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Original Article

The Influence of *INSIG2* rs7566605 polymorphism on serum lipid traits in the Han and Mulao ethnic groups

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Abstract: The influence of the insulin-induced gene 2 (*INSIG2*) rs7566605 single nucleotide polymorphism (SNP) on blood lipid traits in ethnic minorities of China is very little-known. The purpose of this study lies in detecting the influence of *INSIG2* rs7566605 SNP on blood lipid traits in the Han and Mulao ethnic groups. Genotyping of the *INSIG2* rs7566605 SNP was carried out in 830 Han and 861 Mulao subjects employed polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP), finally confirmed by sequencing. The minor allele carriers had higher ApoA1/ApoB ratio and lower serum low-density lipoprotein cholesterol (LDL-C) and apolipoprotein (Apo) B levels in Han, and higher high-density lipoprotein cholesterol (HDL-C), ApoA1 levels and the ApoA1/ApoB ratio and lower triglyceride (TG) in Mulao than the minor allele non-carriers. Gender subgroups researches revealed that the minor allele carriers had lower ApoB levels in Han male, and higher HDL-C and lower TG level in Mulao females than the minor allele non-carriers. Serum lipid traits were also associated with several environmental factors in the Han and Mulao ethnic groups, or in males and females in both ethnic groups. These findings suggest that the influence of the *INSIG2* rs756605 SNP and serum lipid traits might have ethnic- and/or sex-specificity in the Han and Mulao ethnic groups.

Keywords: Lipid, insulin-induced gene 2, single nucleotide polymorphism, environmental factors

Introduction

Cardiovascular disease (CVD) is a major cause of morbidity, mortality and loss of disability adjusted life years (DALYs) worldwide [1, 2]. Apart from the health burden, CVD will exert a significant economic burden, on the order of approximately $15 trillion over the next 20 years [3]. Availability of total cholesterol (TC) concentration [4], triglyceride (TG) concentration [5] along with high density lipoprotein cholesterol (HDL-C [6]), low density lipoprotein cholesterol (LDL-C [7]), apolipoprotein (Apo) A1 [8] and B [9] has always been a major of cardiovascular risk assessment and the main target of therapeutic intervention [10]. Estimates of heritability of the interindividual differences in blood lipid traits from both twin and family studies are within the range of 0.48 to 0.87 [11], meaning that there is considerable genetic influence. Therefore, understanding the influence of genetic variant characteristics of blood lipid traits may provide CVD preventive intervention goals of new insights.

In the past years, candidate genes of researches as well as genome-wide association studies (GWASs) discovered thousands of single nucleotide polymorphisms (SNPs) associated with human diseases related including dyslipidemia and CVD. These researches based on a large number of normal blood lipid individual showed that a few new SNPs can affect blood lipid traits including insulin-induced gene 2 (*INSIG2*) polymorphisms [12, 13]. INSIG2 protein, coded by *INSIG2*, mediates sterol regulation of sterol-regulatory element-binding proteins, cleavage-activating protein, and 3-hydroxy-3-methylglutaryl-coenzyme a reductase. INSIG2 plays important roles in cholesterol metabolism, lipogenesis, and glucose homeostasis [14, 15]. Previous researches have revealed that *INSIG2*

polymorphisms can influenced blood lipid traits. An *in vitro* research of allele specific expression in human adipose tissue demonstrated that *INSIG2* variants were involved in blood lipid traits regulation in human beings and in the general population [16, 17]. Previous studies elucidated *INSIG2* rs7566605 influenced dyslipidemia by a cluster of genome-wide scans in four unrelated samples of European origin, African American living in the U.S.A. and some Western European countries [18-20]. They looked into 86,604 SNPs in the analysis of family by 694 individuals from the Framingham Heart Study, and using two levels of testing strategy to avoid multiple comparisons. Susceptibility of the top 10 SNPs analysis the second from the first screening using transmission disequilibrium test validation. Only rs7566605 reached statistical significance. Rs7566605 is located ~10 kb upstream of *INSIG2*. On the other hand, many studies in other populations did not support this finding owing to the different genetic background of the studied populations [21-29]. The ethnic composition of Malaysia is comprised of 52.4% Malays, 28.6% Chinese, 6.4% Indians, 10.8% Indigenous, and 1.8% other ethnic groups from different genetic pools. In particular, Asians including Chinese (who have a lower plasma cholesterol levels and the incidence of heart attacks is lower than the population of the west) [30], seems to be different dyslipidemia genetic background. Lately, some studies have reported a lack of replication rs7566605 in > 4,000-10,000 European on a large scale [31-33]. The same situation happens in Afro-Caribbean and Indian groups [23, 34]. In order to further understand *INSIG2* rs7566605 susceptibility, we interpreted the influences of *INSIG2* rs7566605 on blood lipid traits in Chinese Han and Mulao ethnic group without known history or symptoms of disease especially dyslipidemia and CVD [35].

Since ancient times China is a multi-ethnic country. Among 56 nationalities in China, the Han nationality is the biggest one. In 2000 the fifth national census statistics of China showed Mulao (also known as Mulam) is one of 55 ethnic minorities, with a population of 207,352. Ninety percent of Mulao populations live in Luocheng Mulao Autonomous County, Guangxi Zhuang Autonomous Region. The history of Mulao ethnic minority can be dated back to the Jin dynasty (AD 265-420). One previous research had shown that the genetic relationship between Han and Mulao nationalities was closer than that between Han and Uighur nationalities [36]. In several previous researches, we have shown influence of several SNPs [37-39] on blood lipid traits in the Han and Mulao ethnic groups. As far as we known, influence of *INSIG2* rs7566605 SNP on blood lipid traits has not been explored in the south Chinese population. Hence, the purpose of this study lies in detecting the influence of *INSIG2* rs7566605 SNP and several environmental factors on blood lipid traits in the Han and Mulao ethnic groups.

