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RESEARCH ARTICLE

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ABCA1 variants rs2230806 (R219K), rs4149313 (M8831I), and rs9282541 (R230C) are associated with susceptibility to coronary heart disease

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Background: To investigate the association between three single nucleotide polymorphisms (SNPs) of ABCA1 gene and susceptibility to coronary heart disease (CHD) in Chinese Han population.

Methods: A total of 484 CHD patients and 488 controls were included in the study. Three SNPs rs2230806 (R219K), rs4149313 (M8831I), and rs9282541 (R230C) in ABCA1 gene were genotyped by SNaPshot.

Results: Single nucleotide polymorphism rs1800977 was associated with susceptibility to CHD (AA vs GG, $P = 0.013$; A vs G, $P = 0.029$; recessive model, $P = 0.020$). Rs4149313 (AA vs GG, $P = 0.010$; recessive model, $P = 0.011$) and rs9282541 (T vs C, $P = 0.029$; dominant model, $P = 0.039$) were also risk factor for CHD.

Conclusion: This study suggests that three SNPs rs2230806, rs4149313, and rs9282541 in ABCA1 gene are significantly associated with susceptibility to CHD; further mechanism should be performed to be applied to drug research and development.

KEYWORDS
ABCA1, coronary heart disease, SNP

1 | INTRODUCTION

Coronary heart disease (CHD) is one of the most common diseases of human life and health and is regarded as a killer that harms the health of middle-aged and elderly people.[1] In western developed countries, CHD has become the leading cause of death in human. Therefore, the prevention and treatment of CHD has become a major public health problem facing all mankind.[2] The main pathological basis is coronary atherosclerosis. Abnormal blood lipids and lipoprotein metabolism are one of the important causes of CHD.[3] The relationship between blood lipids and CHD is highly valued. Epidemiological evidence shows that there is a significant correlation between plasma cholesterol level and CHD occurrence.[4] Elevated cholesterol is a major risk factor for atherosclerosis. Clinical drug research shows that lipid-lowering therapy can stabilize or even reduce plaque and reduce the incidence of cardiovascular events. Gene-level studies have also shown that the LDL receptor mutation can lead to familial hypercholesterolemia, and the level of LDL-C increases significantly, which can increase or advance the occurrence of atherosclerosis and CHD.[5]

It is generally believed that the accumulation of cholesterol and cholesterol in macrophages plays an important role in the formation of atherosclerosis. The factors that affect the balance between the inflow and the outflow of cholesterol are necessarily the promotion of atherosclerosis or anti-atherosclerosis factors. Of these genes, The

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ATP-binding cassette transporter A1 (ABCA1) is an important one because it has the function of regulating the metabolism of cholesterol.[6,7] ABCA1 is a membrane transporter protein that plays an essential role in the secretion of cellular-free cholesterol and phospholipids, from cell membrane to lipid-poor apolipoprotein AI, creating nascent high-density lipoprotein (HDL).[8,9] Some genetic and related studies have shown that common polymorphisms in the *ABCA1* gene can influence the function of ABCA1 transporter resulting in the altered biosynthesis of HDL-C particles.[9] Among the normal people and the CHD population, the most common way of *ABCA1* gene mutation is single nucleotide polymorphism (SNP), and the DNA sequence polymorphism caused by single nucleotide variation is the most common type of human genetic variation, accounting for more than 90% of the known polymorphism.[10] In recent years, a number of studies have shown that the distribution frequency of specific *ABCA1* gene SNPs in different regions and populations is significantly different, and the effects on plasma HDL-C level and the incidence and severity of CHD are also different.[11] Even the same *ABCA1* gene SNPs have similar or opposite effects.

The three loci of the *ABCA1* gene, rs2230806 (R219K), rs4149313 (M8831I), and rs9282541 (R230C), are located in the two major extracellular rings of the ABCA1 protein, which is an important part of the role of APO-I and cholesterol efflux.[12] Therefore, the change in these two loci may lead to the variation of the disease and ultimately affect the level of HDL-C.[13] Several studies attached the importance to the role of the three SNPs of *ABCA1* gene in the risk of CHD, but with conflicting results.[12,14] Therefore, we aimed to investigate the association between three SNPs of *ABCA1* and susceptibility to CHD in Chinese Han population.

2 | MATERIALS AND METHODS

2.1 | Study participants

A total of 484 hospitalized CHD patients and 488 age- and sex-matched controls recruited from Changzhou No. 2 People's Hospital were included in the study. The study protocol was approved by the local ethics review board, and written informed consent was provided by all participants. Clinical data including age, gender, BMI, TG (triglyceride), TC (total cholesterol), LDL (low-density lipoprotein), and HDL (high-density lipoprotein) were measured using standard laboratory techniques. The statistical analysis of clinical data was blinded for the genotype data.

2.2 | Inclusion and exclusion criteria for cases

Patients with CHD and fully conscious and well oriented with person and place were included, and patients with previous revascularization, liver cirrhosis, and end-stage renal diseases were excluded.

2.3 | Inclusion and exclusion criteria for controls

Controls with no evidence of CHD and fully conscious and well oriented with person and place were included, and individuals with CHD family history and cancers were excluded.

