ABSTRACT - Several recently published studies showed the existence of an association between the allele ε4 of the apolipoprotein E and Alzheimer’s disease (AD) in developed countries. We examined this association in 55 patients with possible or probable AD and 56 elderly controls referred to outpatient clinics at the “Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo” and “Centro de Saúde Escola da Faculdade de Saúde Pública da Universidade de São Paulo”. The allele ε4 was significantly more frequent among patients than controls (20.9% vs 8.9%, p=0.038). Thirty-six percent of the cases presented with at least one allele ε4 compared with only 17.9% of the controls (p=0.027). The presence of at least one ε4 allele increased by 2.63 times the risk of subjects being diagnosed as suffering from AD. All three ε4ε4 patients were male and had a pre-senile onset of the disease. There was no significant difference between senile and pre-senile cases (41.9% vs 29.2%, p=0.326) nor between men and women (36.0% vs 36.7%, p=0.959) regarding their risk of being ε4. The age at onset of symptoms did not differ among the different genotype groups, although ε4ε4 cases showed a consistent trend for earlier onset. When only patients with the diagnosis of “probable AD” were included in the analysis (n=43), we observed that 22.1% of the alleles were ε4, a rate that was significantly higher than the 8.9% of controls (p=0.024). This study supports the association between the presence of the ε4 allele and AD and extend this finding to Brazilian patients. Nonetheless, the presence of this allele is not necessary nor sufficient for the development of the disease and it is possible that its contribution to the pathogenesis of the disorder depends on the subject’s ethnic group.

KEY WORDS: dementia, Alzheimer's disease, risk factors, apolipoprotein E, ApoE4.

PALAVRAS-CHAVE: demência, doença de Alzheimer, fatores de risco, apolipoproteína E, ApoE4.
Alzheimer's disease is the most frequent cause of dementia, with an estimated prevalence of 5-10% for those aged 65 years or older and as high as 47% for those above the age of 85. The disease progresses insidiously with impairment of memory, language, praxis, and visuo-spatial and other cognitive abilities. The neuropathological substrate of the disorder includes the deposition of amyloid in senile plaques and the development of neurofibrillary tangles. Environmental and genetic factors have been considered as possible causes of the disorder.

Familial cases with early-onset AD were recently associated with a number of mutations of the amyloid precursor protein (APP) gene on chromosome 21, mutations on chromosome 12, and, more often, with the S182/PS1 gene on chromosome 14. Furthermore, a strong association between the apolipoprotein E (ApoE) locus on chromosome 19 was described for the more common late onset disease form.

ApoE is associated with the metabolism and clearance of cholesterol and other low-density lipoproteins (LDL) and has a central role in the regeneration of the nervous system. The ApoE gene is located on the long arm of chromosome 19 and can present with three allelic variants (types 2, 3, and 4) and five common genotypes (2/3, 2/4, 3/3, 3/4, and 4/4). The ApoE type 4 allele (ε4) seems to increase the risk of late-onset AD in a dose-related fashion, with the disease starting earlier in patients with two ε4 alleles. The association between the allele ε4 and AD has been considered so strong that St. George-Hyslop and colleagues suggested that the absence of ε4 may delay or even avoid the manifestation of AD in subjects with pathological mutations on chromosome 21. Others suggested that it is not so much the presence of ε4 but the absence of ε2 that contributes to the development of the disease.

There are reports suggesting that the distribution of ε4 varies according to the ethnic group, in which case one would expect different prevalence rates of AD or patterns of ε4 distribution across the World. This study aimed to evaluate the association between ApoE alleles and AD in a heterogeneous ethnic sample of Brazilian subjects.

METHODS

Subjects

Patients with diagnosis of possible or probable AD according to NINCDS/ADRDA criteria were recruited from two outpatient clinics at the "Hospital das Clínicas da Faculdade de Medicina da USP". Elderly controls aged 65 or older were selected from the Geriatric Outpatient Clinic at the Primary Care Unit of the "Faculdade de Saúde Pública da USP". Those with previous history of mental disorder or with Abbreviated Mental Test (AMTS) scores of 7 or less were excluded from the study. This criteria was chosen to decrease the risk of including early AD cases among controls.

Procedures

Clinical assessment

Subjects were assessed systematically and all clinical information was then used to select patients classified as possible or probable AD by the NINCDS/ADRDA criteria.

