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# *APOE* gene ε4 allele (388C-526C) effects on serum lipids and risk of coronary artery disease in southern Chinese Hakka population

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## Abstract

**Objective:** To analyze the relationship of Apolipoprotein E (*APOE*) and solute carrier organic anion transporter family member 1B1 (*SLCO1B1*) gene polymorphisms with coronary artery disease (CAD).

**Methods:** 1,129 CAD patients and 1,014 non-CAD controls were included in the study, and relevant information and medical records were collected. The single-nucleotide polymorphisms (SNPs) were analyzed, including rs429358, rs7412 in *APOE* gene and rs2306283, rs4149056 in *SLCO1B1* gene.

**Results:** The CAD patients' average age was 66.3 ± 10.7 years, while 65.5 ± 12.0 years in controls. The frequencies of *APOE* allele ε3, ε4, and ε2 were 83.01%, 10.08%, and 6.91% respectively. There were statistically significant differences in genotype ε3/ε4 ($\chi^2 = 8.077$, $p = 0.005$) in CAD patients compared with the controls. The *SLCO1B1* genotype *1b/*1b and haplotype *1b showed the highest frequency in the study sample. Moreover, ε4 carriers had significantly lower HDL-C, Apo-A1 levels than ε3 carriers among CAD patients, while ε2 carriers showed lower LDL-C, Apo-B level, and higher Apo-A1/Apo-B level than ε3 and ε4 carriers. In controls, ε2 carriers showed lower LDL-C and Apo-B level, higher Apo-A1, and Apo-A1/Apo-B level than ε4 carriers. Logistic regression analysis showed that high LDL-C and Apo-B level, low HDL-C level, smoking, and the ε4 allele were risks for the presence of CAD.

**Abbreviations:** Apo-A1, apolipoprotein A1; Apo-B, apolipoprotein B; APOE, apolipoprotein E; HDL-C, high-density lipoprotein-cholesterol; LDL-C, low-density lipoprotein-cholesterol; SLCO1B1, solute carrier organic anion transporter family member 1B1; TC, total cholesterol; TG, triglyceride.

Qinghua Liu and Heming Wu are Contributed equally to this work.

**Conclusions:** *APOE* ε4 allele may be associated with susceptibility to CAD in southern Chinese Hakka population. It indicated that the *APOE* SNPs rs429358 and rs7412 are associated with CAD, but not SNPs rs2306283 and rs4149056 of *SLCO1B1* gene.

**KEYWORDS**
apolipoprotein E, coronary artery disease, gene polymorphism, Hakka, solute carrier organic anion transporter family member 1B1

## 1 | INTRODUCTION

Coronary artery disease (CAD) is a kind of coronary artery atherosclerosis, narrowing or occlusion of vascular lumen, resulting in myocardial ischemia and hypoxia or necrosis caused by heart disease, and is one of the main causes of death in developed and developing countries.[1,2] CAD is an atherosclerotic inflammatory disease characterized by stable angina, unstable angina, myocardial infarction, or sudden cardiac death.[3] Smoking, drinking, obesity, hypertension, bad living habits, and genetic factors are considered to be closely related to the incidence of CAD. So far, more than 60 genetic variants have been confirmed to be associated with increased susceptibility to CAD using genome-wide association studies (GWAS).[4] Previous studies have shown that genes related to CAD mainly include genes related to vascular structure and function, lipid metabolism, and inflammatory cytokines.[5-7]

Apolipoprotein E (ApoE) is one of the apolipoproteins of chylomicron, high-density lipoprotein-cholesterol (HDL-C), low-density lipoprotein-cholesterol (LDL-C), and very low-density lipoprotein-cholesterol (VLDL-C), which plays an important role in regulating lipoprotein metabolism. ApoE is a multifunctional protein that plays an important role in lipid metabolism by binding to LDL receptors and mediating the removal of chylomicron and VLDL from serum.[8] *APOE* (OMIM 107741) is the gene that codes for ApoE, which its cytogenetic location is 19q13.32. There are 2 common single-nucleotide polymorphisms (SNPs) in *APOE* gene: rs429358 (388T > C) and rs7412 (526C > T). And 3 alleles (ε2(388T-526T), ε3(388T-526C), and ε4(388C-526C)) and 6 genotypes (ε2/ε2, ε2/ε3, ε2/ε4, ε3/ε3, ε3/ε4, and ε4/ε4) can be formed by the 2 SNPs.[9] ApoE2, -E3, and -E4 are 3 major isoforms of human ApoE, which coded by 3 alleles (epsilon (ε) 2, 3, and 4).