2.4 | DNA extraction and genotyping

Genomic DNA was extracted from 200 μL whole blood according to the manufacturer's protocol (QIAamp blood kit, Qiagen, Germany). Genotyping was assessed using the SNaPshot method in keeping with the manufacturer's protocol. Twenty duplicate samples were genotyped for assessing the quality of genotype.

2.5 | Statistical analyses

SPSS 20.0 software (IBM-SPSS, Inc, Chicago, IL) was used to perform statistical analyses. Hardy-Weinberg equilibrium of controls was assessed using the chi-square test. Odds ratios (ORs) with 95% confidence intervals (95% CIs) were calculated to assess the associations under genetic models, including allele frequency model, dominant model, and recessive model. Clinical data between cases and controls were performed by Student's *t* test and chi-square test. $P < 0.05$ was considered as statistically different. Power calculation was performed by Quanto software version 1.2.3 (University of Southern California, Los Angeles, CA).

3 | RESULTS

3.1 | Population characteristics and genotype distributions

There were no differences between the two groups in age and sex. Clinical baseline characteristics between the two groups are shown in Table 1. The genotype distributions of three SNPs of *ABCA1* gene are shown in Table 2. The chi-square test showed that there was no SNP deviated from the Hardy-Weinberg equilibrium.

3.2 | Association between rs2230806 and susceptibility to CHD

As shown in Table 3, there was a significant association between rs2230806 and CHD in different genetic models (AA vs GG, OR = 0.594 (0.393-0.896), $P = 0.013$; A vs G, OR = 0.816 (0.680-0.980), $P = 0.029$; recessive model, OR = 0.639 (0.438-0.931), $P = 0.020$).

TABLE 1 Characteristics between coronary heart disease (CHD) and control individuals

Clinical data	CHD (n = 484)	Control (n = 488)	*P* value
Gender (M/F)	256/228	261/227	0.854
Age (y)	59.26 ± 7.62	60.15 ± 7.84	0.073
BMI (kg/m^2)	25.44 ± 2.21	24.63 ± 2.42	<0.001
TG (mmol/L)	1.61 ± 0.62	1.43 ± 0.63	<0.001
TC (mmol/L)	4.56 ± 0.98	4.50 ± 0.93	0.328
LDL (mmol/L)	2.81 ± 1.02	2.39 ± 1.13	<0.001
HDL (mmol/L)	1.26 ± 0.61	1.21 ± 0.58	0.191

TABLE 2 Genotype distributions of three SNPs in *ABCA1* gene and Hardy-Weinberg equilibrium test

SNP	Genotype	CHD	Control	*P*[a]
rs2230806	GG	182	161	0.179
	GA	251	251	
	AA	51	76	
rs4149313	GG	259	245	0.932
	GA	203	201	
	AA	22	42	
rs9282541	CC	460	476	0.783
	CT	23	12	
	TT	1	0	

[a]*P* for Hardy-Weinberg test in control individuals.

3.3 | Association between rs4149313 and susceptibility to CHD

As shown in Table 4, there was a significant association between rs4149313 and CHD in different genetic models (AA vs GG, OR = 0.495 (0.290-0.847), *P* = 0.010; recessive model, OR = 0.506 (0.300-0.854), *P* = 0.011).

3.4 | Association between rs9282541 and susceptibility to CHD

As shown in Table 5, there was a significant association between rs9282541 and CHD in different genetic models (T vs C, OR = 2.130 (1.080-4.198) *P* = 0.029; dominant model, OR = 2.070 (1.037-4.130), *P* = 0.039).

4 | DISCUSSION

ABCA1 is expressed in various tissues and organs, and the function of ABCA1 is not similar in different organ tissues.[15] The decrease in ABCA1 expression in the local part of the vessel wall is related to the deposition of lipid in the vessel wall and the vascularization of atherosclerosis.[16] The expression at the fork or bend is significantly lower than the vertical. SNPs are DNA sequence polymorphisms caused by mutations at the genomic nucleotide level, including single base transformations, transversions, and single base deletions and insertions.[17,18] The susceptibility may be related to race, genetic, environmental, or other factors such as age, sex, diet, and smoking. In normal persons and CHD patients, the most common way of *ABCA1* gene mutation is single nucleotide polymorphism.[20,21]

In this study, a total of 484 hospitalized CHD patients and 488 age- and sex-matched controls were investigated the associations between three SNPs rs2230806 (R219K), rs4149313 (M8831I), and rs9282541 (R230C) in *ABCA1* gene and susceptibility to CHD in Chinese Han population. The results suggested that *ABCA1* rs2230806 G allele and GG genotype confer 0.594- to 0.816-fold decreased risks for the development of CHD. Likewise, the results also investigated that *ABCA1* rs4149313 GG genotype confers 0.495-fold decreased risks for the development of CHD. Interestingly, the results suggested that *ABCA1* rs9282541 T allele confers 2.130-fold increased risks for the development of CHD. Three SNPs located in the exon, which are missense mutation, may directly change the protein of the gene. Changes in the function of ABCA1 proteins will lead to decreased levels of HDL-C in the patient, which in turn increases the lack of cholesterol efflux, and resulting in CHD risk increase. According to gender, we conducted a subgroup analysis (Tables S1-S3). The results were basically consistent with the overall results, and partial results are negative according to small size due to subgroup, but we observed that there was a trend toward association.

