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

Yi-Jiang Zhou | Rui-Xing Yin | Shao-Cai Hong | Qian Yang | Xiao-Li Cao | Wu-Xian Chen

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 (r2 > 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) 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. 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. Both serum lipid metabolism abnormality and genetic variants are associated with CVD involving the heart, brain and peripheral circulation. 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.\textsuperscript{7,8} CAD may share some common risk factors with IS, such as hypertension, dyslipidemia and genetic variants.\textsuperscript{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.\textsuperscript{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.\textsuperscript{10} Mutations in the HNF1A cause maturity onset diabetes of the young\textsuperscript{10–12} and affect plasma concentrations of C-reactive protein.\textsuperscript{13} In addition, the SNPs in the HNF1A have been associated with levels of low-density lipoprotein cholesterol (LDL-C), total cholesterol (TC)\textsuperscript{14,15} and triglyceride (TG),\textsuperscript{16} as well as high plasma concentrations of high-density lipoprotein cholesterol (HDL-C) and apolipoprotein (Apo) A1.\textsuperscript{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 rs11692288, rs2259820, rs2464196, rs2650000 SNPs and serum lipid levels and the risk of CAD and IS.

\section{MATERIALS AND METHODS}

\subsection{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.\textsuperscript{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.

\subsection{SNP selection}

We selected four SNPs in the HNF1A on the basis of: (1) selected SNPs being established by Haploviz, 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.

\subsection{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 reaction (PCR) products were obtained by Qiagen Company (CA, 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.

\subsection{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).\textsuperscript{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 disequilibrium and haplotype frequencies among the SNPs were analyzed using Haplovie, 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 multi-factor 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...
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-C-A (OR = 1.61, 95% CI = 1.05–2.47) in nondrinkers was associated with an increased risk for IS.

| Characteristic                        | Control (n = 594) | CAD (n = 562) | IS (n = 521) | pCAD | pIS |
|--------------------------------------|------------------|---------------|--------------|------|-----|
| 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/m²)              | 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  | 0.036| 0.361|
| Apolipoprotein (Apo) A1 (g/l)        | 1.41 ± 0.28      | 1.03 ± 0.52   | 1.12 ± 0.60  | 0.014| 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.

| 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  |        |      |

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-C-A (OR = 1.61, 95% CI = 1.05–2.47) in nondrinkers was associated with an increased risk for IS.
| SNP/genotype | n | TC(mmol/l) | TG(mmol/l) | HDL-C(mmol/l) | LDL-C(mmol/l) | ApoA1(g/l) | ApoB(g/l) | p     |
|--------------|---|------------|------------|----------------|----------------|-------------|------------|-------|
| 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| 0.163 |
| 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| 0.004 |
| 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| 0.624 |
| p            |    |            |            |                |                |             |            | 0.624 |
| 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| 0.343 |
| 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| 0.343 |
| 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| 0.149 |
| p            |    |            |            |                |                |             |            | 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| 0.294 |
| 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| 0.343 |
| 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| 0.343 |
| p            |    |            |            |                |                |             |            | 0.765 |
| 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| 0.034 |
| 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| 0.042 |
| 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| 0.042 |
| p            |    |            |            |                |                |             |            | 0.765 |
| 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| 0.039 |
| 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| 0.039 |
| 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| 0.039 |
| p            |    |            |            |                |                |             |            | 0.175 |
| 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| 0.039 |
| 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| 0.039 |
| 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| 0.039 |
| p            |    |            |            |                |                |             |            | 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| 0.039 |
| 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| 0.039 |
| 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| 0.039 |
| p            |    |            |            |                |                |             |            | 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| 0.039 |
| 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| 0.039 |
| 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| 0.039 |
| p            |    |            |            |                |                |             |            | 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| 0.039 |
| 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| 0.039 |
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) | p  | OR (95% CI) | p  |
|------------|----------|-------------|----|-------------|----|
| 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 |
| 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 |
| Dominant   | GG       | 1           |    | 1           |    |
|            | GA + AA  | 0.81 (0.62–1.05) | 0.116 | 0.86 (0.64–1.14) | 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 |
| 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) | pCAD | IS, n (%) | OR (95% CI) | pIS |
|-----------|----------------|------------|-------------|------|-----------|-------------|------|
| 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.23 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 ApoCII.24

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 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 gene 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 CAD31 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.

