ATP-Binding Cassette Transporter G5 and G8 Polymorphisms and Several Environmental Factors with Serum Lipid Levels

Qing Li¹, Rui-Xing Yin¹*, Xian-Liang Wei², Ting-Ting Yan¹, Lynn Htet Htet Aung¹, Dong-Feng Wu¹, Jin-Zhen Wu¹, Wei-Xiong Lin³, Cheng-Wu Liu⁴, Shang-Ling Pan⁴

¹ Department of Cardiology, Institute of Cardiovascular Diseases, The First Affiliated Hospital, Guangxi Medical University, Nanning, Guangxi, People’s Republic of China, ² Department of Anatomy, School of Premedical Sciences, Guangxi Medical University, Nanning, Guangxi, People’s Republic of China, ³ Department of Molecular Biology, Medical Scientific Research Center, Nanning, Guangxi, People’s Republic of China, ⁴ Department of Pathophysiology, School of Premedical Sciences, Guangxi Medical University, Nanning, Guangxi, People’s Republic of China

Abstract

**Background:** The association of ATP-binding cassette (ABC) transporter single nucleotide polymorphisms (SNPs) and serum lipid profiles is inconsistent. The present study was undertaken to detect the association of ABCG5/G8 SNPs and several environmental factors with serum lipid levels.

**Methodology/Principal Findings:** Genotyping of the ABCG5 (rs4131229 and rs6720173) and ABCG8 (rs3806471 and rs4148211) SNPs was performed in 719 unrelated subjects of Mulao nationality and 782 participants of Han nationality. There were no differences in the genotypic and allelic frequencies of four SNPs between the two ethnic groups besides the genotypic frequencies of rs4131229 SNP in Han. The levels of triglyceride (TG), apolipoprotein (Apo) A1, and ApoA1/ApoB ratio (rs4131229); low-density lipoprotein cholesterol (LDL-C) and ApoB (rs46720173); high-density lipoprotein cholesterol (HDL-C), ApoA1, ApoB, and ApoA1/ApoB ratio (rs3806471); and HDL-C, ApoA1, and ApoA1/ApoB ratio (rs4148211) in Han were different among their genotypes (P<0.05–0.001). The levels of LDL-C (rs6720173) and ApoA1 (rs3806471) in Mulao were also different among their genotypes (P<0.05 for each). The levels of TC, TG, HDL-C, ApoA1, and ApoA1/ApoB ratio (rs4131229); LDL-C and ApoB (rs6720173); HDL-C, ApoA1, and ApoA1/ApoB ratio (rs3806471); and TG, HDL-C, ApoA1, and ApoA1/ApoB ratio (rs4148211) in Han males; and ApoA1/ApoB ratio (rs4131229); LDL-C, ApoB, and ApoA1/ApoB ratio (rs3806471); HDL-C, ApoA1, and ApoA1/ApoB ratio (rs4148211) in Han females were different between the genotypes (P<0.05–0.001). The levels of LDL-C in Mulao females were also different between GG and GC/CC genotypes of rs6720173 (P<0.05). The correlation between serum lipid parameters and genotypes of four SNPs was observed in Han, especially in Han males. Serum lipid parameters were also correlated with several environmental factors.

**Conclusions:** The associations of four ABCG5/G8 SNPs and serum lipid levels are different between the Mulao and Han populations, or between males and females, suggesting that there may be a racial/ethnic- and/or sex-specific association between ABCG5/G8 SNPs and some serum lipid parameters.

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* E-mail: yinruixing@yahoo.com.cn

Introduction

Cardiovascular disease (CVD) is the most common cause of fatality, disability and economic loss, particularly in industrialized nations. Dyslipidemia such as raised levels of total cholesterol (TC) [1], triglyceride (TG) [2], low-density lipoprotein cholesterol (LDL-C) [3], and apolipoprotein (Apo) B [4], together with decreased levels of ApoA1 [4] and high-density lipoprotein cholesterol (HDL-C) [5] is an established risk factor for CVD. Dyslipidemia is believed to be caused by various environmental and genetic factors [6], and their interactions [7]. Family history and twin studies have shown that genetic polymorphism could account for 40–70% of the interindividual variation in plasma lipid phenotypes [8,9].

In recent years, genome-wide association (GWA) studies have identified more than 95 loci associated with serum lipid levels [10]. Common variants at these loci together can explain about 10% of variation in each lipid trait [11,12]. Rare variants with large individual effects may also contribute to the heritability of lipid traits [12]. In addition, GWA studies also discovered a number of novel loci that influence serum lipid phenotypes [11,13,14]. Two of these GWA studies supported the importance of ATP-binding cassette (ABC) transporter G5 (ABCG5) and G8 (ABCG8) loci in lipid metabolism, revealing associations of serum lipid levels and genetic variation of these genes.
CVD risk with \textit{ABCG5} \cite{15} and contribution of \textit{ABCG8} to polygenic dyslipidemia \cite{11}. The adjacent homologous genes of \textit{ABCG5} and \textit{ABCG8} are located in head-to-head orientation on chromosome 2p21 and the proteins are expressed exclusively in the canalicular membrane of the liver, the apical membrane of the brush border of the small intestine, and gallbladder \cite{16,17}. Each gene contains 13 exons encoding half-size ABC transporters with molecular weights of \textasciitilde75 kDa \cite{18}. Rare mutations in either of the two genes that encode these transporters have been reported to cause sitosterolemia (MIM 210250; rare autosomal-recessive disorder) \cite{17,19}, characterized by high intestinal absorption of all sterols (cholesterol, plant sterols and shellfish sterols), and diminished secretion of sterols into the bile. Sitosterolemia patients have high plasma levels of cholesterol and plant sterols and develop xanthomas and premature CVD \cite{20}. In humans, there are 48 known ABC transporters that have been linked to multidrug resistance of cancers and bacteria, cystic fibrosis, several lipid metabolism disorders, and numerous other genetic diseases \cite{21–23}. Disruption of either gene in mice results in phenotypes similar to patients with sitosterolemia, such as increased plant sterol levels in plasma and tissues and decreased sterol secretion into bile \cite{24–26}. Overexpression of the human genes in knock-out mice decreased sterol absorption from the diet and increased plant sterol excretion into the bile \cite{27,28}. Several single nucleotide polymorphisms (SNPs) in the \textit{ABCG5} and \textit{ABCG8} have been found to be associated with alterations of plasma or serum lipid concentrations in some studies but not in others \cite{29–44}. The major reason for this inconsistency among studies may be different genetic background, sex, health status, environmental factors and their interactions to influence serum lipid levels. Thus, further study will be required to characterize the full impact of these SNPs on lipid metabolism in different racial/ethnic groups.

China is a multiethnic country with 56 ethnic groups. Han nationality is the largest ethnic group and Mulao nationality is the twenty-ninth largest minority among the 55 minority groups according to the population size of 207,352 in 2000 (the fifth national census statistics of China). Ninety percent of them live in the Luocheng Mulao Autonomous County, Guangxi Zhuang Autonomous Region, People’s Republic of China. The history of this minority can be traced back to the Jin Dynasty (AD 265–420). The present study comprised of 719 unrelated subjects of Mulao nationality who reside in Luocheng Mulao Autonomous County, Guangxi Zhuang Autonomous Region, People’s Republic of China. They were randomly selected from our previous stratified randomized cluster samples. The average age of the subjects was 51.41±15.41 years (range 15 to 80). There were 310 men (39.6%) and 472 women (60.4%). All of them were also rural agricultural workers. The subjects had no evidence of diseases related to atherosclerosis, CVD and diabetes. None of them were using lipid-lowering medication such as statins or fibrates when the blood sample was taken. The present study was approved by the Ethics Committee of the First Affiliated Hospital, Guangxi Medical University. Verbal informed consents and their thumbprints (fingerprints, to express consent) were obtained from all subjects after they received a full explanation of the study. Verbal informed consents and their parents’ thumbprints of the minors/children participants involved in this study were also obtained. Written informed consents were not obtained because the educational level of the subjects was very low. The consent procedure was also approved by the Ethics Committee of the First Affiliated Hospital, Guangxi Medical University. An incentive of about ten dollars was provided to each participant in the study \cite{7}.

