Absence of Alpha-1 Antitrypsin Deficiency Alleles (S and Z) in Japanese and Korean Patients With Aneurysmal Subarachnoid Hemorrhage

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Background and Purpose—A possible association has been proposed for the formation of intracranial aneurysm (IA) and deficiency alleles (S and Z) of the α1-antitrypsin (AAT) gene. We extensively screened this gene in Japanese and Korean patients with aneurysmal subarachnoid hemorrhage.

Methods—Seven allelic variants, including S and Z alleles, were genotyped by direct sequencing of genomic DNA obtained from 195 and 189 ruptured IA patients and 195 and 94 controls in Japanese and Koreans, respectively. The haplotype in phase-unknown samples was constructed with the expectation–maximization method. Differences in allelic frequencies between patients and controls were evaluated by Fisher exact test.

Results—No significant differences in allelic frequencies were observed at all 7 variants between ruptured IA patients and controls. We could not detect the S and Z alleles of the AAT gene in Japanese and Korean populations.

Conclusions—AAT deficiency may not be a common genetic risk factor for aneurysmal subarachnoid hemorrhage in Japanese and Koreans. (Stroke. 2004;35:e376-e378.)

Key Words: aneurysm ■ genetics ■ subarachnoid hemorrhage

It has recently been recognized that genetic factors are associated with the formation of intracranial aneurysms (IAs) and subarachnoid hemorrhage (SAH).1–3 However, no susceptible genes have been identified as underlying the cause of IA. The relationship between alpha-1 antitrypsin (AAT) deficiency and IA formation has been suggested through an imbalance of protease and AAT, a major circulating inhibitor of proteases.4–6 Although general populations in Japan and Korea rarely have AAT deficiency alleles,7,8 we wanted to verify whether AAT contributes to the susceptibility to IA formation in Japanese and Koreans.

Subjects and Methods
The University Ethical Committees approved the study, and all the participants gave written informed consent. The DNA samples for the association study and genotyping were from 195 ruptured IA patients and 195 controls from Japan, and 189 ruptured IA patients and 94 controls from Korea (Table 1). The presence of IA was confirmed by surgical findings in patients with SAH. The age- and sex-matched controls were patients with diseases other than SAH. The case-to-control ratio was maintained at 1:1 among Japanese and 2:1 among Koreans. All subjects were of Japanese and Korean ethnicity.

Single Nucleotide Polymorphisms in the AAT Gene and Haplotype Construction
Five common single nucleotide polymorphisms (SNPs) on the AAT gene in Table 2 were researched in the NCBI database (http://www.ncbi.nlm.nih.gov/SNP/). These common SNPs have led to 7 common allelic variants (M1Ala213, M1Val213, M2, M3, M4, S, and Z).9 The phase-unknown allele (haplotype) frequencies of common allelic variants were estimated by an expectation–maximization algorithm based on a maximum likelihood approach using the ARLEQUIN (http://anthro.unige.ch/arlequin) or SNIPALyze (Dynamode) program.

Genotyping
Genomic DNA was extracted from peripheral blood according to a standard method. All primer sets were designed on genomic sequences obtained through the GenBank database, accession number GE: 20543860, to amplify each segment of the AAT gene with GeneAmp PCR system 9700 (PE Applied, Tokyo, Japan) using 10 ng of genomic DNA derived from each of the samples. The primer sequences are available on request. Direct sequencing was performed on polymerase chain reaction-amplified segments, including SNPs, by using ABI 3700 Automated Sequencers (PE Applied Biosystems) under standard conditions.

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e376
Allelic Association
Allelic association with IA was evaluated by Fisher exact test for each SNP between the cases and controls.

Results
The subjects in this study are summarized in Table 1. The 5 SNPs were genotyped in all our samples (Table 2). No significant differences of genotype frequency were detected in any SNPs between the cases and controls. The genotype distributions agreed with those predicted by the Hardy–Weinberg equilibrium. The estimated allele frequencies in cases and controls are shown in Table 3. No significant differences were identified in the allelic frequencies between groups. S and Z alleles were not observed in this study.

Discussion
The results of this study suggest that specific alleles, S and Z, of the AAT gene are not common genetic risk factors for IA in Japanese and Koreans, because they were not observed. In this study, we identified common alleles (M1–M4, S, and Z) of the AAT gene by direct sequencing for genomic DNA from aneurysm and control patients. Although M alleles are widely dispersed and have been described in several populations, the occurrence of S and Z alleles is virtually restricted to whites of European descent.10 It may be difficult to find patients with AAT-deficiency alleles in our Japanese samples, because the mean frequencies of PiS and PiZ are 0.0004 and 0.0002 in Japan.7,8 An analysis of published genetic epidemiologic surveys reported that those in South Korea are 0.0031 and 0.0061, which are higher frequencies than those in Japan.7,8 If AAT were a genetic risk factor in Koreans, we would have found several alleles of S and Z in our Korean patients with ruptured IA.

