The Study of Synergy between the *BchE-k* Variant and the *ApoE* Gene in the Alzheimer Dementia of the Korean Population

Eun-Sim Shin[1], Song-Ro Yoon[1], Soo-Kyung Choi[2] and Soo-Young Kim[3]

The Apolipoprotein E type 4 allele (*ApoE* ε4) is genetically associated with the common late onset familial and sporadic forms of Alzheimer's disease. The *BchE-k* variant, which is the common variant of the *BchE* gene, has been reported to show allelic association with AD in subjects who are also carriers of the ε4 allele of the *ApoE*, especially in subjects over the age of 75. This study was performed to evaluate the distribution of the *ApoE* and the *BchE* genotypes in the healthy and AD groups and to evaluate the synergy between the *BchE-k* variant and the *ApoE* ε4 in AD. The *ApoE* and the *BchE* genotypes were determined in DNA samples from 610 healthy people and 60 LOAD patients by using ARMS by standard agarose gel electrophoresis. The effect of the *ApoE* ε4 was closely related to AD($p<0.05$). A comparison between the AD patients and the healthy individuals, both with the ε4 allele, indicated an interaction between the *BchE-k* and the *ApoE* ε4($p<0.05$). The association of the *BchE-k* with AD was limited to carriers of the *ApoE* ε4 allele, among whom the presence of the *BchE-k* gave an odds ratio of AD 3.48 (95% C.I. 1.3-9.2). Therefore, these results suggested that further evidence of an association between the *ApoE* ε4 and LOAD, and the *BchE-k* acts in synergy with the *ApoE* ε4 as a susceptibility gene for AD.

Key word : Alzheimer dementia, *ApoE* genotyping, Butyrylcholinestesrase k variant

INTRODUCTION

Alzheimer's disease (AD) is the leading cause of dementia among the elderly (Breteler et al., 1992). Apolipoprotein E (ApoE) is associated with AD neurofibrillary tangles and β-amyloid protein in senile plaques. The *ApoE* gene is located on the chromosome 19q13.2 (Pericak-Vance et al., 1991), and codes for a mature protein of 299 amino acids. The three common alleles in humans are distinguished by amino acid changes at position 112 and 158 (Mahley, 1988). The *ApoE* is defined by three alleles, ε2, ε3, ε4. This polymorphism results in six possible ApoE phenotypes, E2/2, E2/3, E2/4, E3/3, E3/4 and E4/4. The *ApoE* ε4 allele has been associated with AD at a very higher rate (Strittmatter *et al.*, 1993). The *ApoE* was examined as a candidate for familial and sporadic Late-Onset Alzheimer's Disease (LOAD) and the ε4 allele was associated with increased AD risk in a dose-dependent fashion (Strittmatter *et al.*, 1993 ; Corder *et al.*, 1993 ; Saunders *et al.*, 1993). Initial estimates suggested that individuals with one copy of the *ApoE* ε4 had a three-fold risk of AD, while the *ApoE* ε4 homozygotes have an eight-fold risk compared to non-*ApoE* ε4 genotypes (Corder *et al.*, 1993).

Recently, LOAD has also been thought to be associated with the k variant of the butyrylcholinesterase gene situated in the chromosome 3 (Lehmann *et al.*, 1997 ; Jensen *et al.*, 1996). The k variant arised from a G→A substitution at nucleotide 1615 causes an Ala539Thr missense substitution which reduces the catalytic activity of the BchE enzyme (Bartels *et al.*, 1992). The BchE activity in the brain increases at age 60 and is elevated in AD (Perry *et al.*, 1978 ; Perry *et al.*, 1980). The association of the *BchE-k* with LOAD was limited to carriers of the ε4 allele of the *ApoE* gene, and the *BchE-k* is thought to act in synergy with the *ApoE* ε4 as a susceptibility gene for LOAD (Lehmann *et al.*, 1997).

1 Genetic Research Laboratory, Hyundae Medical Institute, Seoul
2 Department of Molecular Biology, University of Southern California, CA, USA
3 Department of Biology, Konkuk University, Seoul
Correspondence: Eun-Sim Shin, Genetic Research Lab., Hyundai Medical Laboratory, Seoul 133 - 170, Korea, Tel: 82-2-2210-1358, Fax: 82-2-2210-1359

Table 1. The frequencies of the *ApoE* genotypes and the allele distribution of healthy and AD groups shown in percentage.

