Int J Clin Exp Pathol 2018;11(5):2887-2900
www.ijcep.com /ISSN:1936-2625/IJCEP0073216

Original Article

Association of *CPS1* rs1047891 SNP and serum lipid levels in two Chinese ethnic groups

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Received January 22, 2018; Accepted March 8, 2018; Epub May 1, 2018; Published May 15, 2018

Abstract: Carbamoyl-phosphate synthase 1 gene (*CPS1*) rs1047891 single nucleotide polymorphism (SNP) has been associated with a number of metabolic disorders including obesity, insulin resistance, and hyperhomocysteine (HCY). Studies on association between this SNP and prevalence of dyslipidemia have been few, with no report from Chinese subjects. This study was to investigate association of rs1047891 SNP and several environment factors with serum lipid levels in Chinese Han and Maonan populations. Genotypes of rs1047891 SNP in 810 individuals of Maonan and 795 participants of Han nationality were determined by polymerase chain reaction-restriction fragment length polymorphism and then confirmed by direct sequencing. Frequencies of CC, CA, and AA genotypes were 71.32%, 25.16%, and 3.52% in Han and 61.36%, 31.85%, and 6.79% in Maonan populations ($P < 0.01$), respectively. The frequency of A allele was 16.10% in Han and 22.72% in Maonan individuals ($P < 0.001$), respectively. Subjects with CA/AA genotypes had lower high-density lipoprotein cholesterol (HDL-C) and apolipoprotein (Apo) A1 levels in Han. They had higher low-density lipoprotein cholesterol (LDL-C) levels and lower HDL-C levels in Maonan than subjects with CC genotype ($P < 0.05$-0.01). Subgroup analyses revealed that subjects with CA/AA genotypes had lower HDL-C and ApoA1 levels in Han females, higher LDL-C levels in Maonan males, and lower HDL-C levels in both Maonan males and females than subjects with CC genotype ($P < 0.05$-0.01). Serum lipid parameters were also correlated with several environmental factors in both ethnic groups. The difference in serum lipid profiles between Han and Maonan populations may partly result from different polymorphisms of *CPS1* rs1047891 and SNP-enviromental interactions.

Keywords: Carbamoyl-phosphate synthase 1, single nucleotide polymorphism, lipids, environmental factor

Introduction

Clinical and epidemiological studies have demonstrated that serum lipid and lipoprotein concertrations are closely related to coronary heart disease (CHD), which is the leading cause of morbidity and mortality worldwide [1, 2]. There are numerous known risk factors for CHD, of which dyslipidemia, in particular low-density lipoprotein cholesterol (LDL-C) elevation [3] and high-density lipoprotein cholesterol (HDL-C) depression [4], is mainly involved in development and progression of CHD. Previous studies have proven an inverse and independent association between plasma concentrations of HDL-C and the incidence of CHD [5, 6]. HDL-C is believed to play a key role in the process of reverse cholesterol transport, removing cholesterol from peripheral tissues and returning it to the liver for biliary excretion [7]. There are significant negative correlations between fasting and postprandial plasma triglyceride (TG) levels and HDL-C concentrations, suggesting a close link between TG and HDL metabolism in human body [8]. Serum lipid levels are complex phenotypes. It has been well established that serum lipid levels are significantly influenced by body weight, current smoking habits, alcohol use, and dietary fat intake [9, 10]. In addition to environmental factors, strong evidence has shown that serum lipid levels in the general population are also heritable traits. The heritability of HDL-C levels has been estimated to be greater than 50% in many studies [8, 11]. Genome wide association studies (GWASes) have identified numerous genetic polymorphsims that influence plasma lipid levels but related vairants in GWAS have accounted for only

5-8% of variation in serum HDL-C levels [12]. Collectively, specific genetic variants that contribute to serum HDL-C levels have remained unexplained.

Carbamoyl-phosphate synthase 1 gene (*CPS1*; also known as: PHN; CPSASE1, Gene ID: 1373, OMIM: 608307, chromosoma location: 2q34), which is highly expressed in liver, encodes the mitochondrial enzyme and plays a pivotal role in catalyzing the first committed step of the hepatic urea cycle [13]. Previous GWASes have identified 62 loci associated with lipid levels in humans including association of *CPS1* rs1047891 SNP and HDL-C in Europeans [14]. *CPS1* rs1047891 SNP is located in the 3' untranslated region and is in near perfect linkage disequilibrium (LD, $r^2 = 0.93$) with rs715 in subjects of northern European ancestry. This region of *CPS1* has been reported to play a key role in glycine and serum homocysteine (Hcy) metabolism. However, the biological function of *CPS1* rs1047891 SNP on serum lipid metabolism remains unclear. As we known, the human genetic variation on serum lipid levels has different magnitudes of effects in different ethnicities. To the best of our knowledge, information on association between *CPS1* rs1047891 SNP and serum lipid levels has not been previously reported in the Chinese populations. Thus, the aim of our present study was to detect association of *CPS1* rs1047891 SNP and several environmental factors with serum lipid profiles in Chinese Han and Maonan populations.

Materials and methods

Subjects

Participants in the present study included 810 unrelated individuals of Maonan (325 males, 40.12% and 486 females, 59.88%) and 795 unrelated participants of Han (305 males, 38.36% and 490 females, 61.64%) descent. They were randomly selected from previous stratified randomized samples. All participants were agricultural workers living in Huanjiang Maonan Autonomous County, Guangxi Zhuang Autonomous Region, People's Republic of China. Age of the participants ranged from 25 to 80 years, with a mean age of 56.05±11.67 years in Han and 57.14±14.99 years in Maonan ($P > 0.05$), respectively. All study subjects were essentially healthy with no history of cardiovascular disease such as CHD and stroke, diabetes, hyper-or hypo-thyroids, and chronic renal disease. We excluded subjects who had a history of taking medications known to affect serum lipid levels (lipid-lowering drugs such as statins or fibrates, beta blockers, diuretics, or hormones) before blood samples were drawn. The present study was approved by the Ethics Committee of the First Affiliated Hospital, Guangxi Medical University (No. Lunshen-2014-KY-Guoji-001, Mar. 7, 2014). Informed consent was obtained from all participants.

