Minor Allele C of Chromosome 1p32 Single Nucleotide Polymorphism rs11206510 Confers Risk of Ischemic Stroke in the Chinese Han Population

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Background and Purpose—Genome-wide association studies found that the common allele T of single nucleotide polymorphism rs11206510 on chromosome 1p32 was associated with increased low-density lipoprotein-cholesterol levels (LDL-C) and with risk of coronary artery disease (CAD) in white populations. The goals of this study are to determine whether rs11206510 is associated with LDL-C and CAD in a different ethnic population, namely a Chinese cohort, and to investigate whether rs11206510 is associated with ischemic stroke.

Methods—The association of rs11206510 with LDL-C was analyzed in 1415 Chinese Han subjects. The CAD study utilized a GeneID cohort with 1543 CAD patients and 1240 controls. For stroke studies, 2 independent cohorts were used and included the GeneID North cohort, with 1205 cases and 1205 controls, and the GeneID Central cohort, with 692 cases and 882 controls.

Results—Different from white populations, the minor allele C of rs11206510 was associated with increased LDL-C levels in the Chinese Han population (adjusted P=0.002) and conferred risk of early-onset CAD (380 cases vs 1240 controls; adjusted P=0.002, odds ratio, 1.89), but not with overall CAD (adjusted P=0.82). The allelic association with ischemic stroke was highly significant in 2 independent cohorts, with adjusted P=1.13×10^-5 (odds ratio,1.71) in the GeneID North cohort and adjusted P=9.32×10^-5 (odds ratio, 1.70) in the GeneID Central cohort. Genotypic association was also significant for both early-onset CAD and ischemic stroke.

Conclusions—Our results indicate that single nucleotide polymorphism rs11206510 is associated with LDL-C levels and early-onset CAD in the Chinese Han population. For the first time to our knowledge, this study also demonstrates that rs11206510 confers a significant risk of ischemic stroke. (Stroke. 2010;41:1587-1592.)

Key Words: coronary artery disease  genome-wide association study  low-density lipoprotein cholesterol  single nucleotide polymorphism  stroke

Stroke is one of the most common causes of mortality and a leading cause of adult disability worldwide. In China, 1.5 to 2 million new strokes occur each year. Ischemic stroke, a main type of stroke, is a complex diseases that is caused by genetic factors, environmental factors, and their interactions. During the past few years, epidemiological studies have identified multiple genetic factors for ischemic stroke. Recent genome-wide linkage analysis and genome-wide association studies identified several candidate genes associated with stroke, including PDE4D, ALOX5AP, AGTRL1, PRCKH, and NINJ2. However, the identified genetic factors explain only a small fraction of the inherited risk of ischemic stroke, and the remaining heritability or genetic variants need to be investigated.

In 2008, a genome-wide association study identified several single nucleotide polymorphisms (SNP) associated with low-density lipoprotein cholesterol (LDL-C) levels, and several of these SNPs, including rs11206510, on chromosome 1p32 were associated with increased LDL-C and risk of CAD in white populations. However, it is not known whether these SNPs are associated with ischemic stroke in different ethnic populations, such as the Chinese Han population.
1p32, were also associated with coronary artery disease (CAD).10 In a 2009 genome-wide association study for early-onset myocardial infarction (MI), the association of SNP rs11206510 was further replicated.11 Therefore, we selected rs11206510 for follow-up studies for association with LDL-C levels and CAD in the Chinese populations. Considering that ischemic stroke may share the similar pathogenic atherosclerotic mechanism with CAD,12 and that serum LDL-C concentrations are a risk factor for both ischemic stroke and CAD,13 we hypothesized that rs11206510 also conferred risk of stroke. This hypothesis was tested in this study.

Subjects and Methods

Study Populations

The study subjects were from the GeneID population, which is a large ongoing Chinese database with clinical data and tissue samples from >15,000 Chinese patients and controls and is used for identification of susceptibility genes for various cardiovascular diseases in the Chinese Han population.14 All subjects were of the ethnic Han origin by self-description. The studies were approved by appropriate local institutional review boards on human subject research and conformed to the guidelines set forth by the Declaration of Helsinki. Written informed consent was obtained from the participants.

The study for the association with serum LDL-C levels was performed in a cohort with 1415 subjects who had available data on a lipid profile, no history of dyslipidemia, and were not using lipid-lowering drugs 2 weeks before the measurement of lipid concentrations or not using treatment for chronic renal failure or hepatitis.11 Total cholesterol, high-density lipoprotein cholesterol, and triglyceride concentrations were analyzed enzymatically on an Olympus AU2700 Clinical Analyzer after overnight fasting, and LDL-C concentrations were calculated using the standard Friedewald formula.

