Research Reports

SERPINA3 Polymorphism Is Not Associated With Primary Intracerebral Hemorrhage in a Polish Population

Joanna Pera, MD; Agnieszka Slowik, MD; Tomasz Dziedzic, MD; Andrzej Szczudlik, MD, PhD

Background and Purpose—Genetic factors involved in the pathogenesis of primary intracerebral hemorrhage (PICH) remain unknown. One of the candidate genes is SERPINA3. Results of a Spanish study suggested that TT genotype of the A/T SERPINA3 polymorphism in the signal peptide sequence was a risk factor for PICH in normotensive subjects. The aim of the present study was to investigate whether SERPINA3 A/T polymorphism is associated with PICH in a Polish population.

Methods—We analyzed 95 PICH patients and 190 unrelated healthy controls matched for age and sex. A/T polymorphism of the SERPINA3 gene was investigated using polymerase chain reaction restriction fragment length polymorphism method.

Results—The distribution of SERPINA3 genotypes was similar among PICH patients (AA 27.4%; AT 46.3%; TT 26.3%) and controls (AA 23.7%; AT 50.5%; TT 25.8%; P=NS). There were also no significant differences in genotype distribution when analyzing separately hypertensive and normotensive PICH patients as well as patients with lobar and deeply located hemorrhage.

Conclusions—We failed to find an association between SERPINA3 A/T polymorphism and PICH in a Polish population.

Key Words: genetics ■ intracerebral hemorrhage

The pathogenesis of primary intracerebral hemorrhage (PICH) remains unknown, and genetic factors may play an important role. The polymorphism of the SERPINA3 (previously known as α1-antichymotrypsin) gene is one of the candidates for being a genetic factor associated with PICH. Recently, an A/T polymorphism in the signal peptide sequence of the SERPINA3 gene has been reported to be associated with PICH in normotensive patients in a Spanish population, whereas in a Chinese study, with hemorrhagic stroke in hypertensive subjects. There probably are ethnic differences in alleles and genotypes distribution of this polymorphism.

In the present study, we investigated whether SERPINA3 A/T polymorphism is associated with PICH in a Polish population.

Materials and Methods

This prospective case-control study involved 95 unrelated patients with PICH selected from 173 patients with hemorrhagic stroke consecutively admitted to the neurology department, University Hospital, Krakow, between October 2002 and April 2003. We excluded patients with head trauma (n=3), vasculitis (n=1), intracranial aneurysms (n=5), arteriovenous malformations (n=9), hematologic disorders (n=2), malignancy (n=3), patients in whom the angiography could not be performed because of a moribund status (n=19) or technical problems (n=10), and those who did not agree to participate in the study (n=26). In all included patients, brain computed tomography (CT) and angiography (digital subtractive angiography or angio-CT or angio–magnetic resonance) were performed. Sex- and age-matched (±1 year) control subjects (CS) were recruited from spouses of the patients of the stroke unit (30%), relatives of hospital staff (35%), and patients of the University Hospital hospitalized for any reason other than neurological diseases (35%). Case-to-control ratio was 1:2. All subjects were white, and all gave informed consent before inclusion into the study. The ethical committee approved the study.

Demographic data and risk factor profile was collected using a questionnaire described previously. Genomic DNA was extracted from peripheral blood using a commercially available kit from Boehringer Mannheim. SERPINA3 polymorphism was studied using polymerase chain reaction restriction fragment length polymorphism method.

Differences between groups were studied using unpaired Student t test (continuous variables) or χ² test (categorical variables). Hardy-Weinberg equilibrium was tested by χ² test in cases and CS separately. The association of the SERPINA3 A/T genotype with PICH was tested using logistic regression analysis under assumptions of recessive (TT versus AT+AA) or dominant (TT versus AA) effect for the T allele. P value <0.05 was considered statistically significant.

Results

Baseline characteristics of study subjects are summarized in Table 1.

Genotype distribution both in PICH patients and CS was in Hardy-Weinberg equilibrium (P>0.05). Allele and genotype frequencies did not differ significantly between studied groups. There were also no significant differences when compared the alleles and genotypes distribution separately in hypertensive and normotensive PICH patients as well as patients with deep (periventricular white matter, basal ganglia, thalamus, internal capsule, brain stem, cerebellum) and lobar hemorrhages with CS (Table 2).

Both crude and adjusted (for hypertension, ischemic heart disease, diabetes, hypercholesterolemia, and smoking) logistic regression analysis failed to reveal a significant association between studied polymorphism and PICH neither under assumptions of recessive nor dominant effect for the T allele (Table 3).
**TABLE 1. Characterization of Study Subjects**

<table>
<thead>
<tr>
<th>Patients (n=90)</th>
<th>Controls (n=190)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (SD) age, y</td>
<td>59.7 (12.2)</td>
<td>59.4 (12.4)</td>
</tr>
<tr>
<td>Female, %</td>
<td>47.4</td>
<td>47.4</td>
</tr>
<tr>
<td>Hypertension, %</td>
<td>85.3</td>
<td>46.3</td>
</tr>
<tr>
<td>Ischemic heart disease, %</td>
<td>23.2</td>
<td>30.0</td>
</tr>
<tr>
<td>Diabetes, %</td>
<td>14.7</td>
<td>12.6</td>
</tr>
<tr>
<td>Hypercholesterolemia, %</td>
<td>49.5</td>
<td>33.2</td>
</tr>
<tr>
<td>Smokers, %</td>
<td>33.7</td>
<td>38.4</td>
</tr>
</tbody>
</table>

**Discussion**

We failed to find an association between the A/T SERPINA3 gene polymorphism and a risk of PICH in a Polish population.

Compared with the Spanish study, the frequency of TT genotype in our control group is higher (25.6% versus 15.0%). The borderline value of the difference (P=0.053) results from relatively small sample sizes. Moreover, our population is younger, with significantly higher frequency of hypertension, ischemic heart disease, and smoking. That suggests the presence of ethnic differences in genetic background and resulting susceptibility for PICH. This is in agreement with recently demonstrated racial variations in risk factors of hemorrhagic stroke. Additionally, studies analyzing the same SERPINA3 gene polymorphism in neurodegenerative disorders have also shown significant racial/ethnic differences in the distribution of alleles and genotypes.

Nevertheless, despite negative results of the current study, we cannot exclude the possible role of SERPINA3 in PICH. As an inhibitor of proteases, it might prevent the degradation of extracellular matrix in the vessel wall. Unfortunately, data concerning the association between the A/T SERPINA3 gene polymorphism and the plasma protein levels are inconsistent.

It is possible that this polymorphism is in linkage disequilibrium with another functional mutation(s) of the SERPINA3 gene, and there are various frequencies of those mutations among different populations.

Our study has some potential limitations. We cannot exclude the selection bias. The inclusion of the patients’ spouses and relatives of hospital staff into the control group could be responsible for the “healthy worker effect.” Also, the loss of patients who died before admission to the hospital or who refused to participate in the study could influence the final result. Next, our sample size was limited. Planning the study, we calculated that given the frequencies of the alleles observed previously, our sample would have a statistical power of 0.61 at the 0.05 significance level (for the power of 0.80 and 95 PICH patients, we would need 23 000 controls). However, considering the alleles frequencies found in our population, the power of the study decreases substantially. That could also influenced the study result.

**Acknowledgments**

A.S. was supported by the State Committee for Scientific Research (Poland) grant 6 P05B 049 20.

**References**

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Stroke. 2006;37:906-907; originally published online January 19, 2006;
doi: 10.1161/01.STR.0000202655.64413.0c
Stroke is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0039-2499. Online ISSN: 1524-4628

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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