No Association of Paraoxonase Genotype or Atherosclerosis With Cerebral Amyloid Angiopathy

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Background and Purpose—Both cerebral amyloid angiopathy (CAA) and paraoxonase have been reported to be related to lipid metabolism and atherosclerosis. We investigated whether the paraoxonase gene (PON1) polymorphism and atherosclerosis are associated with risk of CAA.

Methods—Associations of the PON1 polymorphism and atherosclerosis of the aorta and coronary and cerebral arteries with the severity of CAA were investigated in 154 elderly Japanese individuals, including 47 patients with Alzheimer’s disease.

Results—The PON1 polymorphism or severity of atherosclerosis of the arteries was not associated with the severity of CAA.

Conclusions—The PON1 polymorphism and atherosclerosis would not appear to be associated with risk of CAA in the elderly, although further study with larger samples is necessary for confirmation. (Stroke. 2002;33:896-900.)

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

Cerebrovascular amyloid angiopathy (CAA) is cerebrovascular amyloid deposition and is related to intracerebral hemorrhage and other cerebrovascular disorders. Several cerebrovascular amyloid proteins have been identified, including amyloid β protein (Aβ), cystatin C, prion protein, transthyretin, gelsolin, and Abri; classification of CAA is based on the amyloid proteins (see review in Reference 2). Among the several types of CAA, sporadic CAA of the Aβ type is most commonly found in elderly individuals and in patients with Alzheimer’s disease (AD). Mutations in the genes encoding amyloid precursor proteins are associated with hereditary CAA; eg, a point mutation at codon 693 in the Aβ precursor protein is associated with hereditary cerebral hemorrhage with amyloidosis–Dutch type.

Although the pathomechanism underlying cerebrovascular amyloid deposition remains unclear, several lines of studies suggest that lipid metabolism and atherosclerosis may be implicated in pathogenesis of CAA. Cholesterol depletion has been shown to inhibit the generation of Aβ in hippocampal neurons, suggesting that cholesterol affects Aβ secretion from neurons, and a recent study with transgenic mice has provided evidence that a neuronal source of Aβ is sufficient to induce cerebrovascular amyloid deposition. It has been proposed that Aβ in the brain extracellular fluid or cerebrospinal fluid is bound to apolipoprotein E (ApoE) and that cerebrovascular smooth muscle cells internalize ApoE-Aβ complexes via a lipoprotein pathway, leading to accumulation of Aβ within lysosomes, followed by degeneration of the smooth muscle cells and deposition of Aβ within the extracellular space of the vessel wall. Genotype of the ApoE gene (APOE) may be associated with the development of CAA and CAA-related hemorrhage, although this association is not clear in some populations. One study indicates that atherosclerosis of cerebral arteries may be associated with severity of CAA. Thus, a common pathomechanism related to lipid metabolism may contribute to the development of both atherosclerosis and CAA. In addition, in the pathogenesis of AD, there may be interactions between ApoE, atherosclerosis, and cholesterol level, and recent studies have suggested that statins, cholesterol-lowering agents, may reduce the risk of AD.

The paraoxonase gene family contains at least 3 members, including PON1, PON2, and PON3, which are located on chromosome 7q21.3-22.1. Serum paraoxonase, a gene product of PON1, is believed to be synthesized by the liver. Serum paraoxonase hydrolyzes organophosphate insecticides and nerve gases such as sarin and is responsible for determining the toxicity of these compounds. Furthermore, paraoxonase is involved in lipid metabolism, especially phospholipid metabolism; it circulates on a subtraction of...
high-density lipoprotein (HDL), appears to use phospholipids on both low-density lipoprotein (LDL) and HDL particles as a physiological substrate, and has an antithromogenic effect by protecting lipoproteins against oxidative modification. Various diseases, including amyloidosis, are associated with decreased serum paraoxonase activities. Also appears to be an important determinant of the ability of heart disease and ischemic stroke in some populations, the A-type allele has been reported to be associated with coronary atherosclerosis.17–19 PON1 polymorphism and atherosclerosis are associated with the risk of sporadic AD, in which the neuropathological findings satisfied the criteria of the Consortium to Establish a Registry for Alzheimer’s Disease,20 and 107 subjects without AD or other neurodegenerative disorders. All AD patients clinically showed dementia on the basis of the criteria of American Psychiatric Association.21 There was no significant difference in age at death between the AD (84.6 ± 7.3 years) and non-AD (85.3 ± 7.9 years) groups. No familial case of AD or CAA was included in this series.

