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RESEARCH ARTICLE

Association of the *HNF1A* polymorphisms and serum lipid traits, the risk of coronary artery disease and ischemic stroke

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Abstract

Background Hepatocyte nuclear factor-1α gene (*HNF1A*) single nucleotide polymorphisms (SNPs) have been associated with serum lipid traits in several previous genome-wide association studies. However, little is known about such associations in the Chinese populations. The present study aimed to determine the association of the *HNF1A* rs1169288, rs2259820, rs2464196 and rs2650000 SNPs and serum lipid traits, the risk of coronary artery disease (CAD) and ischemic stroke (IS).

Methods The genotypes of the four SNPs in 562 CAD and 521 IS patients, as well as 594 healthy controls, were detected using the Snapshot technology.

Results The genotype and allele distribution of the four SNPs was not different between controls and CAD or IS patients ($p > 0.05$ for all). rs1169288, rs2259820 and rs2464196 SNPs were significantly associated with serum lipid levels in both controls and CAD patients ($p < 0.004$–0.009). rs2259820 and rs2464196 SNPs were significantly associated with a lower risk of CAD [odds ratio (OR) = 0.64, 95% confidence interval (CI) = 0.44–0.91, $p = 0.015$ and OR =0.62, 95% CI = 0.43–0.89, $p = 0.010$, respectively]. Significant linkage disequilibrium was noted among the four SNPs ($r^2 > 0.5$, $D' > 0.8$). The haplotype of rs1169288A-rs2259820C-rs2464196G-rs2650000A was associated with an increased risk of CAD (OR =1.95, 95% CI: 1.13–3.37, $p = 0.015$). Interactions of SNP–SNP (rs1169288–rs2464196–rs2650000) and haplotype–environment on the risk of CAD (A-C-G-A–smoking) or IS (A-C-G-A–sex and A-T-A-C–alcohol consumption) were also observed among these SNPs.

Conclusions These findings suggest that the *HNF1A* polymorphisms may be the genetic risk factors for CAD and IS.

KEYWORDS

coronary artery disease, hepatocyte nuclear factor-1α (HNF1A), ischemic stroke, serum lipid traits, single nucleotide polymorphism

1 | INTRODUCTION

Cardiovascular disease (CVD) is the leading cause of fatalities globally.[1] In recent decades, rates of death attributable to CVD have declined, although the burden of disease remains high, particularly that of coronary artery disease (CAD) and cerebrovascular disease, in both developing and developed regions.[2–4] Both serum lipid metabolism abnormality and genetic variants are associated with CVD involving the heart, brain and peripheral circulation.[5,6] Accordingly, genetic and functional analyses of the susceptibility genes could uncover the pathogenesis and provide therapy or preventive methods for these diseases.

We have currently searched for the susceptibility genes in serum lipid levels, CAD and ischemic stroke (IS) with the positional candidate gene genotyping approach. One of the chromosomal regions that we

have focused on in the present study is chromosome 12q, especially hepatocyte nuclear factor-1a gene (*HNF1A*), because this chromosomal arm is associated with CAD.[7,8] CAD may share some common risk factors with IS, such as hypertension, dyslipidemia and genetic variants.[6] However, it is unknown whether the single nucleotide polymorphisms (SNPs) associated with the risk of CAD are also associated with the risk of IS.

The *HNF1A* (also known as *TCF1)* product, HNF-1a, is a transcriptional activator of several hepatic genes. This gene is expressed in the pancreas, liver, intestine and kidneys.[9] HNF-1a binds to genes whose products are involved in hepatic biochemistry, including carbohydrate synthesis and storage, lipid metabolism, detoxification, and synthesis of serum proteins.[10] Mutations in the *HNF1A* cause maturity onset diabetes of the young[10–12] and affect plasma concentrations of C-reactive protein.[13] In addition, the SNPs in the *HNF1A* have been associated with levels of low-density lipoprotein cholesterol (LDL-C), total cholesterol (TC)[14,15] and triglyceride (TG),[16] as well as high plasma concentrations of high-density lipoprotein cholesterol (HDL-C) and apolipoprotein (Apo) A1.[17,18] However, it is not known whether the variants in the *HNF1A* are also associated with the risk of dyslipidemia, CAD and IS in the Chinese populations. According to previous genome-wide association studies (GWAS), some loci in the *HNF1A* have pleiotropic effects on several metabolic traits. Therefore, in the present study, we assessed the association of the *HNF1A* rs1169288, rs2259820, rs2464196, rs2650000 SNPs and serum lipid levels and the risk of CAD and IS.