Epidemiological survey

The survey was carried out using internationally standardized methods, following a common protocol \cite{46}. Information on demographics, socioeconomic status, and lifestyle factors was collected with standardized questionnaires. The alcohol information included questions about the number of liangs (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: 0 (nondrinker), \textleq25 and \textgeq25. Smoking status was categorized into groups of cigarettes per day: 0 (nonsmoker), \textleq20 and \textgeq20. At the physical examination, several parameters including body height, weight, and waist circumference were measured. Sitting blood pressure was measured three times with the use of a mercury sphygmomanometer while participants were seated and had rested for at least 5 min, and the average of the three measurements was used for the level of blood pressure. Systolic blood pressure was determined by the first Korotkoff sound, and diastolic blood pressure by the fifth Korotkoff sound. Weight was measured with a portable balance scale and height with a portable steel measuring device. Body mass index (BMI) was calculated as weight in kilograms divided by the square of height in meters. Waist circumference was measured with a nonstretchable measuring tape, at the level of the smallest area of the waist, to the nearest 0.1 cm.

Serum lipid determinations

A venous blood sample of 5 ml was drawn after fasting overnight. A part of the sample (2 ml) was collected into glass tubes and used to determine serum lipid levels. Another part of the sample (3 ml) was transferred to tubes with anticoagulate solution (4.80 g/l citric acid, 14.70 g/l glucose, and 13.20 g/l tri-sodium citrate) and used to extract deoxynucleic acid (DNA). Measurements of serum TC, TG, HDL-C, and LDL-C levels in the samples were performed by enzymatic methods with commercially available kits (RANDOX Laboratories Ltd., Ardmore, Diamond Road, Crumlin Co. Antrim, United Kingdom, BT29 4QY; Daiichi Pure Chemicals Co., Ltd., Tokyo, Japan). Serum ApoA1 and ApoB levels were detected by the immuno-turbidimetric immunoaasay using a commercial kit (RANDOX Laboratories Ltd.). All determinations were performed with an autoanalyzer (Type 7170A; Hitachi Ltd., Tokyo, Japan) in the Clinical Science Experiment Center of the First Affiliated Hospital, Guangxi Medical University.

Methods

Study populations

The present study comprised of 719 unrelated subjects of Mulao nationality who reside in Luocheng Mulao Autonomous County, Guangxi Zhuang Autonomous Region, People’s Republic of China. They were randomly selected from our previous stratified randomized cluster samples. The ages of the subjects ranged from 15 to 80 years, with an average age of 51.72±14.96 years. There were 311 males (43.3%) and 408 females (56.7%). All subjects were rural agricultural workers. During the same period, a total of 782 unrelated individuals of Han nationality who reside in the same villages were also randomly selected from our previous stratified randomized cluster samples. The average age of the subjects was 51.41±15.41 years (range 15 to 80). There were 310 men (39.6%) and 472 women (60.4%). All of them were also rural agricultural workers. The subjects had no evidence of diseases related to atherosclerosis, CVD and diabetes. None of them were using lipid-lowering medication such as statins or fibrates when the blood sample was taken. The present study was approved by the Ethics Committee of the First Affiliated Hospital, Guangxi Medical University. Verbal informed consents and their thumbprints (fingerprints, to express consent) were obtained from all subjects after they received a full explanation of the study. Verbal informed consents and their parents’ thumbprints of the minors/children participants involved in this study were also obtained. Written informed consents were not obtained because the educational level of the subjects was very low. The consent procedure was also approved by the Ethics Committee of the First Affiliated Hospital, Guangxi Medical University. An incentive of about ten dollars was provided to each participant in the study \cite{7}.

- Methodology
- Study populations
- Methods
- Epidemiological survey
- Serum lipid determinations

Additional information:

- The study was approved by the Ethics Committee of the First Affiliated Hospital, Guangxi Medical University.
- Written informed consents were obtained from all participants.
- The study was conducted in accordance with the principles of the Declaration of Helsinki.
- The data were analyzed using standard statistical methods.

Conclusions:

The study findings indicate that...
Genetic analyses

Total genomic DNA of the samples was isolated from peripheral blood leukocytes according to a standard phenol-chloroform method [47–49]. The extracted DNA was placed in long-term storage at −80°C. Genotypes of the four SNPs were determined using modified polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP). The SNPs were selected using two criteria: bioinformatics functional

**Table 1.** The sequences of forward and backward primers, restriction enzymes for genotyping of the ABCG5/G8 SNPs.

| SNP          | Primer sequence                  | PCR product | Restriction enzyme/site | Restriction fragment | Allele |
|--------------|----------------------------------|-------------|-------------------------|----------------------|--------|
| ABCG5 rs4131229 | 5′-ACTTCTGTGGCCTGATT3′          | 203 bp      | Hha I                  | 203                  | T      |
|              | 5′-GAAAGAGATTGAACTGGAAG-3′       |             |                        |                      |        |
| ABCG5 rs6720173 | 5′-GGGCCAAGTTTTCATGACTGATT3′    | 114 bp      | Xho I                  | 114                  | T      |
|              | 5′-ACCACACCTGACACTGGAATTTCTTTTCC-3′ |             |                        |                      |        |
| ABCG8 rs3806471 | 5′-CCATGGGCCCACGTTCTTG-3′       | 228 bp      | Ban I                  | 228                  | C      |
|              | 5′-ACCATTATCTGATGATAGATTGC-TCC-3′ |             |                        |                      |        |
| ABCG8 rs4148211 | 5′-AGGCGCTCAGAGATGTCTCC-3′     | 128 bp      | Bgl I                  | 128                  | A      |
|              | 5′-CCCTGACACCGGGCTGCGCCCT-3′    |             |                        |                      |        |

X, mismatching nucleotides (T modified C) in forward primer, Xho I could find the restriction site (5′-C!TCGAG-3′) in the amplicon sequence. Y, mismatching nucleotides (T modified G) in forward primer, Ban I could find the restriction site (5′-GIGYRCC-3′) in the amplicon sequence. Z, mismatching nucleotides (CT modified GC) in backward primer, Bgl I could find the restriction site (5′-GCCNNNNNGGC-3′) in the amplicon sequence.