Solute carrier organic anion transporter family member 1B1 (SLCO1B1) is an intake transporter for the transport of substances from the blood to the liver. SLCO1B1 is encoded by *SLCO1B1* gene (located on chromosome 12p12.1). The rs2306283 (388A > G) and rs4149056 (521T > C) are 2 common SNPs in *SLCO1B1* gene.[10,11] 4 haplotypes can be formed by these two SNPs: *1a (388A-521T), *1b (388G-521T), *5 (388A-521C), and *15 (388G-521C).[12-14] To date, most studies on *SLCO1B1* have focused on the effect of *SLCO1B1* polymorphisms on the pharmacokinetics, efficacy and side effects of glucose-lowering drugs, statins, and antitumor drug.[15,16]

*APOE* and *SLCO1B1* gene polymorphisms are associated with the efficacy and side effects of statin lipid-lowering drugs, and also affect the occurrence and development of some diseases. However, most patients with CAD will have lipid metabolism dysfunction. Whether these SNP sites are associated with the susceptibility to CAD has not been systematically reported. Although some studies have analyzed the relationship between *APOE* gene polymorphisms and the risk of cardiovascular and cerebrovascular diseases, the results are inconsistent in different regions and populations. In this study, the relationship between *SLCO1B1* and *APOE* polymorphisms and CAD was analyzed in southern Chinese Hakka population.

## 2 | MATERIALS AND METHODS

### 2.1 | Population samples

A total of 2,143 subjects were recruited from Meizhou People's Hospital (Huangtang Hospital), China, between September 2016 and May 2020, including 1129 CAD patients and 1014 individuals with non-CAD as controls. The diagnosis of CAD was based on the American College of Cardiology/American Heart Association (ACC/AHA) classification. The patients have chest pain, ischemic changes in electrocardiograph (ECG), and increased myocardial enzymes by clinical evaluation and more than 50% reduction of coronary artery diameter in at least one of the major arteries proved by coronary angiography. Patients with severe liver, kidney, brain diseases, malignant tumors, and hematological diseases were excluded. Information recorded included demographic data (age, sex), history of major chronic diseases (hypertension, diabetes), smoking history, and alcohol consumption history. This study was performed in accordance with the ethical standards laid down in the updated version of the 1964 Declaration of Helsinki and approved by the Human Ethics Committees of Meizhou People's Hospital (Clearance No.: 2016-A-29). Informed consent was obtained from the patients or their families, and participants' privacy was carefully protected.

### 2.2 | Serum lipid measurements

About 3ml of blood was taken from each subject, serum was rapidly separated and tested, and samples that could not be immediately tested were stored at −80°C. Serum lipid levels of samples were evaluated in the Olympus AU5400 system (Olympus Corporation, Tokyo, Japan), test indicators including total cholesterol (TC),

triglyceride (TG), LDL-C, HDL-C, apolipoprotein B (Apo-B), and apolipoprotein A1 (Apo-A1). Serum lipid levels were measured by the corresponding detection methods following the manufacturers' instructions.

### 2.3 | DNA extraction and genotyping assay

Genomic DNA was extracted from whole blood using a TIANamp Blood DNA Kit (Tiangen, Beijing, China) according to the manufacturer's instructions. *SLCO1B1* and *APOE* gene polymorphisms were detected by TaqMan probe fluorescent PCR method through different channels in the reaction system with different primers and probes combinations (Youzhiyou Medical Technology Co., Ltd, Hubei, China). PCR was used to amplify the target fragments using the Roche LightCycler 480 II system: initial denaturation at 95℃ for 5 min, followed by 40 cycles of denaturation at 95℃ for 15s, annealing, and extension at 60℃ for 1 min. FAM (SLCO1B1*1b 388A, SLCO1B1*5 521T, ApoE2 526C, ApoE4 388T), VIC (SLCO1B1*1b 388G, SLCO1B1*5 521C, ApoE2 526T, and ApoE4 388C) and ROX (internal standard) fluorescence signals were collected.