2 | MATERIALS AND METHODS

2.1 | Study subjects

A total of 562 patients with CAD and 521 patients with IS were recruited from hospitalized patients in the First Affiliated Hospital, Guangxi Medical University. All of the enrolled CAD patients were evaluated by coronary angiography because of suspected CAD or unrelated conditions requiring angiographic evaluation; the coronary angiograms were analyzed by two experienced interventional cardiologists. CAD was defined as significant coronary stenosis (≥ 50%) in at least one of the three main coronary arteries or their major branches (branch diameter ≥ 2 mm). Subjects with congenital heart disease and type I diabetes mellitus were excluded. All of the enrolled IS patients received a strict neurological examination and brain magnetic resonance imaging. The diagnosis of IS was made according to the International Classification of Diseases (9th Revision). Patients with a transient ischemic attack, embolic brain infarction, stroke caused by inflammatory disease, cardio-embolic stroke, autoimmune disease or serious chronic diseases were excluded from the study. Subjects with a past history of CAD were also excluded from the study.[19]

A total of 594 healthy controls matched by age, sex and geographical area were also included. The controls were judged to be free of CAD and IS by questionnaires, medical history and clinical examination. All individuals enrolled were from the Han population in Guangxi, China. A standard questionnaire was used to ascertain general information and medical history from all participants. The study protocol was approved by the Ethics Committee of the First Affiliated Hospital, Guangxi Medical University. Informed consent was obtained from all subjects after they had received a full explanation of the study.

2.2 | SNP selection

We selected four SNPs in the *HNF1A* on the basis of: (1) selected SNPs being established by Haploview, version 4.2 (Broad Institute of MIT and Harvard, Cambridge, MA, USA); (2) SNP information obtained from NCBI dbSNP Build 132 (http://www.Ncbi.nlm.nih.gov/SNP/); (3) a known minor allele frequency higher than 1%; and (4) SNPs possibly being associated with the plasma lipid levels or cardiovascular disease in recent studies.

2.3 | Genotyping

Genomic DNA was isolated from peripheral blood leukocytes using the phenol–chloroform method. Genotyping of the four SNPs was performed using the Snapshot technology. All experimental manipulations were completed in the Center for Human Genetics Research, Shanghai Genesky Bio-Tech Co. Ltd (Shanghai, China). Primers were designed with online Primer3 software (http://frodo.wi.mit.edu). Polymerase chain raction (PCR) products were obtained by Qiagen Company Hot Star *Taq* multiple PCR and purified by shrimp alkali enzyme (SAP) (Promega, Madison, WI, USA) and external enzyme (exobiology I; Epicentre, Madison, WI, USA). Purified products were extended with ABI SNaPshot Multiplex kit. Products of extension were sampled with SAP (Promega) on ABI3130xl after purification. We analyzed the SNP genotypes with GeneMapper, version 4.0 (Applied Biosystems, Foster City, CA, USA). The primers were: rs1169288F: 5′-GCGGCTAGCGTGGTGGAC-3′; rs1169288R: 5′-CGCAGGACTCC CCCTTGTC-3′; rs2259820 and rs2464196F: 5′-CTCCACGCAGG CACAGAGTGT-3′; rs2259820 and rs2464196R: 5′-CTGCTGTGTG GGGCACAGG-3′; rs2650000F: 5′-GGTTTTGTAGCATGTACATTT TCAGTGC-3′; rs2650000R: 5′-CAGGCAGAATCCCAGCAATAGG-3′; extension of primers: rs1169288SF: TTTTTTTTTTTTTTTTTTTT TTTTTTTTTTGGGCTG-AGCAAAGAGGCACTG; rs2259820SF: TTT TTTTTTTTTTTTT-TTTTTTTTTTTTTTTTTTGGCAGCAGCCTGACCA CC; rs2464196SR: TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT TTTTTTTTCATG-GTGGCCATGAAGGGG; rs2650000SR: TTTTT TTTTTTTTTTTTTTT-TTTTTTTTTTTTTTTTTGCTGTGTCTTGAGAC AAAAGTTGTCAGA.

2.4 | Biochemical analysis

All of the biochemical assays and genotyping in CAD and IS patients were performed after hospitalization, and all of the venous blood samples were obtained from the patients and controls after at least 12 h of fasting. The levels of TC, TG, HDL-C and LDL-C in the samples were determined by enzymatic methods with commercially available kits. Serum apolipoprotein A1 (ApoA1) and apolipoprotein B (ApoB) levels were detected by an immunoturbidimetric immunoassay using a commercial kit (RANDOX Laboratories Ltd, Crumlin, UK).[20] The normal values of serum TC, TG, HDL-C and LDL-C; ApoA1 and ApoB; and the ApoA1/ApoB ratio at our Clinical Science Experiment Center were 3.10–5.17, 0.56–1.70, 0.91–1.81 and 2.70–3.20 mmol/l; 1.00–1.78 and 0.63–1.14 g/l; and 1.00–2.50, respectively.

