Original Article

Variants on Chromosome 9p21 Confer Risks of Noncardioembolic Cerebral Infarction and Carotid Plaque in the Chinese Han Population

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Aims: Considering that cerebral infarction (CI) may share a common etiological basis with coronary artery disease (CAD), we evaluated six CAD-related single-nucleotide polymorphisms (SNPs) on 9p21 for investigating the effect of 9p21 on CI or carotid plaque in the Chinese Han population.

Methods: Altogether, 528 patients with noncardioembolic CI (375 with carotid plaque and 153 without carotid plaque) and 258 control subjects were genotyped. Six SNPs previously shown to be associated with CAD were sequenced and assessed for association with CI and carotid plaque using odds ratio (OR) and 95% confidence interval (CI) from logistic regression models.

Results: The G allele frequencies of rs2383206 (OR = 1.472, p = 0.021) and rs4977574 (OR = 1.519, p = 0.013) significantly increased in patients with CI without carotid plaque compared with middle-aged patients in the control group. The CI risk was higher among the GG genotype carriers than among GA and AA genotype carriers (OR = 1.794, 95% CI = 1.059 – 3.039, p = 0.030 for rs2383206; OR = 1.866, 95% CI = 1.088 – 3.201, p = 0.023 for rs4977574). In comparison with the non-GG genotype, the GG genotype of rs2383206 and rs4977574 combined had a 1.733-fold greater risk of CI in the middle-aged group. SNPs rs2383206 and rs4977574 were also associated with a risk of carotid plaque among patients with CI aged > 65 years (OR = 2.329, p = 0.018 and OR = 1.997, p = 0.049, respectively). Moreover, six SNPs were strongly correlated with linkage disequilibrium.

Conclusions: Genetic variations of rs2383206 and rs4977574 on 9p21 are potentially associated with CI and carotid plaque in the Chinese Han population. Our results provide further evidence that the 9p21 region represents a major risk locus for cerebrovascular diseases.


Key words: Cerebral infarction, Carotid plaque, Single-nucleotide Polymorphism, 9p21

Introduction

With increasing age, cerebrovascular diseases are increasing more rapidly and occurring earlier. The number of inpatients with stroke is higher than many other conventional diseases in both urban and rural areas. At present, stroke has been ranked the first life-threatening disease affecting female, ahead of cancer and heart disease. Among the 7 million stroke population in China, 75% have sequelae of physical mobility, along with a growing economic burden upon both family and the entire society1). Approximately half of the strokes can be explained...
by traditional risk factors, including ischemic heart disease, hypertension (HTN), diabetes mellitus (DM), dyslipidemia, and smoke. Cerebral infarction (CI), or ischemic stroke, caused by vascular occlusion, is characterized by the loss of blood flow to an area of the brain. It is a complex entity associated with heredity, environment, and vascular risk factors, mainly including large-vessel disease (LVD), cardiac embolism (CE), and small-vessel disease (SVD); however, the pathogenesis remains unclear. Previous twin and family-based studies suggested heredity as a risk factor for CI. Genome-wide analysis conducted by Bevan et al. suggested that 37.9% of all stroke risk could be attributed to hereditary factors, while heritability was 40.3% for the LVD subtype. Therefore, it is vital to control those modifiable risk factors for preventing CI.

Atherosclerosis (AS) plays a major role in the pathogenesis of coronary artery disease (CAD) and non-cardioembolic CI. Data accumulated from genome-wide association studies (GWAS) have identified links between variants at chromosome 9p21 and a risk of cardiovascular disease; homozygous variants increased risk by 40% in Caucasians. Although subsequent studies indicated that CI, particularly LVD, was influenced by variants at 9p21.3, its role among the Chinese Han population remains unclear. Moreover, carotid atherosclerosis, contributing to at least 20% of the total CI risk, can lead to plaque progression, which is gradually regarded as a strong predictor of CAD. However, vascular risk factors were responsible for only 19%–22% of carotid plaque conditions, while hereditary factors explained another 23%–60%. Therefore, we hypothesized that CI and carotid plaque share similar genetic mechanisms. A rapid, sensitive, and effective identification method called polymerase chain reaction–ligase detection reaction (PCR–LDR) has been designed for multiplex genotyping in our study.