Epidemiological survey

The survey was carried out using internationally standardized methods, following a common protocol [15]. Information on demographics, socioeconomic status, and lifestyle factors was collected with standardized questionnaires. Alcohol consumption was categorized into groups of grams of alcohol per day: 0 (non-drinker), < 25, and ≥ 25. Smoking status was categorized into groups of cigarettes per day: 0 (non-smoker), < 20, and ≥ 20. Several parameters such as blood pressure, height, weight, waist circumference, and body mass index (BMI) were measured or calculated. Methods of measuring the above parameters were referred to a previous study [16].

Biochemical measurements

A fasting venous blood sample of 5 mL was drawn from the participants. Part of the sample (2 mL) was collected into glass tubes and used to determine serum lipid levels and another part (3 mL) was shifted to tubes with anticoagulants (4.80 g/L citric acid, 14.70 g/L glucose and 13.20 g/L tri-sodium citrate) and used to extract deoxyribonucleic acid (DNA). Measurements of serum total cholesterol (TC), TG, HDL-C, and LDL-C levels in samples were performed by enzymatic methods with commercially available kits (RANDOX Laboratories Ltd., Ardmore, Diamond Road, CrumlinCo. Antrim, United Kingdom, BT29 4QY; Daiichi Pure Chemicals Co., Ltd., Tokyo, Japan). Serum apolipoprotein (Apo) A1 and ApoB levels were measured by immunoturbidimetric immunoassay using a commercial kit (RANDOX Laboratories Ltd.). All determinations were performed with an auto-analyzer (Type 7170A; Hitachi Ltd., Tokyo, Japan) in the Clinical Science Experiment Center of the First Affiliated Hospital, Guangxi Medical University [17].

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

Parameter	Han	Maonan	t (x^2)	P
Number	795	810		
Male/female	305/490	325/485	0.425	0.514
Age (years)	56.05±11.67	57.14±14.99	1.383	0.167
Height (cm)	154.01±7.74	153.79±8.07	0.483	0.629
Weight (kg)	52.91±8.85	53.24±10.64	-0.607	0.544
Body mass index (kg/m^2)	22.28±3.25	23.40±3.58	-0.631	0.528
Waist circumference (cm)	75.13±7.81	76.93±9.28	-3.755	0.000
Smoking status [*n* (%)]				
Non-smoker	662 (83.27)	601 (74.19)		
≤ 20 cigarettes/day	107 (13.46)	161 (19.88)		
> 20 cigarettes/day	26 (3.27)	48 (5.93)	15.209	0.001
Alcohol consumption [*n* (%)]				
Non-drinker	642 (80.75)	634 (78.27)		
≤ 25 g/day	69 (8.68)	99 (12.22)		
> 25 g/day	84 (10.57)	77 (9.51)	4.269	0.118
Systolic blood pressure (mmHg)	129.08±19.55	135.91±24.36	-5.274	0.000
Diastolic blood pressure (mmHg)	81.23±11.53	82.92±12.25	-2.459	0.014
Pulse pressure (mmHg)	47.85±15.36	53.00±18.39	-4.639	0.000
Glucose (mmol/L)	6.04±1.74	6.15±1.41	-1.260	0.208
Total cholesterol (mmol/L)	4.92±1.03	5.01±1.06	-1.419	0.156
Triglyceride (mmol/L)	1.17 (0.52)	1.30 (0.51)	-2.489	0.013
HDL-C (mmol/L)	1.74±0.51	1.60±0.33	5.601	0.000
LDL-C (mmol/L)	2.85±0.78	2.91±0.79	-1.288	0.198
ApoA1 (g/L)	1.37±0.25	1.36±0.23	0.175	0.861
ApoB (g/L)	0.85±0.20	0.88±0.19	-2.879	0.004
ApoA1/ApoB	1.70±0.52	1.63±0.49	2.479	0.013

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

DNA amplification and genotyping

Genomic DNA of the samples was extracted from peripheral blood leucocytes, according to phenol-chloroform method [18]. Extracted DNA was stored at 4°C until analysis. Genotyping of *CPS1* rs1047891 SNP was performed by polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP). PCR amplification was performed using 5'-CATGCCTCTGGACTGTGAGT-3' and 5'-CGGAAACAAGTGAGAGCATGA-3' (Sangon, Shanghai, People's Republic of China) as the forward and reverse primer pairs, respectively. Each 25 μL PCR reaction mixture consisted of 2.0 μL genomic DNA, 1.0 μL each primer (10 μmol/L), 12.5 μL of 2 × *Taq* PCR Master mix (constituent: 0.1 U *Taq* polymerase/μL, 500 μM dNTP each and PCR buffer), and 8.5 μL of ddH_2O (DNase/RNase-free). PCR was performed with an initialization step of 95°C for 5 minutes, followed by 30 seconds denaturing at 95°C, 30 seconds of annealing at 58°C, and 30 seconds of elongation at 72°C for 33 cycles. Amplification was completed by a final extension at 72°C for 7 minutes. Following electrophoresis on a 2.0% agarose gel with 0.5 μg/mL ethidium bromide, the amplification products were visualized under ultraviolet light. Subsequently, each restriction enzyme reaction was performed with 5.0 μL amplified DNA, 8.8 μL nuclease-free water, 1.0 μL of 10 × buffer solution, and 0.2 μL *Oli*I restriction enzyme in a total volume of 15 μL digested at 55°C overnight. After restriction enzyme digestion of amplified DNA, genotypes were identified by electrophoresis on 2% ethidium-bromide stained agarose gels and visualized with UV illumination. Genotypes were scored by an experienced reader blinded to epidemiological and serum lipid results. Six samples (CC, CA and AA genotypes in two, respectively) detected by PCR-RFLP were also confirmed by direct sequencing with an ABI Prism 3100 (Applied Biosystems) in Shanghai Sangon Biological Engineering Technology & Services Co., Ltd., People's Republic of China.