2.5 | Statistical analysis

All statistical analyses were performed using SPSS, version 13.0 (SPSS Inc., Chicago, IL, USA). A standard goodness-of-fit test was used to test the Hardy–Weinberg equilibrium. Chi-squared analysis was used to evaluate the difference in genotype distribution between control and case groups. The general characteristics between the cases and controls were tested using Student's unpaired *t*-test. Pairwise linkage disequilibria and haplotype frequencies among the SNPs were analyzed using Haploview, version 4.2 (Broad Institute of MIT and Harvard). The association between genotypes and serum lipid parameters was tested by analysis of covariance. Any variants associated with the serum lipid parameter at $p < 0.013$ (corresponding to $p < 0.05$ after adjusting for four independent tests by Bonferroni correction) were considered statistically significant. Unconditional logistic regression was used to assess the correlation between the risk of CAD or IS and genotypes. Sex, age, body mass index (BMI), blood pressure, alcohol consumption and cigarette smoking were adjusted for the statistical analysis. $p < 0.05$ (two-tailed) was considered to be statistically significant.

The interlocus interaction was analyzed by the generalized multifactor dimensionality reduction (GMDR) method, using GMDR software (http://ibi.zju.edu.cn/bcl/software/gmdr/download.html). The cross-validation consistency score provides the degree of consistency when the selected interaction is identified as the best model among all possibilities considered. The testing balanced accuracy provides the degree of interaction, which accurately predicts the case–control status with scores between 0.50 and 1.00. A sign test or a permutation test provides a *p*-value for predicting accuracy when measuring the significance of an identified model. The best model is selected as the combination of marker with maximum cross-validation consistency and minimum prediction error.

3 | RESULTS

3.1 | Characteristics of the subjects

Table 1 compares the general characteristics and serum lipid levels between the healthy controls and CAD or IS patients. Mean age, sex distribution, serum LDL-C and ApoB levels were not different between controls and CAD or IS patients ($p > 0.05$ for all). CAD patients had higher BMI, pulse pressure and serum TG levels, but lower diastolic blood pressure, serum TC, HDL-C, ApoA1 levels and ApoA1/ApoB ratio. The IS patients had higher BMI, systolic blood pressure, pulse pressure and serum TG levels, as well as lower serum TC, HDL-C, ApoA1 levels and ApoA1/ApoB ratio.

3.2 | Genotype and allele frequencies

Table 2 describes the genotype and allele frequencies of the *HNF1A* SNPs. The genotype distribution of four SNPs agreed with Hardy–Weinberg equilibrium ($p > 0.05$ for all). The genotype and allele frequencies of the rs1169288, rs2259820, rs2464196 and rs2650000 SNPs were no different between controls and CAD or IS patients ($p > 0.05$ for all). Significant linkage disequilibrium (LD) was noted among the rs1169288, rs2259820, rs2464196 and rs2650000 SNPs ($r^2 > 0.5$, $D' > 0.8$).

3.3 | Genotypes and serum lipid levels

As shown in Table 3, after Bonferroni correction of *p*-values, the minor C allele of rs1169288 was associated with high TG concentrations in controls ($p < 0.01$); the minor T allele of rs2259820 and the minor of A allele of rs2464196 were associated with high ApoA1 concentrations in CAD patients ($p < 0.01$), respectively. We found that four SNPs were not associated with lipoprotein or lipid-related traits in IS patients ($p > 0.05$), respectively.

3.4 | *HNF1A* SNPs and the risk of CAD and IS

Table 4 shows the association of the *HNF1A* SNPs and the risk of CAD and IS. SNPs of rs2259820 and rs2464196 were significantly associated with a lower risk of CAD in a logistic regression [odds ratio (OR) = 0.64, 95% confidence interval (CI) = 0.44–0.91, $p = 0.015$ and OR = 0.62, 95% CI = 0.43–0.89, $p = 0.010$, respectively] for the TT genotype compared to the CC genotype and the AA genotype compared to the GG genotype, respectively. The four SNPs were not associated with the risk of IS.

3.5 | Haplotypes and the risk of CAD and IS

As shown in Table 5, the haplotype of A-C-G-C (in the order of the rs1169288, rs2259820, rs2464196 and rs2650000 SNPs) was the commonest haplotype and represented approximately 50% of the sample. The haplotype of A-C-G-A, carrying rs2259820C and rs2464196G alleles, was associated with an increased risk of CAD (OR =1.95, 95% CI – 1.13–3.37, $p = 0.015$).

3.6 | Gene–gene interactions for CAD and IS

Table 6 shows the impacts of the *HNF1A* SNPs for CAD and IS. The one- and three-locus models showed a significant association with the risk of CAD ($p < 0.05$–0.02). The three-locus model was chosen as the best because it had the highest level of testing accuracy (53% for CAD) and good cross-validation consistency (10/10). However, we did not find any impact of the *HNF1A* SNPs on IS.