We reviewed almost all the research papers about the association of chromosome 9p21 with atherosclerotic disease in recent years. McPherson, Helgadottir, and Samani et al. first conducted GWAS studies of CAD in Caucasian populations, and they found that the 9p21 locus may be a candidate gene predisposing to CAD. Luke, Cunnington, and Johnson et al. further confirmed that the polymorphisms at the chromosomal region 9p21 can increase the risk of atherosclerotic disease in whites and blacks from different regions, which had been recognized as a powerful predictor of CAD. These findings demonstrate a major genetic risk variant at this locus. Meanwhile, CI may share common etiological basis with CAD. To investigate whether CAD-associated SNPs also affect CI or carotid plaque susceptibility in the Chinese Han population, we selected six SNPs on 9p21 (rs10757278, rs1333049, rs2383206, rs1537378, rs4977574, and rs2383207) that had been validated well in AS from large cohorts.

Methods and Materials

Subjects and Definitions

A total of 528 patients with acute CI from the Chinese Han population (153 without carotid plaque, 96 men and 57 women, mean age 57 ± 8 years, age range 41–69 years; 375 with carotid plaque, 256 men and 119 women, mean age 61 ± 6 years, age range 39–70 years) were enrolled from four hospitals (Rui Jin Hospital, Yue Yang hospital, Min Hang Central Hospital, and Gongli Hospital) in Shanghai based on China’s 2010 Guidelines for acute ischemic stroke. All subjects, recruited from October 2009 to October 2013, agreed to participate. Inclusion criteria: Acute CI was defined as an acute onset at less than 72 h with a neurological deficit, symptoms lasting for more than 24 h but within 3 days, and corresponding brain MRI lesions. According to the Trial of Org 10172 in the Acute Stroke Treatment classification system, only LVD and SVD were selected. Exclusion criteria: (1) cardioembolic stroke; (2) brain MRI revealing cerebral hemorrhage, intracranial space-occupying lesion, infection, and other types of intracranial lesions; (3) coronary heart disease, peripheral vascular disease, malignant tumor, severe liver and kidney dysfunctions, abnormal thyroid function, and other metabolic diseases; and (4) a family history of CI. Carotid plaque, which was detected by carotid Doppler Ultrasound, was defined as a carotid intima–media thickness (IMT) ≥1.5 mm or evidence of neoplasm. Among 797 patients screened in the study, 528 patients were enrolled and 269 were excluded. Controls (127 men and 131 women; mean age, 56 ± 8 years; age range, 40–78 years) were randomly selected from healthy adults who underwent periodic medical check-ups, including brain MRI and carotid ultrasonography, at the four hospitals. Inclusion criteria: healthy volunteers with routine physical examination aged 40–78, with neither abnormal signal in brain MRI nor carotid plaque by ultrasound. Exclusion criteria: (1) a history of cerebrovascular disease; (2) abnormal brain MRI; (3) coronary heart disease, peripheral vascular disease, malignant tumor, severe liver and kidney dysfunctions, abnormal thyroid function, and other metabolic diseases; (4) a family history of CI and formation of carotid plaque by carotid ultrasonography. Among 602 volunteers screened in the study, 258 volunteers...
were enrolled and 344 were excluded. Only patients with CI younger than 70 years were recruited because previous studies revealed that genetic influence on CI is greater in younger patients. Our aim was to investigate whether we could replicate this finding in a Chinese sample of relatively young individuals. To evaluate genetic role conferred by age, we divided subjects into 3 cohorts, the younger (<45 years), middle-aged (45–65 years), and older (>65 years) based on the World Health Organization criteria. This study was approved by the ethics committee of Ruijin Hospital affiliated with Shanghai Jiaotong University School of Medicine. Written informed consent were obtained from subjects.

Genotyping
Fasting blood samples were collected for blood chemistry and genetic tests, while DNA was purified using phenol–chloroform extraction. Demographic information was also recorded. All genotyping was performed using the PCR–LDR method after designing primers and probes (details are available in Table 1) by Primer Express (https://products.appliedbiosystems.com) and Oligo (http://www.oligo.net).