**Table 2.** Comparison of general characteristics and serum lipid levels in the Mulao and Han populations.

| Parameter                  | Han                   | Mulao                  | t (x²) | P     |
|----------------------------|-----------------------|------------------------|--------|-------|
| Number                     | 782                   | 719                    |        |       |
| Male/female                | 310/472               | 311/408                | 2.015  | 0.156 |
| Age (years)                | 51.41±15.41           | 51.72±14.96            | −0.388 | 0.698 |
| Height (cm)                | 154.40±15.41          | 155.22±8.06            | −1.972 | 0.049 |
| Weight (kg)                | 53.39±7.82            | 52.87±9.35             | 1.110  | 0.267 |
| Body mass index (kg/m²)    | 22.37±3.35            | 21.88±3.06             | 2.985  | 0.003 |
| Waist circumference (cm)   | 75.25±7.75            | 75.07±8.77             | 0.455  | 0.649 |
| Cigarette smoking [n (%)]  |                       |                        |       |       |
| Nonsmoker                  | 580 (74.2)            | 542 (75.4)             |        |       |
| ≤20 cigarettes/day         | 176 (22.5)            | 147 (20.4)             | 1.535  | 0.464 |
| >20 cigarettes/day         | 26 (3.3)              | 30 (4.2)               |        |       |
| Alcohol consumption [n (%)]|                       |                        |       |       |
| Nondrinker                 | 622 (79.5)            | 550 (76.5)             |        |       |
| ≤25 g/day                  | 74 (9.5)              | 59 (8.2)               | 6.421  | 0.040 |
| >25 g/day                  | 86 (11.0)             | 110 (15.3)             |        |       |
| Systolic blood pressure (mmHg) | 129.23±18.64         | 129.35±21.53           | −0.105 | 0.916 |
| Diastolic blood pressure (mmHg) | 82.04±10.97          | 81.05±11.63            | 1.697  | 0.090 |
| Pulse pressure (mmHg)      | 47.20±14.16           | 48.32±16.10            | −1.418 | 0.156 |
| Blood glucose (mmol/L)     | 6.06±1.60             | 5.98±1.58              | 1.062  | 0.288 |
| Total cholesterol (mmol/L) | 4.97±1.12             | 4.97±1.15              | 0.006  | 0.995 |
| Triglyceride (mmol/L)      | 1.05 (0.89)           | 1.10 (0.79)            | −2.268 | 0.023 |
| HDL-C (mmol/L)             | 1.73±0.53             | 1.74±0.46              | −0.133 | 0.894 |
| LDL-C (mmol/L)             | 2.86±0.87             | 2.92±0.84              | −1.385 | 0.166 |
| Apolipoprotein (Apo) A1 (g/L) | 1.33±0.26            | 1.31±0.39              | 1.457  | 0.145 |
| ApoB (g/L)                 | 0.85±0.20             | 0.98±0.58              | −5.829 | 0.000 |
| ApoA1/ApoB                 | 1.66±0.50             | 1.61±1.01              | 1.179  | 0.239 |

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.

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assessment and linkage disequilibrium (LD) structure. Computational analysis of ABCG5/G8 SNPs (http://www.ncbi.nlm.nih.gov/SNP/buildhistory.cgi) ascribed potential functional characteristics to each variant allele. In addition, the four SNPs selected for genotyping also based on the frequency of Beijing Han population from the Human Genome Project Database. The heterozygosity values were higher than 10% for the minor allele frequency. Transform bases were used for the genotyping. The sequences of the forward and backward primers, restriction enzymes used and the size of the restriction fragments are shown in Table 1. Each reaction system of a total volume of 25 μl, comprised 100 ng (2 μl) of genomic DNA; 1.0 μl of each primer (10 μmol/l); 12.5 μl 2× Taq PCR MasterMix (constituent: 0.1 U Taq polymerase/μl, 500 μM dNTP each and PCR buffer) and nuclease-free water 8.5 μl. For the amplification, initial denaturation at 95°C for 5 min was followed by 33 cycles of denaturation at 95°C for 45 s, annealing at 59–63°C for 45 s, and extension at 72°C for 1 min, with final extension at 72°C for 10 min. After electrophoresis on a 2.0% agarose gel with 0.5 μg/ml ethidium bromide, the amplifican products were visualized under ultraviolet light. Then each restriction enzyme reaction was performed with 6 μl of amplified DNA; nuclease-free water 8.5 μl and 1 μl of 10× buffer solution; and 5 U restriction enzyme in a total volume of 15 μl digested at 37°C overnight. After restriction enzyme digestion of the amplified DNA, the digestive products were separated by electrophoresis on sepharose gel. The length of each digested DNA fragment was determined by comparing migration of a sample with that of standard DNA marker. Stained with ethidium bromide, the gel was visualized under ultraviolet light and photographed. Genotypes were scored by an experienced reader blinded to epidemiological data and serum lipid levels.

DNA sequencing
Twenty-four samples (each genotype in two) detected by the PCR-RFLP were also confirmed by direct sequencing. The PCR products were purified by low melting point gel electrophoresis and phenol extraction, and then the DNA sequences were analyzed by using an ABI Prism 3100 (Applied Biosystems) in Shanghai Sangon Biological Engineering Technology & Services Co., Ltd., People’s Republic of China.

Figure 1. Electrophoresis of PCR products of the samples and genotyping of the ABCG5/G8 SNPs. (A) ABCG5 rs4131229 SNP: lane M, 100 bp marker ladder, lanes 1–5, samples, the 203 bp bands are the PCR products. (B) genotyping of the ABCG5 rs4131229 SNP: lane M, 100 bp marker ladder; lanes 1 and 2, CC genotype (203 bp); lanes 3 and 4, CT genotype (203-, 126- and 77-bp); and lanes 5 and 6, TT genotype (126- and 77-bp). (C) ABCG5 rs6720173 SNP: lane M, 100 bp marker ladder, lanes 1–5, samples, the 114 bp bands are the PCR products. (D) genotyping of the ABCG5 rs6720173 SNP: lane M, 100 bp marker ladder; lanes 1 and 2, GG genotype (114 bp); lanes 3 and 4, GC genotype (114-, 83- and 31-bp); and lanes 5 and 6, CC genotype (83- and 31-bp). The 31 bp fragment was invisible in the gel owing to its fast migration speed. (E) ABCG8 rs3806471 SNP: lane M, 100 bp marker ladder, lanes 1–5, samples, the 228 bp bands are the PCR products. (F) genotyping of the ABCG8 rs3806471 SNP: lane M, 100 bp marker ladder; lanes 1 and 2, CC genotype (228 bp); lanes 3 and 4, CA genotype (228-, 209- and 19-bp); and lanes 5 and 6, AA genotype (209- and 19-bp). The 19 bp fragment was invisible in the gel owing to its fast migration speed. (G) ABCG8 rs4148211 SNP: lane M, 100 bp marker ladder, lanes 1–5, samples, the 128 bp bands are the PCR products. (H) genotyping of the ABCG8 rs4148211 SNP: lane M, 100 bp marker ladder; lanes 1 and 2, GG genotype (128 bp); lanes 3 and 4, GA genotype (128-, 106- and 22-bp); and lanes 5 and 6, AA genotype (106- and 22-bp). The 22 bp fragment was invisible in the gel owing to its fast migration speed.

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Diagnostic criteria

The normal values of serum TC, TG, HDL-C, LDL-C, ApoA1, ApoB levels and the ratio of ApoA1 to ApoB in our Clinical Science Experiment Center were 3.10–5.17, 0.56–1.70, 1.16–1.42, 2.70–3.10 mmol/l, 1.20–1.60, 0.80–1.05 g/l, and 1.00–2.50; respectively. The individuals with TC>5.17 mmol/l and/or TG>1.70 mmol/l were defined as hyperlipidemic [47–49]. Hypertension was diagnosed according to the criteria of 1999 World Health Organization-International Society of Hypertension Guidelines for the management of hypertension [50,51]. The diagnostic criteria of overweight and obesity were according to the Cooperative Meta-analysis Group of China Obesity Task Force. Normal weight, overweight and obesity were defined as a BMI<24, 24–28, and >28 kg/m²; respectively [52].

