was performed in a total volume of 25 μL, containing 2 μL of genomic DNA, 1 μL of each primer (10 pmol/L), 12.5 μL of 2 × *Taq* PCR Mastermix (constituent: 20 mM Tris-HCl, pH 8.3, 100 mM KCl, 3 mM $MgCl_2$, 0.1 U *Taq* Polymerase/μL, 500 μM dNTP each; Sangon, Shanghai, People's Republic of China), and 8.5 μL of ddH_2O (DNase/RNase-free), processing started with 94°C for 5 min and followed by 45 s of denaturing at 94°C, 45 s of annealing at 51°C and 1 min of extension at 72°C for 30 cycles. The amplification was completed by a final extension at 72°C for 7 min. Then 10 U of *Tai*I enzyme was added directly to the PCR products (10 μL) and digested at 65°C overnight. After restriction enzyme digestion of the amplified

Table 2. Comparison of the genotype and allele frequencies of rs6782181 polymorphism in the Mulao and Han populations [n (%)]

Group	N	Genotype			Allele		HWE (*P*)
		AA	AG	GG	A	G	
Han	632	359 (56.8)	227 (35.9)	46 (7.3)	945 (74.8)	319 (25.2)	0.226
Mulao	629	346 (55.0)	235 (37.4)	48 (7.6)	927 (73.7)	331 (26.3)	0.360
χ^2			0.414		0.380		
P			0.813		0.537		
Han							
Male	267	149 (55.8)	97 (36.3)	21 (7.9)	395 (74.1)	139 (25.9)	0.355
Female	365	210 (57.5)	130 (35.6)	25 (6.8)	550 (75.3)	180 (24.7)	0.429
χ^2			0.322		0.308		
P			0.851		0.579		
Mulao							
Male	290	151 (52.1)	117 (40.3)	22 (7.6)	419 (72.2)	161 (27.8)	0.919
Female	339	195 (57.5)	118 (34.8)	26 (7.7)	508 (74.9)	170 (25.1)	0.175
χ^2			2.129		1.162		
P			0.345		0.281		

HWE: Hardy-Weinberg equilibrium.

DNA, genotypes were identified by electrophoresis on 2% ethidium-bromide stained agarose gels and visualizing with ultraviolet illumination. Genotypes were scored by an experienced reader blinded to the epidemiological and lipid results.

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, 0.91-1.81, 2.70-3.20 mmol/L, 1.00-1.78, 0.63-1.14 g/L, and 1.00-2.50; respectively [23]. Hypertension was assessed according to the criteria of 1999 World Health Organization-International Society of Hypertension Guidelines for the management of hypertension [25].

Statistical analyses

Statistical analyses were performed by the statistical software package SPSS 16.0 (SPSS Inc., Chicago, Illinois). Qualitative variables were expressed as raw count and percentage. The quantitative variables were presented as mean ± standard deviation (serum TG levels were presented as medians and interquartile ranges). General characteristics between Mulao and Han were compared by Student's unpaired *t*-test. Genotypic and allelic frequencies were calculated by direct counting, and the standard goodness-of-fit test was used to investigate departures from Hardy-Weinberg equilibrium. The difference in genotype distribution and sex ratio between the populations was test by chi-square analysis. Factors that may influence serum lipid concentrations: sex, age, BMI, blood pressure, alcohol consumption, cigarette smoking were adjusted for the statistical analysis. Relationship between serum lipid levels and genotypes and several environment factors were assessed by multiple linear regression analysis with stepwise modeling. A *P* value less than 0.05 (two-tailed) was regarded as statistically significant.

Results

General characteristics and serum lipid levels

The comparison of general characteristics and serum lipid levels between the Mulao and Han populations is summarized in **Table 1**. The levels of BMI and diastolic blood pressure was lower in Mulao than in Han ($P < 0.01$), whereas the level of height and ApoB were higher in Mulao than in Han ($P < 0.05$-0.001). There were no significant differences in the levels of age structure, weight, waist circumference, systolic blood pressure, pulse pressure, glucose, TC, TG, HDL-C, LDL-C, ApoAI and the ratio of ApoAI to ApoB between the two ethnic groups ($P > 0.05$ for all).

