Lack of Association of Urotensin II Gene Polymorphism with Atherosclerotic Cerebral Infarction Risk in the Southern Han Population

Research Article

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Summary

Background: As an endogenous vasoactive peptide, urotensin II is involved in the development of atherosclerosis. However, there have been no related reports focusing on the association between polymorphisms in urotensin II and stroke risk thus far.

Material/Methods: A total of 308 patients with atherosclerotic cerebral infarction (ACI) and 351 healthy controls were enrolled in this study. The S89N polymorphism in the urotensin II gene was genotyped by polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) analyses.

Results: The genotypic and allelic frequencies of urotensin II S89N revealed no significant difference between the ACI group and the control group. Blood pressure, fasting blood sugar (FBS) and blood lipids did not significantly differ among subjects with all three genotypes of S89N.

Conclusions: In the Southern Han population of China, the urotensin II gene polymorphism S89N may be not associated with ACI risk and did not affect blood pressure, FBS or blood lipids.
I. Introduction:

Stroke is the leading cause of death in China; it is a serious threat to human health and a heavy burden on the community as well as the patient’s family (Sun et al., 2013). However, effective treatments for stroke are still lacking. Atherosclerotic cerebral infarction (ACI) is the most common subtype of stroke in China. ACI is generally regarded as a complex disease resulting from interactions between genetic factors and multiple environmental factors (Duan et al., 2012; Malik et al., 2014). Susceptibility genes most likely play an important role in the development of ACI (Hassan A & Markus HS, 2000; Tuttolomondo A et al., 2012). Hypertension, hyperlipidemia, diabetes, heart disease, atherosclerosis, abnormal coagulation and metabolic syndrome are recognized risk factors for ACI. These risk factors have also been found to be related to genetic backgrounds (Alberts et al., 1991; Falcone et al., 2014). Therefore, it is especially vital to determine susceptibility genes for ACI.

Growing evidence indicates that inflammatory processes likely contribute to the development and progression of atherosclerosis (Pinto A et al., 2006; Licata G et al., 2006; Davì G et al., 2009; Pinto A et al., 2009; Tuttolomondo A et al., 2012), which is considered to be vital to the pathogenesis of ACI. Urotensin II is one type of vasoactive peptide, and the gene encoding urotensin II is located on 1p36. Urotensin II is mainly expressed in the cardiovascular system, central nervous system and endocrine tissues (Douglas et al., 2000; Ong et al., 2005). At present, studies have confirmed that urotensin II is an endogenous mitogen involved in the processes of vascular inflammation and atherosclerosis, possibly via the following mechanisms: promotion of endothelial cell proliferation as well as smooth muscle cell proliferation and migration, inhibition of apoptosis (Shi et al., 2006), upregulation of collagen-I expression, reduction of matrix metalloproteinase-I expression (Wang et al., 2004), activation of NADPH oxidase and plasminogen activator inhibitor-I (Djordjevic et al., 2005), or acceleration of macrophase-derived foam cell formation (Watanabe et al., 2005; Allahverdian et al., 2012). Upregulation of urotensin II and its receptors was observed in sections of human aortic atherosclerotic lesions (Hillier et al., 2001). A previous study found that urotensin II can promote the development of atherosclerosis in cholesterol-fed rabbits (Li et al., 2014). Further, animal studies indicated that the development of atherosclerotic plaques can be attenuated by urotensin II receptor antagonism (You et al., 2012; Watson et al., 2013).

S89N and T21M are two common single nucleotide polymorphisms (SNPs) of the urotensin II gene. Studies have shown that S89N, but not T21M, was associated with type 2 diabetes (Zhu et al., 2002; Wenyi et al., 2003; Suzuki S et al., 2004), essential hypertension (Yi L et al., 2006; Peng et al., 2013), and myocardial infarction (Nishihama K et al., 2007; Oguri M et al., 2009) in Asian populations. On the other hand, T21M, but not S89N, was associated with Behcet’s disease (Oztuzcu S et al., 2013) and systemic sclerosis (Pehlivan Y et al., 2012).

The AC haplotype (-11640A and -8515C) of the urotensin II receptor gene was associated with insulin resistance (Ong KL et
al, 2006). However, there are no related reports focusing on urotensin and ACI risk thus far. Therefore, in this study, we attempted to determine whether the S89N polymorphism of the urotensin II gene is associated with ACI risk in the Southern Han Chinese population.