The case-control association study for CAD involved 1543 CAD cases and 1240 controls. All subjects were evaluated by coronary angiography. Subjects with congenital heart disease, childhood hepatitis,11 total cholesterol, high-density lipoprotein cholesterol, and triglyceride concentrations were analyzed enzymatically on an Olympus AU2700 Clinical Analyzer after overnight fasting, and LDL-C concentrations were calculated using the standard Friedewald formula. Hypertension was defined as systolic blood pressure of 140 mm Hg or diastolic blood pressure of 90 mm Hg. Diabetes was defined as ongoing therapy of diabetes or a fasting plasma glucose level of >7.0 mmol/L.

Genotyping

Human genomic DNA was extracted from venous blood samples using the Wizard Genomic DNA Purification Kit (Promega Corporation).

Genotyping for SNP rs11206510 was performed using an LC green Plus (Idaho Technologies) fluorescent dye-based high-resolution melt analysis on a Rotor-gene 6200 System (Corbett Life Science) according to the protocols from the manufacturers.19 Briefly, a fragment flanking rs11206510 was amplified with the forward primer 5'-GGGCCCAGCATCCATCTTCCCTGT-3' and the reverse primer 5'-ATGCAGGAAGGAGCCAAAGAC-3', and a final concentration of 5 μmol/L LC green Plus fluorescent dye. The polymerase chain reaction products were genotyped by high-resolution melt analysis. DNA samples with previously known genotypes were used as positive controls, and appropriate negative controls were also included in genotyping to ensure the quality. The quality of genotyping was ensured by direct DNA sequence analysis of 50 randomly selected samples (100% consistent rate between the 2 methods).

Statistical Analysis

Statistical analysis was performed as previously reported.20–22 For case-control association studies, χ² tests with Pearson 2×2 and 2×3 contingency tables as implemented in PLINK version 1.06 (http://pngu.mgh.harvard.edu) were used to compute the P values and corresponding odds ratios (OR) with 95% confidential intervals for allelic association and genotypic association assuming different genetic models (dominant, recessive, or additive). Hardy-Weinberg linkage disequilibrium test was performed using PLINK Multivariate logistic regression analysis was performed using SPSS version 17.0 by adjusting for some risk factors (age, gender, smoking, hypertension, diabetes mellitus, and lipid concentrations). Analysis of variance was used to assess the association between a quantitative trait and SNP genotypes by SPSS version 17.0. Empirical P values were calculated using 100,000 Monte Carlo simulations using Haploview version 3.0 (http://www.broad.mit.edu/mpg/haploview/).

Results

Association of SNP rs11206510 With Serum LDL-C Levels

The association between SNP rs11206510 and LDL-C levels was analyzed in a total of 1415 study subjects with a mean age of 59.5±10.2 years (Table 1). The mean concentration of serum LDL-C was 2.42±0.51 mmol/L. No deviation from the Hardy-Weinberg equilibrium was observed for rs11206510 in this cohort (P>0.05). Significant association for rs11206510 was identified with LDL-C (observed P [P-obs]=0.0006). After adjusting for significant covariates of age, gender, and smoking, an adjusted P value (P-adj) of 0.002 was obtained for an effect of 0.117 mmol/L per the C risk allele. The mean serum LDL-C for subjects with genotype TT (n=1264) and TC+CC (n=151) were 2.55±0.70 mmol/L and 2.72±0.72 mmol/L, respectively, which was statistically different (P=0.007). SNP rs11206510 can explain 1.6% proportion of the residual variance of the LDL-C concentration in this cohort using 1-way analysis of variance. After adjusting for significant covariates of age, gender, and smoking, nested analysis of variance showed that rs11206510 could explain 4.0% of the residual variance.
Table 1. Clinical Characteristics of Study Populations

<table>
<thead>
<tr>
<th>GeneID</th>
<th>CAD Study</th>
<th>Ischemic Stroke Study</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total CAD Case (n=1543)</td>
<td>Early-Onset CAD Case (n=380)</td>
</tr>
<tr>
<td></td>
<td>Age, y*</td>
<td>59.5±10.2</td>
</tr>
<tr>
<td></td>
<td>Female, n (%)</td>
<td>549 (38.8)</td>
</tr>
<tr>
<td></td>
<td>Hypertension‡ n (%)</td>
<td>297 (20.9)</td>
</tr>
<tr>
<td></td>
<td>Diabetes n (%)</td>
<td>108 (7.6)</td>
</tr>
<tr>
<td></td>
<td>Total cholesterol, mmol/L</td>
<td>3.89±0.94</td>
</tr>
<tr>
<td></td>
<td>Triglyceride, mmol/L</td>
<td>1.61±0.66</td>
</tr>
<tr>
<td></td>
<td>High-density lipoprotein cholesterol, mmol/L</td>
<td>1.41±0.19</td>
</tr>
<tr>
<td></td>
<td>LDL-C, mmol/L</td>
<td>2.42±0.51</td>
</tr>
<tr>
<td></td>
<td>Smoker n (%)</td>
<td>481 (34.0)</td>
</tr>
</tbody>
</table>