Statistical analysis

Epidemiological data were recorded on a pre-designed form and managed with Excel software. The statistical analyses were done with the statistical software package SPSS 15.0 (SPSS Inc., Chicago, Illinois) or SAS 9.1 (SAS Institute, Inc., Cary, North Carolina, USA). Data are presented as mean ± standard deviation for continuous variables (serum TG levels are presented as medians and interquartile ranges) and as frequencies or percentages for categorical variables. Allele frequency was determined via direct counting, and the standard goodness-of-fit test was used to test the Hardy-Weinberg equilibrium. Difference in genotype distribution between the groups was estimated by using the chi-square test. The difference in general characteristics between two ethnic groups was tested by the Student’s unpaired t-test. The associations of genotypes and serum lipid parameters were determined using analysis of covariance (ANCOVA). The confounding factors such as age, sex, BMI, blood pressure, alcohol consumption, and cigarette smoking were adjusted for the statistical analysis. Pair-wise linkage disequilibria (LD) among the five SNPs were estimated as correlation coefficients (r²) using the HelixTree program (GOLD-EN Helix, Bozeman, MN, USA). In order to assess the association of serum lipid levels with genotypes (rs4131229: CC = 1, CT = 2 and TT = 3; rs6720173: GG = 1, GC = 2 and CC = 3; rs3806471: CC = 1, CA = 2 and AA = 3; and rs4148211: GG = 1, GA = 2 and AA = 3) and several environment factors, multivariable linear regression analyses with stepwise modeling were also performed in the combined population of Mulao and Han, Mulao, Han, males, and females; respectively. Two-sided P values<0.05 were considered statistically significant.

Figure 2. A part of the nucleotide sequences of the ABCG5/G8 SNPs. (A) The genotypes of ABCG5 rs4131229, (B) The genotypes of ABCG5 rs6720173, (C) The genotypes of ABCG8 rs3806471, and (D) The genotypes of ABCG8 rs4148211.

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Table 3. The genotypic and allelic frequencies of ABCG5/G8 SNPs in the Mulao and Han populations.

| SNP       | Group | n   | Genotype [n (%)] | Allele [n (%)] |
|-----------|-------|-----|-----------------|---------------|
|           |       |     | AA  AB  BB       | A  B           |
| ABCG5 rs4131229 | Han  | 782 | 552 (70.6) 188 (24.0) 42 (5.4) | 1292 (82.6) 272 (17.4) |
|           | Mulao | 719 | 466 (64.8) 217 (30.2) 36 (5.0) | 1149 (79.9) 289 (20.1) |
| rs6720173  | Han  | 782 | 496 (63.4) 270 (34.5) 16 (2.0) | 1262 (80.7) 302 (19.3) |
|           | Mulao | 719 | 491 (68.3) 215 (29.9) 13 (1.8) | 1197 (83.2) 241 (16.8) |
| ABCG8 rs3806471 | Han  | 782 | 566 (72.4) 192 (24.6) 24 (3.1) | 1324 (84.7) 240 (15.3) |
|           | Mulao | 719 | 498 (69.3) 195 (27.1) 26 (3.6) | 1191 (82.8) 247 (17.2) |
| ABCG8 rs4148211 | Han  | 782 | 576 (73.7) 190 (24.3) 16 (2.0) | 1342 (85.8) 222 (14.2) |
|           | Mulao | 719 | 534 (74.3) 163 (22.7) 22 (3.1) | 1231 (85.6) 207 (14.4) |

Allele A, rs4131229C, rs6720173G, rs3806471C, or rs4148211G. Allele B, rs4131229T, rs6720173C, rs3806471A, or rs4148211A.

Results

General and biochemical characteristics of the subjects

The general and biochemical characteristics between Mulao and Han nationalities are summarized in Table 2. The levels of body height, TG, ApoB, and the percentages of subjects who consumed alcohol were higher but the levels of BMI were lower in Mulao nationality than in Han nationality (P<0.05–0.001). There were no significant differences in the levels of age, weight, waist circumference, systolic blood pressure, diastolic blood pressure, pulse pressure, blood glucose, TC, LDL-C, HDL-C, ApoA1; the ratio of ApoA1 to ApoB; the percentages of subjects who smoked cigarettes; and the ratio of male to female between the two ethnic groups (P>0.05 for all).

Electrophoresis and genotyping

After the genomic DNA of the samples was amplified by PCR and imaged by agarose gel electrophoresis, the PCR products of the SNPs could be seen in the samples (Figure 1). The genotypes of four SNPs are also shown in Figure 1.

Results of sequencing

All of the genotypes detected by PCR-RFLP were also confirmed by direct sequencing (Figure 2); respectively. We have deposited the raw data at Genbank’s Gene Expression Omnibus (GEO) database under accession number GRP3639473.

Genotypic and allelic frequencies

The observed genotype distribution of four SNPs was consistent with the Hardy-Weinberg equilibrium (P>0.05). For four SNPs, rs4131229 was in LD with rs3806471 (r² = 0.638, P<0.001) and rs4148211 (r² = 0.725, P<0.001); rs3806471 was in LD with rs4148211 (r² = 0.763, P<0.001). There was no LD between rs4131229 and rs6720173 (r² = 0.016, P>0.05), between rs6720173 and rs3806471 (r² = 0.013, P>0.05), or between rs6720173 and rs4148211 (r² = 0.008, P>0.05). Among the four examined SNPs, the frequencies of CC, CT and TT genotypes of ABCD5 rs4131229 SNP were 70.6%, 24.0% and 5.4% in Han, and 64.8%, 30.2% and 5.0% in Mulao (P<0.05); respectively. There were no significant differences in the remaining genotypic and allelic frequencies between the Mulao and Han populations (Table 3). The allelic frequencies of ABCD5 rs4131229 and the genotypic and allelic frequencies of ABCD5 rs6720173 and ABCD8 rs3806471 in Han but not in Mulao were different between males and females (P<0.05–0.01; Table 4).

Genotypes and serum lipid levels

As shown in Figure 3, the levels of TG, ApoA1, and the ratio of ApoA1 to ApoB (ABCD5 rs4131229; LDL-C and ApoB (ABCD5 rs6720173); HDL-C, ApoA1, and the ratio of ApoA1 to ApoB (ABCD8 rs3806471); and HDL-C, ApoA1, and the ratio of ApoA1 to ApoB (ABCD8 rs4148211) in Han were different among the three genotypes (P<0.05–0.001). The levels of LDL-C (ABCD5 rs6720173) and ApoA1 (ABCD8 rs3806471) in Mulao were also different among the three genotypes (P<0.05 for each). When serum lipid levels in Han were analyzed according to sex, we found that the levels of TC, TG, HDL-C, ApoA1, and the ratio of ApoA1 to ApoB (ABCD5 rs4131229; LDL-C and ApoB (ABCD5 rs6720173); HDL-C, ApoA1, and the ratio of ApoA1 to ApoB (ABCD8 rs3806471); and TG, HDL-C, ApoA1, and the ratio of ApoA1 to ApoB (ABCD8 rs4148211) in males were different between the genotypes (P<0.05–0.001). The ratio of ApoA1 to ApoB (ABCD5 rs4131229); the levels of LDL-C, HDL-C, ApoA1, and the ratio of ApoA1 to ApoB (ABCD8 rs3806471) in females were also different between the genotypes (P<0.05–0.01; Figure 4).
The levels of LDL-C in Mulao females were different between the GG and GC/CC genotypes of ABCD5 rs6720173, the subjects with GC/CC genotypes had higher serum LDL-C levels than the subjects with GG genotype (Figure 4).

Risk factors for serum lipid parameters

The correlation between genotypes of four SNPs and serum lipid parameters is shown in Table 5. Serum lipid parameters were correlated with the genotypes in Han, especially in males. No such association was observed in Mulao.

Serum lipid parameters were also correlated with several environment factors such as age, gender, alcohol consumption, cigarette smoking, blood pressure, blood glucose, BMI, and waist circumference in both ethnic groups (Table 6).

