Original article

Fatty acid desaturase 1 polymorphisms are associated with coronary heart disease in a Chinese population

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Keywords: high-density lipoprotein-cholesterol; low-density lipoprotein-cholesterol; triglyceride; polymorphisms; coronary heart disease; fatty acid desaturase 1

Background A recent genome-wide association study in Caucasians revealed that three loci (rs174547 in fatty acid desaturase 1 (FADS1), rs2338104 near mevalonate kinase/methylmalonic aciduria, cobalamin deficiency, cbIB type (MK/MMAB) and rs10468017 near hepatic lipase (LIPC)) influence the plasma concentrations of high-density lipoprotein-cholesterol (HDL-C) and triglycerides (TG). However, there are few reports on the associations between these polymorphisms and plasma lipid concentrations in Chinese individuals. This study aimed to evaluate the associations between these three polymorphisms with HDL-C and TG concentrations, as well as coronary heart disease (CHD) susceptibility in Chinese individuals.

Methods We conducted a population-based case-control study in Chinese individuals to evaluate the associations between these three polymorphisms and HDL-C and TG concentrations, and also evaluated their associations with susceptibility to CHD. Genotypes were determined using polymerase chain reaction-restriction fragment length polymorphism assays and TaqMan genotyping assays.

Results We found significant differences in TG and HDL-C concentrations among the TT, TC and CC genotypes of FADS1 rs174547 (P=0.017 and 0.003, respectively, multiple linear regression). The CC variant of rs174547 was significantly associated with hyperlipidemia compared with the TT variant (adjusted odds ratio (OR)=1.71, 95% confidence intervals (CI): 1.16–2.54). The FADS1 rs174547 CC variant was also associated with significantly increased CHD risk compared with the TT and TC variant (adjusted OR=1.53, 95% CI: 1.01–2.31), and the effect was more evident among nonsmokers and females. The polymorphisms rs2338104 and rs10468017 did not significantly influence HDL-C or TG concentrations in this Chinese population.

Conclusion rs174547 in FADS1 may contribute to the susceptibility of CHD by altering HDL-C and TG levels in Chinese individuals.

Coronary heart disease (CHD) is one of the leading causes of death worldwide. In China, CHD accounts for 9% of all deaths in urban areas and 4% in rural areas. Approximately 400,000 patients died from CHD and 652,000 patients were diagnosed with CHD in 2004. Studies have consistently reported that lipoprotein-associated lipid concentrations are associated with the risk of cardiovascular diseases, including CHD. 

High plasma concentrations of high-density lipoprotein-cholesterol (HDL-C) reduce the risk of CHD, whereas high concentrations of triglycerides (TG) increase the risk of CHD. Although smoking, diet and physical activity have a role in determining the plasma lipid concentrations, studies of twins and families have shown that approximately half of the variation of this trait is genetically determined. Single nucleotide polymorphisms (SNP) in the genes ATP-binding cassette, sub-family A member 1 (ABCA1), lipoprotein lipase (LPL) and cholesteryl ester transfer protein (CETP) are associated with variations in HDL-C and TG concentrations, but these SNPs accounted for only a small proportion of total genetic variation, and additional SNPs remain to be characterized.

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A recent genome-wide association study identified three SNPs ((rs174547 in fatty acid desaturase 1 (FADS1), rs2338104 near MVK/MMAB and rs10468017 near hepatic lipase (LIPC)) that were associated with the plasma HDL-C and TG concentrations in European individuals.\textsuperscript{19} FADS1 is located on chromosome 11 and encodes fatty acid desaturase 1, a key enzyme in the synthesis of long-chain polyunsaturated fatty acids (LC-PUFAs). \textit{MVK} and \textit{MMAB} are located on chromosome 12 and encode enzymes involved in cholesterol synthesis and degradation. \textit{LIPC} is located on chromosome 15 and encodes an enzyme that hydrolyzes TG and phospholipids present in most of the major classes of lipoproteins. Considering the differences in allele frequency among different populations, it is of great interest to evaluate the associations between these three SNPs with plasma HDL-C and TG concentrations in non-European populations. Therefore, the aim of current study was to evaluate the associations between these three polymorphisms with HDL-C and TG concentrations, as well as CHD susceptibility in Chinese individuals. To achieve this, we conducted a case-control study of 524 cases with low HDL-C and high TG concentrations, as well as 515 CHD cases and 621 controls with normal HDL-C and TG concentrations.

