SHORT REPORT

Apolipoprotein E genotype and hippocampal asymmetry in Alzheimer’s disease: a volumetric MRI study

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Abstract

Asymmetry of brain structures is common to many species and is even present in utero. Some developmental, pathological, and dementing diseases are associated with alterations in normal anatomical asymmetries. Anatomical asymmetries, however, have been only superficially studied in Alzheimer’s disease. Recent evidence indicates that the allele ε4 of the apolipoprotein E (ApoE), a well known risk factor for Alzheimer’s disease, might play a part in determining some brain morphological changes both in normal carriers and in patients with Alzheimer’s disease. This study evaluated the effect of the ApoE genotype on hippocampal asymmetry in patients with Alzheimer’s disease carrying 0, 1, and 2 copies of the allele. Volumetric right-left differences of the hippocampi were computed in 28 right handed patients with Alzheimer’s disease (14 ε/ε, 9 ε4/ε3, and 5 ε4/ε4) and 30 controls without detectable cognitive deficit. In controls, the right hippocampus was larger than the left, whereas in patients with Alzheimer’s disease this asymmetry was progressively reduced with increasing gene dose of the ε4 allele, and the asymmetry was reversed in the ε4/ε4 Alzheimer’s disease group. The mean right-left volume differences were: 1.2, 0.7, 0.2, and -1.0 in controls, -/ε-, ε4/ε-, and ε4/ε4 patients, respectively (sex adjusted p for trend=0.017). The data indicate a dose dependent effect of the ApoE ε4 allele on hippocampal volume asymmetry in Alzheimer’s disease.

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Laterality of brain structure and function occurs across phylogeny and has been present since antiquity.1 Brain asymmetries are even present in utero and do not seem to be affected by the aging process.2 Neuroanatomical and CT studies3 have shown that in right handed people the right frontal lobe is larger than the left, whereas the opposite occurs in the occipital lobe (the left is larger than the right). These asymmetries are present in adults, infants, and foetuses ranging from 20 to 42 weeks of gestation.3 More recently, some MRI studies focusing on the hippocampus of healthy people have found that the right hippocampus is larger than the left.4 Sex and handedness are related to the physiological asymmetry.5 6 Modifications of asymmetry have been described in some pathological conditions that occur in young and adult age such as autism, dyslexia, verbal intelligence deficits, and schizophrenia.7 In addition, certain degenerative conditions such as progressive aphasia and apraxia, Pick’s disease, and cortical-basal degeneration are characterised by striking asymmetry.8 9 Anatomical asymmetry, however, has been only superficially studied in Alzheimer’s disease. This is surprising given that cognitive and functional asymmetries are common to patients with the disease.9 Apolipoprotein E (ApoE) is a lipid carrying protein that may have a role in brain development and function.10 ApoE presents in three allelic forms (ε2, ε3, and ε4). Differences in normal hippocampal asymmetry in relation to ApoE genotype have been described in non-demented subjects.11 With the use of MRI, Soininen et al12 found a reduction of asymmetry in non-demented older persons heterozygous for the ε4 allele (ε4/ε-) and inversion of asymmetry in homoygous subjects (ε4/ε4) when compared with non-carriers (ε4/ε-). Similar findings have been reported by Tohgi et al.13 Besides age, the ε4 allele is the most relevant risk factor for the sporadic form of the disease known to date and seems to modulate several clinical manifestations of Alzheimer’s disease.13 The ε4 allele is associated with younger age at onset,14 greater memory and lower attention deficit,15 and slower progression.16 Although ApoE is known to influence normal hippocampal asymmetry, its role on the pathological process of Alzheimer’s disease is largely unidentified.

The aim of this study was to evaluate the effect of the ApoE genotype on hippocampal...
asymmetry in patients with Alzheimer’s disease as a function of ε4 allele dosage.