Methods and materials

Participants

A total of 861 participants of Mulao populations (424 males, 49.25% and 437 females, 50.75%) and 830 participants of Han Chinese (401 men, 48.31% and 429 women, 51.69%) from our previous stratified randomized samples were randomly selected. They live in Luocheng Mulao Autonomous County, Guangxi Zhuang Autonomous Region in China. Age ranged from 15 to 80 years. Mulao subjects average age was 52.54 ± 14.69 years, whereas 52.39 ± 14.07 years in Han nationality participants. All subjects in nature were healthy rural people, and without known history or symptoms of disease especially atherosclerosis, CVD and diabetes. In addition, we had rule out any subjects with a history of taking medications known to affect blood lipid characteristics (lipid-lowering drugs such as beta-blockers, fibrates, statins, hormones and diuretics). The study informed consent was obtained from all participants and design was approved by the Ethics Committee of the First Affiliated Hospital, Guangxi Medical University.

Epidemiological survey

This survey followed a common protocol using the internationally standardized method [52]. Information related demographics, lifestyle factors and socioeconomic status was made a collection of standardized questionnaires. The alcohol consumption used *liang* (about 50 g) of corn wine, rice wine, beer, rum, and liquor consumed as a unit dose, at least 12 consecutive months. The alcohol consumption was divided

into 0 (nondrinker), ≤ 25 and > 25 grams of alcohol per day. Cigarette smoking was categorized into 0 (nondrinker), ≤ 20 and > 20 cigarettes per day. At the same time, we also measured several parameters including waist circumference, height and weight in the physical examination. After at least 5-minute of rest, sitting blood pressure (BP) was measured with the use of a mercury sphygmomanometer three times, and average of three measurements are used for representing the blood pressure level. Diastolic blood was determined by the fifth Korotkoff sound; nevertheless, systolic blood pressure by the first Korotkoff sound. Weight was estimated by the portable balance scales, and height by stadiometer. Body mass index (BMI, kg/m^2) was calculated from these above two measurements. By the non-stretchable measuring tape waist circumference was estimated.

Biochemical measurements

At least 12 hours of fasting, venous blood samples of 5 mL were collected. 2 mL of the blood samples was taken to determine serum lipid traits. 3 mL was shifted to a tube with anticoagulants (Shanghai Sangon Biological Engineering Technology & Services Co., Ltd., Shanghai, People's Republic of China), then used to extract deoxyribonucleic acid (DNA). Measurements of serum TG, TC, LDL-C and HDL-C levels were performed by enzymatic methods with commercially available kits (RANDOX Laboratories Ltd., Ardmore, Diamond Road, Crumlin Co. Antrim, United Kingdom, BT29 4QY; Daiichi Pure Chemicals Co., Ltd., Tokyo, Japan). Blood ApoA1 and ApoB levels were measured by the immunoturbidimetric immunoassay. Determinations were carried out in the Clinical Science Experiment Center of the First Affiliated Hospital, Guangxi Medical University with the auto-analyzer (Type 7170A; Hitachi Ltd., Tokyo, Japan) [37-39].

DNA amplification and genotyping

Genomic DNA was independent of peripheral blood leukocytes by using phenol-chloroform methods [37-39]. Extracted DNAs were stored at 4°C until DNA amplification and genotyping. Genotyping of *INSIG2* rs7566605 was determined by polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP). PCR amplification was performed using 5'-CCCTCCAATACCCCATCGGA-3' and 5'-GGGAATCGAGAGCTAAGGAT-3' as the forward and reverse primer pairs respectively (Shanghai Sangon Biological Engineering Technology & Services Co., Ltd., Shanghai, People's Republic of China). Each 25 μL PCR reaction system containing genomic DNA 2 μL, 12.5 μL 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; Tiangen, Beijing, China), and 8.5 μL of ddH_2O (DNase/RNase-free), and 1 μL of each primer (10 pmol/L). After initial denaturizing at 95°C for 7 min, the reaction mixture was subjected to 33 cycles of 45 s denaturation at 95°C, 30 s annealing at 65°C and extension 60 s at 72°C, followed by a final 7 min extension at 72°C. After electrophoresis on a 2.0% agarose gel with 0.5 μg/mL ethidium bromide, the amplified products were visualized under ultraviolet light. Then 5 U of *Mob*I (Shanghai Sangon Biological Engineering Technology & Services Co., Ltd., Shanghai, People's Republic of China) restriction enzyme was 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 2.0% agarose gels and visualized with ethidium-bromide staining ultraviolet illumination. Genotypes were scored by an experienced reader blinded to the epidemiological data and serum lipid traits.

DNA sequencing

To confirm the PCR-RFLP results, the PCR products of six samples (each two of GG, GC and CC genotypes) were sequenced with an ABI Prism 3100 (Applied Biosystems) at Shanghai Sangon Biological Engineering Technology & Services Co., Ltd., Shanghai, People's Republic of China.

Diagnostic criteria

Any individual with TC > 5.17 mmol/L and/or TG > 1.70 mmol/L was defined as the hyperlipidemia [53, 54]. Hypertension diagnosis standard is in according to the criteria of 1999 and 2003 World Health Organization-International Society of Hypertension Guidelines for the management of hypertension [55-57]. Overweight and obesity were according to the diagnosis criteria of the Cooperative Meta-analysis Group of China Obesity Task Force. Obesity, overweight and normal weight were defined as

Table 1. Comparison of demographic, lifestyle characteristics and serum lipid traits between the Han and Mulao ethnic groups