Table 3. Comparison of serum lipid levels among the genotypes between the Mulao and Han populations

Ethnic/Genotype	N	TC (mmol/L)	TG (mmol/L)	HDL-C (mmol/L)	LDL-C (mmol/L)	Apo AI (g/L)	Apo B (g/L)	ApoAI/ApoB
Han								
AA	359	4.93 ± 0.81	1.09 (0.78)	1.71 ± 0.40	2.87 ± 0.67	1.33 ± 0.23	0.85 ± 0.18	1.63 ± 0.49
AG	227	5.03 ± 0.88	1.07 (0.77)	1.76 ± 0.68	2.91 ± 0.85	1.33 ± 0.23	0.85 ± 0.17	1.65 ± 0.50
GG	46	5.43 ± 1.09	1.55 (0.87)	1.77 ± 0.59	3.26 ± 1.09	1.36 ± 0.37	0.95 ± 0.26	1.48 ± 0.49
F		6.983	8.080	0.838	5.312	0.401	6.200	2.433
P		0.001	0.018	0.433	0.005	0.670	0.002	0.089
Han/male								
AA	149	5.07 ± 0.87	1.18 (0.98)	1.64 ± 0.41	2.96 ± 0.67	1.32 ± 0.25	0.91 ± 0.19	1.51 ± 0.46
AG	97	5.07 ± 0.86	1.23 (0.83)	1.63 ± 0.35	2.82 ± 0.88	1.32 ± 0.28	0.87 ± 0.17	1.59 ± 0.55
GG	21	5.82 ± 0.81	1.55 (0.70)	1.87 ± 0.37	3.47 ± 0.80	1.57 ± 0.24	1.04 ± 0.19	1.57 ± 0.41
F		7.367	7.407	3.445	6.282	8.757	7.065	0.801
P		0.001	0.025	0.033	0.002	0.000	0.001	0.450
Han/female								
AA	210	4.84 ± 0.76	1.02 (0.60)	1.75 ± 0.38	2.80 ± 0.67	1.33 ± 0.21	0.81 ± 0.16	1.72 ± 0.49
AG	130	5.00 ± 0.89	0.97 (0.86)	1.85 ± 0.84	2.97 ± 0.83	1.34 ± 0.19	0.83 ± 0.17	1.69 ± 0.45
GG	25	5.11 ± 1.20	1.23 (0.85)	1.69 ± 0.72	3.09 ± 1.27	1.19 ± 0.38	0.87 ± 0.28	1.39 ± 0.54
F		2.116	1.871	1.517	2.996	5.220	1.789	5.118
P		0.122	0.392	0.221	0.051	0.006	0.169	0.006
Mulao								
AA	346	5.13 ± 0.94	1.04 (0.71)	1.80 ± 0.47	3.04 ± 0.78	1.35 ± 0.39	1.02 ± 0.58	1.55 ± 0.65
AG	235	4.86 ± 1.17	1.12 (0.81)	1.74 ± 0.43	2.86 ± 0.80	1.31 ± 0.42	0.95 ± 0.57	1.66 ± 0.96
GG	48	5.20 ± 1.07	1.13 (0.74)	1.78 ± 0.38	3.10 ± 0.87	1.27 ± 0.39	1.03 ± 0.57	1.46 ± 0.77
F		5.311	1.527	1.238	4.360	1.227	0.984	2.147
P		0.005	0.466	0.291	0.013	0.294	0.374	0.118
Mulao/male								
AA	151	5.15 ± 0.88	1.12 (0.95)	1.79 ± 0.56	3.02 ± 0.70	1.36 ± 0.41	1.11 ± 0.73	1.52 ± 0.75
AG	117	4.90 ± 1.10	1.17 (0.93)	1.76 ± 0.45	2.80 ± 0.80	1.36 ± 0.43	0.96 ± 0.56	1.63 ± 0.67
GG	22	5.27 ± 1.00	1.14 (1.05)	1.66 ± 0.35	3.15 ± 0.92	1.22 ± 0.36	1.07 ± 0.53	1.27 ± 0.51
F		2.715	1.488	0.709	3.511	1.192	1.683	2.529
P		0.068	0.475	0.493	0.031	0.305	0.188	0.082
Mulao/female								
AA	195	5.12 ± 0.99	1.03 (0.64)	1.81 ± 0.39	3.06 ± 0.83	1.35 ± 0.38	0.94 ± 0.43	1.57 ± 0.57
AG	118	4.83 ± 1.23	1.03 (0.66)	1.73 ± 0.41	2.91 ± 0.81	1.26 ± 0.41	0.94 ± 0.58	1.70 ± 1.17
GG	26	5.13 ± 1.15	1.08 (0.56)	1.89 ± 0.39	3.06 ± 0.85	1.31 ± 0.42	1.00 ± 0.61	1.62 ± 0.92
F		2.787	0.199	2.574	1.240	1.636	0.144	0.845
P		0.063	0.905	0.078	0.291	0.196	0.866	0.431