In 2013, Zargar et al[23] investigated the frequency of SNP rs2230806 in the *ABCA1* gene of 120 patients of CHD and 100 age-matched, healthy controls using restriction fragment length polymorphism and direct sequencing. They found that SNP rs2230806 in the *ABCA1* gene is significantly associated with the incidence of CHD. Homozygosity for the G allelic variant in CHD patients may be associated with an increased risk of CHD/MI. The result was basically in line with our results. In 2010, Acuña-Alonzo et al[24] found that the C230 allele was found in 29 of 36 Native American groups, but not in European, Asian, or African individuals. And then, they performed a more extensive analysis of this variant in 4405 Native Americans and 863 individuals from other ethnic groups to investigate genetic evidence of positive selection, to assess its functional effect in vitro, and to explore associations with HDL-C levels and other metabolic traits. Surprisingly, R230C was found in our sample, albeit in very small quantities. Multiple studies have reported that smoking, type 2 diabetes (T2D), hypertension, and LDL- and HDL-cholesterol (LDL-C and HDL-C) are significant independent risk factors for CHD in African Americans. In

TABLE 3 Association between rs2230806 and susceptibility to coronary heart disease (CHD)

Genetic model	CHD		Control		OR (95% CI)	Adjusted OR (95% CI)[a]	*P*
GA vs GG	251	182	251	161	0.885 (0.672-1.164)	0.892 (0.648-1.176)	0.382
AA vs GG	51	182	76	161	0.594 (0.393-0.896)	0.601 (0.396-0.891)	0.013
A vs G	353	615	403	573	0.816 (0.680-0.980)	0.817 (0.682-0.984)	0.029
Dominant	302	182	327	161	0.817 (0.628-1.063)	0.815 (0.616-1.073)	0.133
Recessive	51	433	76	412	0.639 (0.438-0.931)	0.642 (0.432-0.935)	0.020

[a]OR adjusted for age, BMI, TC, and sex.

TABLE 4 Association between rs4149313 and susceptibility to coronary heart disease (CHD)

Genetic model	CHD		Control		OR (95% CI)	Adjusted OR (95% CI)[a]	*P*
GA vs GG	203	259	201	245	0.955 (0.735-1.241)	0.962 (0.717-1.261)	0.732
AA vs GG	22	259	42	245	0.495 (0.290-0.847)	0.511 (0.293-0.850)	0.010
A vs G	247	721	285	691	0.831 (0.680-1.014)	0.837 (0.674-1.020)	0.068
Dominant	225	259	243	245	0.876 (0.681-1.127)	0.892 (0.687-1.133)	0.302
Recessive	22	462	42	446	0.506 (0.300-0.854)	0.511 (0.307-0.859)	0.011

[a]OR adjusted for age, BMI, TC, and sex.

TABLE 5 Association between rs9282541 and susceptibility to coronary heart disease (CHD)

Genetic model	CHD		Control		OR (95% CI)	Adjusted OR (95% CI)[a]	*P*
CT vs CC	23	460	12	476	1.983 (0.988-3.983)	1.991 (0.982-3.999)	0.054
TT vs CC	1	460	0	476	—	—	0.309
T vs C	25	943	12	964	2.130 (1.080-4.198)	2.137 (1.081-4.203)	0.029
Dominant	24	460	12	476	2.070 (1.037-4.130)	2.074 (1.041-4.136)	0.039
Recessive	1	483	0	488	—	—	0.315

[a]OR adjusted for age, BMI, TC, and sex.

2010, Peloso et al[25] showed that there was no significant association between *ABCA1* and susceptibility. The cause of the analysis may be different from the genetic background chosen, resulting in different allele frequencies.

In 2015, Lu et al[26] investigated the association of *ABCA1* polymorphisms with plasma lipid variability and CHD risk in the Chinese Han population. They found that *ABCA1* polymorphisms influence plasma lipid variability and CHD risk. *ABCA1* polymorphisms could also modify the effects of plasma lipids on CHD risk. Compared with them, we have a larger sample size to confirm this correlation. Overall, our findings are partially different from others and may be related to clinical heterogeneity, including differences in ethnic groups and sample size. We also make several assumptions about different results. First of all, the inequality of choice is unavoidable because all participants are from the Chinese Han population in the same hospital and the same area. Second, because of the lack of relevant information, we did not perform genetic and environmental interaction analysis. Finally, although the sample size has increased compared with most SNP studies, there is still a problem of insufficient sample size. However, we have explored three SNPs of the *ABCA1* gene, which to a certain extent improved the comprehensiveness of the *ABCA1* gene polymorphism and CHD risk study.

In conclusion, our study suggests that three SNPs rs2230806, rs4149313, and rs9282541 in ABCA1 gene are significantly associated with susceptibility to CHD; further mechanism should be performed to be applied to drug research and development.

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SUPPORTING INFORMATION

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