Groups	Genotype frequencies						Allele frequencies		
	ε 2/2	ε 2/3	ε 2/4	ε 3/3	ε 3/4	ε 4/4	ε 2	ε 3	ε 4
H^b (n^b=610)	1	11	1	73	13	1	7	85	8
AM^c (n^a=68)	1	16	3	68	12	0	11	82	7
AD^d (n^a=60)	0	13	8	47	18	14	11	62	27

n^a: Number of Subjects, H^b: Healthy Group, AM^c: Age-Matched Healthy Group
AD^d: Alzheimer Dementia Patient Group.

Table 2. The frequencies of the *BchE* genotypes and the allele distribution of healthy and AD groups shown in percentage.

Groups	Genotype frequencies			Allele frequencies	
	w/w^c	w/k^d	k/k^e	w	k
Healthy Group (n^a=94)	83	16	1	91	9
AD^b Group (n^a=24)	58	38	4	77	23

n^a: Number of Subjects, AD^b: Alzheimer Dementia Patient Group,
w/w^c: *BchE* genotype is wild type, w/k^d: *BchE* genotype is wild & k-variant type,
k/k^e : *BchE* genotype is k-variant type.

In this study, we examined the *ApoE* genotypes and the *BchE* genotypes in healthy and AD patient groups from Korean population to evaluate the relationship between the *ApoE* ε4 allele and AD, and the association between the *BchE-k* variant and AD.

MATERIALS AND METHODS

Subjects and DNA preparation

60 subjects who were clinically diagnosed to have AD, were studied. Controls for this analysis were 610 unrelated subjects. Genomic DNA was isolated from peripheral blood of all subjects by the Blin and Sambrook *et al.* method.

ApoE genotyping by ARMS analysis

The Amplification Refractory Mutation System (ARMS) was applied to detect the *ApoE* genotypes in human DNA as described (Wenham *et al.*, 1991). By using four allele-specific oligonucleotide primers and one common primer, the three common alleles of the *ApoE* genetic polymorphism were identified by standard agarose gel electrophoresis.

BchE genotyping by Allele-Specific Amplification

The *BchE* genotypes were detected as wild-type and k-variant types by using Allele-Specific oligonucleotides (ASOs) and one common primer, were determined by standard agarose gel electrophoresis.

RESULTS

The Relationship between *ApoE* genotypes and AD

The *ApoE* genotyping was performed on 60 AD subjects and 610 healthy controls. The frequency of alleles and genotypes were shown in Table 1. As shown in Fig. 1, a 198 base pair fragment was amplified with the *ApoE* ASOs primers and a 436 base pair fragment was amplified with internal control primers. Of the AD subjects, 40% had at least one copy of ε4 allele, compared to 15.4% of controls. Table 1 shows that there were significant differences in the *ApoE* genotypes and ε4 allele frequencies. Comparison between AD subjects and Controls was significant using the Chi-square test($P<0.05$).

Association of *BchE-k* variant with AD

After 60 AD subjects and 610 healthy controls had been *ApoE* genotyped, the *BchE* genotyping was found in the *ApoE* ε4 carriers. The frequency of alleles and genotypes were shown in Table 2. As shown in Fig. 2, a 267 base pair fragment was amplified with the *BchE* ASOs primers

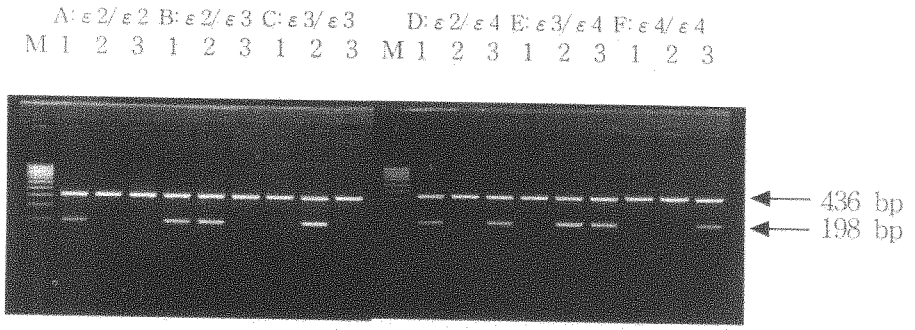

Fig 1. Amplification of the *ApoE* specific alleles and genotypes. : *ApoE* ε2/ε2, ε2/ε3, ε3/ε3, ε2/ε4, ε3/ε4, ε4/ε4, M :100 bp DNA size marker. Each panel A,B,C,D,E and F represent *ApoE* genotype. lane 1: ε2 allele specific lane, lane 2: ε3 allele specific lane, lane 3: ε4 allele specific lane. Each lane has amplified PCR products of Internal Control fragment(436 bp). *ApoE* allele specific DNA fragments(198 bp) are shown.