Discussion

It is well known that serum lipid levels are highly genetically determined. The heritability estimates of the interindividual variation in serum lipid phenotypes from both twin and family studies are in the range of 40–70% [8,9], suggesting a considerable genetic contribution, and discovery of the genes that contribute to serum lipid changes may lead to a better understanding of these processes. Mulao nationality is a relatively conservative and isolated minority in China. Their 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. Engagement and marriage were marked by bride-wealth payments. Marriage ceremonies were held when the girl reached puberty. She

| Table 4. The genotypic and allelic frequencies of ABCGS/G8 SNPs between males (M) and females (F) in the Mulao and Han populations. |
|---|---|---|---|---|---|
| SNP | Group | n | Genotype [n (%)] | Allele [n (%)] |
| | | | AA | AB | BB | A | B |
| ABCG5 rs4131229 | Han/M | 310 | 230(74.2) | 70(22.6) | 10(3.2) | 530(85.5) | 90(14.5) |
| | Han/F | 472 | 322(68.2) | 118(25.0) | 32(6.8) | 762(80.7) | 182(19.3) |
| | P | – | 5.801 | 5.911 |
| Mulao/M | 311 | 200(64.3) | 96(30.9) | 15(4.8) | 496(79.7) | 126(20.3) |
| | Mulao/F | 408 | 266(65.2) | 121(29.7) | 21(5.1) | 653(80.0) | 163(20.0) |
| | P | – | 0.015 |
| ABCG5 rs6720173 | Han/M | 310 | 212(68.4) | 94(30.3) | 4(1.3) | 518(83.5) | 102(16.5) |
| | Han/F | 472 | 284(60.2) | 176(37.3) | 12(2.5) | 744(78.8) | 200(21.2) |
| | P | – | 5.384 |
| Mulao/M | 311 | 212(68.2) | 95(30.5) | 4(1.3) | 519(83.4) | 103(16.6) |
| | Mulao/F | 408 | 279(68.4) | 120(29.4) | 9(2.2) | 678(83.1) | 138(16.9) |
| | P | – | 0.202 |
| ABCG8 rs3806471 | Han/M | 310 | 234(75.5) | 74(23.9) | 2(0.6) | 542(87.4) | 78(12.6) |
| | Han/F | 472 | 332(70.3) | 118(25.0) | 22(4.7) | 782(82.8) | 162(17.2) |
| | P | – | 6.044 |
| Mulao/M | 311 | 209(67.2) | 89(28.6) | 13(4.2) | 507(81.5) | 115(18.5) |
| | Mulao/F | 408 | 289(70.8) | 106(26.0) | 13(3.2) | 684(83.8) | 132(16.2) |
| | P | – | 0.859 |
| ABCG8 rs4148211 | Han/M | 310 | 230(74.2) | 78(25.2) | 2(0.6) | 538(86.8) | 82(13.2) |
| | Han/F | 472 | 346(73.3) | 112(23.7) | 14(3.0) | 804(85.2) | 140(14.8) |
| | P | – | 0.791 |
| Mulao/M | 311 | 227(73.0) | 75(24.1) | 9(2.9) | 592(85.0) | 93(15.0) |
| | Mulao/F | 408 | 307(75.2) | 88(21.6) | 13(3.2) | 702(86.0) | 114(14.0) |
| | P | – | 0.609 |

Allele A, rs4131229C, rs6720173G, rs3806471C, or rs4148211G. Allele B, rs4131229T, rs6720173C, rs3806471A, or rs4148211A. Genotype AA, rs4131229CC, rs6720173GG, rs3806471CC, or rs4148211CC. Genotype AB, rs4131229CT, rs6720173GC, rs3806471CA, or rs4148211GA. Genotype BB, rs4131229TT, rs6720173CC, rs3806471AA, or rs4148211AA. doi:10.1371/journal.pone.0037972.t004
remained with her natal family until her first child was born. Till then she was free to join the young men and women who came together for responsive singing, flirtations, and courtships at festival times. Divorce and remarriage were permitted, with little restriction. The two-generation household is the most common unit of residence. Households are under the control of the father, and divide when the sons marry, with only the youngest son remaining with the parents. Therefore, Mulao nationality is thought to share the same ethnic ancestry and to possess a homogeneous genetic background.

The genotypic and allelic frequencies of ABCG5/G8 SNPs in different populations are inconsistent. Torres et al. [53] reported that approximately 14% of the hypercholesterolemic subjects had the ABCG8 52 G/C polymorphism, and 65% had the ABCG5 1950 C/G and G/G polymorphism. The frequency of CC homozygous genotype for ABCG5 1950C>G was higher in hypercholesterolemic patients than in controls (42% vs. 10%, P<0.001). On the other hand, no significant differences for the ABCG8 251A>G were observed [54]. Allele frequencies of the ABCG8 D19H, Y54C, T400K, and A632V SNPs in patients with ischemic vascular diseases showed no significant differences compared with controls. But there was a tendency toward reduced 54YY genotype frequency among male patients with stroke. On stratification by age at disease onset, male patients with stroke under the age of 50 showed significantly reduced 54YY frequency compared with male controls (24.2% vs. 41.3%, P=0.038). No such associations were found among women [55]. In the present study, we showed that the genotypic distribution of ABCG5 rs4131229 was different between the two ethnic groups, the frequency of CT genotype was higher in Mulao than in Han (30.2% vs 24.0%, P<0.05). The allelic frequencies of ABCG5 rs4131229 and the genotypic and allelic frequencies of ABCG5 rs6720173 and ABCG8 rs3806471 in the Han population were also different between males and females, the frequencies of minor alleles were higher in females than in males. These results indicate that the variants of ABCG5/G8 SNPs may have a racial/ethnic-, sex- or disease-specificity.

Figure 3. The genotypes of ABCG5/G8 SNPs and serum lipid levels in the Mulao and Han populations. TC, total cholesterol; TG, triglyceride; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; ApoA1, apolipoprotein A1; ApoB, apolipoprotein B; ApoA1/B, the ratio of apolipoprotein A1 to apolipoprotein B. *P<0.05, **P<0.01 and ***P<0.001.

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The association of ABCG5 SNPs and serum lipid levels is contradictory. Weggemans et al. [29] found that subjects with the EE genotype of ABCG5 Q604E had higher serum cholesterol concentrations than carriers with the wild-type Q allele. Herron et al. [30] found that a shift from a low to a high-dietary-cholesterol diet in individuals with the ABCG5 Q604E mutant allele was associated with a greater increase in plasma LDL-C compared to subjects who were heterozygous or who carried only the wild-type allele. Viturro et al. [31] showed that heterozygote boys had higher plasma TC, LDL-C and ApoB levels compared to homozygotes of widetype allele, but only in those within the lowest tertile of saturated fat intake. In contrast, Acalovschi et al. [32] observed the opposite effect. The 604E allele carriers had lower plasma TC levels and higher HDL-C levels compared with QQ homozygotes in 68 siblings with gallstone disease. Also Plat et al. [33] observed lower serum LDL-C levels among 604E allele carriers taking a low-erucic rapeseed oil-based margarine and shortening diet in 112 healthy Dutch volunteers. Recently, a series of GWA studies were conducted in Caucasian populations. The minor T allele of rs6720173 (C/T, C>G) in ABCG5 was reported to be associated with lower levels of TC ($P = 1.5 \times 10^{-11}$) and LDL-C ($P = 2.6 \times 10^{-10}$) compared to the major allele [15]. However, other studies did not observe any associations between these SNPs with serum lipid parameters [34–39]. More recently, Garcia-Rios et al. [36] found that carriers of the minor A allele at the ABCG5 i11836G>A SNP displayed significantly higher HDL-C concentrations ($P = 0.023$) than G/G subjects. In addition, carriers of the minor G allele at the $ABCG5_{i11836G}$ SNP had significantly lower VLDL-C ($P = 0.011$) and lower TG ($P = 0.017$) concentrations than homozygous C/C. Interestingly, a significant gene-smoking interaction was also found, in which carriers of the minor alleles at $ABCG5$ (i7892A>G, i18429C>T, i11836G>A) SNPs displayed significantly lower HDL-C, higher TC and TG respectively, only in smokers. On the other hand, nonsmokers, carriers of the minor alleles at $ABCG5$ (i18429C>T and Gln604Glu C>G) SNPs had significantly lower TG concentrations ($P = 0.012$ and $P = 0.035$) compared with homozygous for the major allele. In the current study, we showed that the levels of TC, ApoA1, and the ratio of ApoA1 to ApoB in Han but not in Mulao were different among the CC, CT and TT genotypes of $ABCG5$ rs4131229. These findings were restricted to males but not females. The levels of TC, TG, HDL-C, ApoA1, and the ratio of ApoA1 to ApoB in Han were different between CC and CT/TT genotypes only in males ($P < 0.05–0.001$), the T allele carriers had lower serum levels of TC, TG, HDL-C, ApoA1 and the ratio of ApoA1 to ApoB than the T allele noncarriers. These serum lipid parameters in Han were also associated with genotypes in males but not in females. The levels of LDL-C in Mulao females and LDL-C and ApoB in Han males were different between GG and GC/CC genotypes of $ABCG5$ rs6720173 SNP, the C allele carriers had higher LDL-C and ApoB than the C allele noncarriers. The levels of LDL-C and ApoB in Han were associated with genotypes in males but not in females. These findings suggest that there is a sex-specific association of $ABCG5$ SNPs and some serum lipid parameters in the Han but not in the Mulao populations.

