Associations Between Single Nucleotide Polymorphisms on Chromosome 9p21 and Risk of Coronary Heart Disease in Chinese Han Population

Li Zhou, Xiaomin Zhang, Mei’an He, Longxian Cheng, Ying Chen, Frank B. Hu, Tangchun Wu

Objectives—We aimed to determine whether the single nucleotide polymorphisms (SNPs) on chromosome 9p21 were associated with coronary heart disease (CHD) in a Chinese Han population.

Methods and Results—We determined the genotypes of rs2383206 and rs2383207 on chromosome 9p21 in 1360 CHD patients and 1360 age- and sex-frequency–matched controls from an unrelated Chinese Han population. GG genotypes in rs2383207 occurred more frequently in CHD patients compared to controls, and the odds ratio (OR) was 1.52 (95% CI 1.13 to 2.04), after adjusting for conventional risk factors. In stratified analysis, the risk associated with the GG genotype of the two SNPs was stronger in subjects who were males, less than 60 years old, overweight, and smokers. The SNP rs2383207 had significant interactions with gender and smoking (P=0.018 and 0.037, respectively). The risk allele G of rs2383207 plus family history of CHD had a cumulative association with CHD (P for trend, 1.0×10^-5); the OR for CHD was 4.59 (95% CI 2.52 to 8.37) for those with all the risk factors as compared with subjects without any of the factors.

Conclusions—The SNP rs2383207 on chromosome 9p21 is significantly associated with CHD in Chinese. This SNP combined with family history has a cumulative association with CHD. (Arterioscler Thromb Vasc Biol. 2008;28:2085-2089)

Key Words: coronary heart disease ■ single nucleotide polymorphism ■ family history ■ genetic variation ■ risk factor

Coronary heart disease (CHD) is the leading cause of death worldwide.1,2 The development of CHD is a complex process. It is caused by multiple genetic and environmental factors, and interactions among these factors.3 Many risk factors have been identified for CHD, including smoking, advanced age, male gender, diabetes mellitus, and high blood pressure.4 However, few genetic factors for CHD have been identified. Recently, genome-wide association studies (GWAS) using hundreds of thousands of markers and targeted gene-based resequencing have facilitated the gene discovery for CHD.5,6 In genome-wide association studies, a susceptibility locus for CHD has been mapped to chromosome 9p21, adjacent to the tumor suppressor genes CDKN2A and CDKN2B.7,8 Helgadottir et al7 found that the variant rs2383207 was associated with myocardial infarction (MI). Meanwhile, in another GWAS, McPherson et al8 found that the homozygotes of the risk alleles of rs2383206 were associated with an increased risk of CHD. The same genetic locus was identified by a genome-wide association study from a British population by the Wellcome Trust Case Control Consortium and replicated in a German population.9 However, these associations need to be confirmed by further replication studies, particularly in other ethnic groups. Therefore, we carried out a large case-control association study including 1360 CHD patients and 1360 age- and sex-frequency–matched controls in an unrelated Chinese Han population. We selected 2 SNPs (rs2383206 and rs2383207) in the locus of interest and aimed to determine whether the SNPs on chromosome 9p21 were associated with CHD in a Chinese Han population.

Methods

Study Population

The study population was composed of 1360 case patients and 1360 age- and sex-frequency–matched controls. Patients were consecutively recruited from 3 hospitals (Tongji Hospital, Union Hospital, and Wugang Hospital) in Wuhan (Hubei, China) between May 2004 and October 2006. The diagnostic criteria for CHD cases included 1) the presence of a stenosis ≥50% in at least 1 major segment of coronary arteries (the right coronary artery, left circumflex, or left anterior descending arteries) on coronary angiography; (2) Based on World Health Organization criteria in...
terms of elevations of cardiac enzymes, electrocardiographic changes and clinical symptoms; (3) A documented history of coronary artery bypass graft or percutaneous coronary intervention. Patients with congenital heart disease, cardiomyopathy, and valvular disease were excluded. A total of 1440 patients diagnosed as having CHD were recruited; 1360 of them (94.4%) consented to participate in the study and provided questionnaire information and blood samples. After cases were diagnosed with CHD they were interviewed in person by a trained interviewer within 3 days. The control subjects, residing in the same communities as the cases, were determined to be free of CHD and peripheral atherosclerotic arterial disease by medical history, clinical examinations, and electrocardiography. The response rate for the controls was 90.7% (1360 of 1500). Subjects with severe liver or kidney disease were excluded. Medical history, family history of CHD among first degree relatives, medication use, home environment, and lifestyle factors were obtained through questionnaire interview.

Subjects were classified as smokers and nonsmokers. Those who had smoked less than 100 cigarettes in the lifetime were defined as nonsmokers; otherwise, they were defined as smokers. Body mass index (BMI) was calculated as weight in kilograms divided by the square of height in meters. Subjects were considered to be hypertensive if their systolic blood pressure was \( \geq 140 \) mm Hg or diastolic pressure \( \geq 90 \) mm Hg or they were already being treated with antihypertensive drugs. Diabetes was defined either by 1999 World Health Organization criteria or self-report of being previously diagnosed as diabetic. Family history was positive if first-degree relatives (parents, siblings) had CHD. All subjects gave written consent after receiving a full explanation of the study. The Ethics Committee of Tongji Medical College approved this study.

