A Deletion Polymorphism of $\alpha_2$-Macroglobulin Gene and Cerebral Amyloid Angiopathy

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Background and Purpose—$\alpha_2$-Macroglobulin may be implicated in amyloid $\beta$ protein deposition. A deletion in the exon 18 splice acceptor of the $\alpha_2$-macroglobulin gene (A2M) has been reported to be associated with risk for Alzheimer’s disease (AD). In search of genetic risk factors for cerebral amyloid angiopathy (CAA), we investigated association of the A2M deletion polymorphism with CAA.

Methods—The association between the severity of CAA and A2M deletion polymorphism was investigated in 178 autopsy cases of the elderly including 68 patients with AD.

Results—There was no significant difference in the severity of CAA between individuals with the A2M deletion allele and those without in the AD, non-AD, or total cases. Status for the $\epsilon4$ allele of the apolipoprotein E gene did not influence the results.

Conclusions—Our results suggest that the A2M deletion polymorphism may not be a definitive risk factor of CAA in the elderly, although further study with larger samples is necessary to confirm this. (Stroke. 1999;30:2277-2279.)

Key Words: Alzheimer’s disease ■ amyloid ■ cerebrovascular disorders ■ polymorphism (genetics)

Cerebral amyloid angiopathy (CAA) is commonly found in the elderly as well as in Alzheimer’s disease (AD) and is associated with intracerebral hemorrhage and other cerebrovascular disorders.1-3 Some genetic risk factors for AD have been reported to be associated with sporadic CAA. The $\epsilon4$ allele of the apolipoprotein E gene (apoE), an established risk of AD, has been suggested to be a risk of CAA,4,5 although this was not evident in some populations and the apoE $\epsilon2$ allele may be associated with CAA-related hemorrhage.6-8 We have reported that the polymorphisms in intron 8 of the presenilin-1 gene and in the signal peptide sequence of $\alpha_2$-antichymotrypsin may be associated with sporadic CAA.9,10 AD and CAA would share risk factors in the common pathogenetic process of amyloid $\beta$ protein (A$\beta$) deposition.

$\alpha_2$-Macroglobulin (A2M) is a major protease inhibitor and a ligand of the low-density lipoprotein receptor–related protein (LRP) as apoE. A2M accumulates on senile plaques11 and is implicated in binding, fibril formation, neurotoxicity, degradation, and clearance of A$\beta$.12-16 A2M complexes with and mediates the endocytosis of A$\beta$ through LRP.14 Internalization of A$\beta$ through LRP by cerebrovascular smooth muscle cells may be important to the pathogenesis of CAA.17 Recently, a deletion in the exon 18 splice acceptor of the $\alpha_2$M gene (A2M) has been reported to be associated with risk for AD.18 The association is independent of the effect of APOE $\epsilon4$ and its magnitude is comparable to the association of APOE $\epsilon4$ with AD,18 although biological consequences of the 5’ splice-site deletion in the exon 18 of A2M is unknown. Association of the A2M polymorphism with CAA has not been reported as yet.

In the present study, we investigated whether the A2M polymorphism is associated with the severity of CAA in elderly individuals.

Subjects and Methods

We studied 178 patients (age 62 to 104 years; mean ± SD, 85.8 ± 7.9 years), all Japanese, from the autopsy series at Yokuufukai Geriatric Hospital, Tokyo. The 178 patients included 68 patients with sporadic AD, in which the neuropathological findings satisfied the criteria of the Consortium to Establish a Registry for Alzheimer’s Disease19 and 110 subjects without AD or other neurodegenerative disorders. All the AD patients clinically showed dementia on the criteria of DSM III-R.20 There was no significant difference in the age at death between AD (86.4 ± 7.8 years) and non-AD groups (85.4 ± 7.9 years). No familial case of AD or CAA was included in this series.

Neuropathological examinations and assessment of the severity of CAA were performed as previously described.9,10 Briefly, congoophilic deposits with green birefringence under polarized light were identified as amyloid. With the use of a mouse monoclonal antibody
to Aβ,21 the cerebrovascular amyloid deposits were immunohistochemically confirmed to be Aβ. Four patients with severe CAA were found to have CAA-related cerebral lobar hemorrhage.

For evaluation of the severity of CAA, the number of amyloid-bearing vessels was counted for randomly chosen 100 meningeal and cortical vessels of the occipital lobe in each case (CAA count was equal to the percentage of the amyloid-laden vessels). The occipital lobe was most commonly affected with CAA in the elderly individuals, as shown in our previous study.2 The quantification was performed without knowledge of A2M and APOE genotypes. Severe vascular wall involvement by CAA was commonly found in patients with high CAA counts.

The A2M polymorphism was detected by the amplification-created restriction site method. Genomic DNA was isolated from the frozen brain tissue of all patients. A2M was amplified with primers described by Matthijs and Marynen.22 The amplification product (326 bp) was digested with Hph1 (BioLabs) and electrophoresed on a 2% agarose gel. The A2M polymorphism consists of 2 alleles, the normal allele without deletion (A2M-1) and mutant allele with the 5 nucleotide deletion (A2M-2). The A2M-1 allele was cleaved by Hph1 I to 2 fragments; the A2M-2 allele was not cleaved. The APOE genotype was also examined as reported previously.6

For statistical analyses, the CAA counts were compared between A2M genotypes in AD, non-AD, and total cases. Because the counts did not follow a normal distribution in any group, we used the Mann-Whitney test for comparison as a nonparametric test.

In our previous studies,2,3 intracerebral hemorrhage, a major complication of CAA, was found to be associated only with moderate or severe CAA (affected vessels ≥40%) but not with CAA of the lower degree. Therefore we decided in advance to compare frequencies of the A2M-2 allele between patients with severe or moderate CAA (affected vessels ≥40%) and those with slight or no CAA (affected vessels <40%). The χ² test was used for the comparison.

