Analysis of the butyrylcholinesterase gene and nearby chromosome 3 markers in Alzheimer disease

N. Brindle+, Y. Song+, E. Rogaeva+, S. Premkumar1, G. Levesque, G. Yu, M. Ikeda, M. Nishimura, A. Paterson2, S. Sorbi3, R. Duara4, L. Farrer1 and P. St George-Hyslop*

Centre for Research in Neurodegenerative Diseases, Department of Medicine (Division of Neurology), University of Toronto, and Department of Medicine (Division of Neurology), The Toronto Hospital, Tanz Neuroscience Building, 6 Queen’s Park Crescent, Toronto, Ontario M5S 3H2, Canada, 1Neurogenetics Laboratory, Department of Neurology, Boston University School of Medicine, 80 East Concord Street, Boston, MA 02118, USA, 2Neurogenetics Laboratory, Clarke Institute of Psychiatry, University of Toronto, College Street, Toronto, Ontario, Canada, 3Department of Neurology and Psychiatry, 85 viale Morgagni, 50134 Firenze, Italy and 4Department of Neurology, Wein Center, Mount Sinai Medical Centre, 4300 Alto Road, Miami Beach, FL 33140, USA

Received November 28, 1997; Revised and Accepted January 30, 1998

The K-variant of butyrylcholinesterase (BCHE-K) recently has been reported to be associated with Alzheimer disease (AD) in carriers of the ε4 allele of the apolipoprotein E (APOE) gene. We have re-examined the frequency of the BCHE-K allele in a large data set of both sporadic and familial cases of AD disease, and we have also examined the segregation of three genetic markers on chromosome 3 near BCHE. Our data neither support an association of BCHE-K with sporadic or familial AD, nor do they suggest the existence of another gene nearby on chromosome 3 as a common cause of familial AD.

INTRODUCTION

The butyrylcholinesterase (BCHE) gene contains two common polymorphic variants. One of these polymorphisms, the K-variant (BCHE-K) arises from a G→A substitution at nucleotide 1615 and causes an Ala539Thr missense substitution which reduces the catalytic activity of BCHE enzyme (1). Recently, the BCHE-K variant has been reported to show allelic association with Alzheimer disease (AD) in subjects who are also carriers of the ε4 allele of apolipoprotein E (APOE), especially in subjects over the age of 70 years as the cut-off (2). While the APOE ε4 allele frequency in subjects with AD showed the expected increase in the ε4 allele frequency relative to control subjects (Table 1), there was no difference in the BCHE-K allele frequency between control subjects (n = 165) and subjects with either sporadic AD (n = 138) or unrelated subjects with FAD (n = 50) (Table 1). There was also no significant difference in BCHE-K allele frequency between cases and controls even when each subgroup was stratified either by age (using the mean censoring age of 70 years as the cut-off) or by APOE genotype (Table 2). Power calculations, however, indicate that using the sample sizes in Table 1, we would have at least an 80% power to detect a difference of 7–17% in BCHE-K allele frequency (depending on the actual population frequency) at the 5% significance level. To test for the possibility that an effect of BCHE-K is masked or confounded with APOE genotype, we performed multiple logistic regression analysis including BCHE and APOE genotypes as predictors of disease outcome, adjusting for age and sex (4). While APOE ε4 was found to confer a significant 4.6-fold increase in risk for AD [95% confidence interval (CI) 2.4–8.6], the odds ratio of 1.6 obtained for BCHE-K was not significantly different from one (95% CI: 0.86–3.0). Our data therefore do not confirm an association between BCHE-K and AD, nor do they suggest a synergistic interaction between BCHE-K and the ε4 allele of APOE.