Diagnostic criteria

Normal values of serum TC, TG, HDL-C, LDL-C, ApoA1, ApoB levels, and the ApoA1/ApoB ratio in our Clinical Science Experiment Center were 3.10-5.17, 0.56-1.70, 1.16-1.42, 2.70-3.10 mmol/L, 1.20-1.60, 0.80-1.05 g/L, and 1.00-

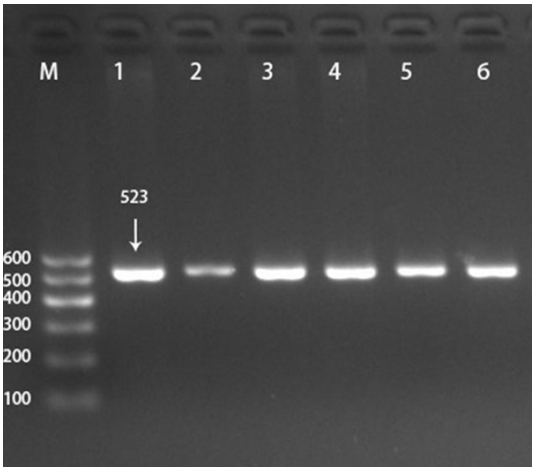

Figure 1. Electrophoresis of polymerase chain reaction products of samples. Lane M is the 100 bp marker ladder; lanes 1-6 are samples, the 523 bp bands are the target genes.

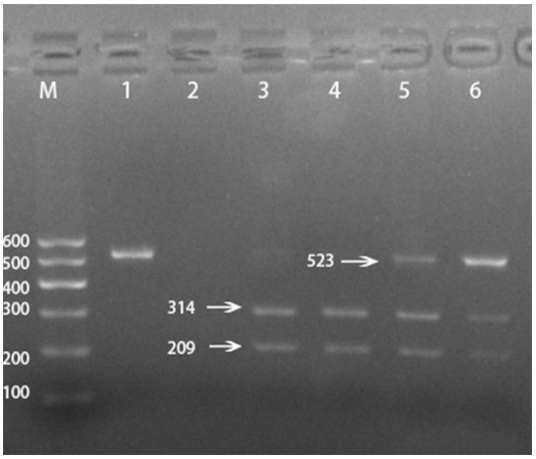

Figure 2. Genotyping of *CPS1* rs1047891 SNP. Lane M, 100 bp marker ladder; lanes 1, AA genotype (523-bp); lanes 2 and 3, CC genotype (314- and 209-bp); and lanes 5 and 6, CA genotype (523-, 314- and 209-bp).

2.50, respectively. Individuals with TC > 5.17 mmol/L and/or TG > 1.70 mmol/L were defined as hyperlipidemic [19]. Hypertension diagnosis standard is according to the cirteria of 1999 and 2003 World Health Organization-International Society of Hypertension Guidelines for management of hypertension [20]. Diagnostic criteria for overweight and obesity were according to the Cooperative Meta analysis Group of China Obesity Task Force. Normal weight, overweight, and obesity were defined as a BMI < 24, 24-28, and > 28 kg/m^2, respectively.

Statistical analyses

Epidemilolgical data were recorded on a predesigned form and managed with Excel software. Data analysis was performed with statistical software package SPSS 22.0 (SPSS Inc., Chicago, Illinois). Quantitative variables were presented as mean ± standard deviation (serum TG levels were presented as medians and interquartile ranges). Allele frequency was determined via direct counting and Hardy-Weinberg equilibrium was verified with the standard goodness-of-fit test. Genotype distribution between the two groups was analyzed by Chi-square test. General characteristics between two ethnic groups were compared by Student's unpaired *t*-test. Association between genotypes and serum lipid parameters was tested by covariance analysis (ANCOVA). Gender, age, BMI, blood pressure, alcohol consumption, and cigarette smoking were adjusted for statistical analysis. Multivariate linear regression analyses with stepwise modeling were used to determine correlation between the genotypes (CC = 1, CA = 2, AA = 3) and several environmental factors with serum lipid levels in combined population of Maonan and Han, Maonan, Han, males, and females, respectively. A *P* value of less than 0.05 was considered statistically significant.

