SHORT REPORT

Failure to confirm a synergistic effect between the K-variant of the butyrylcholinesterase gene and the ϵ 4 allele of the apolipoprotein E gene in Japanese patients with Alzheimer's disease

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Abstract

To confirm a synergistic effect between the polymorphic K variant of the butyrylcholinesterase (BChE-K) gene and the ϵ 4 allele of the apolipoprotein E (APOE) gene in Alzheimer's disease, the frequency of the BChE-K allele was re-examined in a large series of Japanese patients with Alzheimer's disease and controls. Two hundred and three patients with Alzheimer's disease and 288 age and sex matched controls were genotyped by polymerase chain reaction and restriction fragment length polymorphism for BChE-K and APOE. No changes were found in the frequency of BChE-K, either in the Alzheimer's disease group as a whole (0.17 v 0.14; $p=0.36$) or in early (0.16 v 0.16; $p=0.98$) or late (0.17 v 0.13; $p=0.24$) onset patients compared with age matched controls. The study failed to confirm the findings of a previous study which found a significantly higher incidence of BChE-K in patients with Alzheimer's disease with APOE ϵ 4 allele than in controls. In the Japanese population studied here, there was no association between BChE-K and Alzheimer's disease, nor an interaction between BChE-K and APOE ϵ 4 allele.

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At present, the function of butyrylcholinesterase (BChE) is unclear. Its activity, however, is known to increase with age and to be higher in patients with Alzheimer's disease.^{1,2} The normal cerebral cortex contains very little BChE, most of which is located in deep cortical neurons and neuroglia.³ Histochemically reactive BChE is associated with amyloid plaques and neurofibrillary tangles and with amyloid angiopathy in Alzheimer's disease³⁻⁸ and the expression of BChE increases substantially in Alzheimer's disease.⁹ Furthermore, BChE becomes associated with amyloid plaques at approximately the same time that the A β

deposit assumes a compact β -pleated conformation.³

There are at least seven genetic variants of the BChE gene¹⁰⁻¹² with BChE-K being the most common.¹³ BChE-K arises from a guanine to adenine substitution at nucleotide 1615 and causes an Ala539Thr missense substitution which causes a 30% reduction in BChE catalytic activity.¹⁴

Recently, BChE-K has been reported to show an allelic association with Alzheimer's disease in subjects who are also carriers of the ϵ 4 allele of apolipoprotein E (APOE), especially in subjects over the age of 75 years.¹⁵ According to this study, the BChE-K genotype increased the risk of developing Alzheimer's disease about sevenfold for those with one APOE ϵ 4 copy and about 13-fold beyond the risk associated with the APOE ϵ 4 allele alone for those with two APOE ϵ 4 copies. However, this finding was not confirmed in subsequent studies.¹⁶⁻¹⁹ To further clarify this association between BChE-K and Alzheimer's disease, we have genotyped a large series of Japanese patients with Alzheimer's disease for the BChE-K variant and APOE ϵ 4 allele.

Methods

Patients with Alzheimer's disease were recruited from those who were consecutively admitted to Hyogo Institute for Aging Brain and Cognitive Disorders (HI-ABCD) for examination between January 1995 and December 1997. All patients were examined by both neurologists and psychiatrists during an admission of more than 1 month and were given routine laboratory tests, standard neuropsychological examinations, EEG, MRI of the brain, MR angiography of the neck and head, and cerebral perfusion and metabolism studies by PET or single photon emission tomography (SPECT).²⁰

The control group comprised subjects from three sources: 768 subjects between 21 and 90 years of age in the HI-ABCD established population for sex difference study, who had been genotyped for APOE as previously described.²¹ Seventy two subjects between 23 and 80 years of age were volunteers enrolled at

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Proportions of BChE-K allele frequencies in patients with Alzheimer's disease (AD) and control subjects

	All <65 years	APOE ε4 carriers < 65 years	All ≥ 65 years	APOE ε4 carriers ≥ 65 years	All > 75 years	APOE ε4 carriers > 75 years
Controls	28/176 (15.9%)	9/46 (19.6%)	52/400 (13.0%)	10/82 (12.2%)	10/108 (9.3%)	1/12 (8.3%)
AD	17/108 (15.7%)	6/50 (12.0%)	52/298 (17.4%)	31/202 (15.3%)	13/70 (18.6%)	5/42 (11.9%)
Significance	NS	NS	NS	NS	NS	NS

NS = not significant.

the HI-ABCD for a PET study from the community.²⁰ Finally, 37 elderly subjects ranging in age from 70 to 93 years were recruited from a nursing home. These subjects had complete neurological and medical examinations that showed that they were free of significant illness and had mini mental state examination²² scores >28 points. Patients were selected on the basis of having a diagnosis of probable Alzheimer's disease according to the criteria of NINCDS/ADRDA.¹⁹ Another criterion for selection of both patients and controls was the availability of a blood sample.