Parameter	Han	Mulao	*T* (X^2)	*P*
Number	830	861		
Male/female	401/429	424/437	0.147	0.702
Age (years)	52.39 ± 14.07	52.54 ± 14.69	-0.207	0.836
Height (cm)	155.03 ± 7.99	155.08 ± 8.01	-0.124	0.901
Weight (kg)	54.23 ± 9.25	52.63 ± 9.49	3.514	0.000
Body mass index (kg/m^2)	22.53 ± 3.36	21.82 ± 3.13	4.551	0.000
Waist circumference	75.82 ± 7.98	75.04 ± 8.55	1.949	0.051
Cigarette smoking (n %)				
Nonsmoker	570 (68.68)	661 (76.77)		
≤ 20 cigarettes/day	106 (12.77)	72 (8.36)	-3.536	0.000
> 20 cigarettes/day	154 (18.55)	128 (14.87)		
Alcohol consumption [n (%)]				
Nondrinker	621 (74.82)	667 (77.47)		
≤ 25 g/day	55 (6.63)	81 (9.41)	-1.686	0.092
> 25 g/day	154 (18.55)	113 (13.12)		
Systolic blood pressure (mmHg)	130.85 ± 18.71	129.32 ± 21.99	1.542	0.123
Diastolic blood pressure (mmHg)	82.74 ± 10.76	80.86 ± 11.55	3.462	0.001
Pulse pressure (mmHg)	48.11 ± 14.35	48.46 ± 17.11	-0.460	0.646
Glucose	6.21 ± 1.99	6.04 ± 1.69	1.893	0.059
Total cholesterol (mmol/L)	5.06 ± 0.95	5.05 ± 1.10	0.236	0.814
Triglyceride (mmol/L)	1.11 (0.82)	1.03 (0.75)	-3.823	0.000
HDL-C (mmol/L)	1.73 ± 0.41	1.79 ± 0.45	-2.990	0.003
LDL-C (mmol/L)	2.93 ± 0.80	2.97 ± 0.86	-0.854	0.393
Apolipoprotein (Apo) A1 (g/L)	1.36 ± 0.25	1.31 ± 0.43	2.848	0.004
ApoB (g/L)	0.87 ± 0.20	1.02 ± 0.61	-6.869	0.000
ApoA1/ApoB	1.63 ± 0.51	1.51 ± 0.68	4.264	0.000

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.

BMI > 28, 24-28, and < 24 kg/m^2, respectively [58]. The normal values of serum TC, TG, HDL-C, LDL-C, ApoA1, ApoB levels, and the ratio of ApoA1/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.

Statistical analyses

Epidemiological data were recorded on a predesigned form and managed with Excel software. The statistical analysis was performed using the statistical software package SPSS 19.0 (SPSS Inc., Chicago, Illinois). The quantitative variable were presented as the means ± standard deviation (serum TG levels were presented as medians and interquartile ranges) and as frequencies or percentages for categorical variables. Fisher's exact test or chi-square test was used to determine difference in genotype distribution between the groups. General characteristics between Han and Mulao populations were tested by the Student's unpaired *t*-test. The association of genotypes and serum lipid traits (TG excepted, by non-parametric tests) was tested by ANCOVA. Multivariate linear regression analysis with stepwise modeling was performed to assess the association of serum lipid traits with genotypes (GG = 1, GC = 2 and CC = 3) or alleles (the minor allele non-carrier = 1 and the minor allele carrier = 2) with the adjusting of potential confounders including sex, age, blood pressure, cigarette smoking, alcohol consumption and BMI. Any statistical result at a value of $P < 0.05$ was considered statistical significance.

Results

General and biochemical characteristics of the participants

As shown in **Table 1**, the comparison of general and biochemical characteristics and serum lipid traits between the Han and Mulao ethnic groups is summarized. The levels of body weight, BMI, the percentage of cigarette smoking, diastolic blood pressure, serum TG, ApoA1 levels and the ApoA1/ApoB ratio were higher in Han than in Mulao ($P < 0.05$-0.001), whereas the levels of HDL-C and ApoB was lower in Han

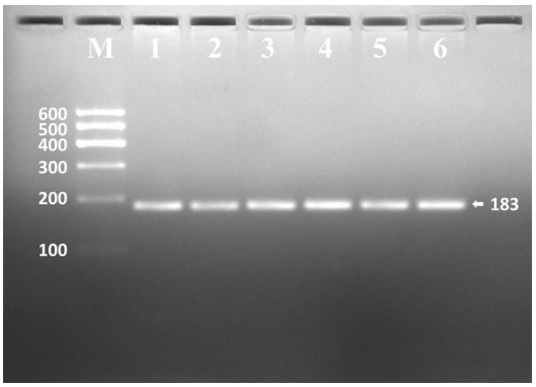

Figure 1. Electrophoresis of PCR products of the samples. Lane M is the 100 bp Marker ladder; lanes 1-6 are samples, the 183 bp bands are the target genes.

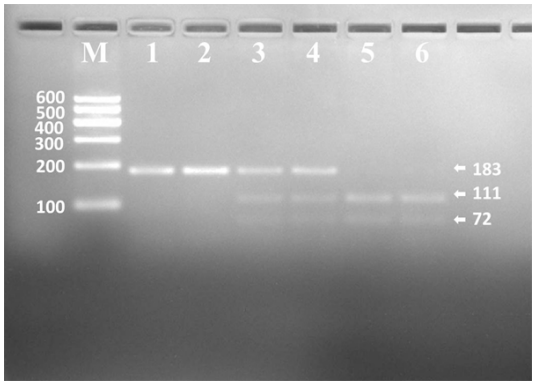

Figure 2. Genotyping of the *INSIG2* rs7566605 SNP. Lane M is the 100 bp Marker Ladder; lanes 1 and 2, GG genotype (183-bp); lanes 3 and 4, GC genotype (183-, 111- and 72-bp); and lanes 5 and 6, CC genotype (111- and 72-bp).

than in Mulao ($P < 0.05$-0.001). There were no significant differences in the gender ratio, age structure, body height, waist circumference, the percentage of Alcohol consumption, systolic blood pressure, pulse pressure, blood glucose, TC and LDL-C levels between the two ethnic groups ($P > 0.05$ for all).

Results of electrophoresis

The PCR products of *INSIG2* rs7566605 were 183-bp nucleotide sequences (**Figure 1**). After the RFLP reaction combined with electrophoresis, the genotypes identified were named according to the presence (C allele) or absence (G allele) of the enzyme restriction sites. Thus, CC genotype was homozygote for the presence of the site (bands at 111- and 72-bp), GC genotype was heterozygote for the presence and absence of the site (bands at 183-, 111- and 72-bp), and GG genotype was homozygote for the absence of the site (bands at 183-bp; **Figure 2**).

DNA sequencing

The results were shown as GG, GC and CC genotypes by PCR-RFLP, the GG, GC and CC genotypes were also confirmed by forward sequencing (**Figure 3**), respectively.