Results

General characteristics and serum lipid levels

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

Results of electrophoresis and genotyping

After genomic DNA of the samples was amplified using PCR and visualized with 2% agarose

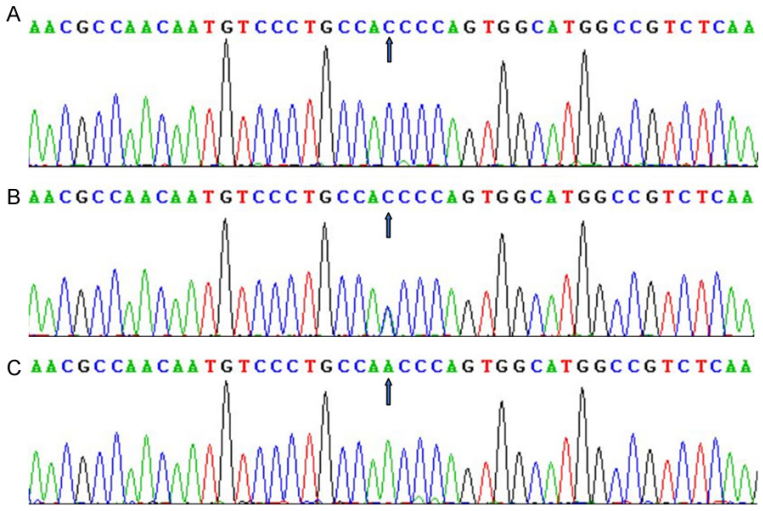

Figure 3. A part of the nucleotide sequence of the *CPS1* rs1047891 SNP. A. CC genotype; B. CA genotype; C. AA genotype.

gel electrophoresis, the products of 523 bp nucleotide sequences were observed in all samples (**Figure 1**). Genotypes identified were termed according to the presence (C allele) or absence (A allele) of enzyme restriction sites. Thus, CC genotype was homozygous for the presence of the site (bands at 314 bp and 209 bp). CA genotype was heterozygous for the presence and absence of the site (bands at 523-, 314- and 209-bp) and AA genotype was homozygous for the absence of the site (bands at 523 bp; **Figure 2**). CC, CA, and AA genotypes detected by PCR-RFLP were also confirmed by direct sequencing (**Figure 3**), respectively.

Genotypic and allelic frequencies

Genotypic and allelic frequencies of *CPS1* rs1047891 SNP are shown in **Table 2**. Frequencies of C and A alleles were 83.90% and 16.10% in Han and 77.28% and 22.72% in Maonan ($P = 0.000$), respectively. Frequencies of CC, CA, and AA genotypes were 71.32%, 25.16%, and 3.52% in Han and 61.36%, 31.85%, and 6.79% in Maonan ($P = 0.002$), respectively. There was no significant difference in genotypic and allelic frequencies between males and females in both ethnic groups ($P > 0.05$ for all).

Genotypes and serum lipid levels

As shown in **Tables 3** and **4**, serum levels of HDL-C and ApoA1 in Han were different between CC and CA/AA genotypes ($P < 0.05$-0.001). A allele carriers had lower serum HDL-C and ApoA1 levels than A allele non-carriers. Serum HDL-C and LDL-C levels in Maonan were different between CC and CA/AA genotypes ($P < 0.05$-0.001). A allele carriers had lower serum HDL-C and higher LDL-C levels than A allele non-carriers. Gender-subgroup analysis showed that serum HDL-C and ApoA levels in Han females but not in males were different between the genotypes ($P < 0.05$-0.01). A allele carriers had lower serum HDL-C and ApoA1 levels ($P < 0.05$-0.01) than A allele non-carriers. In contrast, serum HDL-C levels in both Maonan males and females were different between genotypes ($P < 0.05$ for each), serum LDL-C levels in Maonan males but not in females were different between genotypes ($P < 0.05$). A allele carriers had lower serum HDL-C and higher LDL-C levels ($P < 0.05$-0.01) than A allele non-carriers.

Relative factors for serum lipid parameters

Multiple linear regression analysis showed that serum HDL-C and ApoA1 in Han and HDL-C and LDL-C in Maonan were correlated with genotypes of *CPS1* rs1047891 SNP ($P < 0.05$-0.01; **Table 5**). When the correlation of serum lipid parameters and genotypes was analyzed according to gender, we found that serum HDL-C and ApoA1 levels were assocated with genotypes in Han females and HDL-C levels in both Maonan males and females and LDL-C levels in Maonan males were correlated with the genotypes ($P < 0.05$-0.01 for all; **Table 6**). Serum lipid phenotypes were also associated with environmental factors such as age, gender, BMI, waist circumference, blood pressure, blood glucose, cigarette smoking, and alcohol consumption in both ethnic groups ($P < 0.05$-0.001; **Tables 5** and **6**).

Discussion

CHD is the most common cause of fatality, disability, and economic loss, particularly in industrialized nations. Serum lipid levels are heritable, modifiable, and risk factors for CHD.

Table 2. Comparison of genotype and allele frequencies of *CPS1* rs1047891SNP in Han and Maonan populations [*n* (%)]

Group	*n*	Genotype			Allele	
		CC	CA	AA	C	A
Han	795	567 (71.32)	200 (25.16)	28 (3.52)	1334 (83.90)	256 (16.10)
Maonan	810	497 (61.36)	258 (31.85)	55 (6.79)	1252 (77.28)	368 (22.72)
x^2		12.367			22.424	
P		0.002			0.000	
Han						
Male	305	206 (67.54)	87 (28.52)	12 (3.93)	499 (81.80)	111 (18.20)
Female	490	358 (73.06)	116 (23.67)	16 (3.27)	832 (84.90)	148 (15.10)
x^2		1.359			0.028	
P		0.507			0.868	
Maonan						
Male	325	192 (59.08)	109 (33.54)	24 (7.38)	493 (75.85)	157 (24.31)
Female	485	305 (62.89)	149 (30.72)	31 (6.39)	759 (78.25)	211 (21.75)
x^2		0.949			1.278	
P		0.622			0.258	