Data are shown as mean±SD for quantitative variables and n (%) for qualitative variables.

*Age at the first diagnosis of the disease.
†Hypertension was defined as a systolic blood pressure of ≥140 mm Hg or a diastolic blood pressure of ≥90 mm Hg.
‡Diabetes was defined as ongoing therapy of diabetes or a fasting plasma glucose level of ≥7.0 mmol/L.
§Late-onset CAD was defined as males aged older than 50 years and females aged older than 50 years at the first diagnosis of the disease.

Allelic Association of SNP rs11206510 With Early-Onset CAD

For the case-control study for CAD, we genotyped rs11206510 in 1543 cases (mean age, 61.4±15.8 years) and 1240 controls (mean age, 63.4±7.8 years; Table 1). The genotypes did not deviate from the Hardy-Weinberg equilibrium in the controls (P=0.409). In contrast to the earlier reports,10,11 we did not detect any allelic association between rs11206510 and CAD (P-obs=0.41; empirical P=0.31) in the Chinese Han GeneID population (Table 2). Furthermore, no significant association was identified even after adjusting for potential confounders, including age, gender, smoking, hypertension, diabetes mellitus, and lipid concentrations (total cholesterol, triglycerides, high-density lipoprotein cholesterol, and LDL-c; P-adj=0.82; Table 2).

Further statistical analysis was performed for the patients with early-onset CAD who met the criteria of males age 50 years and younger and females age 55 years and younger at the time of the first diagnosis of the disease.10 A total of 380 CAD patients satisfied a diagnosis of early-onset CAD. The mean age of this group was 43.5±8.3 years (Table 1). The result showed that allelic frequencies of rs11206510 were significantly different between the 380 patients with early-onset CAD and 1240 non-CAD controls (OR, 1.49 for P-obs=0.018; OR, 1.89 for P-adj=0.0023; P-emp=0.019; Table 2). We also performed association analysis for late-onset CAD (older than 50 years in men and older than 55 years in women). No significant association was detected (P-obs=0.91; P-adj=0.50; P-emp=0.28; Table 2).

Table 2. Analysis of Allelic Association of SNP rs11206510 With Overall CAD and Early-Onset CAD

<table>
<thead>
<tr>
<th>Cohort (n, Case/Control)</th>
<th>Allele C Frequency (Case/Control)</th>
<th>Without Adjustment‡</th>
<th>With Adjustment†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>P-obs*</td>
<td>OR (95% CI)*</td>
</tr>
<tr>
<td>Overall CAD (1543/1240)</td>
<td>0.055/0.049</td>
<td>0.41</td>
<td>1.15 (0.88–1.48)</td>
</tr>
<tr>
<td>Early-onset CAD (380/1240)</td>
<td>0.071/0.049</td>
<td>0.02</td>
<td>1.49 (1.07–2.08)</td>
</tr>
<tr>
<td>Late-onset CAD§ (1163/1240)</td>
<td>0.050/0.049</td>
<td>0.91</td>
<td>1.01 (0.78–1.31)</td>
</tr>
</tbody>
</table>

*Uncorrected P value and OR using χ² tests with Pearson ²x².
†Adjusted P value by multivariate logistic regression analysis for potential confounders, including age, gender, smoking, hypertension, diabetes mellitus, and lipid concentrations (total cholesterol, triglycerides, high-density lipoprotein cholesterol, and LDL-C).
‡Early-onset CAD was defined as males age 50 years or younger and females age 55 years or younger at the first diagnosis of the disease.
§Late-onset CAD was defined as males aged older than 50 years and females aged older than 55 years at the first diagnosis of the disease.
Table 3. Significant Allelic Association of SNP rs11206510 With Ischemic Stroke in Two Independent Cohorts