### 2.4 | Statistical analysis

SPSS statistical software version 21.0 (IBM Inc., State of New York, USA) was used for data analysis. Continuous variable data are represented by mean ± SD and analyzed using Student's *t* test or the Mann-Whitney U test. The Chi-square test was used for analyzing categorical variables, which were presented as percentages. Logistic regression analysis was used to evaluate the interactions between *SLCO1B1* and *APOE* polymorphisms and various factors (age, gender, smoking history, drinking history, prevalence of hypertension, and diabetes, etc.) in CAD. $p < 0.05$ was considered statistically significant.

## 3 | RESULTS

### 3.1 | Population characteristics

In this study, there were 2,143 subjects (65.9 ± 11.4 years), with the youngest is 20 years, the oldest is 97 years, consisted of 1,129 CAD patients (694 (61.47%) males and 435 (38.53%) females), and 1014 non-CAD controls (595 (58.68%) males and 419 (41.32%) females). The CAD patients' average age was 66.3 ± 10.7 years, while 65.5 ± 12.0 years in controls. There were statistically significant differences in percentage of smokers (CAD patients vs. non-CAD controls: 31.62% vs. 19.72%, $p < 0.001$), TG level (1.949 ± 1.573 mmol/L vs. 1.642 ± 1.184 mmol/L, $p < 0.001$), HDL-C level (1.237 ± 0.318 mmol/L vs. 1.307 ± 0.357 mmol/L, $p < 0.001$), and Apo-B level (0.898 ± 0.284 g/L vs. 0.861 ± 0.270 g/L, $p = 0.002$) between the patients and controls, while no statistically significant differences in age, TC, LDL-C, Apo-A1, gender composition ratio, and percentage of alcoholics, hypertension, and diabetes (Table 1).

### 3.2 | Genotype and haplotype frequencies of *APOE* gene

The frequencies of genotype ε3/ε3, ε3/ε4, ε2/ε3, ε2/ε4, ε4/ε4, and ε2/ε2 were 69.16%, 16.61%, 11.11%, 1.21%, 1.17%, and 0.75%, respectively, in all subjects. The frequencies of allele ε3, ε4, and ε2 were 83.01%, 10.08%, and 6.91% respectively. The genotype distribution in controls was consistent with Hardy-Weinberg equilibrium ($\chi^2 = 0.515$, $p = 0.992$). The results showed that the most common *APOE* genotype was ε3/ε3, and the frequencies of alleles in order from high to low were ε3, ε4, and ε2 (Table 2).

There were statistically significant differences in genotype ε3/ε4 ($\chi^2 = 8.077$, $p = 0.005$) in CAD patients compared with the controls. The frequency of allele ε4 ($\chi^2 = 8.931$, $p = 0.003$) showed statistically significant difference in the patients compared with controls. The differences in other genotypes (ε2/ε2, ε2/ε3, ε2/ε4, ε3/ε3, and ε4/ε4) and alleles (ε2 and ε3) of *APOE* gene between the CAD patients and the controls were not statistically significant (all $p > 0.05$) (Table 2).

### 3.3 | Genotype and haplotype frequencies of *SLCO1B1* gene

Of all the participants, the frequencies of genotype *1b/*1b, *1a/*1b, *1b/*15, *1a/*15, *1a/*1a, *15/*15, and *1a/*5 were 39.06%, 31.64%, 15.59%, 6.16%, 5.83%, 1.63%, and 0.09%, respectively. The genotypes frequencies in the CAD patients were 38.35%, 31.36%, 16.56%, 5.76%, 6.20%, 1.77%, and 0%, and 39.84%, 31.95%, 14.50%, 6.61%, 5.42%, 1.48%, and 0.20% in the controls. The genotype distribution in controls was consistent with Hardy-Weinberg equilibrium ($\chi^2 = 1.397$, $p = 0.968$). There were no statistically significant differences in the frequencies of these genotypes between CAD patients and controls. The *1b haplotype (62.67%) presented the highest frequency, followed by haplotype *1a (24.78%), *15 (12.51%), and *5 (0.05%). The frequencies of *SLCO1B1* haplotypes between CAD patients and controls showed no statistically significant differences (Table 3).