**Genotyping**

Fasting venous blood was collected in 5-mL EDTA tubes, and genomic DNA was isolated with a Puregene kit (Gentra Systems Inc). Genotyping was performed with TaqMan SNP allelic discrimination by means of an ABI 7900HT (Applied Biosystems), in 384-well format. The TaqMan assay kit was purchased from Applied Biosystems. It included the forward target-specific polymerase chain reaction (PCR) primer, the reverse primer, and the TaqMan MGB probes labeled with 2 special dyes: FAM and VIC. PCR reactions were carried out in reaction volume of 5 \( \mu \)L containing 5 ng DNA, 2.5 \( \mu \)L 2X Taqman universal PCR MasterMixNo AmpErase UNG (Applied Biosystems), 0.125 \( \mu \)L 40X Assay MIX. PCR conditions included 95°C for 10 minutes followed by 40 cycles of 15s at 92°C and 1 minute at 60°C. Two blank controls (DNA hydration) and 2 replicate quality control samples were included in each 384-well format, and 2 replicate samples were genotyped with 100% concordance. Automatic allele calling, with the default settings (the quality value of auto caller \( \geq 95.0 \)), was carried out by ABI 7900HT data collection and analysis software version 2.2.1 (SDS 2.2.1).

**Statistical Analysis**

Continuous variables were reported as the mean value±SD. Normal distribution of data was checked using the Kolmogorov-Smirnov normality test. Data with a normal distribution were compared by Student t test, and those with unequal variance or without a normal distribution were analyzed by a Mann-Whitney rank sum test. Categorical values were compared by the \( \chi^2 \) test, which was also used to test for deviation of genotype distribution from Hardy-Weinberg equilibrium. The association between variants in the two SNPs and CHD risk was estimated by computing odds ratios (ORs) and 95% confidence intervals (CIs) from the multivariate logistic regression analyses. An unconditional logistic model was used to adjust for multiple cardiovascular risk factors. The probability level accepted for significance was \( P<0.05 \). The significance of multiplicative interactions between the selected SNPs and covariates was determined by the likelihood ratio test using the logistic regression model. All data analyses were carried out with the statistical analysis software package SPSS12.0 (SPSS Inc).

### Results

**General Characteristics of the Subjects**

The general characteristics of the study subjects are presented in Table 1. The traditional CHD risk factors such as hypertension, diabetes, BMI, smoking, and family history of CHD were significantly different between the cases and controls. However, total cholesterol levels were significantly lower in cases than in controls, which could be the result of cholesterol-lowering medication in the patients after diagnosis (4.4±1.1 mmol/L versus 5.1±1.3 mmol/L, \( P<0.01 \)). The proportion of subjects reported taking cholesterol-lowering medications such as a statin in the cases and controls in our study were 67.1% and 0.3%, respectively.

**Relation of the SNPs and CHD Risk**

The observed genotype frequencies of the 2 SNPs on chromosome 9p21 were in Hardy-Weinberg equilibrium among the controls (\( P=0.63 \) and 0.64, respectively). As shown in Table 2, univariate analyses indicated that the 2 SNPs rs2383206 and rs2383207 were significantly associated with CHD. The risk genotypes of \( G \) were found in both SNPs, with odds ratio (OR) were 1.29 (95% CI 1.05 to 1.58) and 1.40 (95% CI 1.09 to 1.80), respectively. In multivariate analyses, after adjusting for conventional CHD risk factors such as age, gender, smoking, BMI, hypertension, diabetes, and family history of CHD, only the \( GG \) genotype of rs2383207 had a significant association with CHD (OR=1.52, 95% CI 1.13 to 2.04). When we examined MI separately, the association with this SNP became even stronger (OR of MI for the \( GG \) genotype=2.64, 95% CI 1.35 to 5.17; \( P=0.005 \)). For the risk allele \( G \) of the both SNPs, the univariate ORs of CHD were 1.14 (95% CI 1.03 to 1.27) and 1.26 (95% CI 1.12 to 1.41), respectively (Table 2).