<table>
<thead>
<tr>
<th>Cohort</th>
<th>Group (n, Case/Control)</th>
<th>Allele C Frequency (Case/Control)</th>
<th>Without Adjustment*</th>
<th>With Adjustment†</th>
</tr>
</thead>
<tbody>
<tr>
<td>GeneID North stroke</td>
<td>Early-onset CAD‡ (294/1205)</td>
<td>0.090/0.051</td>
<td>0.0003</td>
<td>1.84 (1.31–2.57)</td>
</tr>
<tr>
<td></td>
<td>Late-onset CAD§ (911/1205)</td>
<td>0.081/0.051</td>
<td>7.16×10⁻⁵</td>
<td>1.64 (1.28–2.10)</td>
</tr>
<tr>
<td></td>
<td>Overall (1205/1205)</td>
<td>0.083/0.051</td>
<td>7.23×10⁻⁶</td>
<td>1.69 (1.34–2.13)</td>
</tr>
<tr>
<td>GeneID Central stroke</td>
<td>Early-onset CAD‡ (117/882)</td>
<td>0.107/0.053</td>
<td>0.0009</td>
<td>2.15 (1.35–3.42)</td>
</tr>
<tr>
<td></td>
<td>Late-onset§ (765/882)</td>
<td>0.085/0.053</td>
<td>0.0005</td>
<td>1.67 (1.25–2.25)</td>
</tr>
<tr>
<td></td>
<td>Overall (692/882)</td>
<td>0.089/0.053</td>
<td>6.54×10⁻⁵</td>
<td>1.76 (1.33–2.33)</td>
</tr>
<tr>
<td>Combined</td>
<td>Combined stroke (1897/2087)</td>
<td>0.085/0.052</td>
<td>2.29×10⁻⁸</td>
<td>1.71 (1.43–2.05)</td>
</tr>
<tr>
<td></td>
<td>Combined early-onset stroke‡ (411/2087)</td>
<td>0.095/0.052</td>
<td>1.85×10⁻⁶</td>
<td>1.91 (1.46–2.50)</td>
</tr>
<tr>
<td></td>
<td>Combined late-onset stroke§ (1468/2087)</td>
<td>0.083/0.052</td>
<td>1.96×10⁻⁷</td>
<td>1.64 (1.36–1.98)</td>
</tr>
</tbody>
</table>

*Uncorrected P value and OR using χ² tests with Pearson 2×2.
†Adjusted P value by multivariate logistic regression analysis for potential confounders, including age, gender, smoking, hypertension, diabetes mellitus, and lipid concentrations (total cholesterol, triglycerides, high-density lipoprotein cholesterol, and LDL-C).
‡Early-onset CAD was defined as males age 50 years or younger and females age 55 years or younger at the first diagnosis of the disease.
§Late-onset CAD was defined as males age 50 years or younger and females age 55 years or younger at the first diagnosis of the disease.

For the combined population of GeneID North and GeneID Central cohorts, the P values for the association between SNP rs11206510 and ischemic stroke became much more significant (OR, 1.71–1.75; P-obs=2.29×10⁻⁸, P-adj=5.05×10⁻⁹, P-emp=1.00×10⁻⁶; Table 3). We also performed association analysis for early-onset stroke (age of diagnosis: males age 50 years or younger and females age 55 years or younger). For the GeneID North cohort, significant association was detected, with an OR of 1.84 (P-obs=0.0003; Table 3). For the GeneID Central cohort, the association was also significant, with an OR of 2.15 (P-obs=0.0009). The OR for early-onset stroke are greater than that for overall stroke (1.69–1.76 for overall stroke; Table 3).

Genotypic Associations of SNP rs11206510 With Early-Onset CAD and Ischemic Stroke

Studies on genotypic association allow assessment of risk of rs11206510 for CAD or ischemic stroke under different genetic models. When the whole CAD cohort was analyzed, similar to allelic association studies, no genotypic association was detected between rs11206510 and CAD under any of the 3 genetic models (Supplemental Table I, available online at http://stroke.ahajournals.org). However, significant genotypic association was identified for early-onset CAD under a dominant model or additive model (OR, 1.76; P-adj=0.015 and OR, 1.89, P-adj=0.002, respectively; Supplemental Table I).

Highly significant genotypic association was identified for ischemic stroke in GeneID North, GeneID Central, and combined populations under both dominant and additive models (Supplemental Table II, available online at http://stroke.ahajournals.org).