### 3.4 | Relationships between serum lipid levels and *APOE* alleles, *SLCO1B1* genotypes and logistic regression analysis of the risks for CAD

Relationships between *APOE* alleles (ε2, ε3, and ε4) and serum lipid levels were analyzed. Because the ε2 and ε4 alleles play opposite roles in lipid metabolism, subjects with both ε2 and ε4 alleles (ε2/ε4 genotype) were excluded (n = 26, 15 patients, and 11 controls). In CAD patients, ε4 carriers had significantly lower HDL-C

**TABLE 1** Comparison of clinical characteristics of coronary artery disease (CAD) patients and non-CAD controls

| | CAD Patients (n = 1129) | Non-CAD controls (n = 1014) | *p* Values |
|---|---|---|---|
| Age, years | 66.3 ± 10.7 | 65.5 ± 12.0 | 0.112 |
| Gender | | | |
| Male | 694 (61.47%) | 595 (58.68%) | 0.188 ($\chi^2 = 1.737$) |
| Female | 435 (38.53%) | 419 (41.32%) | |
| History of smoking, n(%) | 357 (31.62%) | 200 (19.72%) | <0.001 ($\chi^2 = 39.307$) |
| History of alcoholism, n(%) | 58 (5.14%) | 55 (5.42%) | 0.772 ($\chi^2 = 0.088$) |
| Hypertension, n(%) | 645 (57.13%) | 561 (55.33%) | 0.408 ($\chi^2 = 0.707$) |
| Diabetes, n(%) | 333 (29.50%) | 271 (26.73%) | 0.163 ($\chi^2 = 2.024$) |
| TG, mmol/L | 1.949 ± 1.573 | 1.642 ± 1.184 | <0.001 |
| TC, mmol/L | 4.948 ± 1.242 | 4.959 ± 1.218 | 0.831 |
| HDL-C, mmol/L | 1.237 ± 0.318 | 1.307 ± 0.357 | <0.001 |
| LDL-C, mmol/L | 2.737 ± 0.881 | 2.791 ± 0.869 | 0.156 |
| Apo-A1, g/L | 1.146 ± 0.274 | 1.172 ± 0.334 | 0.052 |
| Apo-B, g/L | 0.898 ± 0.284 | 0.861 ± 0.270 | 0.002 |
| Apo-A1/ Apo-B | 1.385 ± 0.493 | 1.479 ± 0.610 | <0.001 |

*Note:* Values for age expressed as n (%) or mean ± SD.

Abbreviations: Apo-A1, apolipoprotein A1; Apo-B, apolipoprotein B; HDL-C, high-density lipoprotein-cholesterol; LDL-C, low-density lipoprotein-cholesterol; TG, triglycerides; TC, total cholesterol;.

(1.193 ± 0.321 mmol/L vs. 1.255 ± 0.322 mmol/L, $p = 0.029$), Apo-A1 (1.106 ± 0.266 g/L vs. 1.156 ± 0.278 g/L, $p = 0.047$) levels than ε3 carriers, while ε2 carriers showed lower LDL-C ($p = 0.004$) and Apo-B ($p = 0.008$) and higher Apo-A1/Apo-B ($p < 0.001$) than ε3 and ε4 carriers. In controls, ε2 carriers showed lower LDL-C and Apo-B, higher Apo-A1 and Apo-A1/Apo-B (all $p < 0.05$) than ε4 carriers (Table 4).

Logistic regression analysis was performed to determine independent predictors for CAD. The results indicated significantly higher risks of CAD in the presence of high LDL-C level (adjusted OR 2.885, 95% CI 2.016–4.129, $p < 0.001$), and Apo-B level (adjusted OR 1.680, 95% CI 1.296–2.178, $p < 0.001$), low HDL-C level (adjusted OR 0.459, 95% CI 0.498–0.839, $p = 0.005$), smoking (adjusted OR 2.043, 95% CI 1.619–2.577, $p < 0.001$), and the ε4 allele (adjusted OR 1.354, 95% CI 1.068–1.717, $p = 0.012$) (Table 5).