To examine the possibility that another nearby gene on chromosome 3 might be associated with increased risk for FAD, we examined the segregation of two informative microsatellite markers (D3S1744 and D3S1763) in 56 FAD pedigrees. These markers have been genetically mapped in the order D3S1744–17.7cM–D3S1763 (data obtained from the Genome

*D To whom correspondence should be addressed. Tel: +1 416 978 7460; Fax: +1 416 978 1878; Email: p.hyslop@utoronto.ca

These authors contributed equally to this manuscript.© 1998 Oxford University Press Human Molecular Genetics, 1998, Vol. 7, No. 5 933–935
DISCUSSION

While our data confirm the well-established association between APOE ε4 and AD (5), our results do not support the notion that the K-variant of BCHE is a risk factor for AD either independently or in association with APOE ε4. The explanation for the disparity between our data and the previous report is not entirely clear. It is of note, however, that the BCHE-K allele frequency observed in our sporadic AD cases (0.20) and our unrelated FAD cases (0.19) is very similar to that observed in AD cases in the previous report (0.17) (2). In contrast, the BCHE-K frequency in control subjects in our study (0.19) was considerably higher than that in the control group of the previous report (0.09) (2). It is unlikely that this difference reflects differing ethnic origins of our control and AD populations because the majority of controls were either unaffected spouses of AD-affected subjects or were normal controls drawn from the same communities as the AD patients. Furthermore, two other studies of normal subjects have shown BCHE-K frequencies similar to those reported here (0.13–0.18) (1,3).

One potential explanation for the failure to discover an allelic association between BCHE and AD is that the BCHE-K allele might be in weak linkage disequilibrium with a causative sequence change in another nearby gene. However, the segregation data in our FAD pedigrees using nearby anonymous, informative, microsatellite markers does not support this hypothesis because neither the maximum likelihood nor the non-parametric analyses detect any significant evidence for allele sharing amongst affected relatives. Cumulatively, our data therefore argue for caution in the application of the BCHE-K variant in identification of elderly subjects who are at high risk of developing AD.

MATERIALS AND METHODS

A total of 138 subjects with sporadic AD diagnosed using NINCDS/ADRDA criteria and/or CERAD neuropathological criteria (128 probable AD; 10 definite AD) were collected from clinics specializing in memory disorders in Toronto or Miami (6–8). The 165 normal control subjects who had no evidence of neurologic disease were collected from the same sources, and were generally either spouses of AD-affected subjects, or were subjects specially recruited from the same communities to serve as normal controls.

Fifty six FAD pedigrees with samples from at least three living AD-affected family members, and usually with AD in at least two contiguous generations, were collected from Caucasian families living in Europe or North America. Mutations in PS1 (9), PS2 (10,11) or APP (12–14) previously have been excluded in these pedigrees.

Genotypes at APOE were determined as previously described (5). The genotype at BCHE was determined using the method previously described (3), and also by ASO methods. Briefly, exon 4 of BCHE was amplified by PCR using the primers 1478, 5′-TCA GTT AAT AAC CAT AAA AAT, and 1479, 5′-TAA GTT AAA GAT GTG AGG AAT CAA in a reaction volume of 10 µl containing 100 ng of genomic DNA, 10 pmol of each primer 250 µM dNTPs, 1.5 mM MgCl2 and 0.5 U of Taq polymerase, for 35 cycles of 94°C for 20 s, 54°C for 20 s and 72°C for 20 s. The 331 bp PCR product was denatured in 100 µl of 0.4 M NaOH, 25 mM EDTA, and slot-blotted to duplicate Hybond-N+ nylon membranes. The ASOs 1480, 5′-TCA GTT AA T GAA ACA GA T AAA AA T, and 1479, 5′-TAA GTT AAA GAT GTG AGG AAT CAA were radiolabeled at the 5′ end and hybridized at 45°C for 2 h in hybridization buffer (5x Denhardt’s, 5x SSC, 0.5% SDS), washed to a final stringency of 2x SSC, 0.1% SDS at 58°C, and then exposed to autoradiographic film.

The primer sequences and PCR conditions for the anonymous markers D3S1744 and D3S1763 were obtained from the Genome Database.

Table 1. Age, sex, APOE and BCHE allele frequencies in AD, FAD and control subjects

<table>
<thead>
<tr>
<th>Group</th>
<th>No.</th>
<th>Mean age</th>
<th>F:M ratio</th>
<th>APOE allele frequency (%)</th>
<th>BCHE allele frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alzheimer</td>
<td>138</td>
<td>75.4</td>
<td>1.8</td>
<td>ε2: 23.3, ε3: 31.4, ε4: 79.7</td>
<td>20.3</td>
</tr>
<tr>
<td>FAD</td>
<td>50</td>
<td>67.2</td>
<td>2.2</td>
<td>ε2: 21.3, ε3: 49.0, ε4: 76.0</td>
<td>18.8</td>
</tr>
<tr>
<td>Control</td>
<td>165</td>
<td>63.4</td>
<td>0.8</td>
<td>ε2: 78.5, ε3: 14.0, ε4: 81.2</td>
<td>18.8</td>
</tr>
</tbody>
</table>