Discussion

In this study, we provide strong genetic evidence that the minor allele C of SNP rs11206510 on chromosome 1p32 confers a significant risk of ischemic stroke (OR, 1.69–1.75). First, in a large Chinese GeneID North cohort with 1205 patients with ischemic stroke and 1205 matched controls, highly significant allelic association was identified (Table 3). The association was also highly significant, assuming a model of dominant or additive model (Supplemental Table II). Second, the initial finding of significant association between rs11206510 and ischemic stroke was replicated in an independent Chinese GeneID Central cohort with 692 ischemic stroke patients and 882 controls. Both allelic association and genotypic association under the dominant and additive models were highly significant (Table 3 and Supplemental Table II). Third, when the GeneID North and GeneID central cohorts were combined, the P values for the association were improved markedly (Table 3 and Supplemental Table II). To the best of our knowledge, this is the first time that SNP rs11206510 is found to be a significant risk factor for ischemic stroke.

In our multivariate analysis, LDL-C was included as a covariate. Because rs11206510 was associated with LDL-C, we repeated multivariate analysis without LDL-C. For early-onset CAD, the OR was increased from 1.89 (P=0.002) to 1.92 (P=0.009). For stroke, the OR was increased from 1.71 (P=1.13×10⁻⁵) to 1.77 (P=5.07×10⁻⁵) in the GeneID North cohort, and from 1.70 (P=3.65×10⁻⁴) to 1.75 (P=9.32×10⁻⁵) in the GeneID Central cohort. Thus, inclusion of LDL-C in the multivariate analysis appears to slightly decrease OR for risk of early-onset CAD or stroke.

In our studies, rs11206510 showed association with early onset CAD, but not in the total CAD cohort (Table 2). This may be attributable to the possibility that genetic factors account for more heritability in early-onset CAD than in late-onset or overall CAD. It is interesting to note that OR for early-onset stroke were higher than OR for overall stroke (1.84–2.15 vs 1.69–1.76, respectively; Table 3), which may also be attributable to the possibility that genetic factors play...
a more important role in early-onset stroke than in late-onset stroke. Further studies are needed to reveal why the OR for stroke were higher than for early-onset CAD (OR, 1.49).

Based on the sample size of CAD cohort, power analysis showed that there was >80% power to detect an association with an OR of >1.39 at a level of 0.05. One limitation of the present study is that the sample size for the CAD cohort was underestimated if the power analysis assumed that the OR for SNP rs11206510 in the Chinese population would be identical to the previously reported OR of 1.13 to 1.15 in white populations. Furthermore, a Breslow-Day test showed that there was a trendy, but not significant, difference between the OR of 1.49 for early-onset CAD and OR of 1.01 for late-onset CAD ($P=0.078$). Thus, we could not exclude the possibility that the significant association between rs11206510 and early-onset CAD may represent a false-positive finding attributable to the small sample size of 380 patients and 1240 controls. In addition, the risk allele appeared to be different between the Chinese population (C allele) and white populations (T allele). The discrepancy that the risk allele in white populations (common allele T) was different from that in the Chinese populations (the minor allele C) may reflect the differences between the Chinese population and white populations. It is also possible that SNP rs11206510 serves as a marker for the disease, and that the true risk allele of a causative variant is in linkage disequilibrium with different alleles of SNP rs11206510 in different populations.

The other limitation of the present study is that population stratification may be a confounding factor for association studies with SNP rs11206510 because the minor allele frequency in the HapMap database varies from 6.7%, 4.4%, 15%, to 10% for the Chinese, Japanese, white, and black populations, respectively. However, for our studies all study subjects belonged to the Han ethnic group, and the observed minor allele frequency for rs11206510 in our study cohorts ranged from 4.9% to 5.3%, which is comparable to the data from the HapMap database. Also, our cases and controls were matched for gender and for the same geographical area. Thus, our study design would have minimized the effect of population stratification, although it could not be fully excluded.

The chromosomal location of SNP rs11206510 is chromosome 1p32 chr1:55 268 377 bp (http://genome.ucsc.edu), and it is not located in any known gene. Therefore, the underlying gene for LDL-C, early-onset CAD, and ischemic stroke at the rs11206510 locus is not known and warrants future studies. Future studies are also needed to determine whether rs11206510 is the actual causative SNP or in linkage disequilibrium with the true causative SNP nearby.

**Conclusion**

In conclusion, the data in this study provide strong evidence to support the novel finding that the minor allele C of SNP rs11206510 is a highly significant risk factor for ischemic stroke. Our study also showed that the minor allele C of rs11206510 was a risk factor for increased LDL-C levels and early-onset CAD in the Chinese population. Together, the association of SNP rs11206510 to both ischemic stroke and early-onset CAD may suggest an important role of the chromosome 1p32 rs11206510 locus in atherosclerosis associated with both cardiovascular and cerebrovascular systems.

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**Disclosures**

None.

**References**

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