

The butyrylcholinesterase K variant and susceptibility to Alzheimer's disease

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Abstract

Previous work has shown an association between the K variant of the butyrylcholinesterase (BCHE) gene and Alzheimer's disease (AD) in patients carrying the $\epsilon 4$ allele of ApoE. We attempted to replicate this finding in 181 UK white AD cases and 71 controls. No difference was found in BCHE-K genotypes ($p=0.75$) or alleles ($p=0.70$) between patients and controls. Moreover, despite a significant excess of ApoE $\epsilon 4$ in patients versus controls ($p<0.0001$), we found no evidence to support previous reports of an interaction between ApoE and BCHE-K ($\chi^2=1.49$, $df=4$, $p=0.83$).

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Four genes have been implicated in the aetiology of Alzheimer's disease (AD). Mutations in three of these, APP, PS1, and PS2, account for most cases of familial autosomal dominant AD.^{1,2} The great majority of cases of AD do not show clear autosomal dominant patterns of inheritance and these probably reflect the co-action or interaction of several or many genes together with environmental factors. One gene that is clearly implicated in this form of the disorder is ApoE. The $\epsilon 4$ allele of ApoE, although neither necessary nor sufficient to cause AD, is associated with increased risk of early and late onset disease.³

Recently Lehmann *et al*⁴ reported an association between the so-called K variant (BCHE-K) of the butyrylcholinesterase (BCHE) gene and late onset AD in carriers of

ApoE $\epsilon 4$ with an odds ratio of 6.9 (95% CI 1.65-29) in people over 65 years of age and of 12.8 (1.9-86) in those over 75 years.

We attempted to replicate this finding in 94 AD cases confirmed at necropsy (PMAD), 87 clinically diagnosed probable AD patients (CLAD), and 71 age matched controls (AMC). The PMAD group ($n=94$), comprising 32 males and 62 females, diagnosed clinically for probable AD under NINCDS-ADRDA criteria⁵ and later confirmed at necropsy,⁶ had an average age at death of 81.8 years (SD 7.5). Using available age of onset and age at death information ($n=78$) the overall mean onset of PMAD was 73.7 years (SD 8.9). This included 11 early (<65 years) onset cases (mean onset 58.0 years, SD 4.4) and 67 late (≥ 65 years) onset cases (onset 76.3 years, SD 6.5). Samples were collected from brain banks in Bristol ($n=39$), the Institute of Psychiatry, London ($n=30$), and Cardiff ($n=25$). No data on family history were available.

The CLAD group ($n=87$), comprising probable AD cases (NINCDS-ADRDA),⁵ had an average age of onset of 67.4 years (SD 9.2); 27 were early onset cases (onset 56.7 years, SD 6.4) and the remainder ($n=60$) late onset (onset 72.2 years, SD 5.6). The group (41 males, 46 females) were UK white subjects and 43 had a family history of AD (defined by having at least one first degree relative with the disease). The AMC group (34 males, 37 females) were collected locally having scored 28 or higher on the Mini Mental State Examination⁷ and had an average age of 73.46 years (SD 6.2) at collection. An additional group (PC) of unrelated white subjects ($n=262$) obtained locally were genotyped

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Table 1 Genotype and allele distributions of the K variant of the BCHE gene

Sample	Genotype			Allele	
	wt/wt	wt/k	k/k	wt	k
CLAD (all cases) (n=87)	56 (0.65)	29 (0.33)	2 (0.02)	141 (0.81)	33 (0.19)
EOAD (onset <65 y) (n=27)	18 (0.66)	8 (0.30)	1 (0.04)	44 (0.81)	10 (0.19)
LOAD (onset ≥ 65 y) (n=60)	38 (0.63)	21 (0.35)	1 (0.02)	97 (0.81)	23 (0.19)
PMAD (all cases) (n=94)	57 (0.61)	30 (0.32)	7 (0.07)	144 (0.77)	44 (0.23)
EOAD* (onset <65 y) (n=7)	4 (0.57)	3 (0.43)	0 (0.0)	11 (0.79)	3 (0.21)
LOAD† (onset ≥ 65 y) (n=42)	27 (0.64)	12 (0.29)	3 (0.07)	66 (0.79)	18 (0.21)
CAD (n=181)	113 (0.62)	59 (0.33)	9 (0.05)	285 (0.79)	77 (0.21)
EOAD (onset <65 y) (n=34)	22 (0.65)	11 (0.32)	1 (0.03)	55 (0.81)	13 (0.19)
LOAD (onset ≥ 65 y) (n=102)	65 (0.64)	33 (0.32)	4 (0.04)	163 (0.80)	41 (0.20)
AMC (n=71)	45 (0.64)	24 (0.33)	2 (0.03)	114 (0.80)	28 (0.20)
PC (n=262)	163 (0.64)	83 (0.32)	16 (0.06)	409 (0.78)	115 (0.22)

Numbers in parentheses represent corresponding percentages.

wt = wild type (normal) BCHE alleles.