**Neuropathological Evaluation of CAA and Atherosclerosis**

Neuropathological examination and assessment of the severity of CAA were performed as previously described. Briefly, congophilic deposits with green birefringence under polarized light were identified as amyloid. With the use of a mouse monoclonal antibody to αβ, the cerebrovascular amyloid deposits were immunohistochemically confirmed to be αβ. 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 100 randomly chosen meningeal and cortical vessels of the occipital lobe in each case (CAA count is the percentage of the amyloid-laden vessels). The occipital lobe was most commonly affected by CAA in the elderly individuals, as shown in our previous study. Quantification was performed without knowledge of PON1 and APOE genotypes. Severe vascular wall involvement by CAA was commonly found in patients with high CAA counts.

Atherosclerosis of systemic arteries was evaluated as previously described. Briefly, aortic atherosclerosis was classified into 4 categories according to the percentage of surface area involvement with atherosclerotic lesions: score = 0 (none), 1 (<50%), 2 (50% to 70%), and 3 (>70%). Coronary atherosclerosis was scored as 4 classes by the severity of stenosis: score = 0 (none), 1 (<25% stenosis), 2 (25% to 75% stenosis), and 3 (>75% stenosis). For cerebral arteries, atherosclerosis of bilateral internal carotid arteries and the basilar artery was rated as in the evaluation of coronary atherosclerosis (score = 0 to 3), and the average score was calculated.

**Identification of the PON1 Polymorphism**

Genomic DNA was isolated from the frozen brain tissue of all patients and amplified by polymerase chain reaction as described by Humbert et al. The polymerase chain reaction product was digested with AlwI (New England Biolabs) and resolved in agarose gel. The Q allele (A-type allele) of the PON1 Q/R192 polymorphism was characterized by the single 99-bp fragment and the R allele (B-type allele) by 2 fragments of 69 and 30 bp. The APOE genotype was also examined as reported previously.

**Statistical Analyses**

CAA counts were compared between PON1 genotypes (A/A, A/B, and B/B) in AD, non-AD, and total cases. Because the counts did not

**TABLE 1. Average CAA Counts in the PON1 Genotype With APOE ε4 Status**

<table>
<thead>
<tr>
<th>PON1 Genotype</th>
<th>A/A</th>
<th>A/B</th>
<th>B/B</th>
<th>Total</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>10.2 ± 3.6 (33)</td>
<td>20.2 ± 3.3 (92)</td>
<td>16.6 ± 5.0 (29)</td>
<td>17.3 ± 2.3 (154)</td>
<td>NS</td>
</tr>
<tr>
<td>AD or non-AD</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AD</td>
<td>19.4 ± 8.3 (11)</td>
<td>38.2 ± 6.5 (28)</td>
<td>35.3 ± 12.0 (8)</td>
<td>33.3 ± 4.8† (47)</td>
<td>NS</td>
</tr>
<tr>
<td>Non-AD</td>
<td>5.6 ± 3.2 (22)</td>
<td>12.3 ± 3.3 (64)</td>
<td>9.5 ± 4.5 (21)</td>
<td>10.4 ± 2.3 (107)</td>
<td>NS</td>
</tr>
<tr>
<td>APOE ε4 status</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>With ε4</td>
<td>12.6 ± 9.0 (9)</td>
<td>27.0 ± 6.7 (22)</td>
<td>15.0 ± 13.5 (3)</td>
<td>22.1 ± 5.1‡ (34)</td>
<td>NS</td>
</tr>
<tr>
<td>Without ε4</td>
<td>9.3 ± 3.8 (24)</td>
<td>18.0 ± 3.7 (70)</td>
<td>16.8 ± 3.4 (26)</td>
<td>16.0 ± 2.6 (120)</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are mean ± SD. Values in parentheses are numbers.

*Statistical difference in CAA counts between the PON1 genotypes by the Kruskal-Wallis test.
†P < 0.0001 (AD vs non-AD) by the Mann-Whitney U test.
‡P = 0.0541 [ε4(+) vs ε4(–)] by the Mann-Whitney U test.
follow a normal distribution in any group, we used the Kruskal-Wallis test for the comparison as a nonparametric test. Similar analyses were performed in the subgroups divided by APOE ε4 status. We also used the Mann-Whitney U test to compare CAA counts between carriers of the PON1 A allele (A carriers) and non-A carriers and between B carriers and non-B carriers.

Associations between the aortic and coronary atherosclerosis scores and CAA counts were analyzed with the Spearman’s rank correlation test.

In our previous studies, 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 a lesser degree. Therefore, we decided in advance to compare the PON1 polymorphism and severity of atherosclerosis between patients with moderate or severe CAA (affected vessels ≥40%) with those with no or slight CAA (affected vessels <40%). The comparison was made by use of the χ² test for frequencies and Mann-Whitney U test for values.

Statistical significance was defined as P<0.05. Statistical analyses were performed with the computer software StatView J-7.5 (Abacus Concepts).