Methods
Subjects and methods of this study have been previously described in reports on MRI measures of atrophy in the degenerative dementias. Briefly, the Alzheimer’s disease group consisted of 28 outpatients with mild to moderate clinically probable disease. Neuropsychological assessment was carried out following a standard protocol. The control group consisted of 30 patients’ relatives (mostly spouses) without detectable cognitive deficit. All patients and controls were right handed. The mini mental state examination (MMSE) was administered to patients and controls shortly before MRI. Blood samples for ApoE phenotyping were obtained from all patients and 28 of the 30 control subjects. ApoE phenotyping was performed with isoelectric focusing on delipidated plasma samples, as previously described. Written informed consent was obtained from controls and patients or their primary caregivers. The study was reviewed and approved by the local ethics committee.

Brain MRI was performed with a 1.5 Tesla scanner (Siemens, Magnetom). A gradient echo 3D technique was employed for image acquisition. The ROIs of the hippocampi were manually traced from contiguous coronal, 2.0 mm thick T1 weighted images, as previously described (intraclass correlation coefficient: 0.95). The intracranial area (in mm$^2$) was used for normalisation to cranial size according to the formula: \( \text{volume/intracranial area} \times 100 \). Hippocampal asymmetry was computed for each subject as the difference between the volumes of the right and the left side. An asymmetry index (figure) was also computed as the ratio between the difference and the sum of hippocampal volumes, representing the proportion of the volume difference between the right and the left side adjusted by total hippocampal volume. This index allows for taking into account the effect of greater atrophy on the reduction of asymmetry.

As both age and sex may influence hippocampal size and asymmetry, initial correlation analyses tested these relations. Correlation and locally weighted regression analyses failed to show any significant relation of hippocampal volume asymmetry with age. Men had greater hippocampal asymmetry than women (right–left difference of normalised volumes: 2.0 (SD 1.5) in men vs 0.8 (SD 1.3) in women, \( p=0.03 \) on \( t \) test for independent samples). The sex-age interaction was not significant in an analysis of variance (ANOVA) model including sex, age, and their interaction. Further analyses, therefore, controlled for the effect of sex on volume asymmetries.

To test the significance of the inverse relation between ε4 gene dose and right–left asymmetry, a test for trend (sex adjusted) was used. This was carried out in a linear regression model in which asymmetry was the dependent and group (coded as a 4 level variable: 0=controls, 1=AD ε4/–, 2=AD ε4/–, and 3=AD ε4/4) and sex were the independent variables. For the test for trend, the group variable was treated as a continuous variable. The significance of the test for trend was that of the coefficient of the group variable.

The critical value for statistical significance was set at \( p<0.05 \) for all comparisons.

Results
The prevalence of ε4 genotype was significantly different between patients with Alzheimer’s disease and controls. Five patients with Alzheimer’s disease and two controls were homozygous, and nine patients with Alzheimer’s disease and four controls were heterozygous for the ε4 allele (allelic frequency: 0.34 and 0.14 in the Alzheimer’s disease and control groups, respectively; \( \chi^2=5.9; df=1; p=0.01 \)).

The table summarises demographic and clinical information. Age, sex, and educational achievement were similar among study groups. In the Alzheimer’s disease group clinical severity of the disease as estimated by the MMSE did not differ between the subgroups according to the ApoE genotype (F(2,29)=0.8; \( p=0.51 \) on ANOVA).