Genotypic and allelic frequencies

As shown in **Table 2**, the frequency of GG, GC and CC genotypes in males and females 66.27% *vs.* 62.46%, 23.59% *vs.* 32.55%, and 10.14% *vs.* 4.99% in Mulao, respectively. The frequency of G and C allele in males and females was 78.07% *vs.* 72.08% and 21.93% *vs.* 27.92% in Mulao ($P < 0.05$-0.001); respectively. There were no differences in the genotypic and allele frequencies of *INSIG2* rs7566605 SNP between Han and Mulao, or between males and females in Han populations, respectively ($P > 0.05$ for all).

Genotypes and serum lipid traits

Serum LDL-C, ApoB levels and the ApoA1/ApoB ratio in Han were different among the genotypes (**Tables 3** and **4**, $P < 0.05$-0.001), the minor allele carriers had lower serum LDL-C and ApoB levels and higher the ApoA1/ApoB ratio than the minor allele non-carriers. Serum TG, HDL-C, ApoA1 levels and the ApoA1/ApoB ratio were different among the genotypes in Mulao ($P < 0.05$-0.001), the minor allele carriers had lower serum TG and higher HDL-C, ApoA1 levels and the ApoA1/ApoB ratio than the minor allele non-carriers. Subgroup analyses showed that serum ApoB levels in Han males were different ($P < 0.05$); the minor allele carriers had lower serum ApoB levels than the minor allele non-carriers. There was no significantly difference in serum lipid traits among the genotypes in Han females. Accordingly, serum TG and HDL-C in levels Mulao females were different among the genotypes ($P < 0.05$ for each). The minor allele carriers had lower serum TG and higher serum HDL-C levels than the minor allele non-carriers in Mulao females. There was no significantly difference in serum

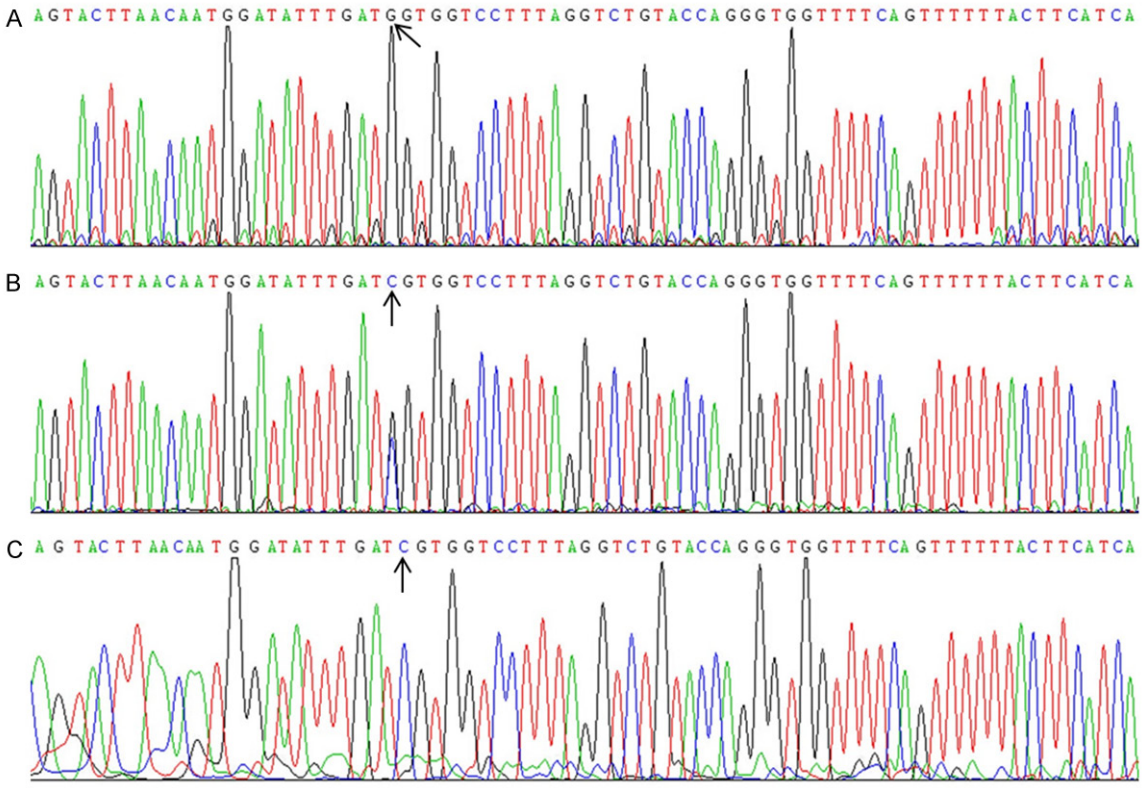

Figure 3. A part of the nucleotide forward sequence of the *INSIG2* rs756605 SNP. A: GG genotype; B: GC genotype; C: CC genotype.

Table 2. Comparison of the genotype and allele frequencies of *INSIG2* rs7566605 SNP in the Han and Mulao ethnic groups [n (%)]

Group	n	Genotype			Allele	
		GG	GC	CC	G	C
Han	830	534 (64.34)	197 (23.73)	99 (11.93)	1265 (76.20)	395 (23.80)
Mulao	861	535 (62.14)	222 (25.78)	104 (12.08)	1292 (75.03)	430 (24.97)
X^2	–		1.048		0.634	
P			0.592		0.426	
Han						
Male	401	263 (65.59)	98 (24.44)	40 (9.97)	624 (77.81)	178 (22.19)
Female	429	271 (63.17)	99 (23.08)	59 (13.75)	641 (82.67)	217 (17.33)
X^2			2.830		2.192	
P			0.243		0.139	
Mulao						
Male	424	281 (66.27)	100 (23.59)	43 (10.14)	662 (78.07)	186 (21.93)
Female	437	254 (62.46)	122 (32.55)	61 (4.99)	630 (72.08)	244 (27.92)
X^2			6.463		69.478	
P			0.039		0.000	

lipid traits among the genotypes in Mulao males.