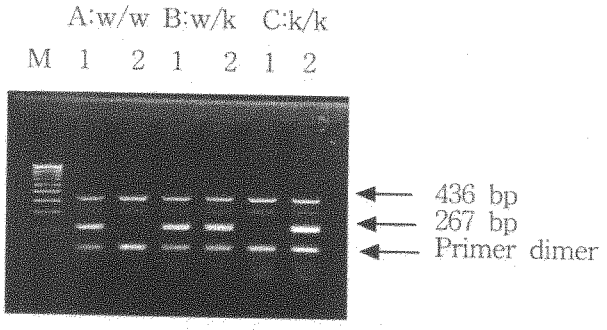

Fig. 2. Amplifications of the *BchE* specific alleles and genotypes. M : 100 bp DNA size marker, Each panel A,B and C represent *BchE* genotype. lane 1: w allele specific lane, lane 2: k allele specific lane. Each lane has amplified PCR products of Internal Control fragment(436 bp). *BchE* allele specific DNA fragments(267 bp) are shown.

and a 436 base pair fragment was amplified with internal control primers. In the *ApoE* ε4 carriers, 22.9% AD subjects compared with 9% controls, had the *BchE-k* variant. This difference is highly significant($P<0.05$).

Synergy between the *BchE-k* variant and the *ApoE* ε4 gene in AD

60 AD subjects and 610 healthy controls were genotyped for the *BchE-k* and for the *ApoE* alleles. The *BchE-k* was more common in AD subjects with ε4 allele. In subjects without the *ApoE* ε4 and the *BchE-k* variant, gave an odds ratio of AD of 1.3 (95% C.I. 0.7-2.3). In subjects that carry both the *ApoE* ε4 allele and the *BchE-k* variant, the odds ratio of AD was 3.5 (95% C.I. 1.3-9.2).

DISCUSSION

This study shows that the previously reported association between AD and the *ApoE* ε4 is also present in Korean subjects, with 40% of AD subjects having at least one ε4 allele compared with 15.4% of controls. In two previous studies having at least oneε4 allele frequencies were 45% and 35% in AD group, and 11% and 16% in control group, respectively (Kuusisito *et al.*, 1994 ; Lehtovirta et al., 1995). These frequencies are quite similar to ours in either group. The healthy group was divided into subgroups according to the age-matched with AD patients group. There were significant differences in the *ApoE* genotypes and ε4 allele frequencies: the AD patients differed significantly from the healthy group and healthy subgroup (age-matched with AD, Table 1). This suggests that the ε4 allele frequency is significantly high in AD ($Chi^2=22.76$ on 1 df, $P<0.001$). The *ApoE* ε4 is neither necessary nor sufficient to cause LOAD, and recent estimates suggest that this locus accounts for about 50% of the genetic aetiology of LOAD (Roses *et al.*, 1995), other genes are likely to impact the AD risk (Rao *et al.*, 1996). We found significant differences in genotype frequencies between the AD patient group and healthy individuals. Our results suggest that the *ApoE* ε4 allele impacts the LOAD risk.

The *BchE-k* variant has been demonstrated to have an elevated frequency in AD patients carrying the ε4 allele of the *ApoE* gene when compared to the control population. We genotyped AD patients and controls who are carriers of the *ApoE* ε4 to confirm this association. We found a difference in the frequency of the *BchE-k* variant in AD who are ε4 carriers when compared to the controls ($Chi^2=6.76$ on 1 df, $P<0.009$). A study from Lehmann *et al.* suggested that the *BchE-k* acts in synergy with the *ApoE* ε4 as a susceptibility gene for LOAD. These findings are with the high *BchE-k* variant frequency observed in AD in the present study. Two previous studies reported no association between the *BchE-k* variant frequency and AD (Brindle *et al.*, 1998 ; Singleton *et al.*, 1998). The association of the *BchE-k* with LOAD was limited to carriers of the ε4 allele of the *ApoE*, among whom the

presence of the *BchE-k* gave an odds ratio of AD of 3.5 (95% C.I. 1.3-9.2). Our data supports the suggestion that there is strong synergy between these two alleles, the *BchE-k* variant and the *ApoE.* ε4 in AD, we could not determine whether a biological effect was present though. We concluded that in the population studied here, the *BchE-k* acts in synergy with the *ApoE* ε4 as a susceptibility gene for AD.

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