PCR was performed on 20 μL of reaction mixture, containing 50 ng of genomic DNA, 2 μL of each primer, 0.6 μL Mg²⁺, 1 unit of Taq DNA polymerase, 2 μL 1× buffer, and 2 mM dNTP. Each reaction mixture was heated at 95°C for 2 min, followed by 35 cycles of 94°C for 30 s, 50°C for 30 s, and 65°C for 30 s, with a final extension at 72°C for 10 min. Further, LDR contained 4 μL PCR products, 1 μL 1× buffer, 2 pmol/μL of each probe, and 2 units of NEB Taq DNA ligase in a final volume of 10 μL. Each reaction mixture was heated at 95°C for 10 min, followed by 40 cycles of 94°C for 15 s, 50°C for 25 s. The PCR and LDR products were treated with gel electrophoresis and sent for direct sequencing to the Biowing Applied Biotechnology Company, Shanghai, China.

Statistical Analysis
Data were analyzed using SPSS (PASW Statistics version 18, SPSS Inc., Chicago, IL, USA). Continuous variables were evaluated by t test or Mann–Whitney U test according to distribution (normal or skewed). Categorical variables were compared using χ² test to analyze the distribution of genotype and allele. Moreover, Bonferroni correction was applied to analyze the distribution characteristics of various clinical data. SNP associations were evaluated using logistic regression models, which were further performed after adjustment for age, sex, and other risk factors (hypertension, diabetes, coronary heart disease, and blood glucose and lipid levels) through odds ratios (OR) with 95% confidence intervals (CI). Genetic models and gene–gene interaction were also assessed. The significance level was defined as α = 0.05 (two-tailed), and Lewomin coefficient (D') of linkage disequilibrium (LD) analysis was conducted using the SHEsis software, while strong LD was admitted when D' > 0.729.

Results

Background Characteristics

Tables 2 and 3 summarize the demographic characteristics of the recruited subjects. The HTN prevalence and blood pressure levels (both systolic and diastolic) of patients with CI without carotid plaque were significantly higher than those of controls (p = 0.003, 0.004, and 0.001, respectively) (Table 2). Patients with CI with carotid plaque had a higher ratio of HTN or DM than those without carotid plaque (p = 0.019 and 0.158, respectively). Meanwhile, age, systolic pressure, and high-density lipoprotein cholesterol (HDL-C) significantly associated with carotid plaque (p < 0.001, p = 0.015, p = 0.008, respectively, Table 3). After re-building stepwise multiple regression analysis, we found that CI tended to occur in males and in subjects with higher systolic blood pressure (p = 0.005 and p < 0.001, respectively), while age and lower HDL-C significantly associated with carotid plaque (p < 0.001 and p = 0.003, respectively; data not described in Table 3).
were more in patients with CI without carotid plaque than in healthy controls, as revealed using \( \chi^2 \) test for patients aged 45-65 years. These associations remained significant when adjusting for age, sex, HTN, and other risk factors by the binary logistic regression model (OR = 1.472, 95% CI = 1.060-2.044, \( p = 0.021 \) for rs238320; OR = 1.519, 95% CI = 1.094-2.110, \( p = 0.013 \) for rs4977574). The GG genotype showed a modest association with CI risk in the middle-aged (31.1% vs 20.5%, \( p = 0.059 \) for rs2383206; 29.4% vs 19.0%, \( p = 0.038 \) for rs4977574, data not described in Table 4).

