# ARTICLE

# Evaluation of polymorphisms in the presenilin-1 gene and the butyrylcholinesterase gene as risk factors in sporadic Alzheimer's disease

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**The ε4 allele of the apolipoprotein E gene (*APOE*) is a major risk factor for late-onset Alzheimer's disease (LOAD) but is neither necessary nor sufficient to cause the disease. In this study, we investigated polymorphisms in the presenilin-1 (*PS-1*), and butyrylcholinesterase (*BChE*) genes, which have been implicated as risk factors for LOAD. Our data-set comprised 177 AD and 118 control patients, all of whom had been histopathologically confirmed following autopsy. We have tested homozygosity for the PS-1 allele 1 and possession of the BChE-K variant in association with APOE ε4 as risk factors in LOAD. Our findings support an association between the PS-1 polymorphism and LOAD, finding homozygosity for allele 1 associated with an approximately two-fold increased risk. Our data also show that in subjects greater than 75 years of age possession of both BChE-K and APOE-ε4 alleles is associated with an increased risk of LOAD, whilst the risk associated with APOE-ε4 allele alone is not significant.**

**Keywords: Alzheimer's disease; polymorphisms; *PS-1* gene; *BChE* gene**

## Introduction

Alzheimer's disease (AD) is the most common cause of dementia in the elderly. Genetic studies have shown that the apolipoprotein E (APOE) ε4 allele is a major risk factor for both familial and sporadic AD,[1] but it is neither necessary nor sufficient for the expression of AD.[2] Other genetic factors have also been implicated, acting either in conjunction with APOE ε4, or alone, to increase risk of late-onset AD (LOAD).

Mutations in the presenilin-1 (*PS*-1) gene are known to be responsible for the majority of early-onset familial AD cases, but several studies also suggest that *PS-1* may play a role in LOAD. Homozygosity for allele 1 of an intronic polymorphism in the *PS-1* gene has been reported to be associated with a doubling of the risk for LOAD.[3] This association has been confirmed by subsequent studies,[4–8] but others have failed to find a significant association.[9–12]

A recent report has also implicated the butyrylcholinesterase (BChE) gene in LOAD.[13] A common variant of this gene, known as the K-variant, has been demonstrated to modify the risk of LOAD in APOE ε4 carriers. The risk associated with possession of both APOE ε4 and BChE-K was found to be 36-fold higher than individuals with neither allele. However, two subsequent studies have failed to confirm an association between the BChE-K variant and LOAD.[14,15]

In this study we aimed to test two reported risk factors for LOAD, namely, homozygosity for the PS-1 allele 1, and possession of both BChE-K and APOE ε4 alleles.

## Materials and Methods

### Patient Material
All subjects were Caucasian. Material was obtained from the Nottingham and Cambridge brain banks. AD patients, (n = 177; 67 male, 110 female, age 81.6 ± 8.6 years, range 55–101) and controls, (n = 118; 81 male, 37 female, age 72.8 ± 8.7 years, range 58–94) were histopathologically confirmed following autopsy. Diagnosis of AD was based on the CERAD protocol.[16] DNA was prepared from brain tissue as previously described.[17]

### Butyrylcholinesterase (BChE) Genotyping
The BChE-K variant was detected using the method previously described by Jensen *et al*.[18] Primers 5' ATATTTTA-CAGGAAATATTGATGTA 3' and 5' ATTAGAGACCCA-CACAACTT 3' are used to introduce a Mae III restriction site when amplifying the K allele. PCR was conducted in a final volume of 20 µl, containing 200 ng of genomic DNA, 0.44 µM of each primer, 10 mM Tris-HCl, pH 8.8, 50 mM KCl, 0.08% Nonidet P40, 1.5 mM MgCl$_2$, 200 µM dNTPs, 0.5 U of Taq polymerase. Reactions were overlaid with mineral oil and subjected to 33 cycles of 1 min at 94°C, 1.5 min at 53°C, and 1.5 min at 72°C, followed by 10 min at 72°C, and cooling to 4°C. Subsequently, PCR products (5 µl) were digested with one unit of Mae III restriction enzyme at 55°C for three hours, then electrophoresed through 10% polyacrylamide gel at 20 mA for one hour and stained with ethidium bromide. The wild-type allele (U) gives a PCR product of 137 bp, whereas the K variant, because of the introduction of the Mae III site, gives 115 bp and 22 bp bands.