Relative factors for serum lipid traits

The multiple linear regression statistics revealed that serum TC, HDL-C, LDL-C and ApoA1 levels in both ethnic groups, LDL-C, ApoA1 and the ApoA1/ApoB ratio in Han and TC, HDL-C, LDL-C, ApoA1 levels and the ApoA1/ApoB ratio in Mulao were correlated with the genotypes (P < 0.05-0.001; **Table 5**).

Subgroup analyses according to gender showed that serum ApoB levels in Han males, LDL-C, ApoA1 levels and the ApoA1/ApoB ratio in Han females,

Table 3. Comparison of the genotypes and serum lipid traits in the Han and Mulao ethnic groups

Ethnic/ Genotype	N	TC (mmol/L)	TG (mmol/L)	HDL-C (mmol/L)	LDL-C (mmol/L)	Apo A1 (g/L)	ApoB (g//L)	ApoA1/ApoB
Han								
GG	534	5.10 ± 0.99	1.14 (0.86)	1.71 ± 0.40	2.98 ± 0.82	1.34 ± 0.24	0.88 ± 0.20	1.60 ± 0.49
GC	197	5.02 ± 0.86	1.12 (0.78)	1.73 ± 0.41	2.91 ± 0.76	1.36 ± 0.26	0.87 ± 0.19	1.63 ± 0.51
CC	99	4.92 ± 0.94	0.98 (0.70)	1.82 ± 0.47	2.72 ± 0.72	1.40 ± 0.28	0.82 ± 0.21	1.81 ± 0.61
F		4.833	5.680	4.017	6.926	3.953	11.852	9.155
P		0.089	0.058	0.134	0.031	0.139	0.003	0.010
Mulao								
GG	535	5.08 ± 1.03	1.08 (0.79)	1.75 ± 0.40	3.01 ± 0.85	1.16 ± 0.38	1.00 ± 0.55	1.27 ± 0.62
GC	222	5.01 ± 1.08	0.98 (0.73)	1.84 ± 0.46	2.91 ± 0.84	1.22 ± 0.47	1.08 ± 0.70	1.39 ± 0.74
CC	104	4.98 ± 1.46	0.95 (0.61)	1.88 ± 0.60	2.88 ± 0.96	1.24 ± 0.55	1.05 ± 0.68	1.46 ± 0.80
F		0.291	11.527	9.007	2.175	9.030	0.162	7.440
P		0.865	0.003	0.011	0.337	0.011	0.922	0.024

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.

Table 4. Comparison of the genotypes and serum lipid traits between males and females in the Han and Mulao ethnic groups

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								
GG	263	5.21 ± 0.95	1.22 (0.93)	1.66 ± 0.42	3.00 ± 0.82	1.36 ± 0.28	0.93 ± 0.20	1.53 ± 0.48
GC	98	5.07 ± 0.82	1.18 (0.78)	1.74 ± 0.42	2.89 ± 0.69	1.39 ± 0.29	0.90 ± 0.18	1.61 ± 0.51
CC	40	5.01 ± 0.83	1.00 (0.79)	1.77 ± 0.51	2.81 ± 0.63	1.40 ± 0.30	0.86 ± 0.20	1.73 ± 0.60
F		4.519	4.433	3.598	2.705	2.249	8.293	4.430
P		0.104	0.109	0.165	0.259	0.325	0.016	0.109
Han/female								
GG	271	5.00 ± 1.01	1.02 (0.80)	1.76 ± 0.37	2.97 ± 0.82	1.33 ± 0.20	0.84 ± 0.18	1.67 ± 0.48
GC	99	4.98 ± 0.91	1.05 (0.71)	1.71 ± 0.40	2.92 ± 0.82	1.33 ± 0.23	0.86 ± 0.19	1.64 ± 0.51
CC	59	4.86 ± 1.01	0.86 (0.64)	1.86 ± 0.44	2.67 ± 0.77	1.40 ± 0.26	0.80 ± 0.21	1.87 ± 0.61
F		1.154	2.587	4.115	5.053	3.359	4.141	5.045
P		0.562	0.274	0.128	0.080	0.186	0.126	0.080
Mulao/male								
GG	281	5.09 ± 0.97	1.12 (0.83)	1.74 ± 0.43	2.96 ± 0.80	1.36 ± 0.40	1.03 ± 0.62	1.55 ± 0.66
GC	100	4.95 ± 1.08	1.05 (0.70)	1.76 ± 0.45	2.86 ± 0.82	1.23 ± 0.45	1.10 ± 0.72	1.36 ± 0.67
CC	43	4.99 ± 1.43	1.03 (0.63)	1.79 ± 0.60	2.74 ± 0.95	1.31 ± 0.51	1.00 ± 0.65	1.57 ± 0.72
F		0.477	4.420	0.795	2.756	4.224	0.612	5.298
P		0.788	0.110	0. 672	0.252	0.121	0.736	0.071
Mulao/female								
GG	254	5.07 ± 1.09	1.01 (0.76)	1.77 ± 0.37	3.05 ± 0.90	1.35 ± 0.35	0.96 ± 0.46	1.59 ± 0.58
GC	122	5.05 ± 1.09	0.94 (0.74)	1.90 ± 0.45	2.96 ± 0.86	1.22 ± 0.49	1.07 ± 0.68	1.41 ± 1.79
CC	61	4.98 ± 1.48	0.88 (0.59)	1.94 ± 0.60	2.98 ± 0.97	1.19 ± 0.57	1.07 ± 0.70	1.39 ± 0.86
F		0.010	6.059	9.650	0.456	4.554	0.097	5.494
P		0.995	0.048	0.008	0.796	0.103	0.952	0.064

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.