## 4 | DISCUSSION

CAD is a common chronic disease in the world. In recent years, the incidence of CAD has gradually increased, and it has become one of the main causes of death.[17,18] The incidence of CAD is increasing among younger individuals.[19] The relationship between gene polymorphisms and genetic susceptibility to CAD has been the focus of clinical and epidemiological studies in recent years. Many studies have shown that the etiologies of CAD are complex, including genetic and environmental factors.[4,20,21] The relationship between genetic polymorphisms of *APOE* and *SLCO1B1* and CAD in Hakka population was analyzed in this study.

Atherosclerosis is an important pathophysiological basis of CAD. The main cause of CAD is the formation of atherosclerotic plaque, and the increase of serum lipid level is the main factor of the formation of atherosclerotic plaque.[22,23] ApoE is a major lipid-binding protein that serves as a carrier for chylomicron, HDL-C, LDL-C, and VLDL-C.[24] However, the results on the relationship between *APOE* gene polymorphisms and serum lipid level are not consistent. Rajesh Chaudhary et al evaluated the effect of *APOE* on lipids has shown that carriers of the ε2 allele have lower TC level and higher TG level, while carriers of the ε4 allele have higher TC and LDL levels.[25] Another study showed that the *APOE* ε4 allele is associated with higher serum lipid levels, whereas the ε2 allele is associated with the lower levels.[26] A Pablos-Méndez et al reported that the presence of ε2 has been associated with lower LDL-C level but with no influence on the HDL-C level.[27]

In the present study, ε4 carriers had significantly lower HDL-C and Apo-A1 levels than ε3 carriers among CAD patients, while ε2 carriers showed lower LDL-C, Apo-B levels, and higher Apo-A1/Apo-B level than ε3 and ε4 carriers. In controls, ε2 carriers showed lower LDL-C and Apo-B levels, higher Apo-A1 and Apo-A1/Apo-B levels than ε4 carriers. ApoE is most important for lipid and lipoprotein metabolism. ApoE is the ligand for the LDL receptor family of proteins and itself assimilates and transfers lipids. The aspartic acid at 154 forms a salt bridge with arginine at position 158 in ApoE3 and ApoE4. In the absence of arginine at position 158, aspartate at position 154 of ApoE2 forms a salt bridge with arginine at position 150. It disrupted the binding of ApoE to the LDL receptor significantly.[28] In this study, there is less effect of *APOE* gene polymorphisms on TC, LDL-C levels in CAD, and control. The effects of *APOE* alleles

**TABLE 2** Genotypes and alleles distribution of *APOE* gene in CAD patients and control participants

| Genotypes | ε2/ε2 | ε2/ε3 | ε2/ε4 | ε3/ε3 | ε3/ε4 | ε4/ε4 |
|---|---|---|---|---|---|---|
| All subjects(n = 2143) | 16(0.75%) | 238(11.11%) | 26(1.21%) | 1482(69.16%) | 356(16.61%) | 25(1.17%) |
| Patients(n = 1129) | 10(0.89%) | 113(10.01%) | 15(1.33%) | 764(67.67%) | 212(18.78%) | 15(1.33%) |
| Controls(n = 1014) | 6(0.59%) | 125(12.33%) | 11(1.08%) | 718(70.81%) | 144(14.20%) | 10(0.99%) |
| *p* Values (Patients vs. controls) | 0.463($\chi^2$ = 0.623) | 0.098($\chi^2$ = = 2.909) | 0.695($\chi^2$ = 0.265) | 0.122($\chi^2$ = 2.466) | 0.005($\chi^2$ = 8.077) | 0.548($\chi^2$ = 0.543) |
| **Alleles** | **ε2** | **ε3** | **ε4** | | | |
| All subjects(n = 4286) | 296(6.91%) | 3558(83.01%) | 432(10.08%) | | | |
| Patients(n = 2258) | 148(6.55%) | 1853(82.06%) | 257(11.38%) | | | |
| Controls(n = 2028) | 148(7.30%) | 1705(84.07%) | 175(8.63%) | | | |
| *p* Values (Patients vs controls) | 0.338($\chi^2$ = 0.918) | 0.087($\chi^2$ = 3.059) | 0.003($\chi^2$ = 8.931) | | | |

*Note*: Numbers in parentheses are percentages.

on differential lipid profile modulation depend on both genetic and environmental factors. Variations in other lipid metabolism-related genes based on population genetic differences may influence differences in lipid spectrum regulation.[29-31] In addition, one factor that may modulate the relationship between *APOE* gene variations and lipid levels is body mass index (BMI).[32] BMI information was not collected and analyzed, which was one of the shortcomings of this study.