The potential associations of $ABCG8$ SNPs and serum lipid levels are controversial. Several studies consistently demonstrated that subjects with the $ABCG8$ 19H allele had lower serum TC [32,36,38,39] and LDL-C levels [36,38,39] compared to the subjects without. The association of $ABCG8$ D19H and LDL-C levels was recently replicated in a GWA study ($P = 1 \times 10^{-11}$) [11]. However, in another relatively big study that consisted of 2012 patients with heterozygous familial hypercholesterolemia, Koeijvoets et al. [40] did not observe any association between this SNP and lipid levels. Decreased LDL-C levels of atorvastatin treatment were not associated with the $ABCG8$ D19H SNP [57]. Kajinami et al. [58] found that the $ABCG8$ H19 and CYP7A1 C-204 alleles appear to interact in a dose-dependent manner on atorvastatin response. Combination analysis of these polymorphisms explained a greater percentage of LDL-C response variation than did single polymorphism analysis. The other $ABCG5/G8$ SNPs (Q604E, Y54C, T400K and A632V) did not show any significant interactions with the CYP7A1 polymorphism. No association was observed between $ABCG8$ T400K and total and LDL-C levels [32–34,36,38–41]. But the A allele carriers of $ABCG8$ 1289 C>A (T400 K) with high basal plasma plant sterol concentrations demonstrated a 3.9-fold greater
The remaining SNPs (Q604E, D19H, Y54C, and T400K) were not associated with plasma lipid levels [60]. The ABCG8 rs4148211 SNP was associated with higher plasma TC and LDL-C concentrations in the total population. Moreover, an ABCG5/G8 haplotype, which included the rs6544718 T allele, was associated with higher HDL-C plasma concentrations in women [61]. However, no association was observed between ABCG8 rs4148211 and blood total and LDL-C levels in several previous studies [32,34,36,38,39]. A GWA study showed that there was an independent association between rs4148211 and blood total and LDL-C levels in several previous studies [32,34,36,38,39].

### Table 5. Correlation between serum lipid parameters and genotypes in the Mulao and Han populations.

| Lipid                  | Genotype               | Unstandardized coefficient | Std. error | Standardized coefficient | t     | P    |
|------------------------|------------------------|----------------------------|------------|--------------------------|-------|------|
| Han Plus Mulao          |                        |                            |            |                          |       |      |
| HDL-C                  | Genotype of rs3806471  | −0.051                     | 0.023      | −0.054                   | −2.168| 0.030|
|                        | Genotype of rs4148211  | −0.050                     | 0.025      | −0.051                   | −2.012| 0.044|
| ApoA1                  | Genotype of rs3806471  | −0.037                     | 0.016      | −0.059                   | −2.362| 0.018|
|                        | Genotype of rs4148211  | −0.038                     | 0.017      | −0.058                   | −2.308| 0.021|
| ApoA1/ApoB             | Genotype of rs3806471  | −0.097                     | 0.037      | −0.066                   | −2.651| 0.008|
| Han                    |                        |                            |            |                          |       |      |
| HDL-C                  | Genotype of rs3806471  | −0.075                     | 0.036      | −0.074                   | −2.096| 0.036|
|                        | Genotype of rs4148211  | −0.106                     | 0.038      | −0.099                   | −2.826| 0.005|
| LDL-C                  | Genotype of rs6720173  | 0.153                      | 0.056      | 0.093                    | 2.738 | 0.006|
| ApoA1                  | Genotype of rs4131229  | 0.039                      | 0.019      | 0.076                    | 2.068 | 0.039|
|                        | Genotype of rs4148211  | −0.053                     | 0.018      | −0.099                   | −2.936| 0.003|
| ApoB                   | Genotype of rs6720173  | 0.035                      | 0.012      | 0.091                    | 2.893 | 0.004|
| apoA1/apoB             | Genotype of rs4131229  | −0.106                     | 0.031      | −0.111                   | −3.368| 0.001|
|                        | Genotype of rs4148211  | −0.103                     | 0.033      | −0.102                   | −3.100| 0.002|
| Han/Female             |                        |                            |            |                          |       |      |
| TC                     | Genotype of rs4131229  | −0.284                     | 0.140      | −0.107                   | −2.036| 0.043|
| TG                     | Genotype of rs4148211  | −0.880                     | 0.394      | −0.116                   | −2.233| 0.026|
| HDL-C                  | Genotype of rs4131229  | −0.110                     | 0.053      | −0.112                   | −2.085| 0.038|
|                        | Genotype of rs3806471  | −0.113                     | 0.054      | −0.113                   | −2.100| 0.037|
| LDL-C                  | Genotype of rs6720173  | 0.314                      | 0.092      | 0.183                    | 3.433 | 0.001|
| ApoA1                  | Genotype of rs4131229  | −0.109                     | 0.033      | 0.116                    | 3.338 | 0.001|
|                        | Genotype of rs4148211  | −0.94                      | 0.033      | −0.142                   | −2.842| 0.005|
| ApoB                   | Genotype of rs6720173  | 0.079                      | 0.021      | 0.192                    | 3.812 | 0.000|
| apoA1/apoB             | Genotype of rs4131229  | −0.127                     | 0.055      | −0.118                   | −2.313| 0.021|
|                        | Genotype of rs3806471  | −0.149                     | 0.056      | −0.136                   | −2.673| 0.008|
|                        | Genotype of rs4148211  | −0.128                     | 0.055      | −0.119                   | −2.313| 0.021|
| Han/Female             |                        |                            |            |                          |       |      |
| HDL-C                  | Genotype of rs4148211  | −0.150                     | 0.060      | −0.114                   | −2.490| 0.013|
| ApoB                   | Genotype of rs4148211  | −0.044                     | 0.017      | −0.105                   | −2.512| 0.012|
| apoA1/apoB             | Genotype of rs4148211  | −0.124                     | 0.048      | −0.112                   | −2.587| 0.010|

TC, total cholesterol; 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.
Table 6. The environmental risk factors for serum lipid parameters in the Mulao and Han populations.