II. Materials and Methods:

II.A. Subjects

ACI group: Cerebral infarction patients hospitalized at the Department of Neurology, Xiangya Hospital, from December 2012 to December 2013 were included in our study. All the cases were examined by cranial CT and/or MRI scanning and conformed to the diagnostic criteria established by the Fourth National Cerebrovascular Diseases Conference in 1995. The exclusion criteria were as follows: arteritis, trauma, blood diseases, tumors, vascular malformations or aneurysm, serious liver or kidney disease, autoimmune disease, pregnancy, and receiving lipid-lowering therapy for half a year. After screening, the final sample consisted of 308 ACI patients (194 males and 114 females, average age: 64.3±10.2 years).

Control group: A total of 351 healthy volunteers (219 males and 132 females, average age: 64.1±9.4 years) were recruited from the Health Management Center of Xiangya Hospital. Clinical and imaging examinations were conducted to exclude stroke. Patients with liver and kidney diseases or autoimmune diseases, patients who were pregnant, and patients receiving lipid-lowering therapy for half a year were excluded.

All subjects were non-consanguineous Southern Han individuals. This study was approved by the Ethics Committee of Xiangya Hospital, Central South University (Changsha, Hunan province, China). All cases and controls provided written informed consent.

II.B. Blood biochemistry tests and genomic DNA extraction

Peripheral venous blood (10 mL) was collected from subjects who had been fasting for 12 hours: 5 ml was used for determination of blood lipids and blood sugar, and 5 ml (EDTA-anticoagulant) was used to extract DNA via the conventional phenol/chloroform method.

II.C. Analysis of polymorphisms

Primers were designed as described previously (Wenyi et al., 2003) and synthesized by Sangon Biotech Co., Ltd (Shanghai, China). The primer sequences were as follows: 5'-gagtcctgtaaaaccagtacag-3' (upstream); 5'-gtgcctgtctgtctgcattca-3' (downstream). The PCR amplification parameters were as follows: denaturation at 95°C for 5 min, followed by 34 cycles of denaturation at 95°C for 45 s, annealing at 60°C for 45 s, and extension at 72°C for 45 s. A final extension step was performed at 72°C for 10 min. The PCR products were digested at 37°C for 3 h using the restriction enzyme Rsal (Promega, Madison, WI, USA).

The digested products were electrophoresed on a 2% agarose gel containing ethidium bromide (0.5×TBE, 120 volts). Enzyme-digested homozygous SS products showed three bands of 161 bp, 84 bp and 18 bp. Enzyme-digested homozygous NN products showed two bands of 245 bp and 18 bp, whereas enzyme-digested heterozygous SN products showed four bands of 245 bp, 161 bp, 84 bp and 18 bp. We note that the 18 bp band was too small to be visualized using agarose gel electrophoresis.

II.D. Statistical analysis

The direct counting method was used to calculate the frequencies of genotypes and alleles. The chi-square test was used to analyze count data, examine Hardy-Weinberg equilibrium, and compare genotype and allele frequencies between groups. Comparisons were made between two
groups using the t test, and comparisons between there groups were made using analysis of variance. The SPSS 18.0 statistical software package for Windows (SPSS Inc., Chicago, IL, USA) was used for statistical processing. P<0.05 was considered statistically significant.

III. Results:

III.A. Clinical characteristics of the subjects

The main clinical data from the ACI group and the control group are shown in Table 1.