Heritability estimates of interindividual variations in blood lipid levels from both twin and family studies are in the range of 40-70% [8, 21]. Therefore, human genetic studies of lipid levels contribute to identifying targets for new therapies for cholesterol management and prevention of heart disease. The Maonan ethnic group is a relatively conservative minority with a population of 107,166, according to China's sixth national census in 2010. Approximately 80% of total Maonan people live in Huanjiang Maonan Autonomous County in Guangxi Zhuang Autonomous Region. In spite of a very small population, this Maonan ethnic group is well known in China for its long history and unique culture. Maonan nationality preserves their custom of consanguineous marriage to cousins of maternal side, suggesting that the genetic background of Maonan population may be less heterogeneous within the population. Recent phylogenetic and principal component analyses have revealed that Maonan people belong to the southeastern Asian group and are most closely related to Buyi people [22]. Recently, Wang et al. showed that prevalence of hypercholesterolemia, hypertriacylglycerolemia, and hyperlipidemia was higher in Maonan than in Han populations [23]. These data indicate that different genetic backgrounds may have important influence on serum lipid levels. Therefore, we can speculate that some hereditary characteristics and genotypes of lipid metabolism-related genes in this population may be different from those in Han nationality.

In our current study, we showed that allelic and genotypic distribution of rs1047891 SNP was different between the two ethnic groups. A allele frequency of *CPS1* rs1047891 SNP was lower in Han than in Maonan populations (16.10% *vs* 22.72%; $P < 0.001$). The distribution of CC, CA, and AA genotypes was also different between the two ethnic groups ($P < 0.01$). Gender subgroup analysis showed that there were no conspicuous differences in genotypic and allelic frequencies between males and females in Maonan and Han nationalities. Minor A allele frequencies in Han (16.10%) and Maonan (22.72%) were in close proximity to those of Chinese Han Beijing (13.41%) reported in international haplotype map (HapMap) project. According to HapMap data, the frequency of A allele was 29.46% in European, 28.32% in Yoruba, and 15.29% in Japanese. These results suggest that genotype and allele frequencies of *CPS1* rs1047891 SNP are inconsistent among diverse ethnic groups.

The potential association of *CPS1* rs1047891 SNP and serum lipid levels has not been previously reported in different ethnic groups. There were hardly any previous studies presenting a direct relationship between *CPS1* rs1047891 SNP and serum lipid levels in humans, except a new GWAS which showed that *CPS1* rs1047891 was significantly associated with HDL-C concentrtions in populations of European descent [14]. In the present study, we found that *CPS1* rs1047891 SNP was significantly associated with multiple serum lipid parameters in Maonan and Han populations. A allele carriers in Han females had lower HDL-C and ApoA1 levels whereas A allele carriers had lower HDL-C levels in both Maonan sexes and higher LDL-C levels in Maonan males than A allele non-carriers. These findings suggest that association of *CPS1* rs1047891 SNP and serum lipid profiles

Table 3. Comparison of genotypes and serum lipid levels in Han and Maonan populations

Genotype	*n*	TC (mmol/L)	TG (mmol/L)	HDL-C (mmol/L)	LDL-C (mmol/L)	ApoA1 (g/L)	ApoB (g/L)	ApoA1/ApoB
Han								
CC	567	4.84±0.99	1.15 (0.57)	1.76±0.54	2.83±0.78	1.37±0.23	0.85±0.19	1.72±0.54
CA/AA	228	4.98±0.84	1.20 (0.49)	1.64±0.43	2.90±0.70	1.31±0.28	0.86±0.22	1.62±0.46
F		1.382	1.195	5.108	0.074	4.870	0.204	3.331
P		0.168	0.232	0.024	0.380	0.028	0.651	0.069
Maonan								
CC	313	4.94±0.98	1.20 (0.49)	1.64±0.33	2.81±0.79	1.37±0.25	0.86±0.19	1.67±0.49
CA/AA	497	5.01±1.29	1.29 (0.54)	1.54±0.30	2.94±0.78	1.35±0.20	0.88±0.21	1.61±0.42
F		0.554	1.670	16.233	4.775	1.706	0.908	2.387
P		0.457	0.095	0.000	0.030	0.123	0.341	0.123

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 between the genotypes was determined by the Kruskal-Wallis test.

Table 4. Comparison of genotypes and serum lipid levels between males and females in Han and Maonan populations

Ethnic/Genotype	*n*	TC (mmol/L)	TG (mmol/L)	HDL-C (mmol/L)	LDL-C (mmol/L)	ApoA1 (g/L)	ApoB (g/L)	ApoA1/ApoB
Han/male								
CC	206	5.02±0.70	1.19 (0.51)	1.69±0.45	2.91±0.82	1.37±0.27	0.89±0.19	1.59±0.51
CA/AA	99	5.09±1.01	1.24 (0.62)	1.67±0.42	2.95±0.59	1.36±0.28	0.91±0.21	1.58±0.45
F		0.235	0.373	0.052	0.070	0.028	0.127	0.005
P		0.628	0.709	0.819	0.719	0.868	0.722	0.942
Han/female								
CC	358	4.71±0.95	1.13 (0.46)	1.81±0.59	2.78±0.76	1.37±0.20	0.81±0.18	1.76±0.54
CA/AA	132	4.95±0.95	1.25 (0.36)	1.61±0.42	2.86±0.77	1.28±0.27	0.83±0.23	1.64±0.46
F		3.616	1.702	7.407	0.606	8.841	0.286	4.085
P		0.058	0.089	0.007	0.437	0.003	0.593	0.054
Maonan/male								
CC	192	4.82±0.92	1.33 (0.59)	1.61±0.31	2.73±0.65	1.36±0.28	0.86±0.16	1.65±0.47
CA/AA	133	5.06±1.09	1.35 (0.61)	1.50±0.34	2.95±0.73	1.33±0.20	0.90±0.19	1.54±0.42
F		4.043	0.762	6.172	6.219	1.071	2.452	3.195
P		0.055	0.446	0.014	0.013	0.302	0.119	0.075
Maonan/female								
CC	305	4.97±1.43	1.18 (0.45)	1.67±0.34	2.78±0.66	1.38±0.23	0.85±0.20	1.68±0.48
CA/AA	180	5.03±1.01	1.28 (0.47)	1.57±0.26	2.94±0.71	1.36±0.21	0.87±0.21	1.66±0.41
F		0.232	1.563	9.541	1.125	0.587	0.679	0.021
P		0.630	0.118	0.002	0.326	0.444	0.410	0.871

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 between the genotypes was determined by the Kruskal-Wallis test.

may have a racial/ethnic specificity in our study populations. The reason for these discrepancies is not yet known. In addition to different genetic background, sample size, different statistical methods, and different gene-gene or gene-environment interactions may have also contributed to discrepancies within our study and other studies in different populations.