### Results

Among the 154 patients examined, A/A, A/B, and B/B genotypes of the PON1 polymorphism were found in 33, 92, and 29 individuals, respectively (0.51 in A-type allele frequency and 0.49 in B-type allele frequency). Age did not differ significantly between genotypes. The PON1 genotype or allele frequencies were not significantly different between AD (0.53 in A-type allele frequency and 0.47 in B-type allele frequency) and non-AD (0.50 in A-type allele frequency and 0.50 in B-type allele frequency) subjects, as we previously reported in a smaller number of samples, although there was a strong association between AD and the APOE ε4 allele in this population (P=0.0004). When subjects were divided by their APOE ε4 status, there was also no significant difference in the PON1 genotype or allele frequencies between AD and non-AD cases (data not shown).

Average values of the CAA counts in the PON1 genotypes are shown in Table 1. There was no significant difference in CAA counts between the PON1 genotypes (A/A, A/B, and B/B) in the total, AD, or non-AD patients (Table 1). There was also no significant difference between A carriers (A/A and A/B) and non-A carriers (B/B) or between B carriers (B/B and A/B) and non-B carriers (A/A). Furthermore, when subjects were divided by status of APOE ε4, the PON1 genotype was not significantly associated with CAA counts (Table 1). In this population, the CAA count in the AD group was higher than in the non-AD group (P<0.0001; Table 1). CAA counts in the APOE ε4 carriers tended to be higher than

### TABLE 2. Comparison of the Diagnosis of AD, PON1 and APOE Genotypes, and Atherosclerosis Between No or Slight CAA and Moderate or Severe CAA

<table>
<thead>
<tr>
<th>Severity of CAA</th>
<th>No or Slight (n=122)</th>
<th>Moderate or Severe (n=32)</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>85.0±8.1</td>
<td>85.4±5.7</td>
<td>NS</td>
</tr>
<tr>
<td>Diagnosis of AD, frequency</td>
<td>0.24</td>
<td>0.56</td>
<td>0.0009</td>
</tr>
<tr>
<td>PON1 genotype, frequency</td>
<td>A-type allele</td>
<td>0.52</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>B-type allele</td>
<td>0.48</td>
<td>NS</td>
</tr>
<tr>
<td>APOE genotype, frequency</td>
<td>ε4 Allele</td>
<td>0.20</td>
<td>NS</td>
</tr>
<tr>
<td>Atherosclerosis scores†</td>
<td>Aorta</td>
<td>2.3±0.7</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Coronary artery</td>
<td>1.7±0.9</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Cerebral arteries</td>
<td>1.9±0.9</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are mean±SD when appropriate.

*Statistical difference by the Mann-Whitney U test (for values) or χ² tests (for frequencies).
†See text for scoring of atherosclerosis in the aorta and coronary and cerebral arteries.
Discussion

Our results show no association between severity of CAA and the PON1 polymorphism or atherosclerosis scores of the systemic arteries, suggesting that the PON1 polymorphism and atherosclerosis are not definitive risk factors for CAA in elderly individuals.

Regarding the PON1 polymorphism and CAA, the possibility of a type II error as a result of insufficient statistical power cannot be ruled out; thus, further study with larger samples is required. In addition, our results may be influenced by the character of our sample population; our samples were obtained from autopsy series of a large geriatric hospital. Ages of the participants were relatively high, and dementia was severe in most AD patients.

Our results are not consistent with a previous report by Ellis and colleagues\(^1\) that high CAA scores correlated with the presence of the atherosclerosis. There are several possible explanations for why our results did not support the positive association between CAA and atherosclerosis. The first is that the methods used to evaluate CAA and atherosclerosis are different between the previous report\(^1\) and this study. In the previous report,\(^1\) the severity of CAA was evaluated semiquantitatively as scores ranging from 0 to 3, and atherosclerosis was rated as none to mild or moderate to severe. The second is the possibility that the association of atherosclerosis with CAA may be different among the sample populations with different ethnic backgrounds and dietary habits. In the previous study, the patients were selected from the US population,\(^1\) whereas all our samples were obtained from Japanese individuals. The third explanation is that the previous study selected only AD patients (n=117),\(^1\) whereas our study included both AD (n=47) and non-AD patients (n=107). The fourth is that our patients seem to be older than those in the previous study.\(^1\)

Further studies are necessary to elucidate a possible role of lipid metabolism and atherosclerosis in CAA. They should include analyses of profiles of lipids, lipoproteins, and apolipoproteins using appropriate cerebrospinal fluid and plasma samples in relation to CAA because lipid metabolism within the brain is separated by the blood-brain barrier and is independent of that in the systemic circulation. With regard to relationships between arteriosclerotic changes and CAA, changes in smaller cerebral arteries should be investigated further. In addition, to elucidate a role of arteriosclerotic factors in the development of CAA-related cerebrovascular disorders, a large number of CAA cases complicated with cerebral hemorrhage and other cerebrovascular disorders should be included in further studies.

Acknowledgments

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References

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