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 value of TG was presented as median (interquartile range). The difference among the genotypes was determined by the Kruskal-Wallis test or the Wilcoxon-Mann-Whitney test.

Results of electrophoresis and genotyping

After the genomic DNA of the samples was amplified by PCR and imaged by 2.0% agarose gel electrophoresis, the purpose gene of 240 bp nucleotide sequences could be found in all samples (**Figure 1**). The genotypes identified were named according to the presence or absence of the enzyme restriction sites, when an A to G transversion at rs6782181 SNP. The presence of the cutting site indicates the G allele; while its absence indicates the A allele (cannot be cut). Therefore, the AA genotype is homozygote for the absence of the site (band at 240 bp), AG genotype is heterozygote for the absence and presence of the site (bands at 240-, 210- and 30-bp), and GG genotype is homozygote for the presence of the site (bands at 210- and 30-bp; **Figure 2**).

Genotypic and allelic frequencies

The genotypic and allelic distribution of the rs6782181 SNP is revealed in **Table 2**. The

Table 4. Relationship between the lipid parameters and relative factors in Mulao and Han

Lipid parameter	Risk factor	B	Std. Error	Beta	*t*	*P*
Han and Mulao						
TC	Body mass index	0.049	0.008	0.163	5.928	0.000
	Age	0.010	0.002	0.155	5.525	0.000
	Alcohol consumption	0.093	0.037	0.069	2.504	0.012
	Glucose	0.032	0.015	0.060	2.141	0.032
TG	Waist circumference	0.042	0.004	0.294	11.013	0.000
	Alcohol consumption	0.192	0.043	0.118	4.428	0.000
	Glucose	0.057	0.017	0.089	3.364	0.001
	Ethnic group	-0.165	0.061	-0.071	-2.689	0.007
HDL-C	Waist circumference	-0.008	0.002	-0.134	-3.541	0.000
	Alcohol consumption	0.114	0.023	0.166	5.044	0.000
	Gender	0.123	0.033	0.124	3.707	0.000
	Body mass index	-0.013	0.006	-0.087	-2.347	0.019
LDL-C	Body mass index	0.049	0.007	0.201	7.388	0.000
	Age	0.010	0.002	0.173	6.219	0.000
	Glucose	0.033	0.012	0.074	2.672	0.008
	Ethnic group	0.110	0.043	0.070	2.563	0.010
	Cigarette smoking	-0.094	0.040	-0.064	-2.370	0.018
ApoAI	Alcohol consumption	0.130	0.015	0.278	8.471	0.000
	Gender	0.068	0.022	0.101	3.040	0.002
	Waist circumference	-0.004	0.001	-0.087	-3.103	0.002
	Pulse pressure	0.001	0.001	0.061	2.205	0.028
ApoB	Waist circumference	0.009	0.001	0.179	6.431	0.000
	Ethnic group	0.137	0.023	0.159	5.882	0.000
	Pulse pressure	0.003	0.001	0.092	3.357	0.001
	Glucose	0.019	0.007	0.078	2.860	0.004
	Gender	-0.051	0.024	-0.058	-2.109	0.035
ApoAI/ApoB	Waist circumference	-0.011	0.003	-0.141	-3.743	0.000
	Age	-0.004	0.001	-0.092	-3.335	0.001
	Alcohol consumption	0.151	0.030	0.164	5.040	0.000