### Presenilin-1 (PS-1) Genotyping
The PS-1 intronic polymorphism was analysed using the method previously described by Wragg *et al*,[3] using primers 5' CACCCATTTACAAGTTTAGC 3' and 5' CACTGATTAC-TAATTCAGGATC 3' which introduce a Bam H1 restriction site into allele 2. PCR was conducted in a final volume of 25 µl, containing 200 ng of genomic DNA, 200 µM dNTPs, 0.35 µM of each primer, 10 mM Tris-HCl, pH 8.8, 50 mM KCl, 0.08% Nonidet P40, 1.5 mM MgCl$_2$ 0.5 U Taq polymerase (Helena BioSciences). Reactions were overlaid with mineral oil and subjected to 35 cycles of 30 s at 94°C, 30 s at 45°C, 30 s at 72°C, followed by 3 min at 72°C, and cooling to 4°C. The resulting 199 bp PCR product (7 µl) was digested with five units Bam H1 restriction enzyme at 37°C for three hours, then resolved through 3% agarose gel and stained with ethidium bromide. Allele 2 products yield 182 bp fragment after digest, distinguishable from allele 1 uncut PCR product (199 bp).

### Statistical Methods
Allele frequencies were estimated by allele counting and compared in AD and control groups by $\chi^2$ testing with Yates' continuity correction, or Fisher's exact test for small sample numbers, using Statistical Package for the Social Sciences (SPSS) software. Logistic regression analysis was performed using SPSS. The level of statistical significance was defined as $P < 0.05$.

## Results

Allele and genotype frequencies for apolipoprotein E, the BChE-K variant, and the PS-1 intronic polymorphism are shown in Tables 1–3. Genotype frequencies in each group for all loci tested were not found to deviate significantly from Hardy-Weinberg equilibrium.

The APOE ε4 allele frequency was significantly higher in AD patients than in controls ($\chi^2$ = 32.97, 1df, $p < 0.001$) as previously reported in this data set.[17] There are no significant differences in allele or genotype frequencies of the BChE-K variant between AD

**Table 1** Allele and genotype frequencies of apolipoprotein E in Alzheimer's disease patients and controls

|  | *AD* (n=177) | | *Controls* (n=118) | |
| --- | --- | --- | --- | --- |
|  | n | *(freq.)* | n | *(freq.)* |
| APOE alleles |  |  |  |  |
| ε2 | 16 | (0.05) | 24 | (0.10) |
| ε3 | 229 | (0.65) | 187 | (0.79) |
| ε4 | 109 | (0.31) | 25 | (0.11) |
|  |  |  |  |  |
| APOE genotypes |  |  |  |  |
| ε2/ε2 | – | – | 2 | (0.02) |
| ε2/ε3 | 8 | (0.05) | 20 | (0.17) |
| ε2/ε4 | 8 | (0.05) | – | – |
| ε3/ε3 | 73 | (0.41) | 71 | (0.60) |
| ε3/ε4 | 75 | (0.42) | 25 | (0.21) |
| ε4/ε4 | 13 | (0.07) | – | – |

**Table 2** Allele and genotype frequencies of BChE polymorphism in Alzheimer's disease patients and controls

|  | *AD* (n=177) | | *Controls* (n=118) | |
| --- | --- | --- | --- | --- |
|  | n | *(freq.)* | n | *(freq.)* |
| BChE alleles |  |  |  |  |
| U | 283 | (0.80) | 191 | (0.81) |
| K | 71 | (0.20) | 45 | (0.19) |
|  |  |  |  |  |
| BChE genotypes |  |  |  |  |
| UU | 114 | (0.64) | 76 | (0.64) |
| UK | 55 | (0.31) | 39 | (0.33) |
| KK | 8 | (0.05) | 3 | (0.03) |