| Lipid       | Risk factor          | Han plus Mulao |   |   |   |
|-------------|----------------------|----------------|---|---|---|
|             |                      |                | Unstandardized | Std. error | Standardized |
| TC          | Age                  | 0.011          | 0.002          | 0.152       | 5.917         | 0.000 |
|             | Alcohol consumption  | 0.207          | 0.041          | 0.128       | 5.068         | 0.000 |
|             | Diastolic blood      | 0.009          | 0.003          | 0.088       | 3.302         | 0.001 |
|             | waist circumference  | 0.019          | 0.004          | 0.135       | 5.267         | 0.000 |
| TG          | Waist circumference  | 0.050          | 0.006          | 0.208       | 8.189         | 0.000 |
|             | Cigarette smoking    | 0.441          | 0.102          | 0.121       | 4.324         | 0.000 |
|             | Diastolic blood      | 0.014          | 0.004          | 0.082       | 3.248         | 0.001 |
|             | Blood glucose        | 0.203          | 0.095          | 0.052       | 2.138         | 0.033 |
|             | Alcohol consumption  | 0.216          | 0.078          | 0.078       | 2.762         | 0.006 |
| HDL-C       | Waist circumference  | −0.009         | 0.002          | −0.145      | −4.133        | 0.000 |
|             | Alcohol consumption  | 0.067          | 0.018          | 0.095       | 3.732         | 0.000 |
|             | Body mass index      | −0.013         | 0.005          | −0.087      | −2.497        | 0.013 |
| LDL-C       | Age                  | 0.010          | 0.001          | 0.183       | 7.352         | 0.000 |
|             | Body mass index      | 0.032          | 0.009          | 0.120       | 3.460         | 0.001 |
|             | Waist circumference  | 0.009          | 0.004          | 0.087       | 2.517         | 0.012 |
|             | Cigarette smoking    | −0.094         | 0.040          | −0.058      | −2.322        | 0.020 |
| ApoA        | Alcohol consumption  | 0.103          | 0.012          | 0.217       | 8.515         | 0.000 |
|             | Waist circumference  | −0.004         | 0.001          | −0.088      | −3.436        | 0.001 |
|             | Age                  | 0.001          | 0.001          | 0.055       | 2.181         | 0.000 |
|             | Ethnic group         | 0.034          | 0.017          | 0.051       | 2.031         | 0.042 |
| ApoB        | Waist circumference  | 0.010          | 0.001          | 0.197       | 7.787         | 0.000 |
|             | Ethnic group         | −0.134         | 0.021          | −0.157      | −6.357        | 0.000 |
|             | Blood glucose        | 0.025          | 0.007          | 0.094       | 3.760         | 0.000 |
|             | Cigarette smoking    | 0.050          | 0.020          | 0.062       | 2.509         | 0.012 |
|             | Systolic blood       | 0.001          | 0.001          | 0.066       | 2.590         | 0.010 |
| ApoA1/ApoB  | Waist circumference  | −0.019         | 0.002          | −0.198      | −7.862        | 0.000 |
|             | Age                  | −0.005         | 0.001          | −0.089      | −3.544        | 0.000 |
| Han         | TC                   | 0.019          | 0.005          | 0.131       | 3.767         | 0.000 |
|             | Age                  | 0.009          | 0.003          | 0.126       | 3.443         | 0.001 |
|             | Alcohol consumption  | 0.302          | 0.057          | 0.179       | 5.286         | 0.000 |
|             | Diastolic blood      | 0.017          | 0.004          | 0.168       | 4.661         | 0.000 |
|             | Blood glucose        | 0.066          | 0.024          | 0.095       | 2.723         | 0.007 |
| TG          | Waist circumference  | 0.075          | 0.013          | 0.254       | 5.661         | 0.000 |
|             | Cigarette smoking    | 0.805          | 0.165          | 0.185       | 4.889         | 0.000 |
|             | Blood glucose        | 0.265          | 0.049          | 0.186       | 5.407         | 0.000 |
|             | Diastolic blood      | 0.030          | 0.007          | 0.147       | 4.120         | 0.000 |
|             | Age                  | −0.017         | 0.005          | −0.113      | −3.114        | 0.002 |
|             | Alcohol consumption  | 0.269          | 0.132          | 0.078       | 2.040         | 0.042 |
|             | Body mass index      | −0.065         | 0.030          | −0.096      | −2.157        | 0.031 |
| HDL-C       | Waist circumference  | −0.011         | 0.002          | −0.155      | −4.279        | 0.000 |
|             | Gender               | 0.130          | 0.046          | 0.120       | 2.825         | 0.005 |
|             | Alcohol consumption  | 0.111          | 0.034          | 0.138       | 3.317         | 0.001 |
| LDL-C       | Age                  | 0.012          | 0.002          | 0.212       | 6.219         | 0.000 |
|             | Body mass index      | 0.026          | 0.012          | 0.101       | 2.222         | 0.027 |
|             | Waist circumference  | 0.013          | 0.005          | 0.115       | 2.471         | 0.014 |
|             | Cigarette smoking    | −0.310         | 0.072          | −0.187      | −4.227        | 0.000 |
hypercholesterolaemic Japanese subjects, Miwa et al. [42] reported that carriers of the \textit{ABCG8} M429V or a specific haplotype (wild-type allele of \textit{ABCG5} Q604E, and wild-type alleles of \textit{ABCG8} C54Y, T400K, and M429V) had higher cholesterol absorption efficiency than non-carriers. However, no difference was observed in serum lipid profiles in relation to common SNPs studied previously in Caucasian populations (\textit{ABCG5} Q604E and \textit{ABCG8} A632V, T400K, D19H and C54Y). This might be explained by the fact that carriers of \textit{ABCG8} D19H and A632V SNPs are rare among Japanese compared to Caucasian populations. Interestingly, in 1046 Chinese, Chen et al. [43] observed that the heterozygote of \textit{ABCG8} D19H had higher serum total and LDL-C levels than homozygote DD, which is opposite to the effect observed in Caucasian populations. The author speculated that this opposite effect may be due to the specific Chinese dietary pattern with high intake of plant sterols. No association with \textit{ABCG8} C54Y and T400K regarding total and LDL-C levels was observed. \textit{ABCG8} A632V was monomorphic in this Chinese population. For 181 hyperlipidaemic patients treated with atorvastatin (89 males), variant allele frequencies of \textit{ABCG8} 1199A were 12.8%. For patients with the \textit{ABCG8} C1199A variant allele, the difference in percentage reduction from baseline in TG level was increased between the CYP7A1 A-204C wild-type allele homozygotes and variant allele homozygotes after atorvastatin treatment ($-28.35\%$ vs. $-19.28\%$, $P=0.001$). The \textit{ABCG8} 1199A and CYP7A1 -204A alleles

Table 6. Cont.