<table>
<thead>
<tr>
<th>Clinical data</th>
<th>ACI group (n=308)</th>
<th>Control group (n=351)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean Age (years)</td>
<td>64.3±10.2</td>
<td>64.1±9.4</td>
<td>0.7935</td>
</tr>
<tr>
<td>Male/Female</td>
<td>194/114</td>
<td>219/132</td>
<td>0.8750</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>23.7±2.32</td>
<td>23.4±2.51</td>
<td>0.1258</td>
</tr>
<tr>
<td>Smoking history (Y/N)</td>
<td>201/107</td>
<td>232/119</td>
<td>0.8213</td>
</tr>
<tr>
<td>Drinking history (Y/N)</td>
<td>232/76</td>
<td>202/149</td>
<td>0.2060</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>141.4±19.36</td>
<td>128.6±10.75</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>84.8±11.57</td>
<td>83.2±13.11</td>
<td>0.1126</td>
</tr>
<tr>
<td>FBS (mmol/l)</td>
<td>6.19±2.24</td>
<td>5.41±1.43</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>TC (mmol/l)</td>
<td>4.54±1.14</td>
<td>4.27±0.93</td>
<td>0.009*</td>
</tr>
<tr>
<td>TG (mmol/l)</td>
<td>2.01±1.13</td>
<td>1.89±1.06</td>
<td>0.1602</td>
</tr>
<tr>
<td>HDL (mmol/l)</td>
<td>1.26±0.33</td>
<td>1.36±0.28</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>LDL (mmol/l)</td>
<td>2.69±0.84</td>
<td>2.56±0.63</td>
<td>0.0239*</td>
</tr>
</tbody>
</table>

* P<0.05

Abbreviations: Y/N: yes/no; ACI: atherosclerotic cerebral infarction; BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; FBS: fasting blood sugar; TC: total cholesterol; TG: triglycerides; HDL: high-density lipoprotein; LDL: low-density lipoprotein.
III.B. Distribution of genotypes and alleles

The genotype frequencies in the ACI group were SS 46.8% (144/308), SN 40.3% (124/308) and NN 12.9% (40/308); in the control group, these frequencies were SS 48.1% (169/351), SN 39.9% (140/351) and NN 12.0% (42/351). The allele frequencies were S 66.9% and N 33.1% in the ACI group and S 68.1% and N 31.9% in the control group. The frequencies of genotypes and alleles in both groups were compatible with Hardy-Weinberg equilibrium (P>0.05). As shown in Table 2, genotypic and allelic frequencies between the two groups were not significantly different (P>0.05).

Table 2. Genotypes and alleles of urotensin II polymorphism S89N

<table>
<thead>
<tr>
<th>Group</th>
<th>Genotypes, n (%)</th>
<th>Alleles, n(%)</th>
<th>$P_G$</th>
<th>$P_A$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SS</td>
<td>SN</td>
<td>NN</td>
<td>$P_{HWE}$</td>
</tr>
<tr>
<td>ACI (n=308)</td>
<td>144(46.8)</td>
<td>124(40.3)</td>
<td>40(12.9)</td>
<td>0.1095</td>
</tr>
<tr>
<td>Control (n=351)</td>
<td>169(48.1)</td>
<td>140(39.9)</td>
<td>42(12.0)</td>
<td>0.1240</td>
</tr>
</tbody>
</table>

$P_G$, the $P$ value for the comparision between 3 different genotypes; $P_A$, the $P$ value for the comparision between S and N allele; $P_{HWE}$, the P value for the Hardy-Wenberg equilibrium. All $P >0.05$

Abbreviations: ACI: atherosclerotic cerebral infarction

III.C. Association between S89N genotypes and traditional stroke risk factors

To ascertain the relationship between the S89N polymorphism and blood pressure, FBS and blood lipid levels, we compared the blood pressure and blood lipid levels among three distinct genotypes in the ACI group and control group. We discovered that regardless of the group, the S89N genotype did not affect blood pressure, FBS, TC, TG, LDL or HDL (Table 3).
Table 3. Association between S89N genotypes and traditional stroke risk factors