**Table 3** Allele and genotype frequencies of PS-1 intronic polymorphism in Alzheimer's disease patients and controls

| | AD (n=177) | | Controls (n=110)[a] | |
|---|---|---|---|---|
| | n | (freq.) | n | (freq.) |
| PS-1 alleles | | | | |
| 1 | 202 | (0.57) | 110 | (0.50) |
| 2 | 152 | (0.43) | 110 | (0.50) |
| | | | | |
| PS-1 genotypes | | | | |
| 1:1 | 62 | (0.35) | 24 | (0.22) |
| 1:2 | 78 | (0.44) | 62 | (0.56) |
| 2:2 | 37 | (0.21) | 24 | (0.22) |

[a]Data unavailable for eight control subjects.

and control groups (Table 2) in contrast to previous findings.[13] Allele and genotype frequencies for the PS-1 intronic polymorphism are shown in Table 3. Allele frequencies do not differ significantly between AD and control groups. However, there is an increased frequency of allele 1 homozygotes in AD patients as compared with controls ($\chi^2$ = 5.64, 1df, $P$ = 0.02) in agreement with previous findings.[3]

Logistic regression analysis was used to test previously reported findings of an associated between risk for LOAD and PS-1 allele 1 homozygosity[3] and possession of both BChE-K and APOE ε4 alleles.[13] In order to correct for significant differences in mean age and sex distribution between the AD and control groups, these were entered as co-variants in the logistic regression analysis, along with APOE-ε4 carrier status, PS-1 allele 1 homozygosity, BChE-K carrier status and combined BChE-K/APOE-ε4 carrier status. Since possession of both BChE-K and APOE ε4 alleles has been reported to confer most risk in the over 75 age group, the logistic regression analysis was carried out in all subjects (age ≥ 55 years) and in only those over 75 years of age (140 AD, 52 controls). Estimated odds ratios from the logistic regression analyses are shown in Tables 4 and 5.

**Table 4** Logistic regression for the effects of APOE ε4, PS-1 allele 1 and BChE-K alleles on risk for LOAD in subjects ≥ 55 years of age

| Variable | Estimated OR | 95% CI | P-value |
|---|---|---|---|
| APOE ε4 carrier | 2.77 | 1.32–5.81 | <0.01 |
| PS-1 allele 1 homozygosity | 2.01 | 1.06–3.98 | 0.03 |
| BChE-K carrier | 0.67 | 0.31–1.42 | 0.29 |
| BChE-K/ε4 carriers | 3.54 | 0.94–13.34 | 0.06 |

Variables entered into the logistic regression model were: age, sex, APOE-ε4 carrier status, PS-1 allele 1 homozygosity, BChE-K carrier status, and combined BChE-K/APOE-ε4 carrier status.

**Table 5** Logistic regression for the effects of APOE ε4, PS-1 allele 1 and BChE-K alleles on risk for LOAD in subjects ≥ 75 years of age

| Variable | Estimated OR | 95% CI | P-value |
|---|---|---|---|
| APOE ε4 carrier | 2.36 | 0.93–6.03 | 0.07 |
| PS-1 allele 1 homozygosity | 2.87 | 1.15–7.16 | 0.02 |
| BChE-K carrier | 0.64 | 0.24–1.70 | 0.37 |
| BChE-K/ε4 carriers | 13.84 | 1.28–149.27 | 0.03 |

Variables entered into the logistic regression model were: age, sex, APOE-ε4 carrier status, PS-1 allele 1 homozygosity, BChE-K carrier status, and combined BChE-K/APOE-ε4 carrier status.

As expected, possession of the APOE ε4 allele is associated with a significantly increased risk of LOAD (OR = 2.77, 95% CI, 1.32–5.81, $P$ < 0.01) in subjects ≥ 55 years of age. However, in the ≥ 75 years age group, the APOE ε4 effect becomes non-significant. The reduction of the APOE-ε4 effect with increasing age has been previously reported.[19] Homozygosity for the PS-1 allele 1 is associated with an approximately two-fold increased risk (OR = 2.01, 95% CI, 1.06–3.98, $P$ = 0.03), and this effect remains significant in the over 75 age group. The frequency of PS-1 allele homozygosity does not differ significantly between APOE ε4 carriers and non-carriers in either AD patients or controls (data not shown), suggesting that the PS-1 effect is independent of APOE ε4 status.