**METHODS**

**Study subjects**

This study was approved by the institutional review board of Nanjing Medical University. We designed a population-based case-control study focusing on hyperlipidemia nested within a community-based cross-sectional survey of 30,500 subjects living in Changzhou and Nantong cities, Jiangsu Province. Briefly, we carried out a community-based cross-sectional survey to investigate the prevalence of non-infectious diseases in Changzhou and Nantong cities, Jiangsu Province, from 2004 to 2007, involving about 30,500 participants. After providing informed consent, all subjects participated in face-to-face interviews and completed a questionnaire recording demographic characteristics, risk factors for chronic diseases and disease history. Physical examinations were also performed and included measurement of blood pressure, height and weight, as well as laboratory tests to measure total cholesterol (TC), TG, HDL-C and fasting plasma glucose concentrations. Fasting blood samples for routine laboratory examinations were obtained early in the morning after an overnight fast. All biochemical parameters were measured enzymatically on an auto-analyzer (Hitachi 7180 Biochemistry Auto-analyzer, Japan) according to the manufacturer’s instructions.

According to the 2007 Chinese criteria and the Adult Treatment Panel III guidelines for hyperlipidemia, 524 subjects with TG $\geq 1.70$ mmol/L and HDL-C $< 1.04$ mmol/L were defined as cases with hyperlipidemia (high-risk subjects), while 621 subjects (low-risk subjects) not meeting these criteria were randomly selected and matched with the high-risk subjects for age and sex. Subjects who were taking lipid-lowering medications were excluded.

To evaluate the association between locus rs174547 in FADS1 with CHD risk, we carried out a case-control study with 515 CHD cases from Nanjing ZhongDa Hospital, Jiangsu province, and the low-risk group defined above. In brief, these CHD cases were defined as having angiographic coronary stenosis with $\geq 50\%$ lumen reduction in at least one major epicardial coronary artery. All patients were genetically unrelated ethnic Han Chinese from Nanjing city and surrounding regions in eastern China. Each patient was interviewed after informed consent was obtained, and a questionnaire was administered by interviewers on enrollment to record demographic characteristics, risk factors for CHD, and history of vascular events. A 5-ml venous blood sample was collected from each patient. This component of the study was approved by the Ethical Committee of Clinic Medical College, Southeast University, Nanjing, China.

**Genotyping**

Genomic DNA was isolated from leucocytes of venous blood by proteinase K digestion and phenol/chloroform extraction. The three SNPs (rs174547, rs2338104 and rs10468017) were genotyped by polymerase chain reaction–restriction fragment length polymorphism assays. The restriction enzymes for loci rs174547, rs2338104 and rs10468017 were \\textit{FokI}, \textit{TaqI} and \textit{Spl} (New England BioLabs, Beverly, MA, USA), respectively. The sense and antisense primers were: 5′-CTGTTTGGGGGACTTTTTTT-3′, and 5′-ACTGTTTTGGTGTTGGA-3′ for rs174547; 5′-GAAGCAATGGTTAGGGAAAT-3′ and 5′-ATATTCACAAGTTGTGAGCCATATC-3′ for rs2338104, and 5′-CCTCCAAAGTGCTGATGT-3′ and 5′-GTGGAAACTCTGGAAACC-3′ for rs10468017, respectively. Genotyping was performed with a positive control for the known heterozygous genotype. The investigators were blinded to the group status. Two research assistants independently read the gel images and repeated the assays if they did not reach a consensus on the tested genotype.

The rs174547 locus was further genotyped using pre-designed TaqMan allelic discrimination assays in the CHD cases. The TaqMan primers were 5′-GGGGACCTTTTTGTTTGTCTT-3′ and 5′-CCCCATTTTGTGTCACT-3′, and the probes were FAM-TAGCAGTCCTCAGTCACACT-3′ and HEX-CATGGACCCCTTTT-MGB. Genotyping was unsuccessful in four cases (0.78%) because of insufficient DNA quantity or quality. The rs174547 genotype was tested by both assays on 10% of all samples and the results were all consistent.