*APOE* gene polymorphisms have been shown to be associated with atherosclerosis.[33,34] However, some previous studies on the association between *APOE* gene polymorphisms and CAD have yielded inconsistent results. Bennet et al found that there was an approximate linear relationship between *APOE* genotype, LDL level, and CAD risk. Compared with individuals with ε3/ε3 genotype, the risk of CAD in ε2 carriers was reduced by 20%, while the risk of ε4 carriers was slightly higher.[35] Some studies found that the risk of CAD in carriers of ε3/ε4 and ε4/ε4 genotypes was significantly higher than that in carriers of ε3/ε3 genotype, but there was no evidence to find a difference between the variant genotypes of ε2/ε2, ε2/ε3, ε2/ε4, and the risk of CAD.[36,37] In this study, there was statistically significant difference in genotype ε3/ε4 among CAD patients compared with controls. Logistic regression analysis indicated that participants with ε4 allele had a significantly higher risk of CAD. However, another study has been shown that *APOE* gene polymorphism has no significant association with the occurrence and development of CAD in the study on the relationship between *APOE* gene polymorphism and blood lipid and CAD in African Caribbean people.[38] These inconsistencies may be related to ethnic and regional differences.

Furthermore, this study found ε3 to be the most common allele of the *APOE* gene, accounting for 83.01%, which was consistent with most previous studies.[28,39] This indicates that the *APOE* allele frequencies in the Meizhou area are similar to those of the other studies.[40-43]

The frequencies of *SLCO1B1* genotypes *1b/*1b, *1a/*1b, *1b/*15, *1a/*15, *1a/*1a, *15/*15, and *1a/*5 were 39.06%, 31.64%, 15.59%, 6.16%, 5.83%, 1.63%, and 0.09%, respectively. The *1b haplotype (62.67%) presented the highest frequency, followed by haplotype *1a (24.78%), *15 (12.51%), and *5 (0.05%). Our results are consistent with previous studies.[39,44-47] The most common haplotype in the German (49.5%) and in the Turkish population (46.3%) was *SLCO1B1* *1a.[48] Greek,[49] Roma and Hungarian,[50] Indian (North),[51] and Brazilian[52] populations exhibit relatively lower rates of *1b (<50%), whereas Thai[53,54] and Chinese[55] populations show higher rates, generally above 60%–70%. In contrast, the allele frequency of haplotypes *15 and *5 displayed little difference. Ghassibe-Sabbagh M et al reported that rs4149056 in *SLCO1B1* was positively associated with hyperhomocysteinemia and may increase CAD risk.[56] Rong Lin et al identified significant association of rs4149013 in *SLCO1B1* with male CAD.[57] FF Hao et al reported that *SLCO1B1* c.388A > G and c.521T > C may have no correlation between gene polymorphisms and the incidence of CAD in Yunnan Bai population.[58] In this study, *SLCO1B1* genotypes and haplotypes have no significant relationship with the risk of CAD.

**TABLE 3** Genotypes and alleles distribution of *SLCO1B1* gene in CAD patients and control participants.

| **Genotypes** | ***15/*15** | ***1a/*15** | ***1a/*1a** | ***1a/*1b** | ***1a/*5** | ***1b/*15** | ***1b/*1b** |
|---|---|---|---|---|---|---|---|
| All subjects(n = 2143) | 35 (1.63%) | 132 (6.16%) | 125 (5.83%) | 678 (31.64%) | 2 (0.09%) | 334 (15.59%) | 837 (39.06%) |
| Patients(n = 1129) | 20 (1.77%) | 65 (5.76%) | 70 (6.20%) | 354 (31.36%) | 0 (0) | 187 (16.56%) | 433 (38.35%) |
| Controls(n = 1014) | 15 (1.48%) | 67 (6.61%) | 55 (5.42%) | 324 (31.95%) | 2 (0.20%) | 147 (14.50%) | 404 (39.84%) |
| *p* Values (Patients vs controls) | 0.614 ($\chi^2 = 0.284$) | 0.420 ($\chi^2 = 0.668$) | 0.461 ($\chi^2 = 0.586$) | 0.780 ($\chi^2 = 0.088$) | 0.224 ($\chi^2 = 2.229$) | 0.190 ($\chi^2 = 1.734$) | 0.506 ($\chi^2 = 0.498$) |
| **Alleles** | ***15** | ***5** | ***1a** | ***1b** | | | |
| All subjects(n = 4286) | 536 (12.51%) | 2 (0.05%) | 1062 (24.78%) | 2686 (62.67%) | | | |
| Patients(n = 2258) | 292 (12.93%) | 0 (0) | 559 (24.76%) | 1407 (62.31%) | | | |
| Controls(n = 2028) | 244 (12.03%) | 2 (0.10%) | 503 (24.80%) | 1279 (63.07%) | | | |
| *p* Values (Patients vs controls) | 0.380 ($\chi^2 = 0.791$) | 0.224 ($\chi^2 = 2.228$) | 0.972 ($\chi^2 = 0.001$) | 0.613 ($\chi^2 = 0.261$) | | | |