The SNP of rs1047891 on chromosome 2q34 is located in the 3' untranslated region. It encodes Thr1405Asn in gene *CPS1* and explains 3% of

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

Lipid	Risk factor	B	Std. error	Beta	*t*	*P*
Han and Maonan						
TC	Waist circumference	0.015	0.006	0.125	2.524	0.012
	Age	0.008	0.003	0.111	3.002	0.003
	Alocohol consumption	0.165	0.061	0.100	2.725	0.007
	Height	-0.044	0.019	-0.336	-2.733	0.020
	Weight	0.050	0.025	0.484	1.998	0.046
TG	Alocohol consumption	0.335	0.076	0.152	4.431	0.000
	Height	-0.075	0.024	-0.433	-3.190	0.001
	Weight	0.108	0.031	0.789	3.446	0.001
	Waist cirumference	0.032	0.007	0.206	4.402	0.000
	Glucose	0.078	0.025	0.092	3.086	0.002
	Body mass index	-0.193	0.067	0.496	2.877	0.004
HDL-C	Ethnic group	-0.077	0.032	-0.092	-2.446	0.015
	Gender	0.077	0.036	0.091	2.113	0.035
	Alocohol consumption	0.081	0.023	0.124	3.449	0.001
	Waist circumference	-0.007	0.002	-0.156	-3.184	0.001
LDL-C	Genotype	0.099	0.049	0.075	2.023	0.043
	Age	0.008	0.002	0.134	3.684	0.000
	Waist circumference	0.014	0.004	0.157	3.244	0.001
	Glucose	0.036	0.015	0.076	2.456	0.014
ApoA1	Gender	0.056	0.021	0.116	2.667	0.008
	Cigarette smoking	0.010	0.004	0.080	2.554	0.011
	Alocohol consumption	0.102	0.014	0.273	7.530	0.000
ApoB	Age	0.001	0.001	0.101	2.911	0.004
	Waist circumference	0.007	0.001	0.291	6.238	0.000
	Glucose	0.011	0.004	0.093	3.150	0.002
	Distolic blood pressure	0.001	0.000	0.073	2.364	0.018
ApoA1/ApoB	Gender	0.095	0.041	0.097	2.355	0.019
	Age	-0.003	0.001	-0.094	-2.725	0.007
	Alcohol consumption	0.098	0.026	0.129	3.771	0.002
	Waist circumference	-0.015	0.003	-0.285	-6.128	0.000
Han						
TC	Alcohol consumption	0.266	0.089	0.171	2.987	0.003
	Pulse pressure	0.006	0.003	0.102	2.041	0.043
	Glucose	0.065	0.026	0.126	2.557	0.011
TG	Alcohol consumption	0.263	0.121	0.118	2.173	0.030
	Waist circumference	0.038	0.014	0.217	2.778	0.006
	Distolic blood pressure	0.011	0.005	0.098	2.043	0.042
	Glucose	0.099	0.035	0.134	2.859	0.004
HDL-C	Genotype	0.114	0.055	0.101	2.055	0.041
	Alcohol consumption	0.101	0.050	0.120	2.034	0.043
	Waist circumference	-0.014	0.004	-0.208	-3.094	0.002
LDL-C	Age	0.008	0.003	0.126	2.549	0.011
	Glucose	0.053	0.021	0.128	2.533	0.012
	Waist circumference	0.016	0.008	0.159	2.046	0.041
ApoA1	Genotype	0.053	0.025	0.101	2.115	0.035

the variability in Hcy levels. The minor allele of rs1047891 has been associated with CAD-related traits including increased Hcy and creatinine levels but decreased homoarginine and fibrinogen levels [24-26]. A genome-wide meta-analysis of Hcy metabolism indentified that *CPS1* rs1047891 SNP was strongly associated with circulating Hcy levels [27]. Hcy is a non-protein-forming sulfur amino acid produced during the catabolism of methionine. Methionine is considered to be an important precursor of S-adenosylmethionine (SAM). Hcy concentration may play a pathogenic role in vascular damage by promoting oxidative stress, systemic inflammation, and endothelial dysfunction. Elevated blood Hcy levels has been correlated with risk of CHD atherosclerosis and dyslipdemia. A retrospective study on Hcy and lipids showed that disturbed Hcy metabolism results in decreased SAM, thus affecting synthesis of phospholipids [28]. The hypothesis that high Hcy might reduce production of HDL-C has been tested in mice being fed a methionine rich diet [29], suggesting that its mechanisms are related to downregulation of crucial play-