3.7 | Interactions of the haplotypes and environment factors on the risk of CAD

Interactions of one haplotype and smoking on the risk of CAD were noted in the present study. Compared to the A-C-G-C haplotype in nonsmokers, the haplotype of A-C-G-A (OR =0.51, 95% CI = 0.26–0.98) in nonsmokers was associated with a decreased risk for CAD.

3.8 | Interactions of the haplotypes and environment factors on the risk of IS

The interactions of several haplotypes and alcohol consumption, as well as sex, on the risk of IS were also noted in the present study. For men, compared to the A-C-G-C haplotype, A-C-G-A haplotype

TABLE 1 Characteristics of the participants

Characteristic	Control (n = 594)	CAD (n = 562)	IS (n = 521)	p_{CAD}	p_{IS}
Male/female	434/160	414/148	378/149	0.817	0.617
Age (years)	61.35 ± 9.74	62.14 ± 10.59	62.69 ± 12.43	0.186	0.051
Body mass index (kg/m^2)	22.41 ± 2.86	23.84 ± 3.34	24.82 ± 2.26	0.000	0.010
Systolic blood pressure (mmHg)	130.65 ± 20.02	132.94 ± 23.22	148.17 ± 21.86	0.072	0.000
Diastolic blood pressure (mmHg)	82.96 ± 13.45	79.14 ± 14.28	84.08 ± 12.87	0.000	0.156
Pulse pressure (mmHg)	49.76 ± 14.73	53.80 ± 17.31	64.17 ± 17.81	0.000	0.000
Cigarette smoking, n (%)	65 (10.9)	67 (11.9)	78 (14.8)	0.601	0.053
Alcohol consumption, n (%)	177 (29.8)	151 (27.0)	147 (27.9)	0.460	0.483
Total cholesterol (mmol/l)	4.94 ± 1.11	4.53 ± 1.20	4.53 ± 1.15	0.000	0.000
Triglyceride (mmol/l)	1.01 (0.70)	1.35 (0.94)	1.36 (0.92)	0.000	0.000
HDL-C (mmol/l)	1.90 ± 0.50	1.14 ± 0.34	1.22 ± 0.40	0.000	0.000
LDL-C (mmol/l)	2.74 ± 0.80	2.72 ± 1.00	2.69 ± 0.90	0.637	0.361
Apolipoprotein (Apo) A1 (g/l)	1.41 ± 0.28	1.03 ± 0.52	1.02 ± 0.22	0.000	0.000
ApoB (g/l)	0.91 ± 0.22	0.91 ± 0.27	0.89 ± 0.25	0.962	0.294
ApoA1/ApoB	1.63 ± 0.48	1.36 ± 0.11	1.19 ± 0.60	0.014	0.000

p_{CAD}, the p value of CAD versus control; p_{IS}, the p value of IS versus control.

TABLE 2 Genotype and allele frequencies of the *HNF1A* polymorphisms in cases and controls

SNP/genotype/allele	Control (%)	CAD (%)	IS (%)	p_{CAD}	p_{IS}
Rs1169288					
AA	259 (43.7)	258 (45.9)	237 (45.5)		
AC	263 (44.4)	236 (42)	214 (41.1)		
CC	71 (12)	68 (12.1)	70 (13.4)	0.706	0.504
A	781 (65.9)	752 (66.9)	688 (66)		
C	405 (34.1)	372 (33.1)	354 (34)	0.597	0.964
p_{HWE}	0.736	0.220	0.054		
Rs2259820					
CC	189 (31.9)	194 (34.6)	183 (35.1)		
CT	285 (48.1)	282 (50.3)	247 (47.4)		
TT	119 (20.1)	85 (15.2)	91 (17.5)	0.088	0.387
C	663 (55.9)	670 (59.7)	613 (58.8)		
T	523 (44.1)	452 (40.3)	429 (41.2)	0.070	0.170
p_{HWE}	0.539	0.289	0.627		
Rs2464196					
GG	189 (31.9)	194 (34.6)	181 (34.8)		
GA	284 (47.9)	283 (50.5)	248 (47.7)		
AA	120 (20.2)	83 (14.8)	91 (17.5)	0.053	0.404
G	662 (55.8)	671 (59.9)	610 (58.7)		
A	524 (44.2))	449 (40.1)	430 (41.3)	0.052	0.183
p_{HWE}	0.480	0.218	0.703		
Rs2650000					
CC	217 (36.5)	214 (38.1)	205 (39.3)		
CA	278 (46.8)	260 (46.3)	238 (45.7)		
AA	99 (16.7)	88 (15.7)	78 (15)	0.825	0.560
C	712 (59.9)	688 (61.2)	648 (62.2)		
A	476 (40.1)	436 (38.8)	394 (37.8)	0.551	0.277
p_{HWE}	0.534	0.541	0.513		

p_{CAD}, the p value of CAD versus control; p_{IS}, the p value of IS versus control; p_{HWE}, the p value of the Hardy–Weinberg equilibrium (HWE).