### Table 2. Phenotypic characteristics of patients with carotid infarction without carotid plaque and control subjects

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>CI without CAP</th>
<th>Control</th>
<th>( p )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, mean (SD), year*</td>
<td>57 (8)</td>
<td>56 (8)</td>
<td>0.779</td>
</tr>
<tr>
<td>Men, No, (%)</td>
<td>96 (63)</td>
<td>127 (49)</td>
<td>0.018*</td>
</tr>
<tr>
<td>Hyperlipidemia No, (%)</td>
<td>52 (34)</td>
<td>79 (31)</td>
<td>0.479</td>
</tr>
<tr>
<td>TC, mean (SD), mM*</td>
<td>4.60 (1.12)</td>
<td>4.68 (0.96)</td>
<td>0.760</td>
</tr>
<tr>
<td>HDL-C, mean (SD), mM</td>
<td>1.22 (0.40)</td>
<td>1.35 (1.42)</td>
<td>0.271</td>
</tr>
<tr>
<td>LDL-C, mean (SD), mM</td>
<td>2.86 (0.90)</td>
<td>2.80 (0.80)</td>
<td>0.463</td>
</tr>
<tr>
<td>TG, mean (SD), mM</td>
<td>1.90 (1.21)</td>
<td>1.91 (1.46)</td>
<td>0.992</td>
</tr>
<tr>
<td>DM, No, (%)</td>
<td>23 (15)</td>
<td>29 (11)</td>
<td>0.264</td>
</tr>
<tr>
<td>Glucose, mean (SD), mM</td>
<td>5.78 (2.19)</td>
<td>5.51 (1.46)</td>
<td>0.134</td>
</tr>
<tr>
<td>HTN, No, (%)</td>
<td>81 (53)</td>
<td>84 (36)</td>
<td>0.003*</td>
</tr>
<tr>
<td>SBP, mean (SD), mmHg</td>
<td>138 (21)</td>
<td>132 (16)</td>
<td>0.004*</td>
</tr>
<tr>
<td>DBP, mean (SD), mmHg</td>
<td>83 (12)</td>
<td>79 (10)</td>
<td>0.001*</td>
</tr>
</tbody>
</table>

* \( p < 0.05 \); *Mann–Whitney \( U \) test; CI: cerebral infarction; CAP: carotid plaque; TG: triglyceride; TC: total cholesterol; HDL-C: low-density lipoprotein cholesterol; HTN: hypertension; SBP: systolic pressure; DBP: diastolic pressure; DM: diabetes mellitus

### Table 3. Phenotypic characteristics of patients with carotid infarction with and with carotid plaque

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>CI without CAP</th>
<th>CI with CAP</th>
<th>( p )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, mean (SD), year*</td>
<td>57 (8)</td>
<td>61 (6)</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Men, No, (%)</td>
<td>96 (63)</td>
<td>256 (68)</td>
<td>0.695</td>
</tr>
<tr>
<td>Hyperlipidemia No, (%)</td>
<td>52 (34)</td>
<td>148 (40)</td>
<td>0.277</td>
</tr>
<tr>
<td>TC, mean (SD), mM*</td>
<td>4.60 (1.12)</td>
<td>4.50 (1.33)</td>
<td>0.644</td>
</tr>
<tr>
<td>HDL-C, mean (SD), mM</td>
<td>1.22 (0.40)</td>
<td>1.12 (0.35)</td>
<td>0.008*</td>
</tr>
<tr>
<td>LDL-C, mean (SD), mM</td>
<td>2.86 (0.90)</td>
<td>3.02 (0.93)</td>
<td>0.192</td>
</tr>
<tr>
<td>TG, mean (SD), mM</td>
<td>1.90 (1.21)</td>
<td>2.04 (1.34)</td>
<td>0.869</td>
</tr>
<tr>
<td>DM, No, (%)</td>
<td>23 (15)</td>
<td>86 (23)</td>
<td>0.158</td>
</tr>
<tr>
<td>Glucose, mean (SD), mM</td>
<td>5.78 (2.19)</td>
<td>6.24 (2.82)</td>
<td>0.070</td>
</tr>
<tr>
<td>HTN, No, (%)</td>
<td>81 (53)</td>
<td>246 (66)</td>
<td>0.019*</td>
</tr>
<tr>
<td>SBP, mean (SD), mmHg</td>
<td>138 (21)</td>
<td>142 (19)</td>
<td>0.015*</td>
</tr>
<tr>
<td>DBP, mean (SD), mmHg</td>
<td>83 (12)</td>
<td>83 (11)</td>
<td>0.972</td>
</tr>
</tbody>
</table>