The inhibition of various proteolytic enzymes, such as neutrophil elastase by AAT, may have an important role in maintaining the integrity of connective tissue, including the blood vessel wall.6 A protease–antiprotease imbalance may have a pathogenetic role in the formation and rupture of IA. Acquired AAT deficiency, caused by the oxidation of methi-

**TABLE 1. Clinical Background and Study Samples**

| Group | Case | Control | P *
|-------|------|---------|-----
| Japanese | 195 | 195 | NS
| No. | 60.1 (12.4) | 60.2 (12.9) | NS
| Gender (M/F) | 74/121 | 74/121 | NS
| Site of ruptured IA, no. | ACoA | 69 | 69
| MCA | 60 | 60
| ICA | 42 | 42
| Other | 24 | 24
| Korean | No. | 189 | 94 | NS
| Mean (SD) age, y | 55.4 (11.0) | 56.0 (9.6) | NS
| Gender (M/F) | 53/136 | 26/68 | NS
| Site of ruptured IA, no. | ACoA | 83 | 83
| MCA | 66 | 66
| ICA | 33 | 33
| Other | 7 | 7

ACoA indicates anterior communicating artery; MCA, middle cerebral artery; ICA, internal carotid artery.

**TABLE 2. Genotype Distribution of Each Variant in Both Cases and Controls**

<table>
<thead>
<tr>
<th>Position</th>
<th>Genotype</th>
<th>MM (Case/Ctr)</th>
<th>Mm (Case/Ctr)</th>
<th>mm (Case/Ctr)</th>
<th>Total (Case/Ctr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Japanese</td>
<td>Arg101&gt;His</td>
<td>118/108</td>
<td>63/75</td>
<td>14/12</td>
<td>195/195</td>
</tr>
<tr>
<td>(C/G&gt;C/A)</td>
<td>Ala213&gt;Val</td>
<td>0/0</td>
<td>2/1</td>
<td>192/194</td>
<td>193/195</td>
</tr>
<tr>
<td>(G/C&gt;G/T)</td>
<td>Glu264&gt;Val</td>
<td>194/195</td>
<td>0/0</td>
<td>0/0</td>
<td>194/195</td>
</tr>
<tr>
<td>Glu342&gt;Lys</td>
<td>195/195</td>
<td>0/0</td>
<td>0/0</td>
<td>195/195</td>
<td></td>
</tr>
<tr>
<td>(G/A&gt;G/G)</td>
<td>Glu376&gt;Asp</td>
<td>108/96</td>
<td>68/80</td>
<td>19/19</td>
<td>193/195</td>
</tr>
<tr>
<td>(G/A&gt;G/G)</td>
<td>Korean</td>
<td>Arg101&gt;His</td>
<td>115/59</td>
<td>61/31</td>
<td>8/4</td>
</tr>
<tr>
<td>Ala213&gt;Val</td>
<td>0/0</td>
<td>2/2</td>
<td>186/92</td>
<td>188/94</td>
<td></td>
</tr>
<tr>
<td>Glu264&gt;Val</td>
<td>188/94</td>
<td>0/0</td>
<td>0/0</td>
<td>188/94</td>
<td></td>
</tr>
<tr>
<td>Glu342&gt;Lys</td>
<td>188/94</td>
<td>0/0</td>
<td>0/0</td>
<td>188/94</td>
<td></td>
</tr>
<tr>
<td>Glu376&gt;Asp</td>
<td>97/55</td>
<td>77/33</td>
<td>14/6</td>
<td>188/94</td>
<td></td>
</tr>
</tbody>
</table>

MM indicates major (more frequent) alleles, homozygote; Mm, major and minor (less frequent) alleles, heterozygote; mm, minor alleles, homozygote; Ctr, control.

**TABLE 3. Estimated Allele Frequencies in Cases and Controls**

<table>
<thead>
<tr>
<th>Allele</th>
<th>Japanese</th>
<th>Korean</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
<td>390</td>
<td>378</td>
</tr>
<tr>
<td>M1Ala213</td>
<td>0</td>
<td>0.005</td>
</tr>
<tr>
<td>M1Val213</td>
<td>0.723</td>
<td>0.705</td>
</tr>
<tr>
<td>M2</td>
<td>0.225</td>
<td>0.198</td>
</tr>
<tr>
<td>M3</td>
<td>0.044</td>
<td>0.080</td>
</tr>
<tr>
<td>M4</td>
<td>0.005</td>
<td>0.012</td>
</tr>
<tr>
<td>S</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Z</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

No. indicates the total number of alleles used to calculate allele frequency.

Significant differences of genotype frequency were detected in any SNPs between the cases and controls. The genotype distributions agreed with those predicted by the Hardy–Weinberg equilibrium. The estimated allele frequencies in cases and controls are shown in Table 3. No significant differences were identified in the allelic frequencies between groups. S and Z alleles were not observed in this study.

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The inhibition of various proteolytic enzymes, such as neutrophil elastase by AAT, may have an important role in maintaining the integrity of connective tissue, including the blood vessel wall.6 A protease–antiprotease imbalance may have a pathogenetic role in the formation and rupture of IA. Acquired AAT deficiency, caused by the oxidation of methi-
online in the reactive center, might explain the increased risk of IA and SAH in smokers.6,11

Because the clinical characteristics of SAH are recognized equally in whites, Japanese, and Koreans, genetic factors for SAH may not differ among populations. No significant relationship of S and Z allele frequencies with SAH was reported recently in whites.2 Allelic variants resulting in AAT deficiency may not be associated with the development of IA.

References
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