was associated with a decreased risk for IS (OR =0.26, 95% CI = 0.08–0.80). Compared to the A-C-G-C haplotype in nondrinkers, the haplotype of A-T-A-C (OR =0.43, 95% CI = 0.20–0.93) in drinkers was associated with a decreased risk for IS, whereas the haplotype of A-T-A-C (OR =1.61, 95% CI = 1.05–2.47) in nondrinkers was associated with an increased risk for IS.

TABLE 3 Association of the genotypes and serum lipid levels in the controls and cases

SNP/genotype	n	TC(mmol/l)	TG(mmol/l)	HDL-C(mmol/l)	LDL-C(mmol/l)	ApoA1(g/l)	ApoB(g/l)
Rs1169288							
Control	593						
AA	259	4.86 ± 0.07	0.97 (0.61)	1.87 ± 0.03	2.71 ± 0.05	1.37 ± 0.02	0.90 ± 0.01
CA	263	4.94 ± 0.07	1.07 (0.66)	1.92 ± 0.03	2.79 ± 0.05	1.44 ± 0.02	0.91 ± 0.01
CC	71	5.15 ± 0.13	1.15 (0.84)	1.89 ± 0.05	2.70 ± 0.09	1.42 ± 0.03	0.93 ± 0.03
p		0.163	0.004	0.624	0.535	0.013	0.477
CAD	562						
AA	258	4.46 ± 0.07	1.29 (0.93)	1.12 ± 0.02	2.68 ± 0.06	1.02 ± 0.03	0.90 ± 0.02
CA	236	4.53 ± 0.08	1.37 (0.94)	1.13 ± 0.02	2.69 ± 0.06	1.06 ± 0.03	0.91 ± 0.02
CC	68	4.77 ± 0.14	1.47 (1.09)	1.19 ± 0.04	2.93 ± 0.12	1.00 ± 0.06	0.93 ± 0.03
p		0.149	0.294	0.343	0.171	0.638	0.684
IS	521						
AA	237	4.61 ± 0.07	1.35 (0.91)	1.24 ± 0.03	2.74 ± 0.06	1.03 ± 0.01	0.91 ± 0.02
CA	214	4.43 ± 0.07	1.41 (0.98)	1.19 ± 0.03	2.64 ± 0.06	1.01 ± 0.02	0.87 ± 0.02
CC	70	4.59 ± 0.14	1.36 (1.00)	1.23 ± 0.05	2.70 ± 0.11	1.02 ± 0.03	0.89 ± 0.03
p		0.218	0.609	0.422	0.464	0.639	0.241
Rs2259820							
Control	593						
CC	189	4.91 ± 0.09	0.98 (0.55)	1.92 ± 0.04	2.71 ± 0.06	1.38 ± 0.02	0.90 ± 0.02
CT	285	4.93 ± 0.07	1.01 (0.77)	1.89 ± 0.03	2.75 ± 0.05	1.42 ± 0.02	0.91 ± 0.01
TT	119	4.98 ± 0.10	1.13 (0.76)	1.89 ± 0.04	2.78 ± 0.07	1.42 ± 0.03	0.91 ± 0.02
p		0.835	0.063	0.797	0.766	0.401	0.765
CAD	561						
CC	194	4.46 ± 0.08	1.32 (0.97)	1.12 ± 0.02	2.67 ± 0.07	1.01 ± 0.04	0.91 ± 0.02
CT	282	4.55 ± 0.07	1.40 (0.93)	1.13 ± 0.02	2.74 ± 0.06	1.00 ± 0.03	0.62 ± 0.02
TT	85	4.61 ± 0.13	1.31 (0.93)	1.22 ± 0.04	2.73 ± 0.10	1.19 ± 0.06	0.86 ± 0.03
p		0.583	0.017	0.049	0.739	0.009	0.175
IS	521						
CC	183	4.52 ± 0.08	1.32 (0.92)	1.23 ± 0.03	2.65 ± 0.07	1.03 ± 0.02	0.89 ± 0.02
CT	247	4.57 ± 0.07	1.32 (0.92)	1.22 ± 0.03	2.74 ± 0.06	1.03 ± 0.01	0.91 ± 0.02
TT	91	4.47 ± 0.12	1.32 (0.92)	1.20 ± 0.04	2.66 ± 0.09	1.00 ± 0.02	0.86 ± 0.03
p		0.734	0.337	0.822	0.603	0.506	0.320
Rs2464196							
Control	593						
GG	189	4.91 ± 0.08	1.40 (0.55)	1.92 ± 0.04	2.71 ± 0.06	1.38 ± 0.02	0.91 ± 0.02
GA	284	4.92 ± 0.07	1.41 (0.77)	1.89 ± 0.03	2.75 ± 0.05	1.41 ± 0.02	0.91 ± 0.01
AA	120	5.01 ± 0.10	1.45 (0.73)	1.90 ± 0.04	2.79 ± 0.07	1.42 ± 0.03	0.90 ± 0.02
p		0.723	0.050	0.794	0.738	0.392	0.768
CAD	560						
GG	194	4.46 ± 0.08	1.67 (0.97)	1.12 ± 0.04	2.67 ± 0.07	1.01 ± 0.04	0.91 ± 0.02
GA	283	4.55 ± 0.07	1.63 (0.93)	1.13 ± 0.02	2.74 ± 0.06	1.00 ± 0.03	0.92 ± 0.02
AA	83	4.61 ± 0.13	1.68 (0.94)	1.22 ± 0.04	2.73 ± 0.11	1.20 ± 0.06	0.86 ± 0.03
p		0.577	0.587	0.039	0.738	0.007	0.165
IS	520						
GG	181	4.51 ± 0.08	1.64 (0.90)	1.23 ± 0.03	2.64 ± 0.07	1.03 ± 0.02	0.89 ± 0.02
GA	248	4.58 ± 0.07	1.73 (1.06)	1.22 ± 0.03	2.74 ± 0.06	1.03 ± 0.01	0.91 ± 0.02
AA	91	4.47 ± 0.12	1.50 (0.77)	1.20 ± 0.04	2.66 ± 0.09	1.00 ± 0.02	0.86 ± 0.03
p		0.690	0.259	0.824	0.529	0.512	0.290
Rs2650000							
Control	594						
CC	217	4.89 ± 0.08	1.43 (0.61)	1.91 ± 0.03	2.72 ± 0.05	1.38 ± 0.02	0.89 ± 0.01
CA	278	4.93 ± 0.07	1.40 (0.73)	1.88 ± 0.03	2.74 ± 0.05	1.41 ± 0.02	0.92 ± 0.01