* \( p < 0.05 \); *Mann–Whitney \( U \) test; CI: cerebral infarction; CAP: carotid plaque; TG: triglyceride; TC: total cholesterol; HDL-C: low-density lipoprotein cholesterol; HTN: hypertension; SBP: systolic pressure; DBP: diastolic pressure; DM: diabetes mellitus

### Genotyping Results for Six SNPs on 9p21 between Patients with CI without Carotid Plaque and Controls

Distributions of all SNPs met Hardy–Weinberg equilibrium criteria for all subjects. Allele analysis of the six SNPs rs10757278, rs1333049, rs2383206, rs1537378, rs4977574, and rs2383207 on 9p21 are shown in Table 4. No significant associations were found between controls and patients with CI without carotid plaque aged <45 or >65 years. However, carriers of the G allele of rs2383206 (55.0% vs 45.9%, \( p = 0.024 \)) and rs4977574 (54.2% vs 44.4%, \( p = 0.015 \)) were more in patients with CI without carotid plaque than in healthy controls, as revealed using \( \chi^2 \) test for patients aged 45-65 years. These associations remained significant when adjusting for age, sex, HTN, and other risk factors by the binary logistic regression model (OR = 1.472, 95% CI = 1.060-2.044, \( p = 0.021 \) for rs238320; OR = 1.519, 95% CI = 1.094-2.110, \( p = 0.013 \) for rs4977574). The GG genotype showed a modest association with CI risk in the middle-aged (31.1% vs 20.5%, \( p = 0.059 \) for rs2383206; 29.4% vs 19.0%, \( p = 0.038 \) for rs4977574, data not described in Table 4).
Combined Analyses of 2 SNPs for Middle-Aged Individuals and LD Analyses of Six SNPs

Among subjects aged 45–65 years, we performed three genetic models to discuss the genetic role on CI between patients with CI without carotid plaque and controls (Table 5). Significant differences were detected on CI for rs4977574 and rs10757278 ($p=0.011$ and 0.044, respectively) in a codominant model (AA vs GG). Furthermore, CI risk was higher for the GG genotype than for the A-allele carriers on rs2383206 and rs4977574, respectively (OR=1.794, 95% CI=1.059–3.039, $p=0.030$ and OR=1.866, 95% CI=1.088–3.201, $p=0.023$, respectively).

**SNPs Association with Acute CI Under Genetic Models in Middle-Aged Chinese Han Population**

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**Combined Analyses of 2 SNPs for Middle-Aged Individuals and LD Analyses of Six SNPs**

An essential pathway for exploring the genetic combination or linkage effect on multifactorial diseases has been recently proposed. Joint effects of the SNPs rs2383206 and rs4977574 were studied for middle-aged subjects. The GG genotype carriers at each SNP combined (26.1% for patients with CI without carotid plaque and 17.8% for controls; Table 6) had a 1.733-fold increased risk of CI in the middle-aged...
95% CI 1.002–3.981 for rs4977574). The SNP rs10757278 displayed a higher risk of carotid plaque in older adult patients, as revealed using $\chi^2$ test ($p=0.048$); however, this difference was no longer significant after adjustment for the abovementioned risk factors ($p=0.057$).

**Discussion**

In this study, six CAD/MI-related SNPs (rs10757278, rs1333049, rs2383206, rs1537378, rs4977574, and rs2383207) located within a locus spanning a 64-kb region on chromosome 9p21.3 were studied. We revealed that the G allele of the SNPs rs2383206 and rs4977574 were associated with susceptibility to CI, and the GG genotype on both sites combined increased the risk of CI in middle-aged Chinese HAN population. However, associations were not found in the

### Table 5. SNP associations in patients with carotid infarction without carotid plaque and control under genetic modes in middle-aged adults

| Locus       | genotype | Adjust OR (95%) | $p$adj  
|-------------|----------|-----------------|-------
| rs10757278  | Codominant | 1.996 (1.018–3.922) | 0.044* 
|            | Dominant  | 1.620 (0.948–2.766) | 0.077 
|            | Recessive | 0.652 (0.375-1.136) | 0.131 
| rs2383206   | Codominant | 1.626 (0.929-2.846) | 0.089 
|            | Dominant  | 1.794 (1.059-3.039) | 0.030* 
|            | Recessive | 0.631 (0.365-1.090) | 0.099 
| rs4977574   | Codominant | 2.37 (1.218-4.608) | 0.011* 
|            | Dominant  | 1.634 (0.92-2.907) | 0.094 
|            | Recessive | 1.866 (1.088-3.201) | 0.023* 

