Butyrylcholinesterase K Variant and Cerebral Amyloid Angiopathy

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Background and Purpose—Cholinesterases are found histochemically in the vessels affected with cerebral amyloid angiopathy (CAA). A gene for the K variant of butyrylcholinesterase (BCHE-K) may be associated with late-onset Alzheimer’s disease (AD). In search of genetic risk factors for CAA, we investigated the association of BCHE-K with CAA.

Methods—The association between the severity of CAA and BCHE-K was investigated in 155 autopsy cases of the elderly, including 48 patients with AD.

Results—There was no significant association of BCHE-K with the severity of CAA in the total, AD, or non-AD cases. Status of the ε4 allele of apolipoprotein E gene did not influence the results.

Conclusions—Our results may suggest that BCHE-K is not a definitive risk factor for CAA in the elderly, although further study with larger samples is necessary to confirm this. (Stroke. 1998;29:2488-2490.)

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

Cerebral amyloid angiopathy (CAA) is commonly found in the elderly as well as 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 CAA. The ε4 allele of the apolipoprotein E (apoE) gene (APOE), an established risk factor for AD, has been suggested to be a risk factor for CAA,4,5 although this was not evident in some populations, and the APOE ε2 allele may be associated with CAA-related hemorrhage.6-8 We have recently reported that the polymorphism in the intron 8 of the presenilin-1 gene and in the signal peptide sequence of α1-antichymotrypsin may be associated with sporadic CAA.9,10 AD and CAA would share risk factors in the common pathogenetic process of amyloid β protein (Aβ) deposition.

Vessels affected with CAA as well as senile plaques and neurofibrillary tangles histochemically show intense acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) activities.11-14 Although the origin of the cholinesterases observed in these lesions remains unclear, it has been suggested that the cholinesterases are associated with proteolytic activity that may participate in amyloidogenic processing of the amyloid precursor protein and may play a pathogenetic role in development of Alzheimer-type pathological changes.14 AChE accelerates assembly of Aβ to fibrils.15 BChE activity in the brain increases with age and in AD.16

The gene for BChE (BCHE) is located on the long arm of chromosome 3 at q26, and there are several genetic variants.17 The K variant of BCHE (BCHE-K) is associated with a point mutation at nucleotide 1615 (GCA to ACA), which changes alanine at amino acid 539 to threonine.17 A 30% reduction of serum BChE activity is associated with this mutation.17 The additional threonine residue at amino acid 539 of the K variant has a high propensity for β-sheet formation, which may be related to amyloidogenesis.18-20 It has been recently reported that BCHE-K is associated with late-onset AD in carriers of the APOE ε4 allele.20 Although mechanisms underlying this association remain unclear, the hypothesis has been proposed that BChE and apoE may interact and that this interaction is influenced by their allelic variants.20

In the present study we investigated whether BCHE-K is associated with the severity of CAA.

Methods

We studied 155 patients (age, 62 to 103 years; mean±SD, 85.1±7.7 years), all Japanese, from the autopsy series at Yokufukai Geriatric Hospital, Tokyo, Japan.10 The 155 patients included 48 patients with sporadic AD, in which the neuropathologic findings satisfied the criteria of the Consortium to Establish a Registry for Alzheimer’s Disease,21 and 107 subjects without AD or other neurodegenerative disorders. There was no significant difference in the age at death between AD (84.6±7.3 years) and non-AD (85.3±7.9 years) groups.

Neuropathologic examinations and assessment of the severity of CAA were performed as previously described.3,9,10 Briefly, congo-
philic deposits with green birefringence under polarized light were identified as amyloid. For 16 patients with severe CAA, the cerebrovascular amyloid deposits were immunohistochimically confirmed to be Aβ. Two patients with severe CAA were found to have CAA-related 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=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 BCHE and APOE genotypes. The CAA counts were almost parallel with the severity of the vascular wall involvement by CAA.

BCHE-K was analyzed as described by Jensen et al.22 Briefly, genomic DNA was isolated from the frozen brain tissue of all patients. For detecting the K variant by the amplification-creation restriction site method, a K allele was amplified with the normal and modified primer designed to create an MboIII restriction site. The amplification product was digested with MboIII and electrophoresed on a 2% agarose gel. The K allele was cleaved by MboIII into 2 fragments of 22 bp and 115 bp; the normal (N) allele was not cleaved (137 bp). The BCHE-K allele was also examined as reported previously.6

For statistical analyses, the CAA counts were compared between BCHE genotypes (KK, KN, and NN) and between BCHE-K carriers and noncarriers in AD, non-AD, and total cases. Since the counts did not follow a normal distribution in any group, we used the Kruskal-Wallis test for the comparison between BCHE genotypes and the Mann-Whitney U test for the comparison between BCHE-K carriers and noncarriers as nonparametric tests. Furthermore, correlations between the number of the K allele and the CAA counts were examined by the Kruskal-Wallis test. When Spearman’s rank correlation was applied, no significant correlation was present between the K allele frequency and the CAA counts in the AD group, non-AD group, or total cases. In addition, no significant difference was found in the CAA counts between the K allele frequency and the CAA counts when examined by the Kruskal-Wallis test. When Spearman’s rank correlation was applied, no significant correlation was present between the K allele frequency and the CAA counts in the AD group, non-AD group, or total cases.