	Distolic blood pressure	0.002	0.001	0.120	2.287	0.023
	Cigarette smoking	0.112	0.029	0.215	3.855	0.000
	Alcohol consumption	0.113	0.023	0.283	4.988	0.000
ApoB	Gender	-0.070	0.028	-0.170	-2.487	0.013
	Alcohol consumption	0.040	0.017	0.124	2.315	0.021
	Waist circumference	0.006	0.002	0.233	3.061	0.002
	Glucose	0.018	0.005	0.167	3.634	0.000
ApoA1/ApoB	Gender	0.207	0.072	0.200	2.863	0.004
	Waist circumference	-0.011	0.005	-0.169	-2.179	0.030
	Cigarette smoking	0.197	0.057	0.185	3.445	0.001
	Glucose	-0.031	0.013	-0.113	-2.402	0.017
Maonan						
TC	Age	0.011	0.004	0.154	3.169	0.002
	Height	-0.057	0.026	-0.419	-2.183	0.029
	Weight	0.076	0.035	0.749	2.159	0.031
	Body mass index	-0.155	0.076	-0.520	-2.035	0.042
	Waist circumference	0.017	0.007	0.144	2.288	0.023
TG	Alcohol consumption	0.375	0.098	0.171	3.812	0.000
	Height	-0.090	0.032	-0.515	-2.832	0.005
	Weight	0.129	0.043	0.991	3.012	0.003
	Waist circumference	0.027	0.009	0.183	3.058	0.002
HDL-C	Genotype	-0.121	0.025	-0.184	-4.815	0.000
	Gender	0.087	0.036	0.133	2.430	0.015
	Alcohol consumption	0.071	0.023	0.144	3.151	0.002
	Waist circumference	-0.008	0.002	-0.233	-3.831	0.000
LDL-C	Genotype	0.174	0.062	0.108	2.813	0.005
	Age	0.008	0.002	0.159	3.375	0.001
	Waist circumference	0.016	0.005	0.191	3.157	0.002
ApoA1	Gender	0.085	0.026	0.179	3.211	0.001
	Cigarette smoking	0.008	0.004	0.086	2.141	0.033
	Alcohol consumption	0.093	0.017	0.257	5.530	0.000
	Waist circumference	-0.004	0.002	-0.149	-2.402	0.017
ApoB	Age	0.002	0.001	0.149	3.253	0.001
	Waist circumference	0.007	0.001	0.318	5.342	0.000
ApoA1/ApoB	Waist circumference	-0.017	0.003	-0.335	-5.733	0.000
	Age	-0.004	0.001	-0.112	-2.488	0.013
	Alcohol consumption	0.122	0.032	0.168	3.836	0.000

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

ers in HDL-C production. In line with this, high Hcy was highly correlated with decreased activity of hepatic thiolase and serum lecithin-cholesterol acyltransferase (LCAT), two primary enzymes involved in HDL-C matabolism [30]. Another line of evidence suggested that Hcy inhibits liver expression of ApoA1 and reduces levels of blood ApoA1 and HDL-C [31]. Clinical studies on patients with CHD documented a negative association between Hcy and serum HDL-C levels [18]. Taken together, we speculated that *CPS1* rs1047891 SNP was supposed to have a close connection with serum lipid metabolism but the detailed role of this polymorphism requires further exploration.

The interaction of gene-environment on serum lipid parameters cannot be ignored. In the present study, we summarized that values of waist circumference, blood pressure, and cigarette smoking were significant differences in the two ethnic groups. We also found that gender, weight, waist circumference, BMI, blood pressure, fasting blood glucose levels, alcohol consumption, and cigarette smoking were correlated with lipid profiles in both Maonan and Han populations. These data demonstrate that environmental exposures also play an essential role in determing serum lipid profiles. It is generally recognized that an unhealthy diet is strongly associated with dyslipidemia [32]. The Maonan people prefer to eating pickled sour meat, snails, and animal offals which contain abundant saturated fatty acids. This preference of a high-fat diet can give rise to higher blood pressure, serum TG, ApoB, and lower HDL-C levels in Maonan than in Han people. A meta-analysis revealed that every 1% alteration in total energy from saturated fatty acids

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

Lipid	Risk factor	B	Std. error	Beta	*t*	*P*
Han/male						
TC	Alcohol consumption	0.252	0.096	0.222	2.614	0.010
TG	Waist circumference	0.154	0.047	0.428	3.295	0.001
HDL-C	Cigarette smoking	0.125	0.053	0.185	2.359	0.020
	Alcohol consumption	0.092	0.042	0.175	2.191	0.030
	Height	0.036	0.017	0.639	2.084	0.039
LDL-C	Cigarette smoking	-0.211	0.095	-0.174	-2.209	0.029
ApoA1	Cigarette smoking	0.105	0.033	0.245	3.187	0.002
	Alcohol consumption	0.108	0.026	0.323	4.139	0.000
	Weight	-0.027	0.013	-0.896	-2.137	0.034
ApoB	Diastolic blood pressure	0.003	0.001	0.173	2.046	0.043
	Glucose	0.021	0.009	0.178	2.239	0.027
	Alcohol consumption	0.043	0.020	0.175	2.164	0.032
ApoA1/ApoB	Cigarette smoking	0.163	0.060	0.209	2.701	0.008
	Height	0.040	0.019	0.624	2.061	0.041
Han/female						
TC	Age	0.013	0.005	0.149	2.442	0.015
	Glucose	0.070	0.031	0.140	2.303	0.022
TG	Waist circumference	0.040	0.016	0.240	2.425	0.016
	Diastolic blood pressure	0.016	0.007	0.148	2.302	0.022
	Glucose	0.085	0.038	0.137	2.211	0.028
HDL-C	Genotype	0.193	0.081	0.154	2.384	0.018
	Weight	-0.014	0.005	-0.178	-2.740	0.007
LDL-C	Age	0.012	0.005	0.170	2.309	0.022
	Glucose	0.057	0.025	0.142	2.335	0.020
	Waist circumference	0.022	0.010	0.208	2.247	0.025
ApoA1	Genotype	0.089	0.032	0.177	2.773	0.006
	Waist circumference	-0.005	0.002	-0.142	-2.344	0.020
	Pulse pressure	0.003	0.001	0.167	2.732	0.007
ApoB	Waist circumference	0.006	0.003	0.216	2.258	0.025
	Glucose	0.017	0.006	0.168	2.800	0.006
ApoA1/ApoB	Cigarette smoking	0.590	0.231	0.166	2.560	0.011
	Waist cicumference	-0.014	0.007	-0.193	-1.991	0.048
	Glucose	-0.032	0.016	-0.121	-1.992	0.047
Maonan/male						
TC	Weight	0.031	0.006	0.333	4.897	0.000
	Body mass index	0.082	0.016	0.271	5.011	0.000
	Glucose	0.090	0.042	0.132	2.182	0.030
TG	Alcohol consumption	0.410	0.144	0.173	2.856	0.005
	Glucose	0.202	0.082	0.148	2.496	0.014
HDL-C	Genotype	-0.101	0.039	-0.153	-2.597	0.010
	Alcohol consumption	0.085	0.024	0.216	3.582	0.000
	Waist circumference	-0.010	0.004	-0.304	-2.788	0.006
LDL-C	Genotype	0.206	0.084	0.147	2.449	0.015
	Alcohol consumption	-0.107	0.052	-0.128	-2.075	0.039
ApoA1	Cigarette smoking	0.008	0.004	0.118	2.024	0.044
	Alcohol consumption	0.102	0.018	0.339	5.694	0.000