**Statistical analysis**

Data were shown as mean ± standard deviation (SD) or $n$...
Differences in demographic characteristics, clinical variables, and genotype frequencies between the high-risk and low-risk subjects were calculated using Student’s t test for continuous variables or $\chi^2$ test for categorical variables. Associations between the genotypes with plasma lipid concentrations and risk of CHD were determined by logistic regression analysis with adjustment for age, sex, body mass index (BMI) and smoking status, and odds ratios (ORs) with 95% confidence intervals (CIs) were calculated. Analysis of variance (ANOVA) was used to compare the effects of the rs174547 genotypes on plasma lipid concentrations. The Kruskal-Wallis ANOVA if there was unequal variance (ANOVA) was used to compare the effects of the rs174547 genotypes on plasma lipid concentrations.

**RESULTS**

**Characteristics of the subjects**

The characteristics of the subjects are shown in Table 1. There were no statistically significant differences between the high-risk subjects (cases) and the low-risk subjects (controls) in terms of age, sex, and smoking status. BMI ($P < 0.0001$) and TC ($P < 0.0001$) were significantly greater in the high-risk subjects compared with the low-risk subjects. Mean TG and HDL-C concentrations in high-risk subjects (3.12±1.28 mmol/L and 0.77±0.24 mmol/L, respectively) were significantly higher and lower, respectively, compared with those in the low-risk subjects (0.77±0.24 mmol/L and 1.79±0.34 mmol/L, respectively). The mean age and BMI of the CHD cases were (66.7±10.0) years and (24.84±3.61) kg/m², respectively. Overall, 379 subjects with CHD (73.59%) were males and 258 (50.10%) were smokers.

**Associations between the SNPs and plasma lipid concentrations**

The genotype distributions of rs174547, rs2338104 and rs10468017 in the high-risk and low-risk subjects are shown in Table 2. Single locus analysis revealed that rs174547 variants were significantly associated with hyperlipidemia (i.e., low HDL-C and high TG) ($OR=1.71$, 95% CI: 1.16–2.54 for CC vs. TT). In the dominant genetic model, TC and CC combined was associated with hyperlipidemia (TC and CC vs. TT: adjusted $OR=1.36$, 95% CI: 1.03–1.79). In the recessive genetic model, the CC variant was also associated with hyperlipidemia (CC vs. TC and TT: adjusted $OR=1.49$, 95% CI: 1.05–2.12). Table 3 shows the effects of rs174547 on plasma TC, TG and HDL-C concentrations. There were significant differences in TG and HDL-C concentrations among the TT, TC and CC variants ($P=0.017$ and 0.003, respectively, multiple regression analysis). Individuals with TC/CC genotypes had significantly higher TG and lower HDL-C concentrations than those with TT genotype ($P < 0.05$). Overall, we found no significant associations between rs2338104 and rs10468017 with plasma lipid concentrations in this study.

**Association between rs174547 and CHD risk**

To evaluate the association between rs174547 and CHD risk, we recruited 515 CHD patients as a case group and...
used the same 621 low-risk subjects as the control group. The Logistic regression analysis revealed that the rs174547 CC variant was associated with significantly increased risk for CHD in a recessive genetic model (adjusted OR=1.53, 95% CI: 1.01–2.31) (Table 4). We then evaluate the effect of rs174547 on CHD risk with stratification for age, sex, smoking status and BMI. These analyses revealed that the risk effect of the CC variant was greater in female (OR=1.94, 95% CI: 1.04–3.61) and in nonsmokers (OR=1.88, 95% CI: 1.09–3.22) than in other subjects (Table 5).