	Gender	0.166	0.044	0.126	3.802	0.000
	Body mass index	-0.023	0.008	-0.113	-3.045	0.002
	Ethnic group	-0.079	0.036	-0.060	-2.195	0.028
	Glucose	-0.021	0.010	0.057	-2.028	0.043
Han						
TC	Waist circumference	0.012	0.006	0.109	2.171	0.030
	Age	0.011	0.002	0.177	4.496	0.000
	Genotype	0.138	0.053	0.100	2.617	0.009
	Body mass index	0.034	0.013	0.131	2.596	0.010
	Alcohol consumption	0.114	0.050	0.087	2.270	0.024
	Glucose	0.036	0.018	0.079	2.035	0.042
TG	Waist circumference	0.055	0.006	0.322	8.779	0.000
	Alcohol consumption	0.352	0.074	0.173	4.730	0.000
	Glucose	0.111	0.025	0.158	4.370	0.000
HDL-C	Waist circumference	-0.010	0.003	-0.153	-3.846	0.000
	Gender	0.231	0.058	0.215	3.986	0.000
	Alcohol consumption	0.092	0.037	0.114	2.458	0.014
	Cigarette smoking	0.112	0.052	0.111	2.172	0.030

LDL-C	Age	0.011	0.002	0.206	5.309	0.000
	Body mass index	0.049	0.009	0.211	5.593	0.000
	Cigarette smoking	-0.298	0.072	-0.200	-4.143	0.000
	Gender	-0.209	0.077	-0.132	-2.732	0.006
	Genotype	0.109	0.047	0.087	2.312	0.021
	Glucose	0.034	0.016	0.084	2.171	0.030
ApoAI	Alcohol consumption	0.133	0.016	0.361	8.086	0.000
	Gender	0.130	0.025	0.263	5.167	0.000
	Cigarette smoking	0.086	0.023	0.185	3.805	0.000
	Body mass index	-0.010	0.003	-0.131	-3.486	0.001
ApoB	Waist circumference	0.005	0.001	0.221	4.667	0.000
	Glucose	0.014	0.004	0.146	4.010	0.000
	Gender	-0.082	0.017	-0.220	-4.773	0.000
	Body mass index	0.010	0.003	0.179	3.844	0.000
	Age	0.002	0.000	0.131	3.619	0.000
	Cigarette smoking	-0.038	0.016	-0.108	-2.383	0.017
ApoAI/ApoB	Waist circumference	-0.009	0.003	-0.151	-3.103	0.002
	Alcohol consumption	0.143	0.033	0.193	4.398	0.000
	Gender	0.316	0.050	0.318	6.314	0.000
	Cigarette smoking	0.201	0.044	0.215	4.525	0.000
	Body mass index	-0.027	0.007	-0.187	-3.868	0.000
	Age	-0.004	0.001	-0.108	-2.942	0.003
Mulao						
TC	Age	0.010	0.003	0.134	3.419	0.001
	Body mass index	0.050	0.014	0.144	3.668	0.000
TG	Waist circumference	0.020	0.006	0.182	3.387	0.001
	Body mass index	0.045	0.016	0.147	2.725	0.007
HDL-C	Body mass index	-0.038	0.006	-0.259	-6.760	0.000
	Alcohol consumption	0.108	0.028	0.183	3.896	0.000
	Gender	0.093	0.042	0.104	2.220	0.027
LDL-C	Body mass index	0.049	0.010	0.187	4.816	0.000
	Age	0.008	0.002	0.143	3.680	0.000
ApoAI	Alcohol consumption	0.002	0.021	0.193	4.879	0.000
	Waist circumference	-0.004	0.002	-0.083	-2.096	0.037
ApoB	Waist circumference	0.012	0.003	0.173	4.413	0.000
	Pulse pressure	0.004	0.001	0.116	2.969	0.003
ApoAI/ApoB	Waist circumference	-0.019	0.004	-0.202	-5.144	0.000
	Age	-0.005	0.002	-0.096	-2.430	0.015
	Alcohol consumption	0.136	0.046	0.130	2.936	0.003
	Cigarette smoking	-0.132	0.063	-0.092	-2.097	0.036
	Glucose	-0.040	0.019	-0.085	-2.141	0.033