ACKNOWLEDGEMENTS

The present study was supported by the National Natural Science Foundation of China (No: 30960130) and the Science Foundation of Guangxi Returned Oversea Scholars (No: 0991004).

REFERENCES

1. Jeemon P, Pettigrew K, Sainsbury C, et al. Implications of discoveries from genome-wide association studies in current cardiovascular practice. World J Cardiol. 2011;3:230–247.

2. Fuster V, Kelly BB. Promoting Cardiovascular Health in the Developing World: A Critical Challenge to Achieve Global Health. Washington DC: National Academy of Sciences; 2010.

3. Go AS, Mozaffarian D, Roger VL, et al. Executive summary: heart disease and stroke statistics – 2013 update: a report from the American Heart Association. Circulation. 2013;127:143–152.

4. He J, Neal B, Gu D, et al. International collaborative study of cardiovascular disease in Asia: design, rationale, and preliminary results. Ethn Dis. 2004;14:260–268.

5. Kannel WB, Dawber TR, Kagan A, et al. Factors of risk in the development of coronary heart disease—six year follow-up experience. The Framingham Study. Ann Intern Med. 1961;55:33–50.

6. Wung SF, Hickey KT, Taylor JY, et al. Cardiovascular genomics. J Nurs Scholarsh. 2013;45:60–68.

7. Erdmann J, Grosshennig A, Braund PS, et al. New susceptibility locus for coronary artery disease on chromosome 3q22.3. Nat Genet. 2009;41:280–282.

8. Reiner AP, Gross MD, Carlson CS, et al. Common coding variants of the HNF1A gene are associated with multiple cardiovascular risk phenotypes in community-based samples of younger and older European-American adults: the Coronary Artery Risk Development in Young Adults Study and The Cardiovascular Health Study. Circ Cardiovasc Genet. 2009;2:244–254.

9. Skupien J, Gorczynska-Kosiorz S, Klupa T, et al. Molecular background and clinical characteristics of HNF1A MODY in a Polish population. Diabetes Metab. 2008;34:524–528.

10. Odom DT, Zilisperger N, Gordon DB, et al. Control of pancreas and liver gene expression by HNF transcription factors. Science. 2004;303:1378–1381.

11. Horova E, Prazny M, Kankova K, et al. Genetic and functional analyses of MRAS and HNF1A genes in diabetes and diabetic nephropathy. Folia Biol (Praha). 2012;58:121–127.

12. Fajans SS, Bell GI, Polonsky KS. Molecular mechanisms and clinical pathophysiology of maturity-onset diabetes of the young. N Engl J Med. 2001;345:971–980.

13. Reiner AP, Barber MJ, Guan Y, et al. Polymorphisms of the HNF1A gene encoding hepatocyte nuclear factor-1 alpha are associated with C-reactive protein. Am J Hum Genet. 2008;82:1193–1201.

14. Kathiresan S, Willer CJ, Peloso GM, et al. Common variants at 30 loci contribute to polygenic dyslipidemia. Nat Genet. 2009;41:56–65.

15. Teslovich TM, Musunuru K, Smith AV, et al. Biological, clinical and population relevance of 95 loci for blood lipid traits. Nature. 2010;466:707–713.

16. Giuffrida FM, Furuzawa GK, Kasamatsu TS, et al. HNF1A gene polymorphisms and cardiovascular risk factors in individuals with late-onset autosomal dominant diabetes: a cross-sectional study. Cardiovasc Diabetol. 2009;8:28.

17. Hegele RA, Hanley AJ, Zimman B, et al. Disparity between association and linkage analyses for HNF1A G319S in type 2 diabetes in Canadian Oji-Cree. J Hum Genet. 2000;45:184–187.

18. Babaya N, Ikegami H, Fujisawa T, et al. Association of I27L polymorphism of hepatocyte nuclear factor-1 alpha gene with high-density lipoprotein cholesterol level. J Clin Endocrinol Metab. 2003;88:2548–2551.

19. Wu DF, Yin RX, Cao XL, et al. Scavenger receptor class B type 1 polymorphism of hepatocyte nuclear factor-1 alpha gene with high-density lipoprotein cholesterol level. J Clin Endocrinol Metab. 2008;93:3903.