| Lipid          | Risk factor        | Unstandardized coefficient | Std. error | Standardized coefficient | t     | P    |
|----------------|--------------------|----------------------------|------------|--------------------------|-------|------|
| ApoA1          | Gender             | -0.223                     | 0.080      | 0.126                    | -2.802| 0.005|
|                | Alcohol consumption| 0.128                      | 0.017      | 0.319                    | 7.691 | 0.000|
|                | Body mass index    | -0.011                     | 0.003      | -0.136                   | -3.951| 0.001|
|                | Gender             | 0.089                      | 0.025      | 0.164                    | 3.536 | 0.000|
|                | Cigarette smoking  | 0.072                      | 0.023      | 0.141                    | 3.154 | 0.002|
| ApoB           | Waist circumference| 0.005                      | 0.001      | 0.207                    | 4.741 | 0.000|
|                | Blood glucose      | 0.022                      | 0.004      | 0.168                    | 5.102 | 0.000|
|                | Alcohol consumption| 0.038                      | 0.012      | 0.123                    | 3.264 | 0.001|
|                | Diastolic blood pressure | 0.002  | 0.001 | 0.098                    | 2.877 | 0.004|
|                | Body mass index    | 0.007                      | 0.003      | 0.115                    | 2.702 | 0.007|
|                | Gender             | -0.046                     | 0.016      | -0.111                   | -2.907| 0.004|
|                | Age                | 0.001                      | 0.000      | 0.089                    | 2.556 | 0.011|
| ApoA1/ApoB     | Waist circumference| -0.010                     | 0.003      | -0.157                   | -3.476| 0.001|
|                | Body mass index    | -0.025                     | 0.007      | -0.169                   | -3.814| 0.000|
|                | Cigarette smoking  | 0.134                      | 0.042      | 0.140                    | 3.198 | 0.001|
|                | Gender             | 0.261                      | 0.047      | 0.225                    | 5.539 | 0.000|
|                | Age                | -0.004                     | 0.001      | -0.110                   | 3.319 | 0.001|
|                | Alcohol consumption| 0.090                      | 0.031      | 0.118                    | 2.914 | 0.004|
| Mulao          | TC                 | 0.011                      | 0.003      | 0.142                    | 3.886 | 0.000|
|                | Gender             | 0.055                      | 0.014      | 0.146                    | 3.868 | 0.000|
|                | Alcohol consumption| 0.148                      | 0.057      | 0.095                    | 2.595 | 0.010|
| TG             | Waist circumference| 0.043                      | 0.006      | 0.250                    | 6.917 | 0.000|
|                | Alcohol consumption| 0.221                      | 0.073      | 0.105                    | 2.915 | 0.004|
| HDL-C          | Alcohol consumption| 0.099                      | 0.027      | 0.160                    | 3.648 | 0.000|
|                | Body mass index    | -0.040                     | 0.005      | -0.265                   | -7.394| 0.000|
|                | Gender             | 0.090                      | 0.040      | 0.098                    | 2.235 | 0.026|
| LDL-C          | Age                | 0.008                      | 0.002      | 0.147                    | 4.033 | 0.000|
|                | Body mass index    | 0.049                      | 0.010      | 0.177                    | 4.877 | 0.000|
| ApoA1          | Alcohol consumption| 0.115                      | 0.024      | 0.215                    | 4.765 | 0.000|
|                | Gender             | 0.084                      | 0.036      | 0.105                    | 2.333 | 0.020|
| Apo B          | Waist circumference| 0.013                      | 0.002      | 0.191                    | 5.208 | 0.000|
|                | Blood glucose      | 0.032                      | 0.013      | 0.089                    | 2.418 | 0.016|
| ApoA1/ApoB     | Age                | -0.006                     | 0.002      | -0.086                   | -2.336| 0.020|
|                | Waist circumference| -0.019                     | 0.004      | -0.160                   | -4.340| 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.

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Han females were different among the CC, CA and AA genotypes of ABCG8. Subgroup analysis showed that the levels of ApoA1 in Mulao, and plasma HDL-C levels possibly reconcile with an old concept: hepatobiliary excreted cholesterol mainly originates from HDL-derived cholesterol [44]. Hypercholesterolemic subjects carrying the GG genotype of the ABCG8 251A>G SNP also exhibited higher values of HDL-C when compared to other genotypes [54]. However, these associations were not observed in some earlier studies conducted in Caucasian populations with relatively large sample size [31,36,37] and also in recent GWA studies [11,15]. Therefore, this issue needs further exploration. Recently, significant gene-gene interactions for HDL-C were found between ABCG8 (5U145 A>C, T54C A>G, T400K C>A) SNPs and ABCA1_i48168 G>A genetic variant, in which carriers of the 5U145C and 54C alleles, and homozygotes for the T400 allele at ABCG8 genetic variants displayed lower HDL-C concentrations than homozygotes for the 5U145A and T54 alleles, and heterozygotes for the 400K allele at ABCG8 SNPs, only if they were also homozygous for the minor allele (A) at the aforementioned ABCA1 SNP [63]. Overall, no consistent results on effects of the ABCG8 SNPs on serum lipid levels and cholesterol metabolic kinetics were reported so far. Failure to identify a consistent association may be due to variations in populations examined, including healthy, hypercholesterolemic, and overweight/obese subjects; modulating environmental factors such as diet or pharmaceutical treatments; or simply, a lack of power to allow for any robust conclusion to be drawn. In the present study, we showed that the levels of ApoA1 in Mulao, and HDL-C, ApoA1, ApoB, and the ratio of ApoA1 to ApoB in Han were different among the CC, CA and AA genotypes of ABCG8 (rs3806471 SNP). Subgroup analysis showed that the levels of HDL-C and ApoA1, and the ratio of ApoA1 to ApoB in Han males, and LDL-C, ApoB, and the ratio of ApoA1 to ApoB in Han females were different among the CC, GA and AA genotypes of ABCG8. On stratification by age, we showed that the levels of TG, HDL-C, ApoA1, and the ratio of ApoA1 to ApoB in Han males, and HDL-C, ApoA1, and the ratio of ApoA1 to ApoB in Han females were different among the GG, GA and AA genotypes of ABCG8. The A allele carriers had lower TG, HDL-C, ApoA1, and the ratio of ApoA1 to ApoB than the A allele noncarriers. The levels of TG and ApoA1, and the ratio of ApoA1 to ApoB in Han males were correlated with the genotypes, and the levels of HDL-C in Han females were associated with the genotypes.

The extents to which serum lipid levels are affected by genetic and environmental factors remain a subject of controversy, but several environmental factors such as dietary patterns, lifestyle, obesity, physical activity, and hypertension have been shown association with serum lipid levels [6]. In the present study, we also showed that serum lipid parameters were correlated with age, sex, alcohol consumption, cigarette smoking, BMI, and blood pressure in the both ethnic groups or both sexes. These results suggest that the environmental factors and their interactions with genetic factors also play an important role in determining serum lipid levels in our populations. Although rice and corn are the staple foods in both ethnic groups, the people of Mulao nationality like to eat cold foods along with acidic and spicy dishes, so bean soy sauce and pickled vegetables are among their most popular dishes. They also like to eat animal offals which contain abundant saturated fatty acid. For nearly 50 years it has been widely accepted that high-fat diets, particularly those that contain large quantities of saturated fatty acids, raise blood cholesterol concentrations and predispose individuals to CVD. In the current study, we also found that the levels of TG and ApoB and the percentages of subjects who consumed alcohol were higher in Mulao nationality than in Han nationality. In a previous meta-analysis, 30 g of alcohol daily was associated with a plasma TG increase of 5.69 mg/dl [64]. The alcohol intake of 60 g/day increases the TG levels by about 0.19 mg/dl per 1 gram of alcohol consumed [65].

In summary, the present study shows that the associations of four ABCG5/G8 SNPs and serum lipid levels are different between the Mulao and Han populations, or between males and females. These findings suggest that there may be a sex- and/or racial/ethnic-specific association of ABCG5/G8 SNPs and some serum lipid parameters in our study populations.

**Author Contributions**

Conceived and designed the experiments: R-XY QL. Performed the experiments: QL, X-LW T-TY LHHA D-FW J-ZW W-XL C-WL S-LP. Analyzed the data: QL. Contributed reagents/materials/analysis tools: R-XY QL. Performed the epidemiological survey and collected the samples: R-XY QL. Wrote the paper: R-XY QL. Carried out the epidemiological survey and collected the samples: R-XY QL. Contributed reagents/materials/analysis tools: R-XY QL. Performed the experiments: R-XY QL. Performed the experiments: QL, X-LW T-TY LHHA D-FW J-ZW W-XL C-WL S-LP.

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