$\chi^2$ test by logistic regression

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### Table 6. Association analyses of rs2383206 and rs4977574 after adjustment for clinical covariates at 45–65 years

| rs2383206 | rs4977574 | CI without CAP N, rate (%) | Control, N, rate (%) | OR (95% CI) | $p$ 
|-----------|-----------|-----------------------------|----------------------|-------------|-------
| GG        | GG        | 40 (26.1)                   | 46 (17.8)            | 1.271 (0.072-22.220) | 0.870 
| GA+AA     | GG        | 1 (0.7)                     | 1 (0.4)              | 1.733 (1.056-2.849) | 0.030 
| GG        | GA+AA     | 2 (1.3)                     | 5 (1.9)              | 3.174 (0.566-17.860) | 0.189 
| GA+AA     | GA+AA     | 110 (71.9)                  | 206 (79.8)           | 1.002–3.981 for rs4977574) | 0.057 

CI: cerebral infarction; CAP: carotid plaque; OR (95% CI): test by logistic regression

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compared with the A-allele carriers ($p=0.030$). The six SNPs were in a strong linkage disequilibrium ($D=0.85–0.99$).

**Genotype Frequencies in Patients with CI with or without Carotid Plaque**

We examined the association between chromosome 9p21 and incidence of carotid plaque to explore the genetic influences on the pathogenesis of AS. In all, 528 patients with CI were divided into two cohorts (with or without carotid plaque) based on Doppler Ultrasound (Table 7). G alleles of rs2383206 and rs4977574 were associated with an increased possibility of carotid plaque in patients with CI aged >65 years ($p=0.012$ and 0.043, respectively). The significance level also remained after adjustment for age, sex, HTN, and other traditional risk factors (OR=2.329, 95% CI=1.159–4.681 for rs2383206 and OR=1.997, 95% CI=1.002–3.981 for rs4977574). The SNP rs10757278 displayed a higher risk of carotid plaque in older adult patients, as revealed using $\chi^2$ test ($p=0.048$); however, this difference was no longer significant after adjustment for the abovementioned risk factors ($p=0.057$).

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**Discussion**

In this study, six CAD/MI-related SNPs (rs10757278, rs1333049, rs2383206, rs1537378, rs4977574, and rs2383207) located within a locus spanning a 64-kb region on chromosome 9p21.3 were studied. We revealed that the G allele of the SNPs rs2383206 and rs4977574 were associated with susceptibility to CI, and the GG genotype on both sites combined increased the risk of CI in middle-aged Chinese HAN population. However, associations were not found in the
The potential association between CI and chromosome 9p21 was investigated from genetic efficiency and impact route by logistic regression models with various genetic models and combination analyses. Two SNPs (rs2383206 and rs4977574) on 9p21 conferred a risk of CI in subjects aged 45–65 years (78% of our participants) by several genetic models. The mutant allele on both loci probably exhibited a synergistic effect on the pathogenesis of CI, which may also contribute to AS. Regardless of the adjustment made for conventional risk factors, no significant differences were found among subjects aged $\leq 45$ or $\geq 65$ years in a relatively smaller sample. Therefore, early intervention in CI is important, particularly in the main force of society (the middle-aged group). A strong LD was discovered within the six SNPs. Moreover, patients with cardioembolic stroke were excluded; therefore, the unique mechanism leading to remaining four SNP loci. The same two SNPs confer a risk of carotid plaque among the older patients ($\geq 65$ years).