20. Wu DF, Yin RX, Aung LH, et al. Sex-specific association of ACAT-1 rs1044925 SNP and serum lipid levels in the hypercholesterolemic subjects. Lipids Health Dis. 2012;11:9.

21. Kritis AA, Kistakei E, Barda D, et al. An indirect negative autoregulatory mechanism involved in hepatocyte nuclear factor-1 gene expression. Nucleic Acids Res. 1993;21:5882–5889.

22. Chiu KC, Chuang LM, Ryu JM, et al. The I27L amino acid polymorphism of hepatocyte nuclear factor-1alpha is associated with insulin resistance. J Clin Endocrinol Metab. 2000;85:2178–2183.

23. Nowak M, Hell-reader-Chapman A, Jakel H, et al. Insulin-mediated down-regulation of apolipoprotein A5 gene expression through the phosphatidylinositol 3-kinase pathway: role of upstream stimulatory factor. Mol Cell Biol. 2005;25:1537–1548.

24. Zhao W, Zhao SP. Atorvastatin might inhibit insulin resistance induced by insulin through the triglyceride-lowering role of apolipoprotein A5. Eur Rev Med Pharmacol Sci. 2015;19:3895–3903.

25. Pennacchio LA, Olivier M, Hubacek JA, et al. An apolipoprotein influencing triglycerides in humans and mice revealed by comparative sequencing. Science. 2001;294:169–173.
26. van der Vliet HN, Sammels MG, Leegwater AC, et al. Apolipoprotein A-V: a novel apolipoprotein associated with an early phase of liver regeneration. J Biol Chem. 2001;276:44512–44520.

27. Kathiresan S, Melander O, Guiducci C, et al. Six new loci associated with blood low-density lipoprotein cholesterol, high-density lipoprotein cholesterol or triglycerides in humans. Nat Genet. 2008;40:189–197.

28. Meroufel DN, Mediene-Benchekor S, Lardjam-Hetraf SA, et al. Associations of common SNPs in the SORT1, GCKR, LPL, APOA1, CETP, LDLR, APOE genes with lipid trait levels in an Algerian population sample. Int J Clin Exp Pathol. 2015;8:7358–7363.

29. Willer CJ, Sanna S, Jackson AU, et al. Newly identified loci that influence lipid concentrations and risk of coronary artery disease. Nat Genet. 2008;40:161–169.

30. Zhu L, Lu Z, Ouyang X, et al. Lipoprotein ratios are better than conventional lipid parameters in predicting coronary heart disease in Chinese Han people. Kardiol Pol. 2015;73:931–938.

31. Lundstam U, Herlitz J, Karlsson T, et al. Serum lipids, lipoprotein (a) level, and apolipoprotein (a) isoforms as prognostic markers in patients with coronary heart disease. J Intern Med. 2002;251:111–118.

32. Francis MC, Fröhlich JJ. Coronary artery disease in patients at low risk—apolipoprotein A1 as an independent risk factor. Atherosclerosis. 2001;155:165–170.

33. Hsu LA, Ko YL, Teng MS, et al. Effect of obesity on the association between common variations in the HNF1A gene region and C-reactive protein level in Taiwanese. Clin Chim Acta. 2011;412:725–729.

34. Ley SH, Hegele RA, Connelly PW, et al. Assessing the association of the HNF1A G319S variant with C-reactive protein in Aboriginal Canadians: a population-based epidemiological study. Cardiovasc Diabetol. 2010;9:39.

35. Kleber ME, Grammer TB, Renner W, et al. Effect of the rs2259816 polymorphism in the HNF1A gene on circulating levels of C-reactive protein and coronary artery disease (the ludwigshafen risk and cardiovascular health study). BMC Med Genet. 2010;11:157.

36. Ridker PM, Pare G, Parker A, et al. Loci related to metabolic syndrome pathways including LEPR,HNF1A, IL6R, and GCKR associate with plasma C-reactive protein: the Women’s Genome Health Study. Am J Hum Genet. 2008;82:1185–1192.

How to cite this article: Zhou Y-J, Yin R-X, Hong S-C, Yang Q, Cao X-L, Chen W-X. Association of the HNF1A polymorphisms and serum lipid traits, the risk of coronary artery disease and ischemic stroke. J Gene Med. 2017;19:e2941. https://doi.org/10.1002/jgm.2941