The assessment of family history of dementia involved the collection of information on grandparents, parents, uncles/aunts, brothers/sisters, and children from two qualified informants (spouse, children, brothers/sisters). However, this information was not analysed because of uncertainties and inconsistencies between informants.

Genetic analysis

Blood samples were sent to the Genetic laboratory of the "Instituto de Biociências da USP" for DNA extraction. ApoE allelic type was determined by standard procedures.

Data analysis

The data were analysed with the "Statistical Package for the Social Sciences for Windows 5.0" (SPSS/PC + 5.0 for Windows). Likelihood ratio analysis of contingency tables was used in the investigation of categorical variables (e.g., frequency of the different alleles among patients and controls), the statistical result being distributed
as chi-square ($\chi^2$). The odds ratio was calculated when appropriate. Student’s t-test was used to compare the means of continuous variables of two different groups (e.g., age). Nonparametric Kruskal-Wallis one-way analysis of variance was used to compare ordinal variables of different groups (e.g., age at onset for patients with 0, 1, or 2 $\varepsilon 4$ alleles), the statistical result being distributed as chi-square ($\chi^2$). Nonparametric U test of Mann-Whitney was employed for the analysis of ordinal data of two different groups, the normal statistical result being referred to as “z”. Ninety-five percent confidence intervals were estimated for the mean (CI), difference between the means (CId), and odds ratio (CIOR).

**RESULTS**

Fifty-five patients and 56 controls fulfilled the entry criteria of the study; 54.5% and 69.6% of them respectively were women ($\chi^2=2.70$, df=1, $p=0.100$; OR=1.91, CIOR=0.87 to 4.16). The mean age of patients and controls were 68.31 (CI=65.90 to 70.71; range: 45-89) and 75.02 (CI=73.73 to 76.30; range: 67-84), with patients being significantly younger than controls ($t=4.93$, df=82.60, $p<0.001$, CI$_d$=4.00 to 9.42). The mean age at onset of symptoms was 64.71 (CI=62.29 to 67.12), with the disease starting after the age of 65 (senile AD) in 56.4% of patients.

Of the total number of alleles ($n=222$), 6.3%, 78.8% and 14.9% were $\varepsilon 2$, $\varepsilon 3$ and $\varepsilon 4$ respectively, patients being more likely than controls to display the $\varepsilon 4$ allele (20.9% vs 8.9%; $\chi^2=6.53$, df=2, $p=0.038$). In fact, 36.4% of patients had at least one $\varepsilon 4$ allele compared to only 17.9% of controls ($\chi^2=4.89$, df=1, $p=0.027$; OR=2.63, CIOR=1.09 to 6.31). Table 1 shows the genotype and allelic distribution of patients and controls.

There was no significant difference on the $\varepsilon 4$ frequency distribution between patients with pre-senile (24 cases) and senile dementia (32 cases) (29.2% vs 41.9%; $\chi^2=0.96$, df=1, $p=0.326$; OR=1.75, CIOR=0.56 to 5.44). All three $\varepsilon 4\varepsilon 4$ subjects were classified as cases, with onset of symptoms at the age of 53, 59 and 61 years. Among patients 36.0% of men and 36.7% of women had at least one $\varepsilon 4$ allele, although all $\varepsilon 4\varepsilon 4$ were male ($\chi^2=4.94$, df=1, $p=0.026$). Kruskal-Wallis one-way analysis of variance showed an age at onset difference between patients with 0, 1 and 2 $\varepsilon 4$ alleles ($\chi^2=5.64$, df=2, $p=0.059$). This difference was due to the fact that cases with 2 alleles had an earlier onset than