Variants in our study on chromosome 9p21.3 span a 100-kb region containing the genes that encode tumor suppressors named cyclin-dependent kinase inhibitors (CDKN2A/2B). They are cell cycle regulators of the CDK4 family, p16\textsuperscript{INK4a} (by CDKN2A gene), p15\textsuperscript{INK4b} (by CDKN2B gene), and p14\textsuperscript{ARF} (non-suppressor), affecting cell proliferation, aging, apoptosis, and the pathogenesis of AS \textsuperscript{31, 32}. Moreover, the expression of CDKN2A/B is excessively reduced because of deletion of the repeating homologous sequences on the mouse chromosome 4, along with excessive proliferation of aortic smooth muscle, which is closely associated with the pathogenesis of AS \textsuperscript{33}.

When considering the effect of carotid plaque on CI, approximately 800 patients with acute CI were recruited. The potential association between CI and chromosome 9p21 was investigated from genetic efficiency and impact route by logistic regression models with various genetic models and combination analyses. Two SNPs (rs2383206 and rs4977574) on 9p21 conferred a risk of CI in subjects aged 45–65 years (78% of our participants) by several genetic models. The mutant allele on both loci probably exhibited a synergistic effect on the pathogenesis of CI, which may also contribute to AS. Regardless of the adjustment made for conventional risk factors, no significant differences were found among subjects aged $< 45$ or $\geq 65$ years in a relatively smaller sample. Therefore, early intervention in CI is important, particularly in the main force of society (the middle-aged group). A strong LD was discovered within the six SNPs. Moreover, patients with cardioembolic stroke were excluded; therefore, the unique mechanism leading to

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Fig. 1. Linkage disequilibrium analysis of six sites on 9p21 using the SHEsis software. The top of figure reveals the site's position (kb); red indicates that Lewin coefficients ($D^\prime$) between six loci were $>0.9$.  

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that 9p21 participated in the progression of AS as it stimulates the carotid plaque formation and finally leads to vascular diseases as well as CI.

Limitations of our findings should be considered. First, our results are based on a relatively small sample size. We limited the enrollment of old people for a better age match between groups and replication of findings among relatively young individuals. Therefore, younger patients with CI were included. Considering the CI mortality, rigid inclusion criteria, and CI diagnosis standard, only 528 patients with acute CI (153 plaque-free) were finally included. Second, we are aware of the age limitation of subjects aged 40–70 years. Further, 9p21 was markedly associated with patients with CI aged 45–65 years; therefore, inconsistent results were found in younger patients because of the insufficient samples. As the mean age of patients with CI with carotid plaque had exceeded 60 years, statistical difference was finally found among the older cohort.

In conclusion, genetic variations of rs2383206 and rs4977574 on 9p21 are potentially associated with CI and carotid plaque in the Chinese Han population. The G allele may confer risks of arteriosclerotic diseases. Our findings need to be strengthened by expanding samples from multicenter studies based on varied races. Hence, defining the CI subtypes and characteristics of carotid plaque are intended to be performed based on the common criteria in subsequent studies according to age stratification. The inter-

Subjects with the GG/GA genotypes of rs2383206 had a 2.09-fold increased risk of LVD compared with the AA carriers in Northern Chinese, according to the research by Hu et al. ($p = 0.002$)\(^{35}\). Similarly, Swedish scientists performed a 25 CAD-susceptible SNP study, including 3986 patients with CI and 2459 control volunteers; they found that the SNP rs4977574 significantly associated with the overall CI, with an OR of 1.12 (95% CI: 1.04–1.20, $p = 0.002$), even at a higher risk of LVD subtype (OR = 1.36, 95% CI: 1.13–1.64, $p = 0.001$)\(^{22}\). These findings are in accordance with those of our study, indicating the promising role in CI of both loci. Furthermore, rs4977574 and rs2383206 polymorphisms were associated with a thicker IMT or carotid atherogenesis among the middle-aged Caucasians\(^{36, 37}\). Because asymptomatic or subclinical plaque development is becoming a more accurate predictor of AS than IMT\(^{38}\), variants on chromosome 9p21 probably confer risks of acute CI in the Chinese Han population. Here, we assumed that 9p21 participated in the progression of AS as it stimulates the carotid plaque formation and finally leads to vascular diseases as well as CI.

