Genetic polymorphisms in Sox17 associated with intracranial aneurysm in Chinese Han people: a genotype-phenotype study

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Purpose: Genetic factors play a vital role in intracranial aneurysm (IA) onset and development. Studying the relationship between IA and the Sox17 polymorphisms in diverse populations is essential for establishing credibility.

Patients and methods: We collected blood samples derived from a total of 596 sporadic IA patients and 600 individual controls in several medical institutes in China. We used the Sequenom MassArray system for single nucleotide polymorphisms (SNPs) genotyping after DNA extraction. The SNPs data was tested and analyzed in PLINK (version 1.9). Multiple-testing was performed in PLINK to make the statistics more rigorous and accurate.

Results: We found that the allelic G of rs1072737 (OR = 1.303, genomic-control corrected P-value = 0.001032) is a risk allele, while the allelic G of rs9298506 (OR = 0.7253, genomic-control corrected P-value = 0.01559) is a protective allele in Chinese Han people.

Conclusion: The allelic G of rs1072737 is a risk factor for IA, while the allelic G of rs9298506 serves as a protective factor for IA in Chinese Han people.

Keywords: intracranial aneurysm, Sox17, genetics, cerebral vascular disease

Introduction

Rupture of intracranial aneurysm (IA) is a contributing factor for hemorrhagic stroke which is characterized by high fatality and disability rates.1 A systematic review and meta-analysis found that the prevalence of unruptured IA is about 3.2%.2

The genetic risk factors associated with IA and the strength of their associations with IA remain uncertain.3 Thus, the underlying genetics of IA should be further explored. Moreover, IA-related genetic polymorphisms in diverse populations are not entirely the same. Many initial studies have been carried out in populations of European descent, and it is still a challenge to extend the studies to other populations, especially to populations of Asian and African descent.4 Compared with other populations, genetic studies on IAs have rarely been carried out in Chinese populations, which is ill-matched with the increasing rate of sequencing in China.5 Two genetic variations in the Sox17 gene, rs1072737 (NC_000008.11: g.54501764T>G) and rs9298506 (NC_000008.10: g.55437524A>G), have been shown to be risk factors for IA.6,8 However, there have not been any studies to test these findings in the Chinese people. Genotype-phenotype associations in different populations are essential to establish credibility; thus, it is important to study IA-related single nucleotide polymorphisms (SNPs) in Sox17 in the Chinese population. To further establish the credibility of the relationship between IA and the Sox17 risk allele in diverse populations, we studied the IA-related single nucleotide polymorphisms in diverse populations.
nucleotide variants of Sox17, and discoveries of Sox17 SNPs associated with IA were made in Chinese Han people.

**Patients and methods**

**Ethics approval and informed consent**

All the studies complied with the Declaration of Helsinki and were approved by the Institutional Review Board and Ethics Committee of Tianjin Medical University General Hospital and the collaborating hospitals’ ethics committees. All the participants reported themselves to be of Han nationality and provided written informed consent.

**Patients and blood samples**

This case-control study included 595 patients with sporadic IAs and 600 controls. We recruited patients with IAs between February 2014 and March 2017 from three clinical centers located in Tianjin (Tianjin Medical University General Hospital), Shandong (Linyi People’s Hospital), and Hunan (Xiangya Hospital). All IA cases were confirmed to have IA by imaging or surgery. The IA diagnosis was based on symptoms suggestive of subarachnoid hemorrhage (SAH), subarachnoid blood on computed tomography (CT), computerized tomography angiography (CTA), digital subtraction angiography (DSA), and/or surgery. All cases with confirmed IAs based on angiography were retrospectively confirmed by three independent observers, a neuroradiologist, and two highly qualified cerebrovascular neurosurgeons who were blinded to the clinical history and the imaging results. All the patients included in this research underwent either endovascular embolization or a neurosurgical operation after confirmation of the IA. All patients were asked whether they had a family history of IA. The patients with no family history of IA were included.

A total of 600 controls were randomly selected from the Physical Examination Center in the Tianjin Medical University General Hospital (TJMUGH). For an extended period, the Physical Examination Center collected the clinical information and the blood of healthy patients and built a database that provided information on the sex, age, blood pressure, smoking status, alcohol usage and other IA-related indexes for this study. In 2016, TJMUGH united 28 high-level hospitals from different regions in China and established the Chinese Multicenter Cerebral Aneurysm Database (CMAD). The CMAD is a cloud database that consists of clinical information and blood samples (http://www.cmadtj.com). The CMAD contributed data for IA cases in this study.

**Selection of SNPs**

The Sox17 variants were selected based on the frequency of these variants in the Chinese population and based on the previous genome-wide association study (GWAS) on the Sox17 gene. The rate of Sox17 variants in the Chinese population was determined using the NCBI (National Center for Biotechnology Information) database (http://www.ncbi.nlm.nih.gov/SNP/).