| Table 1. ApoE genotypes and allele frequency among AD patients (possible + probable) and elderly controls. |
|---|---|---|
| Genotype | AD(%) | Controls(%) | p value |
| **n=55** | **n=56** | |
| $\varepsilon 4\varepsilon 4$ | 5.5 | 0 | 0.038 |
| $\varepsilon 4\varepsilon 3$ | 27.3 | 17.9 | 0.234 |
| $\varepsilon 4\varepsilon 2$ | 3.6 | 0 | 0.092 |
| $\varepsilon 3\varepsilon 3$ | 54.5 | 69.6 | 0.100 |
| $\varepsilon 3\varepsilon 2$ | 9.1 | 12.5 | 0.562 |
| $\varepsilon 2\varepsilon 2$ | 0 | 0 | — |
| $\varepsilon 4e^*$ | 36.4 | 17.9 | 0.027 |
| $e^*\varepsilon^*$ | 63.6 | 82.1 | — |
| Allele | **n=110** | **n=112** | |
| $\varepsilon 4$ | 20.9 | 8.9 | — |
| $\varepsilon 3$ | 72.7 | 84.8 | 0.038 |
| $\varepsilon 2$ | 6.4 | 6.3 | — |

*represents alleles 2 or 3 (the 3 $\varepsilon 4\varepsilon 4$ patients were included in the $\varepsilon 4e^*$ group).
Table 2. ApoE genotypes and allele frequency among AD patients (probable) and elderly controls.

<table>
<thead>
<tr>
<th></th>
<th>AD n=43</th>
<th>Controls n=56</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (mean; CI)</td>
<td>69.02</td>
<td>75.02</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>66.28 to 71.76</td>
<td>73.73 to 76.30</td>
<td></td>
</tr>
<tr>
<td>Female sex</td>
<td>62.8%</td>
<td>69.6%</td>
<td>0.474</td>
</tr>
<tr>
<td>Genotype</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ε4ε4</td>
<td>7.0%</td>
<td>0</td>
<td>0.079</td>
</tr>
<tr>
<td>ε4ε3</td>
<td>25.6%</td>
<td>17.9%</td>
<td>0.353</td>
</tr>
<tr>
<td>ε4ε2</td>
<td>4.7%</td>
<td>0</td>
<td>0.186</td>
</tr>
<tr>
<td>ε3ε3</td>
<td>51.2%</td>
<td>69.6%</td>
<td>0.061</td>
</tr>
<tr>
<td>ε3ε2</td>
<td>11.6%</td>
<td>12.5%</td>
<td>0.895</td>
</tr>
<tr>
<td>ε2ε2</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>ε4e*</td>
<td>37.2%</td>
<td>17.9%</td>
<td>0.052</td>
</tr>
<tr>
<td>ε<em>e</em></td>
<td>62.8%</td>
<td>82.1%</td>
<td></td>
</tr>
<tr>
<td>Allele</td>
<td>n=86</td>
<td>n=112</td>
<td></td>
</tr>
<tr>
<td>ε4</td>
<td>22.1</td>
<td>8.9%</td>
<td></td>
</tr>
<tr>
<td>ε3</td>
<td>69.8%</td>
<td>84.8%</td>
<td>0.025</td>
</tr>
<tr>
<td>ε2</td>
<td>8.1%</td>
<td>6.3%</td>
<td></td>
</tr>
</tbody>
</table>

*represents alleles 2 or 3 (the 3 ε4ε4 patients were included in the ε4ε* group).

CI, confidence interval of the mean.

patients with just 1 allele (mean age of onset=57.67 vs 68.17; z=-2.22, p=0.026). However, there was no significant age at onset difference between genotype groups (i.e., ε2ε3, ε3ε3, ε2ε4, ε3ε4, and ε4ε4) (χ²=5.92, df=4, p=0.204).

The exclusion of patients with the diagnosis of “possible AD” from the analysis (12 patients) did not produce significant changes in our results (Table 2).

DISCUSSION

The association between ApoE type 4 allele and AD has been widely replicated in the developed World during the past two years. Our results confirm the consistency of this association in Brazil, with 36.4% of patients exhibiting at least one ε4 allele. Moreover, the presence of this allele increased the risk of subjects being classified as AD by 2.6 times. However, only 20.9% of the total number of alleles of patients were ε4, a rate lower than the 25-40% described by previous studies. There are three possible explanations for this discrepancy:

1) our sample included a large number of patients with pre-senile dementia, which may have contributed to decrease the total number of ε4 alleles in our sample. However, we found no ε4 frequency difference between pre-senile and senile cases in our sample, which suggests that the low prevalence of ε4 cannot be simply explained by age at onset bias. Another possible confounding factor is that the older age of our control group may have led to an overestimation of the difference between patients and controls, as the ε4 frequency has been shown to decrease with increasing age. The problem with this explanation is that our patients were younger than those described in previous studies and should, therefore, present an even higher ε4 rate;