### Table 1. General Characteristics of CHD Patients and Controls

<table>
<thead>
<tr>
<th>Variables</th>
<th>Cases (n=1360)</th>
<th>Controls (n=1360)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex, m/f, (%)</td>
<td>931/429 (68.5/31.5)</td>
<td>931/429 (68.5/31.5)</td>
<td>1.00</td>
</tr>
<tr>
<td>Age, mean±SD</td>
<td>60.9±9.8</td>
<td>60.4±9.8</td>
<td>0.13</td>
</tr>
<tr>
<td>Blood pressure, mm Hg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic</td>
<td>135.7±25.2</td>
<td>132.1±21.5</td>
<td>0.00</td>
</tr>
<tr>
<td>Diastolic</td>
<td>82.2±14.7</td>
<td>82.3±11.5</td>
<td>0.89</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>24.4±3.5</td>
<td>24.1±3.6</td>
<td>0.04</td>
</tr>
<tr>
<td>Fasting glucose, mmol/L</td>
<td>6.6±3.5</td>
<td>5.3±2.0</td>
<td>0.00</td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>4.4±1.1</td>
<td>5.1±1.3</td>
<td>0.00</td>
</tr>
<tr>
<td>Triglyceride, mmol/L</td>
<td>1.7±1.2</td>
<td>1.7±1.3</td>
<td>0.87</td>
</tr>
<tr>
<td>Smoking, no/yes, (%)</td>
<td>567/733 (41.7/58.3)</td>
<td>686/674 (50.4/49.6)</td>
<td>0.00</td>
</tr>
<tr>
<td>Past history</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypertension, (%)</td>
<td>984 (72.4)</td>
<td>473 (34.8)</td>
<td>0.00</td>
</tr>
<tr>
<td>Diabetes (%)</td>
<td>392 (28.8)</td>
<td>87 (6.4)</td>
<td>0.00</td>
</tr>
<tr>
<td>Family history of CHD (%)</td>
<td>110 (8.1)</td>
<td>37 (2.7)</td>
<td>0.00</td>
</tr>
</tbody>
</table>
We conducted stratified analysis for the two SNPs (Table 3). GG carriers of two SNPs (rs2383206 and rs2383207) had higher risk in males (OR = 1.57, 95% CI 1.14 to 2.16 and OR = 1.96, 95% CI 1.32 to 2.90, respectively), younger than 60-year-old subjects (OR = 1.35, 95% CI 1.00 to 1.84 and OR = 1.58, 95% CI 1.02 to 2.47, respectively), smokers (OR = 1.55, 95% CI 1.10 to 2.20 and OR = 2.03, 95% CI 1.34 to 3.08, respectively), and BMI ≥25 kg/m² subjects (OR = 1.52, 95% CI 1.00 to 2.33 and OR = 2.39, 95% CI 1.41 to 4.05, respectively). Furthermore, when multiplicative interaction was tested for each possible pair of these 2 SNPs, we found significant interactions between rs2383207 and gender (P = 0.018) and smoking (P = 0.037).

We evaluated cumulative effects of allele G of the SNP rs2383207 and family history of CHD (Table 4). Subjects who carried 3 risk factors, including 2 risk alleles G and family history of CHD had an OR of 4.59 (95% CI 2.52 to 8.22).

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8.37) for CHD, as compared with subjects who carried none of the risk factors, after adjusting for other conventional risk factors. The $P$ for trend of this cumulative effect was $1.0 \times 10^{-6}$.

**Discussion**

Our large case-control study not only replicated the findings of the SNPs on chromosome 9p21 that were associated with CHD established previously by genome-wide association studies, but also provided several novel findings that were relevant to this locus. Our results showed that the genotype $GG$ of the SNP rs2383207 was associated with increased overall risk of CHD. Especially among males, young subjects (age $\leq$60), smokers, and overweight patients (BMI $\geq$25 kg/m$^2$), the genotypes $GG$ of the 2 SNPs rs2383206 and rs2383207 were strongly associated with higher risk of CHD.

It is possible that genetic factors would exert a greater influence in younger persons and males, and smoking and obesity may exacerbate the influence of the genetic factors. Significant interactions were found between the traditional CHD risk factors (gender and smoking) and rs2383207 in our study. These findings support the notion that many genes, each with a relatively small effect, work in combination with other modifier genes and environmental factors.  

In conclusion, we have demonstrated that the SNP rs2383207 plus family history of CHD have a cumulative, significant association with CHD.

**Table 4. Cumulative Effect of Associated Factors on the Risk of CHD**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Case</th>
<th>Control</th>
<th>OR (95% CI)†</th>
<th>$P$ Value</th>
<th>$P_{\text{trend}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>120</td>
<td>(8.8%)</td>
<td>162 (11.9%)</td>
<td>1.00</td>
<td>...</td>
</tr>
<tr>
<td>1</td>
<td>503</td>
<td>(37.0%)</td>
<td>591 (43.5%)</td>
<td>1.13 (0.85–1.51)</td>
<td>0.399</td>
</tr>
<tr>
<td>2</td>
<td>680</td>
<td>(50.0%)</td>
<td>588 (43.2%)</td>
<td>1.58 (1.19–2.10)</td>
<td>0.010</td>
</tr>
<tr>
<td>3</td>
<td>57</td>
<td>(4.2%)</td>
<td>19 (1.4%)</td>
<td>4.59 (2.52–8.37)</td>
<td>0.000</td>
</tr>
</tbody>
</table>

*Listed is the No. of factors associated with CHD, including the risk allele $G$ of rs2383207 and family history of CHD.†ORs were obtained from a logistic regression model with adjustment for age, sex, smoking, BMI, hypertension, diabetes.
Acknowledgments
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Disclosures
None.

References
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