TC: total cholesterol; TG: triglyceride; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; ApoAI: Apolipoprotein AI; ApoB: Apolipoprotein B.

genotypic distribution was followed Hardy-Weinberg equilibrium (HWE). The frequency of *MARS* rs6782181-G allele was 25.2% in Han and 26.3% in Mulao. There was no significant difference in either genotypic or allelic frequencies between Mulao and Han, or between males and females in both ethnic groups.

Genotypes and serum lipid levels

As shown in **Table 3**, the subjects with GG genotype had higher serum TC, TG, LDL-C, and ApoB levels in Han, and higher serum TC and LDL-C levels in Mulao than the subjects with AA/AG genotypes ($P < 0.05$-0.01). When serum lipid

Table 5. Relationship between the lipid parameters and relative factors in male and female of Mulao and Han population

Lipid parameter	Risk factor	B	Std. Error	Beta	*t*	*P*
Han/male						
TC	Body mass index	0.055	0.014	0.235	3.985	0.000
	Genotype	0.253	0.081	0.184	3.112	0.002
	Cigarette smoking	-0.182	0.087	-0.123	-2.082	0.038
TG	Waist circumference	0.071	0.012	0.334	5.913	0.000
	Alcohol consumption	0.351	0.114	0.174	3.082	0.002
	Glucose	0.102	0.051	0.114	2.013	0.045
HDL-C	Waist circumference	-0.013	0.003	-0.256	-4.467	0.000
	Alcohol consumption	0.109	0.028	0.234	3.968	0.000
	Cigarette smoking	0.110	0.038	0.167	2.903	0.004
	Age	0.003	0.002	0.127	2.164	0.031
LDL-C	Body mass index	0.047	0.012	0.225	3.877	0.000
	Cigarette smoking	-0.315	0.076	-0.241	-4.148	0.000
ApoAI	Alcohol consumption	0.140	0.017	0.436	8.250	0.000
	Cigarette smoking	0.078	0.024	0.173	3.270	0.001
	Waist circumference	-0.006	0.002	-0.170	-3.275	0.001
	Genotype	0.065	0.022	0.154	2.960	0.003
ApoB	Waist circumference	0.006	0.002	0.271	4.101	0.000
	Body mass index	0.011	0.003	0.233	3.345	0.001
	Age	0.002	0.001	0.139	2.464	0.014
	Cigarette smoking	-0.037	0.017	-0.117	-2.112	0.036
ApoAI/ApoB	Waist circumference	-0.013	0.004	-0.214	-3.386	0.001
	Alcohol consumption	0.165	0.031	0.282	5.244	0.000
	Cigarette smoking	0.184	0.044	0.224	4.192	0.000
	Body mass index	-0.027	0.008	-0.209	-3.307	0.001
Han/female						
TC	Age	0.017	0.003	0.283	5.637	0.000
	Waist circumference	0.018	0.006	0.155	3.167	0.002
	Glucose	0.056	0.022	0.129	2.595	0.010
TG	Waist circumference	0.041	0.006	0.308	6.380	0.000
	Glucose	0.117	0.024	0.232	4.805	0.000
LDL-C	Age	0.019	0.003	0.339	6.907	0.000
	Waist circumference	0.020	0.005	0.189	3.905	0.000
	Alcohol consumption	-0.477	0.189	-0.123	-2.525	0.012
ApoAI	Genotype	-0.040	0.019	-0.112	-2.155	0.032
	Cigarette smoking	0.146	0.068	0.111	2.141	0.033
	Body mass index	-0.008	0.004	-0.105	-2.028	0.043
ApoB	Waist circumference	0.007	0.001	0.286	5.947	0.000
	Glucose	0.015	0.004	0.172	3.516	0.000
	Pulse pressure	0.002	0.001	0.122	2.435	0.015
	Age	0.001	0.001	0.123	2.424	0.016
ApoAI/ApoB	Age	-0.008	0.002	-0.220	-4.286	0.000
	Cigarette smoking	0.559	0.143	0.197	3.805	0.000
	Body mass index	-0.034	0.008	-0.209	-4.244	0.000
	Genotype	-0.082	0.039	-0.105	-2.109	0.036
Mulao/male						
TC	Pulse pressure	0.009	0.004	0.141	2.419	0.016