Table 4. Association between rs174547 polymorphisms and CHD risk

<table>
<thead>
<tr>
<th>Genotype</th>
<th>CHD cases (n=515)</th>
<th>Low-risk subjects (n=621)</th>
<th>Crude OR (95% CI)</th>
<th>Adjusted OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs174547 T&gt;C</td>
<td>511 616</td>
<td>230 (37.34)</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>TT</td>
<td>193 (37.77)</td>
<td>7 (26.92)</td>
<td>0.91 (0.70–1.18)</td>
<td>0.92 (0.67–1.28)</td>
</tr>
<tr>
<td>TC</td>
<td>228 (44.62)</td>
<td>299 (48.54)</td>
<td>1.23 (0.87–1.75)</td>
<td>1.46 (0.93–2.30)</td>
</tr>
<tr>
<td>CC</td>
<td>90 (17.61)</td>
<td>87 (14.12)</td>
<td>1.53 (1.01–2.31)</td>
<td>1.00</td>
</tr>
<tr>
<td>CT</td>
<td>23 (20.72)</td>
<td>49 (14.58)</td>
<td>2.05 (0.90–3.27)</td>
<td>1.00</td>
</tr>
</tbody>
</table>

Values are shown as n or n (%). *Adjusted for age, sex, smoking and BMI.

Table 5. Stratified analysis of rs174547 polymorphisms and CHD risk by age, sex, smoking status, and BMI

<table>
<thead>
<tr>
<th>Variables</th>
<th>Genotype</th>
<th>CHD cases (n=515)</th>
<th>Low-risk subjects (n=621)</th>
<th>Crude OR (95% CI)</th>
<th>Adjusted OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;49</td>
<td>TT+TC</td>
<td>19 (37.08)</td>
<td>255 (84.72)</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>CC</td>
<td>7 (26.92)</td>
<td>46 (15.28)</td>
<td>2.05 (0.73–5.77)</td>
<td>1.00</td>
</tr>
<tr>
<td>≥49</td>
<td>TT+TC</td>
<td>402 (82.89)</td>
<td>274 (86.98)</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>CC</td>
<td>83 (17.11)</td>
<td>41 (13.02)</td>
<td>1.43 (0.91–2.24)</td>
<td>1.00</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>TT+TC</td>
<td>311 (82.93)</td>
<td>235 (84.53)</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>CC</td>
<td>64 (17.07)</td>
<td>43 (15.47)</td>
<td>1.28 (0.74–2.23)</td>
<td>1.00</td>
</tr>
<tr>
<td>Female</td>
<td>TT+TC</td>
<td>110 (80.88)</td>
<td>294 (86.98)</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>CC</td>
<td>26 (19.12)</td>
<td>44 (13.02)</td>
<td>1.94 (1.04–3.61)</td>
<td>1.00</td>
</tr>
<tr>
<td>Smoking status</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smoker</td>
<td>TT+TC</td>
<td>214 (83.59)</td>
<td>155 (84.24)</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>CC</td>
<td>42 (16.41)</td>
<td>29 (15.76)</td>
<td>1.15 (0.60–2.21)</td>
<td>1.00</td>
</tr>
<tr>
<td>Nonsmoker</td>
<td>TT+TC</td>
<td>207 (81.18)</td>
<td>374 (86.57)</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>CC</td>
<td>48 (18.82)</td>
<td>58 (13.43)</td>
<td>1.88 (1.09–3.22)</td>
<td>1.00</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 22</td>
<td>TT+TC</td>
<td>88 (79.28)</td>
<td>287 (85.42)</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>CC</td>
<td>23 (20.72)</td>
<td>49 (14.58)</td>
<td>1.72 (0.90–3.27)</td>
<td>1.00</td>
</tr>
<tr>
<td>≥ 22</td>
<td>TT+TC</td>
<td>333 (83.25)</td>
<td>241 (86.38)</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>CC</td>
<td>67 (16.75)</td>
<td>38 (13.62)</td>
<td>1.42 (0.83–2.42)</td>
<td>1.00</td>
</tr>
</tbody>
</table>

Values are shown as n (%). *Adjusted for age, sex, smoking status and BMI (excluding the stratified factor in each stratum).