**Genotyping of SNPs**

Genomic DNA was extracted from peripheral blood. All of the obtained DNA samples were stored at −80°C. All the primers were designed by MassArray Assay Design 3.1 (Sequenom Inc., San Diego, CA, USA). SNPs were genotyped via the Sequenom MassArray system (BioMiao Biotechnology (Beijing) Co. Ltd) and analyzed with the MassArray Typer 4.0 software (Sequenom).

**Statistical analysis**

First, we checked the grouping information using the ID of the blood sample and the SNP data. Second, the data were tested and analyzed in PLINK (version 1.9). Finally, when the outcomes were determined, the data were tabulated for further discussion. Multiple-testing was performed in PLINK to make the statistics more rigorous and accurate.

**Results**

**Case and control characteristics and statistical outcome**

Characteristics of the cases and controls are shown in Table 1. The genotype distribution of the Sox17 variants rs1072737 and rs9298506 in the cases and controls are shown in Table 2.

**Table 1 The distribution of sporadic IA and control cases**

<table>
<thead>
<tr>
<th></th>
<th>Sporadic IA</th>
<th>Control cases</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>595</td>
<td>600</td>
<td></td>
</tr>
<tr>
<td>Women, n (%)</td>
<td>342 (57.5)</td>
<td>219 (36.5)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Age, years</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>60.93±11.93</td>
<td>43.50±11.04</td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>17–91</td>
<td>25–94</td>
<td></td>
</tr>
<tr>
<td>Risk factors</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypertension, n (%)</td>
<td>342 (57.5)</td>
<td>122 (20.3)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Hyperglycemia, n (%)</td>
<td>51 (8.6)</td>
<td>46 (7.7)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Smoke, n (%)</td>
<td>170 (28.6)</td>
<td>191 (31.8)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Drink, n (%)</td>
<td>82 (13.8)</td>
<td>191 (31.8)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Abbreviation: IA, intracranial aneurysm.
The allele G of rs1072737 is a risk factor for IA, while the allele G of rs9298506 is a protective factor for IA. Multiple-testing correction was performed to verify the risk effect of rs1072737 and the protective effect of rs9298506 with. Both rs1072737 and rs9298506 passed the multiple-testing method “genomic control” (GC) (Table 4). The results were clear and definite in the chi-squared test (rs1072737: OR 1.306, genomic-control corrected P-value = 0.001599; rs9298506: OR 0.7121, genomic-control corrected P-value = 0.02359) and in the allelic gene logistic regression (rs1072737: OR: 1.303, genomic-control corrected P-value = 0.01559; rs9298506: OR: 0.7253, genomic-control corrected P-value = 0.001032) (Table 4). Additionally, rs9298506 passed other multiple-test methods such as the Bonferroni adjusted method and the Benjamini and Hochberg adjusted method (Table 4).

**Discussion**

In our study, we found that allele G of rs1072737 is a risk factor for IA, while allele G of rs9298506 is a protective factor for IA. This finding is based on the multiple-testing correction.

A previous GWAS showed that rs1072737 and rs9298506 the Sox17 were associated with IA. However, the present study indicates that they have different roles in IA in Chinese Han population: the allelic C of rs1072737 is a risk allele, while the allelic G is a protective allele in Chinese Han people (Table 4). The GWAS showed rs9298506 (P = 1.3×10^-12, OR: 1.28, posterior probability of association > 0.9999) to be significantly associated with IA. Additionally, rs9298506 was reported to have an IA-related risk effect in another meta-analysis. Nonetheless, in our study, allele G of rs9298506 was a protective factor for IA in Chinese Han people. Recently, the relationship between Sox17 gene polymorphisms and IA was investigated in a homogeneous Korean population. It was shown that allele G of rs1072737 is a protective factor (OR: 0.69, 95%CI: 0.49–0.96, P = 0.03) and that allele G of rs9298506 has no statistical significance. The results of this study in the Korean population are contrary to our results and others.

Some initial findings cannot be replicated in the follow-up studies due to some issues. For risk alleles and the haplotypes, initial studies and follow-up studies may not be the same in different populations. Therefore, the confidence would be higher if we evaluated the genotype-phenotype association in people of diverse ancestries, regardless of whether the genotype-phenotype association is derived from candidate-gene studies or genome-wide association studies. Concordant findings in similar studies are necessary to confirm the genotype-phenotype associations.

Sox17 is primarily expressed in the arterial endothelial cells in the early stages of embryonic development. This expression pattern and distribution continues into adulthood. It suggests that the change in Sox17 expression leads to abnormalities in intracranial artery differentiation.