Table 5. Relationship between serum lipid parameters and relative factors in the Han and Mulao ethnic groups

Lipid parameter	Risk factor	*B*	*Std. error*	*Beta*	*t*	*P*
Han and Mulao						
TC	Genotype	-0.074	0.035	-0.050	-2.119	0.034
	Age	0.008	0.002	0.110	3.464	0.001
	Waist circumference	0.010	0.005	0.084	2.114	0.035
	Diastolic blood pressure	0.008	0.002	0.085	3.347	0.001
TG	Ethnic group	-0.254	0.063	-0.093	-4.035	0.000
	Age	-0.008	0.003	-0.086	-2.830	0.005
	Cigarette smoking	0.188	0.051	0.104	3.680	0.000
	Body mass index	-0.245	0.062	-0.582	-3.963	0.000
	Waist circumference	0.031	0.006	0.186	4.874	0.000
	Diastolic blood pressure	0.006	0.003	0.048	1.973	0.049
	Glucose	0.062	0.018	0.084	3.525	0.000
HDL-C	Ethnic group	0.054	0.020	0.063	2.724	0.007
	Genotype	0.044	0.014	0.071	3.104	0.002
	Gender	0.098	0.030	0.114	3.323	0.001
	Age	0.002	0.001	0.083	2.724	0.007
	Alcohol consumption	0.091	0.016	0.158	5.701	0.000
	Waist circumference	-0.007	0.002	-0.133	-3.489	0.000
LDL-C	Genotype	-0.098	0.028	-0.082	-3.484	0.001
	Age	0.007	0.002	0.121	3.845	0.000
	Diastolic blood pressure	0.005	0.002	0.062	2.448	0.014
ApoA1	Ethnic group	-0.043	0.017	-0.060	-2.530	0.012
	Genotype	-0.027	0.012	-0.054	-2.289	0.022
	Alcohol consumption	0.093	0.014	0.195	6.820	0.000
	Waist circumference	-0.004	0.002	-0.104	-2.622	0.009
	Pulse Pressure	0.002	0.001	0.109	4.162	0.000
ApoB	Ethnic group	0.160	0.022	0.174	7.338	0.000
	Waist circumference	0.009	0.002	0.165	4.184	0.000
	Pulse Pressure	0.003	0.001	0.110	4.243	0.000
	Glucose	0.018	0.006	0.072	2.944	0.003
ApoA1/ApoB	Ethnic group	-0.135	0.028	-0.112	-4.808	0.000
	Gender	0.143	0.042	0.118	3.414	0.001
	Alcohol consumption	0.101	0.023	0.124	4.455	0.000
	Waist circumference	-0.015	0.003	-0.198	-5.147	0.000
Han						
TC	Age	0.008	0.003	0.117	2.525	0.012
	Alcohol consumption	0.115	0.051	0.095	2.281	0.023
	Waist circumference	0.017	0.007	0.138	2.322	0.020
	Diastolic blood pressure	0.014	0.003	0.158	4.404	0.000
	Glucose	0.038	0.017	0.079	2.239	0.025
TG	Gender	0.387	0.175	0.119	2.214	0.027
	Age	-0.013	0.005	-0.108	-2.402	0.017
	Cigarette smoking	0.403	0.086	0.195	4.689	0.000
	Body mass index	-0.226	0.088	-0.467	-2.575	0.010
	Waist circumference	0.051	0.012	0.249	4.323	0.000
	Diastolic blood pressure	0.011	0.005	0.073	2.084	0.037

	Glucose	0.098	0.028	0.119	3.507	0.000
HDL-C	Gender	0.117	0.044	0.142	2.658	0.008
	Age	0.003	0.001	0.103	2.302	0.022
	Alcohol consumption	0.091	0.021	0.174	4.334	0.000
	Body mass index	0.047	0.022	0.385	2.126	0.034
	Waist circumference	-0.009	0.003	-0.165	-2.872	0.004
LDL-C	Genotype	-0.093	0.039	-0.082	-2.418	0.016
	Gender	-0.198	0.089	-0.124	-2.228	0.026
	Age	0.007	0.003	0.129	2.751	0.006
	Cigarette smoking	-0.171	0.044	-0.169	-3.910	0.000
	Diastolic blood pressure	0.008	0.003	0.110	3.043	0.002
	Glucose	0.035	0.014	0.089	2.511	0.012
ApoA1	Genotype	0.024	0.012	0.066	1.978	0.048
	Gender	0.071	0.028	0.140	2.550	0.011
	Cigarette smoking	0.055	0.014	0.173	4.064	0.000
	Alcohol consumption	0.080	0.013	0.249	6.051	0.000
ApoB	Gender	-0.057	0.021	-0.145	-2.761	0.006
	Waist circumference	0.006	0.001	0.248	4.379	0.000
	Diastolic blood pressure	0.002	0.001	0.128	3.750	0.000
	Glucose	0.012	0.003	0.126	3.770	0.000
ApoA1/ApoB	Genotype	0.055	0.024	0.075	2.315	0.021
	Gender	0.252	0.055	0.246	4.595	0.000
	Cigarette smoking	0.102	0.027	0.157	3.785	0.000
	Alcohol consumption	0.065	0.026	0.100	2.482	0.013
Mulao						
TC	Genotype	-0.125	0.054	-0.079	-2.330	0.020
	Age	0.009	0.003	0.116	2.632	0.009
	Pulse Pressure	0.005	0.002	0.079	2.120	0.034
TG	Gender	-0.275	0.095	-0.132	-2.887	0.004
	Body mass index	-0.257	0.111	-0.771	-2.326	0.020
	Waist circumference	0.017	0.006	0.135	2.594	0.010
	Pulse Pressure	0.005	0.002	0.078	2.143	0.032
HDL-C	Genotype	0.043	0.021	0.068	2.050	0.041
	Gender	0.087	0.041	0.097	2.120	0.034
	Alcohol consumption	0.091	0.025	0.142	3.693	0.000
	Waist circumference	-0.006	0.003	-0.109	-2.085	0.037
LDL-C	Genotype	-0.119	0.042	-0.097	-2.854	0.004
	Age	0.007	0.003	0.114	2.602	0.009
ApoA1	Genotype	-0.083	0.021	-0.136	-4.052	0.000
	Alcohol consumption	0.123	0.024	0.200	5.133	0.000
	Waist circumference	-0.008	0.003	-0.168	-3.172	0.002
	Pulse Pressure	0.004	0.001	0.155	4.206	0.000
ApoB	Waist circumference	0.010	0.004	0.142	2.637	0.009
	Pulse Pressure	0.005	0.001	0.147	3.925	0.000
	Glucose	0.030	0.012	0.083	2.386	0.017
ApoA1/ApoB	Genotype	-0.066	0.032	-0.068	-2.040	0.042
	Alcohol consumption	0.161	0.038	0.167	4.304	0.000
	Waist circumference	-0.018	0.004	-0.227	-4.329	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.