*Note:* Numbers in parentheses are percentages.

**TABLE 4** Relationships between serum lipid level and *APOE* allele in CAD patients and control participants.

| | **CAD patients (n = 1114)** | | | | **Controls (n = 1003)** | | | |
|---|---|---|---|---|---|---|---|---|
| **Serum lipid level** | **ε2 (n = 123)** | **ε3 (n = 764)** | **ε4 (n = 227)** | ***p* Values** | **ε2 (n = 131)** | **ε3 (n = 718)** | **ε4 (n = 154)** | ***p* Values** |
| TG, mmol/L | 2.191 ± 1.500 | 1.907 ± 1.587 | 1.873 ± 1.441 | 0.137 | 1.857 ± 1.242◊ | 1.640 ± 1.229◊ | 1.424 ± 0.785* | 0.008 |
| TC, mmol/L | 4.815 ± 1.268 | 4.965 ± 1.170 | 4.953 ± 1.384 | 0.454 | 5.001 ± 1.423 | 4.938 ± 1.178 | 4.986 ± 1.042 | 0.802 |
| HDL-C, mmol/L | 1.218 ± 0.271 | 1.255 ± 0.322◊ | 1.193 ± 0.321* | 0.029 | 1.335 ± 0.351 | 1.304 ± 0.365 | 1.300 ± 0.326 | 0.637 |
| LDL-C, mmol/L | 2.498 ± 0.872*◊ | 2.764 ± 0.828 | 2.792 ± 0.980 | 0.004 | 2.672 ± 0.965◊ | 2.794 ± 0.861 | 2.884 ± 0.767 | 0.117 |
| Apo-A1, g/L | 1.157 ± 0.270 | 1.156 ± 0.278◊ | 1.106 ± 0.266* | 0.047 | 1.227 ± 0.337◊ | 1.171 ± 0.340 | 1.134 ± 0.299 | 0.065 |
| Apo-B, g/L | 0.829 ± 0.285*◊ | 0.902 ± 0.269 | 0.925 ± 0.314 | 0.008 | 0.820 ± 0.295◊ | 0.860 ± 0.265 | 0.900 ± 0.262 | 0.044 |
| Apo-A1/ Apo-B | 1.520 ± 0.526*◊ | 1.380 ± 0.481 | 1.308 ± 0.469 | <0.001 | 1.654 ± 0.697*◊ | 1.477 ± 0.613 | 1.342 ± 0.482 | <0.001 |

*Note: p* Value shows the differences compared between groups (ε2, ε3, and ε4).

*$p < 0.05$ versus corresponding ε3 group.

◊$p < 0.05$ versus corresponding ε4 group.