<table>
<thead>
<tr>
<th>Stroke Risk Factors</th>
<th>ACL Group</th>
<th></th>
<th></th>
<th>P1</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SS (144)</td>
<td>SN (124)</td>
<td>NN (40)</td>
<td></td>
<td>SS (169)</td>
<td>SN (140)</td>
<td>NN (42)</td>
</tr>
<tr>
<td>TC (mmol/l)</td>
<td>4.48±1.04</td>
<td>4.61±1.27</td>
<td>4.74±1.10</td>
<td>0.1975</td>
<td>4.19±0.85</td>
<td>4.32±1.04</td>
<td>4.44±0.83</td>
</tr>
<tr>
<td>LDL (mmol/l)</td>
<td>2.64±0.79</td>
<td>2.69±0.10</td>
<td>2.83±0.86</td>
<td>0.4919</td>
<td>2.58±0.69</td>
<td>2.57±0.76</td>
<td>2.48±0.81</td>
</tr>
<tr>
<td>TG (mmol/l)</td>
<td>1.50±1.10</td>
<td>2.13±1.62</td>
<td>2.01±1.13</td>
<td>0.3745</td>
<td>1.91±0.66</td>
<td>1.84±0.89</td>
<td>1.94±0.93</td>
</tr>
<tr>
<td>HDL (mmol/l)</td>
<td>1.29±0.32</td>
<td>1.25±0.29</td>
<td>1.12±0.41</td>
<td>0.3835</td>
<td>1.35±0.32</td>
<td>1.39±0.30</td>
<td>1.36±0.27</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>142.37±25.20</td>
<td>143.41±19.10</td>
<td>139.49±16.34</td>
<td>0.6135</td>
<td>129.61±14.32</td>
<td>127.13±12.93</td>
<td>129.25±11.65</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>85.59±14.32</td>
<td>83.51±12.23</td>
<td>86.16±11.09</td>
<td>0.3428</td>
<td>84.52±15.91</td>
<td>82.57±12.65</td>
<td>85.08±13.35</td>
</tr>
<tr>
<td>FBS (mmol/l)</td>
<td>6.10±2.58</td>
<td>6.25±1.72</td>
<td>6.33±2.01</td>
<td>0.7804</td>
<td>5.52±1.61</td>
<td>5.27±0.98</td>
<td>5.45±1.46</td>
</tr>
</tbody>
</table>

All P > 0.05

**Abbreviations**: ACI: atherosclerotic cerebral infarction; TC: total cholesterol; LDL: low-density lipoprotein; TG: triglycerides; HDL: high-density lipoprotein; SBP: systolic blood pressure; DBP: diastolic blood pressure; FBS: fasting blood sugar.

**IV. Discussion:**

Urotensin II is a cyclic peptide composed of only 11 amino acid residues, and it elicits a series of physiological effects in the endocrine, cardiovascular, renal, and immune systems. Urotensin II and its receptor are widely expressed throughout the cardiovascular system and central nervous system, and metabolic systems (Vaudry et al., 2010). Upregulation of urotensin II and its receptor has been demonstrated in several disease states, including metabolic syndrome, atherosclerosis, diabetes, and hypertension (McDonald et al., 2007; Ross et al., 2010). Numerous experiments have shown that urotensin II can bind to and activate its receptor to stimulate the proliferation of endothelial cells and smooth muscle cells as well as the chemotaxis of monocytes (Tölle et al., 2008; Xu et al., 2012). Thus, urotensin II and its receptor are thought to play an important role in atherosclerosis development.

Here, we conducted a case-control study to test the association of the S89N polymorphism in urotensin II with ACI risk. However, there were no significant differences found in the genotypic or allelic frequencies of S89N between the ACI group and the control group. However, previously published studies have reported that the S89N polymorphism is related to hypertension (Peng et al., 2013) and type 2 diabetes (Zhu et al., 2002; Wenyi et al., 2003). Additionally, the plasma level of urotensin II was found to be upregulated in hypertensive patients (Suguro et al., 2007; Peng et al., 2013). Urotensin II was also shown to influence blood pressure under pathological conditions in vivo (Gendron et al., 2005; Zemancíková & Török, 2013; Debiec et al., 2013); however, the exact underlying mechanisms are still unclear.
Here, we further analyzed the relationship between three genotypes of the S89N polymorphism and traditional stroke risk factors. However, our results revealed that the S89N genotype did not affect blood pressure or FBS levels in the stroke group or the control group. Next, we explored the association between the S89N polymorphism and lipid metabolism. We found that the blood lipid levels of stroke groups and controls were not significantly different regardless of genotype.

There were some limitations to this study. First, only one SNP (S89N) was examined in this study. T21M and SNPs in the promoter region of the urotensin II gene should be included in future studies. Second, we did not measure the plasma level of urotensin II; thus, the relationship between S89N genotypes and the plasma level of Urotensin II was not clarified. Third, the sample size in this study was relatively small.

In summary, our study indicated that the urotensin II gene polymorphism S89N may be not associated with ACI risk in the Southern Han population of China and also had no effect on blood pressure, FBS or blood lipids. Further studies should be performed with larger samples and more ethnically diverse populations to confirm this finding.

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