LDL-C and ApoA1 levels in Mulao males and TG, HDL-C and ApoA1 levels in Mulao females were correlated with genotypes (**Table 6**, $P < 0.05$-0.001).

As shown in **Tables 5** and **6**, serum lipid traits were also correlated with age, gender, BMI, systolic blood pressure, diastolic blood pressure, blood glucose levels, cigarette smoking and alcohol consumption in both populations ($P < 0.05$-0.001).

Discussions

Several previous studies have explored the variants in the *INSIG2*, but the association of the SNP of rs7566605 near the *INSIG2* locus and serum lipid traits had not been previously systematically reported in the Han and Mulao populations. Although the genotypic and allelic frequencies of the *INSIG2* rs7566605 SNP had been reported previously in different racial/ethnic- and sex- groups, but the results of previous studies were inconsistent. In the present study, we showed that the frequencies of the minor allele of *INSIG2* rs7566605 SNP were 23.80% *vs.* 24.97% in Han and Mulao, 22.19% *vs.* 17.33% in Han males and females, and 21.93% *vs.* 27.92% in Mulao males and females; respectively. As a comparison, the frequencies of the minor allele of the *INSIG2* rs7566605 polymorphism were 44% in the Malaysian Malay population [40], 23% in the Uyghur population, 28% in the White American population, 36% in the Han Chinese and Japanese populations, 41% in the African population, and 37% or 31% in populations with West European ancestry [18]. What's more, in our present study, the frequency of GG, GC and CC genotypes in males and females was 66.27% *vs.* 62.46%, 23.59% *vs.* 32.55%, and 10.14% *vs.* 4.99% in Mulao, respectively. The frequency of G and C allele in males and females was 78.07% *vs*. 72.08% and 21.93% *vs*. 27.92% in Mulao ($P < 0.05$-0.001); respectively. These results suggest that the prevalence of C allele frequency of *INSIG2* rs7566605 SNP may have racial/ethnic- and sex-specificity.

These findings also suggest that there may be a racial/ethnic- and sex-specific association of the *INSIG2* rs7566605 SNP and serum lipid traits. First, the minor allele carriers had lower serum LDL-C and ApoB levels and higher the ApoA1/ApoB ratio than the minor allele non-carriers in Han. Second, the minor allele carriers had lower serum TG and higher serum HDL-C, ApoA1 levels and the ApoA1/ApoB ratio than the minor allele non-carriers in Mulao. Third, subgroup analyses showed that the minor allele carriers had lower serum ApoB levels than the minor allele non-carriers in Han males. Fourth, the minor allele carriers had lower serum TG and higher serum HDL-C level than the minor allele non-carriers in Mulao females. However, several previous studies found that the *INSIG2* rs7566605 SNP was associated with BMI, but not with waist hip ratio (WHR), SBP, DBP, TG and cholesterol levels in the Chinese minority group in Xinjiang Uyghur, Northeast China [20]. Polymorphism on *INSIG2* rs7566605 was not associated with BMI, lipoprotein parameters, and free fatty acid levels in the Utah and Austrian population [24]. Similarly, the *INSIG2* rs7566605 polymorphism had no effect on TC, TG, HDL-C, LDL-C, or blood pressure parameters in the Chinese population [22]. In addition, the *INSIG2* rs7566605 SNP had no effect on TG levels in two UK-based cohorts [34]. No association was observed between this polymorphism and obesity-related traits, except for WHR, in White, Hispanic, and African-American subjects [26]. Similarly, there was no association between the *INSIG2* rs7566605 SNP and BMI or obesity-related traits in Indian subjects [23]. The *INSIG2* rs7566605 SNP was not significantly associated with TC, LDL-C, HDL-C and TG levels in Malaysian Malays. This was similarly observed in Korean and Japanese population [19, 41, 42]. The *INSIG2* rs7566605 SNP was not associated with BMI, WHR, plasma levels of cholesterols, or TG in the Slavonic Caucasian population [43]. Those differences also may be related to variations in examined populations, including healthy, hypercholesterolemia and overweight/obese subjects; modulating environmental factors such as diet or pharmaceutical treatments. Therefore this association needs to be further confirmed with larger sample.

Accordingly, in addition to the *INSIG2* rs7566605 SNP, these data demonstrated that several environmental factors including gender, age, waist circumference, BMI, systolic blood pressure, diastolic blood pressure, blood glucose, cigarette smoking and alcohol consumption, also had some influences on serum lipid traits in both ethnic groups. Although rice and

Table 6. Relationship between serum lipid parameters and relative factors in the males and females of the Han and Mulao ethnic groups