The logistic regression analyses show that possession of the BChE-K allele alone has no significant effect in either age group. However, possession of both BChE-K and APOE ε4 alleles is associated with a significantly increased risk of LOAD in the ≥ 75 year age group only (OR = 13.8, 95% CI, 1.28–149.27, $P$ = 0.03).

## Discussion

Although the APOE ε4 is a well established risk factor for LOAD, it is estimated to account for, at most, only 50% of the total genetic effect in AD.[20] Thus, many studies have been directed towards the identification of further genes which may play a role in LOAD. Various putative AD risk factors have been investigated, but studies have failed to yield consistent results. This may be due to population differences, or heterogeneity in terms of classification of AD, which may cause a problem when diagnosis is made on clinical grounds only. In this study, autopsy-confirmed material was used exclusively for both AD and control groups, resulting in a stringent definition of the phenotype. Two putative LOAD risk factors were investigated in this study:

homozygosity for the PS-1 allele 1, and possession of both BChE-K and APOE ε4 alleles.

The BChE-K variant has recently been implicated in LOAD, with Lehmann *et al* reporting an odds ratio of 36 associated with possession of both the APOE ε4 and BChE-K allele in the ≥ 75 age group, whilst the effect of the APOE ε4 allele alone is not significant.[13] In this study we do not find a significantly increased frequency of the BChE-K allele in AD patients as compared with controls, in contrast to the finding of Lehmann *et al*.[13] However, our findings do support an increased risk of LOAD associated with possession of both APOE ε4 and BChE-K in the over 75 age group, with an odds ratio of 13.8. In our AD patient group, possession of the BChE-K allele produces an apparent reduction in the risk of AD (non-significantly), whereas possession of both APOE ε4 and BChE-K alleles results in an increased risk (Tables 4 and 5) which becomes significant in the older age group. Clearly this effect merits further study. To confirm the role of the BChE-K allele in LOAD, further studies using considerably larger data sets are required, since in both this study and that of Lehmann *et al*,[13] there are very small numbers in the control group possessing both APOE ε4 and BChE-K alleles, resulting in very large confidence intervals; this may be contributing to the apparent dichotomy of the BChE-K allele effect.

Our data also show that homozygosity for allele 1 of the *PS-1* gene is associated with an approximate doubling of LOAD risk, as reported by Wragg *et al*.[3] In this study the effect of PS-1 allele 1 homozygosity is significant both in the ≥ 55 year and ≥ 75 year age groups, and is apparently independent of APOE ε4 status. These results are in agreement with Wragg *et al*[3] who also found that PS-1 allele 1 homozygosity was a significant risk factor in AD patients aged 65 years or less, and for cases over the age of 65 years. However, it should be noted that in our study this is of borderline significance ($0.01 < P < 0.05$) and excess of PS-1 allele 1 homozygotes in our AD group is largely at the expense of heterozygotes; the frequency of PS-1 allele 2 homozygotes is similar in our AD and control groups (Table 3). This is further highlighted by the fact that the odds ratio associated with PS-1 allele 1 homozygosity, taking allele 2 homozygotes as reference (rather than combined heterozygotes and allele 2 homozygotes), is not significant (OR = 1.68, 95% CI, 0.83–3.36). The PS-1 intronic polymorphism may be functionally significant or may be linked to a functional mutation elsewhere in the *PS-1* gene. Wragg *et al*[3] failed to find

any coding sequence changes linked to allele 1 of the *PS-1* gene. However, there may still be genetic variability in the non-coding regions of the gene which remains to be found.

In conclusion, in this study we support an association between the PS-1 intronic polymorphism and LOAD, with an approximate doubling of risk associated with homozygosity for allele 1. The BChE-K variant will require further studies on much larger groups than used hitherto, to confirm its action as a risk modifier in association with the APOE ε4 allele.

# Acknowledgements

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