(*Continues*)

TABLE 3 (Continued)

SNP/genotype	n	TC(mmol/l)	TG(mmol/l)	HDL-C(mmol/l)	LDL-C(mmol/l)	ApoA1(g/l)	ApoB(g/l)
AA	99	5.06 ± 0.11	1.45 (0.79)	1.93 ± 0.05	2.82 ± 0.08	1.44 ± 0.02	0.90 ± 0.02
p		0.457	0.087	0.562	0.569	0.211	0.340
CAD	562						
CC	214	4.52 ± 0.08	1.62 (0.95)	1.13 ± 0.02	2.74 ± 0.07	1.00 ± 0.04	0.92 ± 0.02
CA	260	4.48 ± 0.07	1.62 (0.93)	1.12 ± 0.02	2.62 ± 0.06	1.02 ± 0.03	0.90 ± 0.02
AA	88	4.70 ± 0.13	1.82 (0.90)	1.23 ± 0.03	2.86 ± 0.11	1.15 ± 0.06	0.88 ± 0.03
p		0.284	0.764	0.024	0.229	0.081	0.536
IS	521						
CC	205	4.54 ± 0.08	1.71 (0.91)	1.22 ± 0.03	2.68 ± 0.06	1.03 ± 0.02	0.89 ± 0.01
CA	238	4.53 ± 0.07	1.66 (0.98)	1.22 ± 0.03	2.70 ± 0.06	1.02 ± 0.01	0.92 ± 0.01
AA	78	4.52 ± 0.13	1.54 (0.94)	1.21 ± 0.05	2.71 ± 0.10	1.01 ± 0.03	0.88 ± 0.03
p		0.994	0.764	0.960	0.958	0.767	0.903

The values of TG are presented as the median (interquartile range) and the difference among the genotypes was determined by the Kruskal–Wallis test. $p < 0.013$ (corresponding to $p < 0.05$ after adjusting for four independent tests by the Bonferroni correction) was considered statistically significant.