2) it is possible that other risk factors, such as poor schooling, were more important in the causation of AD in our sample than in the studies from developed countries reported so far. Conversely, the
e4 allele has been recently associated with lower intellectual achievement\(^\text{11,36,47}\) and reduction in regional brain metabolism of non-demented young subjects\(^\text{45}\), which suggests that lower educational achievement may result from the presence of the e4 allele and might be only secondarily associated with AD. There may be other risk factors playing a role in the causation of AD in Brazil, but their characteristics remain elusive;

3) the Brazilian population may have, on the whole, a lower e4 frequency than that found in developed Countries. In fact, Utermann\(^\text{53}\) suggested that the e4 distribution rate varies considerably around the World, so that the use of a heterogeneous ethnic sample may have contributed to produce these results. This is, of course, a highly speculative hypothesis, particularly because the e4 rates among our controls were similar to those described elsewhere\(^\text{16,27,34,40}\).

The presence of two e4 alleles was observed only among the men of our sample. Payami and coworkers\(^\text{31}\) have recently suggested that women are more susceptible to the effects of ApoE4 than men, with only 1 allele being sufficient to increase the risk of AD among women whereas 2 would be necessary in men. This hypothesis was questioned by Corder and colleagues\(^\text{9}\) who reported similar effects of ApoE4 among men and women. The results of this study suggest that the difference of e4 distribution among men and women was due to the higher prevalence of e4e4 among the former, which reinforces the idea that women might be more vulnerable to the deleterious effects of apoE4.

The presence of two e4 alleles was also associated with an earlier age at onset, which is in line with previously published data\(^\text{8,34,52}\). However, there was no difference regarding the age at onset of symptoms between patients with one or no e4 alleles, nor between cases exhibiting different genotypes. These findings contrast with the results of most studies reported to date and can be explained by the relatively small number of patients included in our sample.

Our results indicate that the e4 allele is an important risk factor for AD, although it also shows that its presence is not necessary nor sufficient for the development of the disease\(^\text{35}\). Moreover, the balance between genetic and non-genetic risk factors may vary considerably in different parts of the World and across different ethnic groups. Nonetheless, it is important to clarify the mechanisms by which ApoE4 contributes to the development of the disease. ApoE4 binds to senile plaques\(^\text{42}\) and forms a stable complex with β-amyloid\(^\text{49,50,56}\) and may, therefore, alter the balance between the deposition and clearance of β-amyloid in favour of the formation of senile plaques and vascular amyloid\(^\text{43,57}\). Alternatively, ApoE4 may influence the phosphorylation rate of tau and favour the formation of neurofibrillary tangles\(^\text{38}\). Others\(^\text{48}\) propose that it is not so much the presence of e4, but the absence of e2/e3 that render patients more susceptible to the development of AD. This model suggests that e2/e3 promote the binding of tau to microtubules stabilising the neurone’s cytoskeleton and protecting the protein from abnormal phosphorylation. Conversely, β-amyloid might facilitate the phosphorylation of tau and neurofibrillary tangles. Another possibility is that ApoE4 interferes with the metabolism of cholesterol and other lipids and hinders the integrity of synapses. In other words, the compensatory mechanisms of neuronal regeneration may be impaired in patients with an e4 allele\(^\text{39}\). It is clearly essential that the mechanisms by which ApoE4 contributes to the development of AD are uncovered, as they may represent promising targets for the treatment of the disorder.

Acknowledgements - This study was supported by a grant from FAPESP (94/2158-8) and CNPq. OPA is supported by CNPq. We are grateful to Luciana Vasquez, Prof. Dr. Maria Rita Passos Bueno and Prof. Dr. Mayana Zatz for helping with the molecular analysis of the blood samples; Prof. Dr. Ricardo Nitrini, the staff at the Neurology Outpatient Clinic and at PROTER for helping with the recruitment of patients, and the staff at the “Centro de Saúde Escola da FSP-USP” for helping with the selection of controls.

REFERENCES


51. Thompson P, Blessed G. Correlation between the 37 item Mental Test Score and abbreviated 10-item Mental Test Score by psychogeriatric day patients. Br J Psychiatry 1987;151:206-209.