Table 2. BCHE-K allele frequencies in AD and control subjects

<table>
<thead>
<tr>
<th>Group</th>
<th>≤70 years</th>
<th>&gt;70 years</th>
<th>APOE ε4+</th>
<th>APOE ε4–</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. (f(K)</td>
<td>No. (f(K)</td>
<td>No. (f(K)</td>
<td>No. (f(K)</td>
</tr>
<tr>
<td>AD</td>
<td>32</td>
<td>28.1</td>
<td>104</td>
<td>18.3</td>
</tr>
<tr>
<td>Controls</td>
<td>95</td>
<td>22.1</td>
<td>55</td>
<td>13.6</td>
</tr>
</tbody>
</table>
A \( \chi^2 \) test was used to compare \( BCHE \) allele frequencies between unrelated AD, FAD and control cases. The Fisher’s exact test was used to compare \( APOE \) genotypes. The influence of \( BCHE \) and \( APOE \) genotypes, age group and sex on the odds of developing AD was assessed using logistic regression procedures (4). To accommodate polychotomous classification of the genetic loci in regression analysis, indicator variables were constructed representing two \( BCHE \) genotype classes (K/W and K/K) and two \( APOE \) genotype classes (2/2 or 2/3; and 2/4, 3/4 or 4/4). These variables took on the value of ‘1’ if the subject had the corresponding genotype, and ‘0’ otherwise. According to this scheme, \( BCHE-W/W \) and \( APOE-3/3 \) genotypes were considered as the referents. Interaction between \( BCHE, APOE, \) age and sex was evaluated by deriving product terms for each genotype with each other, age group and sex. Models were evaluated using the LOGISTIC procedure in SAS (15). Comparisons of the relative fit of hierarchical models were carried out by computing the difference in the –2 ln likelihoods for the models, which follows a \( \chi^2 \) distribution.

The hypothesis for the existence of an AD gene adjacent to \( BCHE \) was evaluated by multilocus linkage analysis in 56 FAD pedigrees. Linkage to the interval between \( D3S1744 \) and \( D3S1763 \) was tested using the GENEHUNTER program (16). Marker allele frequencies were obtained from the Genome Database. For parametric analyses, AD was modelled as an autosomal dominant trait with a mutant allele frequency of 0.001. Replication using a disease allele frequency of 0.01 gave similar results. Age- and sex-dependent penetrance was defined as a step function based on 17 age intervals derived from censored data analyses for early-onset (family mean onset age <65 years) and late-onset (family mean onset age >65 years) families (17,18). A second set of analyses were carried out by assigning a constant low penetrance (0.02) to all unaffected at-risk individuals. This represents a conservative ‘affecteds-only’ model where unaffected individuals provide minimal information with regard to the disease but are important for linkage phase determination with respect to marker data. The possibility of linkage in the presence of heterogeneity was assessed using Smith’s admixture test implemented in the HOMOG program (19). Comparison of the null hypothesis of no linkage (H0) and the alternate hypothesis of linkage with heterogeneity (H1, \( \alpha < 1 \)) for the location scores was carried out as a likelihood ratio test with \( P \) values calculated from the asymptotic \( \chi^2 \) distribution with two degrees of freedom. Families having fewer than two informative meioses were excluded from the test. Linkage data were also evaluated using a non-parametric approach implemented in GENEHUNTER.

ACKNOWLEDGEMENTS

This work was supported through grants from the Medical Research Council of Canada, The Canadian Genetic Diseases Network, The Alzheimer Association of Ontario, The Howard Hughes Medical Research Foundation, the EJLB Foundation, the National Institutes of Health (AG09029), the Peter Burgess Fellowship (E.A.R.), the Alzheimer Society of Canada Fellowship (G.L.), the Helen B. Hunter Fellowship (G.Y.), the National Institutes of Health (T32-AG00115) (S.P.) and the Medical Research Council of Canada (A.P.).

REFERENCES