TABLE 4 Association of the *HNF1A* polymorphisms and the risk of CAD or IS in different genetic models

SNP/model	Genotype	OR (95% CI)$_{CAD}$	p_{CAD}	OR (95% CI)$_{IS}$	p_{IS}
Rs1169288					
Codominant	AA	1		1	
	CA	0.81 (0.62–1.05)	0.116	0.83 (0.62–1.10)	0.185
	CC	0.92 (0.62–1.37)	0.685	1.07 (0.70–1.62)	0.759
Dominant	AA	1		1	
	AC + CC	0.84 (0.65–1.07)	0.158	0.88 (0.67–1.14)	0.333
Recessive	CC	1		1	
	AA + AC	0.98 (0.68–1.42)	0.915	0.85 (0.58–1.26)	0.426
Rs2259820					
Codominant	CC	1		1	
	CT	0.89 (0.67–1.18)	0.410	0.91 (0.68–1.23)	0.556
	TT	0.64 (0.44–0.91)	0.015	0.72 (0.49–1.05)	0.090
Dominant	CC	1		1	
	CT + TT	0.81 (0.63–1.06)	0.123	0.85 (0.64–1.13)	0.274
Recessive	TT	1		1	
	CC + CT	1.47 (1.06–2.03)	0.020	1.32 (0.94–1.86)	0.109
Rs2464196					
Codominant	GG	1		1	
	GA	0.89 (0.68–1.18)	0.419	1.30 (0.91–1.87)	0.151
	AA	0.62 (0.43–0.89)	0.010	1.41 (0.96–2.07)	0.082
Dominant	GG	1		1	
	GA + AA	0.81 (0.62–1.05)	0.116	0.86 (0.649–1.14)	0.296
Recessive	AA	1		1	
	GG + GA	1.51 (1.09–2.08)	0.014	1.34 (0.96–1.89)	0.089
Rs2650000					
Codominant	CC	1		1	
	CA	0.84 (0.58–1.20)	0.334	0.90 (0.67–1.20)	0.465
	AA	0.88 (0.67–1.16)	0.370	0.80 (0.54–1.19)	0.273
Dominant	CC	1		1	
	CA + AA	0.87 (0.67–1.12)	0.287	0.87 (0.66–1.15)	0.323
Recessive	AA	1		1	
	CC + CA	1.12 (0.80–1.56)	0.513	0.85 (0.59–1.22)	0.382

TABLE 5 Association of the *HNF1A* haplotypes and the risk of CAD or IS

Haplotype	Control, *n* (%)	CAD, *n* (%)	OR (95% CI)$_{CAD}$	p_{CAD}	IS, *n* (%)	OR (95% CI)$_{IS}$	p_{IS}
A-C-G-C	599.67 (0.51)	574.22 (0.51)	1.10 (0.93–1.30)	0.273	574.22 (0.51)	1.11 (0.94–1.33)	0.223
C-T-A-A	357.70 (0.30)	293.03 (0.26)	0.85 (0.71–1.02)	0.088	293.03 (0.26)	0.94 (0.78–1.13)	0.513
A-T-A-A	73.15 (0.06)	72.84 (0.06)	1.09 (0.78–1.53)	0.605	59.52 (0.06)	0.94 (0.66–1.34)	0.744
A-T-A-C	86.49 (0.07)	66.58 (0.06)	0.83 (0.60–1.15)	0.266	64.93 (0.06)	0.86 (0.62–1.20)	0.391
A-C-G-A	20.69 (0.02)	36.37 (0.03)	1.95 (1.13–3.37)	0.015	19.99 (0.02)	–	–

The haplotypes consist of four alleles in the order: rs1169288, rs2259820, rs2464196 and rs2650000 SNPs.

TABLE 6 Best interlocus interaction models identified by the generalized multifactor dimensionality reduction method

Locus number	Best combination for CAD	Testing accuracy	p	Cross-validation consistency
1	rs2464196	0.5178	0.0107	9/10
3	rs1169288-rs2464196-rs2650000	0.5289	0.0498	10/10

4 | DISCUSSION

In the present study, we showed that the observed genotype and allelic frequencies of four SNPs in the three Chinese populations were almost consistent with those of the International HapMap Chinese Han Beijing samples, although they were lower than those in the International HapMap Utah residents with ancestry from Northern and Western Europe (http://hapmap.ncbi.nlm.nih.gov/cgi-perl/gbrowse/hapmap24_B36/#search).

In the present study, we observed that the *HNF1A* rs2259820 and rs2464196 SNPs were related to ApoA1 concentrations in CAD patients. The *HNF1A* rs2259820T and rs2464196A allele carriers in CAD patients had higher ApoA1 concentrations than the rs2259820T and rs2464196A allele noncarriers. A previous study reported that one *HNF1A* mutation (G319S) was associated with ApoA1 concentration in Canadian Oji-Cree and suggested that the relationship was mediated through differences in plasma insulin concentration.[17] Another direct mechanism could explain this association. HNF-1 strongly downregulated transcription driven by its own promoter in HepG2 cells and repressed the activity of HNF-4 (hepatocyte nuclear factor 4), which is a major positive regulator of a different set of liver genes, including ApoA1 and ApoCIII.[21]

Interestingly, no association of the *HNF1A* SNPs and serum TC, LDL-C and HDL-C levels was found in our study populations, although previous studies had speculated there might be one. We conjecture that these features may have more important determinants rather than the *HNF1A* SNPs in Chinese people. It may be the expression of other gene products or the environmental factors that are involved in lipid metabolism.