The figure (A) shows that in controls hippocampal volumes were 8% greater on the right than on the left side (\( r=4.42, df=29, p \)
Apolipoprotein E genotype and hippocampal asymmetry in Alzheimer’s disease

Table 1  Characteristics of the study groups

<table>
<thead>
<tr>
<th></th>
<th>Controls (n=30)</th>
<th>Alzheimer’s disease patients</th>
<th>p Value</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td>~~/~ (n = 14)</td>
<td>c4~/~ (n = 9)</td>
</tr>
<tr>
<td>Mean (SD) years of age at observation</td>
<td>69 (8)</td>
<td>69 (11)</td>
<td>77 (6)</td>
</tr>
<tr>
<td>[range]</td>
<td>[53–86]</td>
<td>[53–84]</td>
<td>[67–86]</td>
</tr>
<tr>
<td>No. (%) of women</td>
<td>20 (67)</td>
<td>9 (64)</td>
<td>8 (89)</td>
</tr>
<tr>
<td>Mean (SD) years of education</td>
<td>8 (3)</td>
<td>8 (4)</td>
<td>7 (5)</td>
</tr>
<tr>
<td>[range]</td>
<td>[5–19]</td>
<td>[3–18]</td>
<td>[2–17]</td>
</tr>
<tr>
<td>Mean (SD) mini mental state examination</td>
<td>29 (1)</td>
<td>20 (4)</td>
<td>21 (4)</td>
</tr>
<tr>
<td>[range]</td>
<td>[23–30]</td>
<td>[12–27]</td>
<td>[15–27]</td>
</tr>
</tbody>
</table>

p = Significance on one way ANOVA or chi² test.

p<0.0001 on t test for paired sample. In patients with Alzheimer’s disease, this effect was progressively reduced with increasing gene dose of the e4 allele, and in e4/4 patients the effect was reversed (right hippocampus 12% smaller than the left). The effect of the e4 on asymmetry effect is also shown in the figure B. The mean difference between right and left hippocampal volumes was decreasing from controls (1.2) throughout -/- (0.7), e4/- (0.2), to e4/4 (-1.0) Alzheimer’s disease patient groups. The sex adjusted test for trend was significant. As there were few men in the study groups, we could not test the effect separately in men and women. A separate analysis of women, however, showed the same results. The effect in controls alone could not be tested due to the few people carrying the e4 allele.

The effect of the e4 allele on asymmetry was also tested in the whole temporal lobe and entorhinal cortex. The methods of volume measurement for these regions have been described elsewhere.14 No effect of the e4 allele was shown in either region (p>0.2).

Discussion

These data indicate a dose dependent effect of the ApoE e4 allele on hippocampal volume asymmetry in Alzheimer’s disease. In patients without the allele, the normal right greater than left asymmetry is diminished, in those with one copy of the allele it is abolished, and in those with two copies it is reversed (left volumes greater than right).

Although the volumetric asymmetry of the hippocampus by ApoE genotype has never been explicitly investigated in Alzheimer’s disease, previous workers have reported hippocampal volumes separately for the right and left side, and comparisons can be made with our own data. Consistent with our data, Lehtonen et al found that in 16 controls the right hippocampal volume was 11% greater than the left, whereas in five e4/4 patients it was 6% smaller.22

The same effect has been reported in two studies investigating brain asymmetry by ApoE genotype in adult and elderly non-demented people. In a sample of 32 community dwelling elderly subjects,11 Soininen et al have shown a reduction of the physiological asymmetry in the e4/- and an inversion of the asymmetry in the e4/4 subjects. The difference between right and left hippocampal volumes was 401 (SD 181) mm³ in 18~7/~, 132 (SD 209) in 10 e4/-, and ~258 (SD 181) in four e4/4 subjects. Similar findings can be extrapolated from the study of Tohgi et al,12 measuring right and left hippocampal areas in 40 non-demented non-carriers and 14 e4 carriers aged 39 to 80 years. The authors found that the right hippocampus was larger than the left in non-carriers, whereas the opposite was true in carriers.

Overall, these findings seem to indicate that carriers of the e4 allele have left greater than right hippocampi whereas the opposite pattern is present in non-carriers, and that this occurs in both non-demented subjects and patients with Alzheimer’s disease. We speculate that the effect of ApoE on brain structure might operate either during brain development20 or senescence. Further studies on the effect of ApoE on brain function and behaviour in health and disease are warranted.

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