TG	Waist circumference	0.036	0.007	0.295	5.232	0.000
HDL-C	Body mass index	-0.044	0.009	-0.265	-4.755	0.000
	Alcohol consumption	0.117	0.032	0.206	3.688	0.000
LDL-C	Body mass index	0.040	0.015	0.155	2.657	0.008
ApoAI	Alcohol consumption	0.129	0.026	0.277	4.899	0.000
ApoB	Pulse pressure	0.009	0.003	0.220	3.655	0.000
	Age	-0.006	0.003	-0.138	-2.300	0.022
ApoAI/ApoB	Waist circumference	-0.017	0.005	-0.206	-3.628	0.000
	Alcohol consumption	0.145	0.045	0.182	3.200	0.002
Mulao/female						
TC	Age	0.016	0.004	0.208	3.941	0.000
	Body mass index	0.056	0.019	0.155	2.934	0.004
TG	Body mass index	0.067	0.013	0.265	5.039	0.000
HDL-C	Body mass index	-0.033	0.007	-0.258	-4.899	0.000
LDL-C	Body mass index	0.059	0.014	0.218	4.216	0.000
	Age	0.013	0.003	0.235	4.541	0.000
ApoB	Waist circumference	0.013	0.003	0.207	3.922	0.000
	Glucose	0.041	0.019	0.117	2.159	0.032
	Age	0.004	0.002	0.114	2.114	0.035
ApoAI/ApoB	Waist circumference	-0.019	0.006	-0.179	-3.369	0.001
	Age	-0.009	0.003	-0.160	-3.007	0.003

TC: total cholesterol; TG: triglyceride; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; ApoAI: Apolipoprotein AI; ApoB: Apolipoprotein B.

parameters were compared according to the sex subgroups, the subjects with GG genotype had higher TC, TG, HDL-C, LDL-C, ApoAI and ApoB in Han males, lower ApoAI and the ratio of ApoAI to ApoB in Han females; and higher LDL-C levels in Mulao males but not in Mulao females than the subjects with AG/AA genotypes.

Risk factors for serum lipid parameters

As described in **Tables 4** and **5**, multiple linear regression analyses showed that the levels of TC and LDL-C in Han but not in Mulao were correlated with genotypes ($P < 0.05$-0.01). When the regression analysis was performed according to the sex subgroups, we showed that the levels of TC and ApoAI in Han males, LDL-C levels in Han females were associated with genotypes but not in the Mulao population ($P < 0.05$-0.01).