K = the K variant of BCHE with diminished enzymatic activity.

No evidence of any departure from Hardy Weinberg equilibrium for any of the groups.

CLAD = clinical AD; PMAD = post mortem AD; CAD = combined AD; AMC = aged matched controls; PC = population controls.

EOAD* and LOAD† = data only shown for PMAD cases where actual age of onset information was available.

Table 2 APOE ϵ 4 and BCHE-K distributions in unaffected and affected groups

E4	BCHE-K	Controls (AMC)	Cases (CAD)	OR	95% CI
-	-	33	56	Reference	
-	+	18	24	0.79	(0.37, 1.68)
+	-	12	58	2.85	(1.32, 6.16)
+	+	8	46	3.39	(1.40, 8.19)

solely for the BCHE-K polymorphism for a frequency estimate in the general population. This group (149 males, 113 females) had an average age of 38.9 years (SD 12.6) at collection. Genotyping for both ApoE and BCHE-K was performed as previously described.^{8,9} Statistical comparisons were by standard Pearson chi-square tests and logistic regression analyses.

Genotype and allele frequencies for the BCHE gene are shown in table 1. No differences between early and late onset cases in either CLAD (genotype $p=0.77$, allele $p=0.92$) or PMAD (genotype $p=0.62$, allele $p=0.99$) were observed and they were thus combined within each group. This contrasts with the findings of Lehmann *et al.*⁴ who did find differences in K variant frequency between early and late onset cases (0.09 versus 0.16 respectively). Comparing CLAD and PMAD groups showed no significant differences (genotypes $p=0.28$, alleles $p=0.3$) and they were therefore combined. Comparisons between the combined patient group (CAD, $n=181$) and the age matched control group (AMC, $n=71$) showed no significant differences for BCHE-K genotypes ($\chi^2=0.57$, $df=2$, $p=0.75$) or alleles ($\chi^2=0.15$, $df=1$, $p=0.70$). No differences according to gender or family history were found in the CLAD group.

We also used logistic regression to test for any interaction between ApoE ϵ 4 and BCHE-K in our patients. Following Lehmann *et al.*⁴ four categories defined by the presence (+) or absence (-) of an ϵ 4 or K allele with respective numbers and odds ratios are defined (table 2). As expected, ApoE ϵ 4 confers an increased risk but this does not appear to vary in the presence of BCHE-K. Logistic regression showed the significant association with ApoE ($\chi^2=16.95$, $df=1$, $p=0.00004$) and, after allowing for this, no significant association with BCHE-K ($\chi^2=0.08$, $df=1$, $p=0.78$) or interaction between BCHE-K and ApoE ϵ 4 ($\chi^2=0.44$, $df=1$, $p=0.51$) were found. Finally, we found no evidence for an interaction between BCHE-K and ApoE ϵ 4 when patients over 75 years of age were considered alone ($\chi^2=0.024$, $df=1$, $p=0.88$).

Our failure to replicate the findings of Lehmann *et al.*⁴ could be for a number of reasons. First, it is possible that our study represents a type 2 error. However, our results support recent work by other groups¹⁰⁻¹² who

have also failed to replicate the findings of Lehmann *et al.*⁴ Moreover, we were careful to match both groups of cases and the controls for ethnicity and this lessens the likelihood that differences between them were obscured by population stratification, a point which is reinforced by the fact that frequencies of BCHE-K in patients were similar in both studies. However, there was a marked difference in K allele frequency between control groups in the two studies (Oxford 0.09, mean age 78.1 years; Cardiff 0.20, mean age 73.5 years) though this is hard to explain since both contained elderly, non-demented, UK white subjects. Previous frequency estimates for the K polymorphism of 0.12 in UK white subjects were based on enzymatic assays in relatively small groups.^{13,14} We therefore genotyped a large sample and obtained a frequency of 0.22 which closely matches that observed in all our other sample groups and in the patient group of Lehmann *et al.*⁴ This suggests that the original findings may be in fact the result of a type 1 error, to which genetic association studies are known to be prone.¹⁵ Further studies are required to resolve this issue.

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