**Table 2 Genotype statistics**

<table>
<thead>
<tr>
<th>SNP</th>
<th>Mutant type</th>
<th>Wild type</th>
<th>IA group</th>
<th>Control group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A1</td>
<td>A2</td>
<td>A1A1</td>
<td>A1A2</td>
</tr>
<tr>
<td>rs1072737</td>
<td>G</td>
<td>T</td>
<td>21</td>
<td>180</td>
</tr>
<tr>
<td>rs9298506</td>
<td>A</td>
<td>G</td>
<td>30</td>
<td>164</td>
</tr>
<tr>
<td>Note: A1 and A2 are allelic genes, A1 is the mutant type, A2 is wild type. Abbreviations: SNP, single nucleotide polymorphism; IA, intracranial aneurysm.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Table 3 Hardy–Weinberg equilibrium**

<table>
<thead>
<tr>
<th>SNP</th>
<th>Test</th>
<th>A1</th>
<th>A2</th>
<th>Genotype (A1A1/A1A2/A2A2)</th>
<th>O(HET)</th>
<th>E(HET)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs1072737</td>
<td>ALL</td>
<td>G</td>
<td>T</td>
<td>37/332/796</td>
<td>0.285</td>
<td>0.2878</td>
<td>0.7598</td>
</tr>
<tr>
<td>rs1072737</td>
<td>AFF</td>
<td>G</td>
<td>T</td>
<td>21/180/372</td>
<td>0.3141</td>
<td>0.3124</td>
<td>I</td>
</tr>
<tr>
<td>rs1072737</td>
<td>UNAFF</td>
<td>G</td>
<td>T</td>
<td>16/152/424</td>
<td>0.2568</td>
<td>0.2625</td>
<td>0.6376</td>
</tr>
<tr>
<td>rs9298506</td>
<td>ALL</td>
<td>G</td>
<td>T</td>
<td>74/381/686</td>
<td>0.3339</td>
<td>0.3562</td>
<td>0.03755</td>
</tr>
<tr>
<td>rs9298506</td>
<td>AFF</td>
<td>G</td>
<td>T</td>
<td>30/164/363</td>
<td>0.2944</td>
<td>0.3213</td>
<td>0.04868</td>
</tr>
<tr>
<td>rs9298506</td>
<td>UNAFF</td>
<td>G</td>
<td>T</td>
<td>44/217/323</td>
<td>0.3716</td>
<td>0.3859</td>
<td>0.3907</td>
</tr>
</tbody>
</table>

Notes: All cases; *Case group; †Control group. Abbreviations: O(HET), observed heterozygosity; E(HET), expected heterozygosity; SNP, single nucleotide polymorphisms.
A study demonstrated that IA could be generated through induced hypertension and circle of Willis damage by elastase in Sox17-knockdown mice, which further supports the theory that hereditary and acquired risk factors facilitate IA formation. It was shown that Sox17 has specific expression in the cerebral artery. Furthermore, compared with that in the normal cerebral artery, the Sox17 expression in IA is reduced and the endothelial junctions are destabilized. Therefore, we assume that gene Sox17 variations lead to the function changes of Sox17, cause damage to cerebral artery, and finally promote IA formation. However, molecular biology experiments and a reliable animal model are required to explore the specific mechanisms further.

## Conclusion

The allelic G of rs1072737 is a risk factor for IA while the allelic G of rs9298506 serves as a protective factor for IA in Chinese Han people.

## Acknowledgments

The authors thank BioMiao Biotechnology (Beijing) Co. Ltd for providing SNP sequencing and bioinformatics analysis services. The authors appreciate the Chinese Multicenter Cerebral Aneurysm Database (CMAD), which contributed clinical data and blood samples to this study. The authors thank the National Natural Science Foundation of Tianjin City (16JCZDJC35700) and (81571144) and (81501073), and the Natural Science Foundation of China (81571144) and (81501073), and the Natural Science Foundation of Tianjin City (16JCZDJC35700) and (15ZXLC SY00060) for supporting this work.

## Disclosure

The authors report no conflicts of interest in this work.

## References


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## Table 4

<table>
<thead>
<tr>
<th>SNP</th>
<th>UNADJ P-value</th>
<th>OR</th>
<th>GC</th>
<th>BONF P-value</th>
<th>HOLM P-value</th>
<th>SIDAK SS P-value</th>
<th>SIDAK SD P-value</th>
<th>FDR_BH P-value</th>
<th>FDR_BY P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs9298506</td>
<td>0.000679</td>
<td>0.7121</td>
<td>0.001599</td>
<td>0.01696</td>
<td>0.017</td>
<td>0.01683</td>
<td>0.01683</td>
<td>0.01696</td>
<td>0.06473</td>
</tr>
<tr>
<td>rs1072737</td>
<td>0.0148</td>
<td>1.306</td>
<td>0.02359</td>
<td>0.3699</td>
<td>0.3403</td>
<td>0.3111</td>
<td>0.2903</td>
<td>0.1233</td>
<td>0.4705</td>
</tr>
</tbody>
</table>

**Abbreviations:** UNADJ, unadjusted P-value; GC, genomic-control corrected P-values; BONF, Bonferroni single-step adjusted P-values; HOLM, Holm (1979) step-down adjusted P-values; SIDAK SS, Sidak single-step adjusted P-values; SIDAK SD, Sidak step-down adjusted P-values; FDR_BH, Benjamini and Hochberg step-up FDR control; FDR_BY, Benjamini and Yekutieli step-up FDR control.