We also found that the minor rs1169288C allele was associated with high TG concentrations in the controls. In a previous study, Chiu *et al.*[22] showed that the *HNF1A* rs1169288 (I27L) was associated with insulin resistance. Subjects with the LL genotype had higher insulin resistance than those with IL and II genotypes.[22] Furthermore, some studies found that insulin resistance could inhibit ApoA5 mRNA expression in HepG2 cells of humans and rodents.[23,24] ApoA5 was an important determinant of plasma TG levels. Knockout mice without the ApoA5 gene led to increased plasma TG, whereas its overexpression resulted in a reduction of TG.[25,26] By contrast, one cross-sectional study with a sample of Brazilian diabetic individuals demonstrated that the presence of the I27L variant can show a 'protective effect' with respect to the risk of hypertriglyceridemia.[16] Thus, differences in genetic traits among studied populations or statistical methodology are a possible explanation for the association of the variants in the *HNF1A* and serum lipid levels. We could not exclude the possibility that the *HNF1A* was in LD with a functional variant of another gene on chromosome 12 that had been found in association with TG in GWAS.

Some studies found that genetic loci associated with LDL-C, TC, HDL-C and ApoA1 were also associated with the risk of CAD.[27–29] In addition, clinical studies showed that ApoB100/ApoA1 significantly increased the risk of CAD.[30] A low level of ApoA1 could increase the risk of death in patients with CAD[31] and be an independent risk factor for CAD patients.[32] Erdmann *et al.*[7] found a possible association of CAD risk and rs2259816 SNP in a German population. The *HNF1A* rs1169288 and rs2464196 SNPs were also associated with an increased risk of CAD in the general population of younger and older European-American adults.[8] Our findings show for the first time that two SNPs at the *HNF1A* locus (rs2259820T and rs2464196A alleles) associated with higher serum ApoA1 levels consistently decreased the risk of CAD in Chinese people. In addition, we noted that the haplotype of A-C-G-A, carrying rs2259820C and rs2464196G alleles, was associated with an increased risk of CAD. This might be associated with decreased C-reactive protein level, an inflammatory biomarker that predicts future cardiovascular events.[33–36] An interlocus interaction among the *HNF1A* rs1169288, rs2464196 and rs2650000 SNPs was found to be associated with the risk of CAD. In GMDR analyses, a significant association of the *HNF1A* SNPs and CAD was found in one- to three- locus models. Moreover, we also found a strong association of the rs1169288CC genotype and high levels of TG in control subjects. Such an association of raised circulating TG levels and increased CAD risk has been confirmed in 29 Western prospective studies [37]. We concluded that this is because the association depended on levels of other traditional risk factors, such as blood pressure and BMI, as well as other lipid parameters, such as HDL-C, TG and other CAD biomarkers. Moreover, genetic/environmental links, ethnic

difference between the traits and a potential gene–gene interaction should be taken into account.

The interactions of the *HNF1A* haplotypes and some environmental factors on the risk of CAD and IS are not known. In the present study, we found that the haplotype of A-C-G-A in nonsmokers was associated with a decreased risk for CAD. The present study did not show any association between the *HNF1A* SNPs and the risk of IS. However, we firstly found that the A-C-G-A haplotype in men and the A-T-A-C haplotype in drinkers were associated with a decreased risk for IS, whereas the A-T-A-C haplotype in nondrinkers was associated with an increased risk for IS. The reason for this finding is not clear. Further studies with larger sample sizes in the other populations are needed to help assess the association. Our findings may potentially provide new insights into the biological mechanisms with respect to the connection between lipid metabolism and CAD and IS risk.

Although the present study provided interesting findings about the association of the *HNF1A* SNPs and serum lipid phenotypes and the risk of CAD or IS, there are several limitations. First, the number of subjects was too small to interpret the associations of the SNPs and the risk of diseases. Second, other parameters with potential interest could not be determined, including blood sugar, insulin, serum C-reactive protein levels and fibrinogen. Third, we were unable to conduct the concerning the *in vitro* functional impact of the *HNF1A* SNP products. Larger studies should be conducted aiming to assess the potential association of the *HNF1A* SNPs with more complex, clinical-disease-related endpoints, such as diabetes and metabolic syndrome.

In conclusion, to the best of our knowledge, this is the first study to link *HNF1A* polymorphisms with serum lipid levels and the risk of CAD or IS in the Chinese populations. We confirmed that the SNPs in the *HNF1A* were associated with higher serum ApoA1 levels. Our results indicate that the polymorphic variability in the *HNF1A* locus may be a factor involving in the risk of CAD and IS.

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