Statistical significance was defined as P<0.05. The statistical analyses were performed with the use of computer software (StatView 1-7.5, Abacus Concepts).

Results

Among the 178 patients examined, A2M-1/A2M-1 and A2M-1/A2M-2 genotypes were found in 160 and 18 individuals, respectively (0.05 in A2M-2 allele frequency). There was no case of A2M-2/A2M-2 genotype. The age did not differ significantly between the genotypes. The A2M genotype or allele frequencies were not significantly different between AD (0.02 in A2M-2 allele frequency) and non-AD subjects (0.07 in A2M-2 allele frequency), although there was strong association between AD and the APOE e4 allele in this population (P=0.0004). When the subjects were divided by their APOE e4 status, there was also no significant difference in the A2M genotype or allele frequencies between AD and non-AD cases (data not shown).

The average values (mean±SE) of the CAA counts in the A2M genotypes are shown in Table 1. There was no significant difference in the CAA counts between the A2M-1/A2M-2 (A2M-2 carriers) and A2M-1/A2M-1 genotypes (A2M-2 non-carriers). Further, when the subjects were divided by the status of the APOE e4, the A2M genotype was not significantly associated with the CAA counts (Table 1). In this population, the CAA counts in the AD group was significantly higher compared with the non-AD group (P<0.0001 (Table 1). The CAA counts in the APOE e4 carriers was higher than those in non-e4 carriers in the total cases (P=0.0154 (Table 1); within the AD or non-AD group, however, the CAA counts were not significantly different between the e4 carriers and non-e4 carriers.

Severe or moderate CAA was found in 22 (32.4%) of the 68 AD patients and in 16 (14.5%) of the 110 non-AD subjects (AD vs non-AD, P=0.0048 by χ² test). The A2M genotype or allele frequencies were not significantly different between severe or moderate CAA and slight or no CAA (Table 2).

Discussion

There was no significant association between A2M genotype and severity of CAA in the AD, non-AD, or total cases. Further, in the subgroups divided by the APOE e4 status, A2M genotype was not associated with severity of CAA. Our results with elderly Japanese subjects suggest that A2M polymorphism may not be a risk factor of CAA in the elderly. However, because the size of our sample is relatively small, further study with larger samples is necessary to rule out a statistical error. The low frequency of the A2M-2 allele in our

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### Table 1. Average CAA Counts (No. of Amyloid-Laden Vessels per 100 Vessels) (mean±SE) in A2M Genotype With APOE e4 Status

<table>
<thead>
<tr>
<th>A2M Genotype</th>
<th>AD (n=3)</th>
<th>Non-AD (n=65)</th>
<th>Total (n=68)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A2M-1/1-A2M-1</td>
<td>31.7±27.8</td>
<td>29.2±4.0</td>
<td>29.4±3.9*</td>
</tr>
<tr>
<td>A2M-2</td>
<td>18.1±7.8</td>
<td>10.8±2.6</td>
<td>11.8±2.5</td>
</tr>
<tr>
<td>A2M-1</td>
<td>20.4±7.7</td>
<td>18.3±2.3</td>
<td>18.5±2.2</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>APOE e4 status</th>
<th>With e4 (n=2)</th>
<th>Without e4 (n=42)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AD</td>
<td>26.5±25.5</td>
<td>23.4±4.8</td>
</tr>
<tr>
<td>Non-AD</td>
<td>19.6±8.3</td>
<td>16.5±2.6</td>
</tr>
</tbody>
</table>

*P<0.0001 (AD vs non-AD by Mann-Whitney U test). †P=0.0154 (e4[+] vs e4[−] by Mann-Whitney U test).

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### Table 2. A2M Genotypes and Alleles in Individuals With Severe or Moderate CAA (n=38) and Those With Slight or No CAA (n=140)

<table>
<thead>
<tr>
<th>A2M Genotype</th>
<th>A2M Allele</th>
<th>A2M Genotype</th>
<th>A2M Allele</th>
</tr>
</thead>
<tbody>
<tr>
<td>A2M-1/A2M-2 (n=18)</td>
<td>33 (87%)</td>
<td>A2M-1 (n=338)</td>
<td>71 (93%)</td>
</tr>
<tr>
<td>A2M-1/A2M-1 (n=160)</td>
<td>127 (91%)</td>
<td>A2M-2 (n=18)</td>
<td>5 (7%)</td>
</tr>
</tbody>
</table>

Severe or moderate CAA: 5 (13%) | 33 (87%) | P=0.48 | 71 (93%) | 5 (7%) | P=0.49

Slight or no CAA: 13 (9%) | 127 (91%) | 267 (95%) | 13 (5%)
sample population prevented analysis for the association of A2M-2/A2M-2 genotype with the severity of CAA.

Our study confirmed strong association of CAA with AD, as shown in many studies.1-5 Blacker et al18 reported that the A2M-2 allele was associated with AD as comparable to the association of APOE e4 with AD. In our study with pathologically confirmed patients, however, A2M-2 allele was not associated with AD irrespective of the APOE e4 status. It should be noted that the results by Blacker et al18 were from family-based association studies. Recent population-based case-control studies as well as family-based studies failed to replicate the association of the A2M-2 allele with AD,23-25 although a family-based study24 indicated significant but weaker associations than those observed by Blacker et al.18

As mentioned above, the low frequency of the A2M-2 allele in our Japanese population is remarkable compared with those in Europeans and Mediterraneans (0.18)23 and the United Kingdom (0.18).23 There is the possibility that association of the A2M-2 allele with CAA as well as AD may be different between different ethnic groups, requiring further study with larger samples from populations with different ethnic backgrounds.

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References

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