Limitations of our findings should be considered. First, our results are based on a relatively small sample size. We limited the enrollment of old people for a better age match between groups and replication of findings among relatively young individuals. Therefore, younger patients with CI were included. Considering the CI mortality, rigid inclusion criteria, and CI diagnosis standard, only 528 patients with acute CI (153 plaque-free) were finally included. Second, we are aware of the age limitation of subjects aged 40–70 years. Further, 9p21 was markedly associated with patients with CI aged 45–65 years; therefore, inconsistent results were found in younger patients because of the insufficient samples. As the mean age of patients with CI with carotid plaque had exceeded 60 years, statistical difference was finally found among the older cohort.

In conclusion, genetic variations of rs2383206 and rs4977574 on 9p21 are potentially associated with CI and carotid plaque in the Chinese Han population. The G allele may confer risks of arteriosclerotic diseases. Our findings need to be strengthened by expanding samples from multicenter studies based on varied races. Hence, defining the CI subtypes and characteristics of carotid plaque are intended to be performed based on the common criteria in subsequent studies according to age stratification. The inter-

<table>
<thead>
<tr>
<th>SNP</th>
<th>Case</th>
<th>Control</th>
<th>Crude OR (95% CI)</th>
<th>$p$ value</th>
<th>Adjust OR (95% CI)</th>
<th>$p^*$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs10757278 G</td>
<td>99 (57.6)</td>
<td>18 (40.9)</td>
<td>1.959 (0.999-3.839)</td>
<td>0.048</td>
<td>1.932 (0.980-3.809)</td>
<td>0.057</td>
</tr>
<tr>
<td>A</td>
<td>73 (42.4)</td>
<td>26 (59.1)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs1333049 C</td>
<td>97 (56.4)</td>
<td>18 (40.9)</td>
<td>1.868 (0.954-3.659)</td>
<td>0.066</td>
<td>1.852 (0.940-3.650)</td>
<td>0.075</td>
</tr>
<tr>
<td>G</td>
<td>75 (43.6)</td>
<td>26 (59.1)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs2383206 G</td>
<td>95 (55.2)</td>
<td>15 (34.1)</td>
<td>2.385 (1.194-4.765)</td>
<td>0.012</td>
<td>2.329 (1.159-4.681)</td>
<td>0.018</td>
</tr>
<tr>
<td>A</td>
<td>77 (44.8)</td>
<td>29 (65.9)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs1537378 T</td>
<td>25 (14.9)</td>
<td>5 (11.4)</td>
<td>1.326 (0.477-3.689)</td>
<td>0.587</td>
<td>1.332 (0.473-3.754)</td>
<td>0.587</td>
</tr>
<tr>
<td>C</td>
<td>147 (85.5)</td>
<td>39 (88.6)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs4977574 G</td>
<td>92 (53.5)</td>
<td>16 (36.4)</td>
<td>2.012 (1.016-3.986)</td>
<td>0.043</td>
<td>1.997 (1.002-3.981)</td>
<td>0.049</td>
</tr>
<tr>
<td>A</td>
<td>80 (46.5)</td>
<td>28 (63.6)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs2383207 G</td>
<td>124 (72.1)</td>
<td>26 (59.1)</td>
<td>1.788 (0.899-3.556)</td>
<td>0.094</td>
<td>1.766 (0.880-3.544)</td>
<td>0.11</td>
</tr>
<tr>
<td>A</td>
<td>48 (27.9)</td>
<td>18 (40.9)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Case: CI with carotid plaque; Control: CI without carotid plaque; $p$ value: $\chi^2$ test; $p^*$ value: adjusted for age, sex, and other traditional risk factors

CI through 9p21 polymorphisms could be explored, which also explained the inconsistent conclusions compared to other research. To discuss the associations of 9p21 with carotid plaque, we mainly focused on all patients with CI. Susceptibility was found among the older, while age played an important role\(^{34}\). As mentioned above, chromosome 9p21 is conductive to the occurrence and progression of AS, leading to CI or carotid plaque.
relationships between gene and environment remain uninvestigated using *in vitro* or *in vivo* experiments. As expected, our study may provide novel methods to prevent or treat acute CI and carotid plaque.

**Acknowledgments**

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**Conflict of Interest Statement**

The authors declare no financial or other conflict of interests.

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