Discussion

Disorder of lipid metabolism is strongly associated with CVD [5-11]. It is well recognized that dyslipidemia is a complex trait caused by multiple genetic and environmental factors and their interactions [12-14, 26]. Many literatures suggest that about 40-60% of the variation in serum lipid profiles is genetically determined [16, 27]. The prevalence of dyslipidemia continues to increase worldwide, not only causing serious personal health problems but also imposing a substantial economic burden on societies [28, 29]. Therefore, it is very important to understand and control the risk factors of dyslipidemia in some populations.

Little is known about the association between the *MARS* rs6782181 polymorphism and serum lipid levels. Alshahid et al. [19] reported that the rs6782181GG genotype was associated with the risk of obesity, hypercholesterolemia, hypertriglyceridemia and low HDL-C levels in the Saudi population. In the current study, we showed that the *MARS* rs6782181 SNP was associated with high serum TC, TG, LDL-C, and ApoB levels in Han, and higher serum TC and LDL-C levels in Mulao than the subjects with AA/AG genotypes. Subgroup analyses showed that these results were found only in males but not in females in both ethnic groups. The subjects with GG genotype in Han had higher TC, TG, HDL-C, LDL-C, ApoAI and ApoB in males, but lower ApoAI and the ratio of ApoAI to ApoB in females than the subjects with AG/AA geno-

types. The subjects with GG genotype in Mulao had higher LDL-C levels in males but not in females than the subjects with AG/AA genotypes. These results suggested that the prevalence of the *MARS* rs6782181 SNP may have racial/ethnic and/or sex specificity. For Mulao nationality, one of the 55 minorities with population of 207,352 according to the fifth national census statistics of China in 2000, some particular customs must be considered. Firstly, the majority of the people live in Guangxi Zhuang Autonomous Region, People's Republic of China, which are characterized by agriculture economy, and its inhabitants show similar life styles as well as eating habits, they prefer to eat cold foods along with acidic and spicy dishes, local bean soy sauce, pickled vegetables and animal offal's which contain abundant saturated fatty acid. Secondly, there was a strict intra-ethnic marriage in Mulao society, the engagements were family arranged in childhood, usually with the girl being four or five years older than the boy. There was a preference for marriage to mother's brother's daughter. Therefore, for the relatively conservative and isolated minority in China, we believe that the genetic background and some lipid-associated genetic variants in this population may be different from those in Han nationality. The above evidence may partly explain the discrepancies of *MARS* polymorphism and serum lipid levels in Han and Mulao populations.

The reason for these conflicting results is not yet known, probably because of differences in the study population, experiment designs, sample size, and the methods used to measure serum lipid levels. Different racial/ethnic groups have different genetic background or the *MARS* rs6782181 SNP had a linkage disequilibrium with the other genes. Furthermore, environmental factors were also positively correlated with serum lipid levels [12-14]. In the present study, we showed that serum lipid parameters were associated with age, sex, alcohol consumption, cigarette smoking, BMI, and blood pressure in both ethnic groups. These findings suggest that the environmental factors play a key role in determining serum lipid levels in our study populations.

There are several potential limitations in our study. Firstly, the sample size may be not big enough. Secondly, despite we have detected the association of *MARS* rs6782181 SNP and serum lipid levels in this study, there are still many unmeasured genetic and environmental factors. Thirdly, the interactions of gene-gene, gene-environment, and environment-environment were not studied in the present investigation. Therefore, further studies with large sample size, especially with the consideration of gene-gene and gene-environment interactions, will be needed to confirm our findings.

In conclusion, this study showed that the association of the *MARS* rs6782181 SNP and serum lipid levels is different between the Mulao and Han populations, or between males and females in the both ethnic groups. There may be an ethnic- and/or sex-specific association between the *MRAS* rs6782181 SNP and serum lipid levels in our study populations. But further studies with large sample size are needed to confirm our findings.

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Disclosure of conflict of interest

None.

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