Lipid parameter	Risk factor	*B*	*Std. error*	*Beta*	*t*	*P*
Han/male						
TC	Genotype	-0.479	0.154	-0.205	-3.104	0.002
	Alcohol consumption	0.204	0.083	0.156	2.464	0.014
	Weight	-0.085	0.041	-0.706	-2.045	0.042
	Waist circumference	0.047	0.015	0.348	3.053	0.003
	Diastolic blood pressure	0.027	0.007	0.265	4.173	0.000
TG	Cigarette smoking	0.734	0.321	0.145	2.289	0.023
	Waist circumference	0.192	0.046	0.485	4.164	0.000
	Diastolic blood pressure	0.040	0.020	0.131	2.020	0.044
HDL-C	Alcohol consumption	0.095	0.033	0.179	2.919	0.004
	Height	0.054	0.014	0.932	3.841	0.000
	Weight	-0.068	0.016	-1.400	-4.189	0.000
	Body mass index	0.120	0.034	1.076	3.526	0.001
	Diastolic blood pressure	0.008	0.003	0.191	3.111	0.002
LDL-C	Genotype	-0.364	0.124	-0.200	-2.932	0.004
	Cigarette smoking	-0.347	0.086	-0.261	-4.062	0.000
ApoA1	Genotype	-0.084	0.038	0.134	-2.209	0.028
	Cigarette smoking	0.129	0.020	0.366	6.302	0.000
	Alcohol consumption	0.083	0.015	0.269	5.378	0.000
	Height	0.032	0.009	0.835	3.630	0.000
	Weight	-0.046	0.010	-1.438	-4.543	0.000
	Body mass index	0.069	0.021	0.945	3.267	0.001
	Diastolic blood pressure	0.005	0.002	0.182	3.116	0.002
ApoB	Genotype	-0.071	0.028	-0.162	-2.518	0.012
	Alcohol consumption	0.034	0.015	0.137	2.214	0.028
	Waist circumference	0.009	0.003	0.351	3.160	0.002
	Diastolic blood pressure	0.003	0.001	0.145	2.352	0.019
	Glucose	0.016	0.008	0.131	2.052	0.041
ApoA1/ApoB	Cigarette smoking	0.141	0.044	0.188	3.228	0.001
	Alcohol consumption	0.101	0.034	0.177	2.964	0.003
	Height	0.045	0.015	0.718	3.047	0.003
	Weight	-0.056	0.017	-1.077	-3.317	0.001
	Body mass index	0.080	0.035	0.667	2.250	0.025
Han/female						
TC	Age	0.023	0.004	0.296	5.290	0.000
	Waist circumference	0.024	0.011	0.160	2.129	0.034
TG	Waist circumference	0.047	0.013	0.283	3.664	0.000
	Diastolic blood pressure	0.016	0.005	0.146	3.025	0.003
	Glucose	0.137	0.038	0.168	3.618	0.000
HDL-C	Age	0.008	0.003	0.156	2.543	0.011
LDL-C	Age	0.023	0.004	0.358	6.406	0.000
	Height	0.103	0.048	0.703	2.139	0.033
	Weight	-0.184	0.073	-1.462	-2.530	0.012
	Body mass index	0.411	0.164	1.427	2.505	0.013
	Waist circumference	0.021	0.009	0.176	2.339	0.020
ApoA1	Age	0.003	0.001	0.152	2.582	0.010

	Alcohol consumption	0.228	0.075	0.162	3.045	0.002
ApoB	Age	0.002	0.001	0.139	2.589	0.010
	Waist circumference	0.008	0.002	0.309	4.282	0.000
	Pulse Pressure	0.002	0.001	0.103	2.292	0.022
	Glucose	0.013	0.006	0.102	2.352	0.019
ApoA1/ApoB	Alcohol consumption	0.365	0.141	0.129	2.584	0.010
	Waist circumference	-0.017	0.005	-0.262	-3.518	0.000
	Pulse Pressure	-0.005	0.002	-0.131	-2.822	0.005
Mulao/male						
TC	Glucose	-0.277	0.130	-0.142	-2.131	0.034
HDL-C	Alcohol consumption	0.158	0.039	0.274	4.045	0.000
LDL-C	Genotype	-0.124	0.061	-0.101	-2.028	0.043
ApoA1	Genotype	-0.074	0.031	-0.117	-2.422	0.016
	Alcohol consumption	0.151	0.030	0.339	5.054	0.000
ApoB	Pulse Pressure	0.009	0.004	0.161	2.417	0.017
ApoA1/ApoB	Alcohol consumption	0168	0.053	0.216	3.182	0.002
	Pulse pressure	-0.010	0.005	-0.134	-2.023	0.044
Mulao/female						
TC	Age	0.016	0.004	0.199	3.876	0.000
TG	Alcohol consumption	1.026	0.329	0.141	3.119	0.002
	Height	-0.141	0.066	-0.683	-2.146	0.032
	Weight	0.226	0.102	1.455	2.214	0.027
	Body mass index	-0.525	0.236	-1.346	-2.223	0.027
	Waist circumference	0.041	0.012	0.246	3.472	0.001
HDL-C	Age	0.003	0.001	0.112	2.223	0.027
	Waist circumference	-0.009	0.004	-0.177	-2.523	0.012
LDL-C	Age	0.011	0.003	0.169	3.328	0.001
ApoB	Waist circumference	0.011	0.005	0.166	2.332	0.020
	Glucose	0.091	0.030	0.139	3.022	0.003
ApoA1/ApoB	Waist circumference	-0.022	0.008	-0.211	-2.940	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.

corn are the staple foods in both groups, Mulao people living in an enclosed environment shared their typical diet. They eat too many acidic and spicy dishes, local bean soy sauce, pickled vegetables and animal offal's which contain abundant saturated fatty acid. Many studies stated that diet alone can account for the variability on serum lipid traits [44-46]. It has been reported that diet rich in polyunsaturated fatty acids, monounsaturated fatty acids, carbohydrates, and even one saturated fatty acid, stearic acid can reduce LDL-C levels [47, 48]. Our findings also showed that different dietary habits, lifestyles and environmental factors probably further modified the effect of genetic variation on serum lipid traits in our study populations. Many studies have stated that daily eating habits can strongly influence on serum levels of ApoB, ApoA1 and their ratio, and which in turn can come into being the risk of CVD [49-51]. The difference in the association of this SNP and serum lipid traits between the two ethnic groups might be partly attributed to the difference in daily eating habits between the Han and Mulao ethnic groups.

There are still several potential limitations including many unmeasured environmental and genetic factors and their interactions in the present study. In addition, the study size was not enough quantity. Thus, the interactions of gene-gene, environment-environment and gene-environment on serum lipid traits remain to be determined.

Conclusions

This study interpreted that the *INSIG2* rs7566605 SNP and a little environmental factors were influence of some serum lipid parameters in the Han and Mulao ethnic groups, but the associated trends of the SNP and serum lipid parameters are different. The difference in serum lipid traits between the Han and Mulao populations might result from different *INSIG2* rs7566605 SNP.

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Disclosure of conflict of interest

None.

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