**TABLE 5** Logistic regression analysis of risks of CAD in southern Chinese Hakka population.

| | | Unadjusted values | | | Adjusted values | | |
|---|---|---|---|---|---|---|---|
| **Variables** | **Alleles/Genotypes** | ***p* Value** | **OR** | **95% CI** | ***p* Value** | **Adjusted OR** | **95% CI** |
| Gender (male) | | 0.180 | 0.888 | 0.746–1.057 | 0.121 | 1.181 | 0.957–1.456 |
| Smoking | | <0.001 | 1.863 | 1.524–2.276 | <0.001 | 2.043 | 1.619–2.577 |
| TG | | <0.001 | 1.191 | 1.108–1.281 | 0.323 | 0.953 | 0.866–1.049 |
| TC | | 0.880 | 0.995 | 0.927–1.067 | 0.058 | 0.767 | 0.582–1.009 |
| HDL-C | | <0.001 | 0.542 | 0.419–0.700 | 0.005 | 0.459 | 0.498–0.839 |
| LDL-C | | 0.166 | 0.933 | 0.845–1.029 | <0.001 | 2.885 | 2.016–4.129 |
| Apo-A1 | | 0.046 | 0.752 | 0.568–0.996 | 0.293 | 0.788 | 0.505–1.229 |
| Apo-B | | 0.002 | 1.658 | 1.211–2.269 | <0.001 | 1.680 | 1.296–2.178 |
| *APOE* allele | | | | | | | |
| | ε2 carrier[c] | 0.154 | 0.826 | 0.636–1.074 | 0.141 | 0.811 | 0.614–1.072 |
| | ε4 carrier[d] | 0.003 | 1.411 | 1.126–1.767 | 0.012 | 1.354 | 1.068–1.717 |
| *SLCO1B1* 388 genotype | | | | | | | |
| | A/G + G/G[e] | 0.436 | 0.865 | 0.601–1.245 | 0.357 | 0.831 | 0.560–1.232 |
| | A/A + A/G[f] | 0.711 | 0.968 | 0.815–1.150 | 0.576 | 0.947 | 0.784–1.145 |
| *SLCO1B1* 521 genotype | | | | | | | |
| | T/C + C/C[e] | 0.514 | 1.069 | 0.874–1.307 | 0.690 | 1.046 | 0.840–1.301 |
| | T/T + T/C[f] | 0.590 | 0.830 | 0.423–1.631 | 0.737 | 0.883 | 0.429–1.820 |

Abbreviations: CI, confidence interval; LDL-C, low-density lipoprotein-cholesterol; OR, odds ratio.

[a]ε2/ε2 plus ε2/ε3, reference genotype: ε3/ε3 plus ε3/ε4 plus ε4/ε4.

[b]ε3/ε4 plus ε4/ε4, reference genotype: ε2/ε2 plus ε2/ε3 plus ε3/ε3.

[c]Reference genotype: *SLCO1B1* 388 A/A.

[d]Reference genotype: *SLCO1B1* 388 G/G.

[e]Reference genotype: *SLCO1B1* 521 T/T.

[f]Reference genotype: *SLCO1B1* 521 C/C.

There are several strengths of this study. This is the first systematic study of the association between CAD and *APOE* and *SLCO1B1* gene polymorphisms in Hakka population. The lifestyle, lipid levels, *APOE*, and *SLCO1B1* gene polymorphisms were included in the analysis and have excluded the influence of related confounding factors on the results. There are some limitations to this study. First, CAD is a multifactorial diseases caused by genetic and environmental factors. As a retrospective case-control analysis, some records in this study could not be traced and verified, limiting the assessment of potential gene-environment interactions. For example, the study could not trace whether patients were taking statins, lipid-lowering drugs, or traditional Chinese medicine prior to admission. Second, although the sample size included in this study is enough to draw reliable conclusion, the sample size is not particularly large, and the possibility of some deviation in the results cannot be ruled out.

## 5 | CONCLUSIONS

The present study suggests that *APOE* ε4 allele has effects on serum lipids and may be associated with susceptibility to CAD in southern Chinese Hakka population. *SLCO1B1* c.388A > G and c.521T > C may have no relationship between gene polymorphisms and the incidence of CAD. It indicated that the *APOE* SNPs rs429358 and rs7412 are associated with CAD, but not SNPs rs2306283 and rs4149056 of *SLCO1B1* gene. Therefore, *APOE* genotyping and serum lipids testing may be useful to identify individuals at risk for CAD.

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### CONFLICT OF INTEREST

The authors declare that they have no competing interests.

### AUTHORS CONTRIBUTIONS

Zhixiong Zhong and Heming Wu designed the study. Qinghua Liu, Heming Wu, and Qingyan Huang collected clinical data. Heming Wu, Zhikang Yu, and Qingyan Huang analyzed the data. Heming Wu and Qinghua Liu prepared the manuscript. All authors were responsible for critical revisions, and all authors read and approved the final version of this work.

## DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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