Results

BCHE-K and AD
Among the 155 patients examined, BCHE KK, KN, and NN genotypes were found in 4, 45, and 106 individuals, respectively (KK 0.03, KN 0.29, and NN 0.68 in genotype frequency; K 0.17 and N 0.83 in allele frequency). The age did not differ significantly between the genotypes in AD, non-AD, or total cases (data not shown). The BCHE genotype or allele frequencies were not significantly different between AD (KK 0.00, KN 0.31, and NN 0.69 in genotype frequency; K 0.16 and N 0.84 in allele frequency) and non-AD subjects (KK 0.04, KN 0.28, and NN 0.68 in genotype frequency; K 0.18 and N 0.82 in allele frequency).

The frequency of APOE e4 allele was significantly higher in AD (0.21) compared with non-AD subjects (0.08) (P=0.0012 by χ² test). When the subjects were divided by their APOE e4 status, there was also no significant difference in the BCHE genotype or allele frequencies between AD and non-AD cases (data not shown).

In addition, the analyses in the subgroup aged ≥75 years showed no association of BCHE-K with AD in any APOE status (data not shown).

BCHE-K and CAA
The average values (mean±SE) of the CAA counts in the BCHE genotypes are shown in the Table. There was no significant difference in the CAA counts between the BCHE KK, KN, and NN genotypes in total, AD, or non-AD cases when examined by the Kruskal-Wallis test. When Spearman’s rank correlation was applied, no significant correlation was present between the K allele frequency and the CAA counts in the AD group, non-AD group, or total cases. In addition, no significant difference was found in the CAA counts between BCHE-K carriers and noncarriers by Mann-Whitney U test. Furthermore, when the subjects were divided by the status of the APOE e4, the BCHE-K was not significantly associated with the CAA counts (Table). In this

### Average CAA Counts (Number of Amyloid-Laden Vessels per 100 Vessels) in the BCHE Genotype with APOE ε4 Status

<table>
<thead>
<tr>
<th>BCHE Genotype</th>
<th>BCHE-K Allele</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>K+</td>
<td>–</td>
</tr>
<tr>
<td>KK</td>
<td>32.8±9.0 (n=0)</td>
<td>34.7±5.7 (n=33)</td>
</tr>
<tr>
<td>KN</td>
<td>10.0±10.0 (n=4)</td>
<td>13.8±5.2 (n=30)</td>
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<tr>
<td>NN</td>
<td>10.0±10.0 (n=4)</td>
<td>20.1±4.7 (n=45)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>APOE ε4 status</th>
<th>With ε4</th>
<th>Without ε4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>K+</td>
<td>–</td>
</tr>
<tr>
<td>KK</td>
<td>20.5±8.9 (n=0)</td>
<td>22.0±6.2 (n=11)</td>
</tr>
<tr>
<td>NN</td>
<td>10.0±10.0 (n=4)</td>
<td>20.0±5.6 (n=34)</td>
</tr>
</tbody>
</table>

Values are mean±SE.
P<0.001 (AD vs non-AD by Mann-Whitney U test).
P=0.065 (ε4+ vs ε4− by Mann-Whitney U test).

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population, the CAA counts in the AD group was significantly higher than those in the non-AD group \( (P<0.0001) \); the CAA counts in the APOE e4 carriers tended to be higher than those in non-e4 carriers, but this was not significant \( (P=0.065) \) (Table).

Severe or moderate CAA was found in 18 of the 48 AD patients (37.5\%) and in 13 of the 107 non-AD subjects (12.1\%) \( (AD \text{ versus non-AD, } P=0.0003) \) by \( \chi^2 \) test. The BCHE genotype or allele frequencies were not significantly different between severe or moderate CAA (KK 0.33, KN 0.65 in genotype frequency; K 0.19 and N 0.81 in allele frequency) and slight or no CAA (KK 0.02, KN 0.28, and NN 0.69 in genotype frequency; K 0.17 and N 0.83 in allele frequency).

Analyses in the subgroup aged >75 years also showed no significant association of BCHE-K with severity of CAA.

Discussion

CAA is strongly associated with AD,\(^1\) as confirmed in this study. Lehmann et al\(^2\) first reported that BCHE-K was associated with AD and modified the effect of APOE e4 on AD. In our population, BCHE-K was not associated with AD, irrespective of the APOE e4 status. Another recent study also showed a negative association between BCHE-K and pathologically confirmed AD.\(^3\) One possible explanation for the difference of the results is that BCHE-K locus may be in linkage disequilibrium with the relevant variability in BCHE or other adjacent gene on chromosome 3 in some populations, but not in others.

In our study of CAA, there was no significant association between BCHE-K and severity of CAA in the AD, non-AD, or total cases. Analyses in the subgroup aged >75 years also showed no association between BCHE-K and CAA. Furthermore, in the subgroups divided by the APOE e4 status, BCHE-K was not associated with severity of CAA. Our results with elderly Japanese subjects may suggest that BCHE-K is not a definitive risk factor for CAA in the elderly. However, since the size of our sample is relatively small, further study with larger samples is necessary to rule out a type II error; the possibility cannot be ruled out that the KK carriers tend to be higher than that in the elderly white controls of the Oxford study by Lehmann et al \( (0.17) \). \(^{20} \) There is the possibility that the association of BCHE-K 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.

Acknowledgments

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References

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