will lead to a change in TG of 1.9 mg/dl; LDL-C of 1.8 mg/dl, and HDL-C of 0.3 mg/dl [33]. Long term consumption of a high saturated fat diet is a major risk for obesity, lipid metabolic disorder, and atherosclerosis [34]. Moreover, the percentages of subjects who smoked cigarettes were higher in Maonan than in Han. Cigarette smoking has been implicated as a key modifiable risk factor for CHD and dyslipidemia. The adverse effects of smoking on CHD risk are mediated through multiple interrelated mechanisms including increased oxidative stress, endothelial injury, and derangements of lipid metabolism [35]. Cross-sectional studies have revealed that cigarette smoking is associated with a more atherogenic lipid profile and characterized by an increase in concentrations of TC and LDL-C and lower concentrations of HDL-C [36]. Another study also indentified that there was an inverse correlation between HDL-C levels and tobacco users [37]. In conclusion, it is possible that the difference is in dietary habits, lifestyles, and environmental factors between Han and Maonan ethnic groups. These factors could partly contribute to variability in the effects of *CPS1* rs1047891 SNP on serum lipid levels.

	Waist circumference	-0.008	0.003	-0.319	-2.963	0.003
ApoB	Alcohol consumption	-0.026	0.013	-0.111	-1.987	0.048
	Waist cirumference	0.007	0.001	0.353	6.728	0.000
	Glucose	0.016	0.007	0.121	2.311	0.021
ApoA1/ApoB	Age	-0.005	0.002	-0.170	-2.377	0.018
	Alcohol consumption	0.120	0.032	0.217	3.772	0.000
	Waist cirumference	-0.010	0.005	-0.208	-2.003	0.046
Maonan/female						
TC	Age	0.013	0.005	0.156	2.523	0.012
	Waist circumference	0.024	0.010	0.186	2.536	0.014
TG	Waist circumference	0.029	0.006	0.326	4.774	0.000
	Pulase pressure	0.006	0.012	0.122	2.700	0.007
HDL-C	Genotype	-0.129	0.034	-0.199	-3.875	0.000
	Waist circumference	-0.006	0.003	-0.181	-2.482	0.014
LDL-C	Age	0.010	0.003	0.192	3.193	0.002
	Alcohol consumption	0.332	0.168	0.087	1.977	0.048
	Waist circumference	0.021	0.006	0.239	3.337	0.001
	Pulse pressure	0.004	0.002	0.099	2.001	0.046
ApoA1	Weight	-0.003	0.001	-0.112	-2.452	0.015
	Body mass index	-0.007	0.013	-0.129	-2.834	0.005
	Waist circumference	-0.003	0.001	-0.114	-2.426	0.016
ApoB	Age	0.002	0.001	0.154	2.677	0.009
	Waist circumference	0.009	0.002	0.403	5.785	0.000
ApoA1/ApoB	Waist circumference	-0.019	0.004	-0.377	-5.394	0.000
	Pluse pressure	-0.003	0.001	-0.121	-2.177	0.030
	Glucose	-0.029	0.014	-0.093	-2.038	0.042

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

Limitations

There were several potential limitations to our study. First, the sample size was relatively small compared to many GWASes. Replication studies and further studies with larger sample sizes are needed to confirm our results. Second, we were not able to alleviate the effect of diet and several environmental factors during statistical analysis. Third, we recognize the limited power to provide a more significant advance in understanding the full impact of *CPS1* rs1047891 SNP on lipoprotein metabolism. Thus, *CPS1* expression in adipose tissue and serum lipid levels should be further detected in future investigations.

Conclusions

In summary, our present study showed that genotype and allele frequencies of *CPS1* rs1047891 SNP were significantly different between Han and Maonan populations. Association of *CPS1* rs1047891 SNP and serum lipid levels was also different between the two ethnic groups and between males and females in Maonan population. These findings suggest that there may be a racial/ethnic- and/or gender-specific association of *CPS1* rs1047891 SNP and serum lipid profiles.

Acknowledgements

This study was supported by the National Natural Science Foundation of China (No. 81460169).

Disclosure of conflict of interest

None.

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