Before beginning this study, informed consent was obtained for all patients and from all volunteers according to the Declaration of Human Rights, Helsinki, 1975. Subjects were genotyped as previously described—by polymerase chain reaction methods using blood samples—for BChE-K¹³ and APOE.²³ Analyses were conducted using SAS Release 6.10 software (SAS Institute Inc). We classified the patient groups by age of onset (early onset < 65 years, late onset ≥ 65 years) to see if age of onset was confounding a potential genetic association. We also classified the data on the basis of the presence or absence of copies of the APOE ε4 allele and BChE-K genotype. The χ^2 test for homogeneity was used to test for differences in gene and allele frequencies between patients and controls. Significance was adjudged to be at the 5% level. To examine whether the BChE genotype predicts Alzheimer's disease, we used a multiple logistic regression analysis, with diagnosis (Alzheimer's disease *v* control) as a dependent variable and the BChE and APOE genotypes as independent variables.

Results

We examined 491 Japanese patients, of which 203 had Alzheimer's disease (mean age at examination (SD) = 68.2 (8.5) years; 149 were late onset, and 54 were early onset) and 288 were controls (mean age (SD) = 68.5 (7.4) years). There was a significantly higher frequency of the APOE ε4 allele in the Alzheimer's disease group compared with controls (36.9% *v* 11.6%; $p < 0.0001$). However, we found no significant difference in the frequency of BChE-K between the Alzheimer's disease groups tested and the age matched control groups (17.0% *v* 13.9%; $p = 0.36$). The table shows that there was also no significant difference in BChE-K allele frequency between cases and controls even when each subgroup was classified by age of 65 or 75 years as the cut off. Because APOE ε4 is a known risk factor for late onset Alzheimer's disease which may confound a BChE association or interact with BChE, these groups were then classified

according to the APOE ε4 allele frequency and compared with age matched APOE ε4 load matched controls. No significant difference was found between any of these APOE ε4 classified Alzheimer's disease groups and their respective control groups (table). A previous report¹⁵ suggested that the association with BChE-K and Alzheimer's disease, and the synergistic interaction with APOE ε4 were enhanced in patients over the age of 75. We did not find an association in this age group in any of the analyses presented (table). A logistic regression analysis disclosed that there was no effect of the BChE-K allele on predicting Alzheimer's disease when the effects of APOE ε4 allele, age, and sex were adjusted (odds ratios 1.30, 95% confidence interval 0.84–2.02, $p = 0.24$).

Discussion

Lehmann *et al*¹⁵ recently suggested that a common polymorphism in the BChE-K allele modifies the Alzheimer's disease risk associated with the APOE ε4 allele. The results of the present study on patients with early and late onset Alzheimer's disease fail to confirm these findings: in our population the BChE-K allele did not interact with the APOE gene to modify the Alzheimer's disease risk associated with the ε4 allele. In agreement with our findings, some reports^{16–19} found no association between APOE and BChE-K polymorphisms in Alzheimer's disease. However, the race of the subjects (generally white) or the communities (Florida, USA; the United Kingdom) investigated in these studies were very different from those in our study.

One of the central findings in Alzheimer's disease research is the reduction of acetylcholine and the dysfunction and loss of central cholinergic neurons from the basal forebrain.²⁴ The loss of cholinergic neurons is thought to underlie some of the cognitive deficits in Alzheimer's disease. The findings of a genetic link between the cholinergic system and a tendency to develop Alzheimer's disease therefore seemed promising.¹⁵

It is difficult, however, to envision a role for BChE-K in the aetiology of Alzheimer's disease. BChE-K has 30% less catalytic activity than normal BChE¹⁴ and would therefore be expected to lead to increased concentrations of acetylcholine in the brains of patients with Alzheimer's disease. Furthermore, in Alzheimer's disease, cortical BChE increases dramatically,¹ and all histopathological markers of Alzheimer's disease, including amyloid plaques, neurofibrillary tangles, and vessels with amyloid angiopathy, express BChE enzyme activity.^{4 5 9 25 26} Incubation of synthetic Aβ with low doses of BChE blocks aggregation of the Aβ into large fibrils.²⁵

Although our data confirm the well established association between APOE $\epsilon 4$ and Alzheimer's disease, our results do not support the notion that the BChE-K is a risk factor for Alzheimer's disease either independently or in association with APOE $\epsilon 4$. The explanation for the discrepancy between our data and the results of Lehmann *et al* is not entirely clear. However, the differences in sampling strategy, ethnic origins, or other factors might reflect a true genetic difference between the populations. For example, the BChE-K allele frequency in Japanese control subjects (0.14) was considerably higher than that in white control groups (0.09),¹⁵ although four other studies of normal subjects have shown BChE-K frequencies (0.128–0.198) similar to those reported here.^{13–16–18}

In summary, the BChE-K allele might be weakly linked disequilibrium with a causative sequence change in another nearby gene or there might exist another stronger genetic influence on late onset Alzheimer's disease acting in concert with the APOE $\epsilon 4$ allele that masks the effect of BChE-K.

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