**DISCUSSION**

In this molecular epidemiological, case-control study of Chinese individuals, we investigated whether the associations between three SNPs with HDL-C and TG concentrations identified in a previous European genome-wide association study were also apparent in Chinese individuals. We also investigated whether these SNPs influenced the risk of developing CHD. We found that rs174547 T>C in FADS1, but not the other SNPs, were associated with TG and HDL-C concentrations and with CHD risk.

rs174547, which is located in intron 9 of FADS1, was first reported to be associated with TG and HDL-C concentrations in a genome-wide association study of European individuals and was recently confirmed in Japanese individuals. FADS1 encodes the fatty acid delta-5 desaturase, a key enzyme in the synthesis of LC-PUFAs. These fatty acids regulate membrane fluidity and influence the generation of signaling molecules. In addition, LC-PUFAs are also precursors of important inflammatory mediators. The concentration of LC-PUFAs in phospholipids is associated with cardiovascular disease and metabolic syndrome. The expression quantitative trait locus data indicate that rs174547 modulates the expression of FADS1 and FADS3. The major T allele was associated with higher transcript levels and led to higher HDL-C and lower TG concentrations. However, it is unclear how genetic variants influence blood lipid concentrations. One possible reason is that people carrying the minor alleles may have lower desaturase expression, which may result in lower LC-PUFA concentrations and reduced peroxisome proliferator activating receptor (PPAR)-α activation. Endogenous LC-PUFAs are natural ligands of PPARα and PPARα activation can increase HDL-C levels and decrease TG levels by inducing the expression of apolipoprotein (Apo)-AI, Apo-AIL and lipoprotein lipase, and suppressing ApoCIII. However, other mechanisms are also likely because blood lipid concentrations are also influenced by diet and lifestyle.

There have been a few studies discussing associations between FADS polymorphisms and CHD susceptibility. For example, Martinelli et al analyzed the associations between FADS1/2/3 genotypes and CHD risk in a case-control study of 610 CHD cases and 266 controls of Italian origin. They found no single variants that were differently distributed between the CHD cases and CHD-free controls. This result was consistent with that reported by Baylin et al. However, in the present study in a Chinese population, rs174547 variants were associated with low HDL-C and high TG plasma concentrations and the high-risk, C variant allele was associated with increased CHD risk. Interestingly, the effect of the C variant allele was dominant for hyperlipidemia but recessive for CHD. This suggests that the rs174547 CC variant may affect CHD susceptibility by decreasing HDL-C and increasing TG concentrations. Stratified analyses subsequently revealed that the risk effects of the CC variant were greater in females and nonsmokers compared with other individuals. These findings may relate to the fact that most of the nonsmokers in China are females. Smoking is a well-known factor if they do not have a joint effect. Nevertheless, such effects need further investigation.

Willer et al reported that MKM/MMAB is a novel locus for HDL-C concentrations. A genome-wide association study revealed that the T allele of rs10468017 was associated with lower LIPC expression and increased...
HDL-C levels. However, in our study, neither rs2338104 nor rs10468017 were consistent with these earlier studies, but our findings were consistent with the results of a large-scale replication analysis in Japanese individuals. The effects of SNPs on HDL-C concentrations might differ according to ethnic background, as the rs2338104 G allele and the rs10468017 T allele were more frequent in Caucasians than in Asians. Additionally, rs2338104 was located in different blocks between Caucasians and Asians according to the public HapMap SNP database (http://www.hapmap.org/). Therefore, the causative variants may be tagged by SNPs rs2338104 and rs10468017 in Caucasians but not in Asians. Fine mapping and/or functional characterizations are necessary to identify the causal variants associated with plasma lipid concentrations in Asian populations.

Several possible limitations of our study should be discussed. First, the number of subjects in our study was moderate, limiting the statistical power. Second, the CHD cases were recruited from a hospital whereas controls were randomly selected from the community. Furthermore, the CHD cases were not matched to the controls (low-risk subjects) in terms of age or sex. Therefore, inherent selection bias cannot be completely excluded. However, we applied a rigorous epidemiological design and laboratory tests and statistically adjusted for several known risk factors to minimize potential bias. Third, hyperlipidemia usually refers to higher plasma TG, TC and LDL-C concentrations. However, our study was performed to validate the associations between three polymorphisms with HDL-C and TG concentrations that were reported in an earlier genome-wide association study. Therefore, we defined hyperlipidemia as high TG and low HDL-C concentrations. Further studies are required to elucidate the genetic mechanisms underlying hyperlipidemia, particularly regarding TG, TC and LDL-C levels.

In conclusion, we found that rs174547 of FADSJ was associated with lower HDL-C, higher TG and increased CHD risk in a Chinese population. These findings need to be extended by fine mapping and functional studies.

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