SHORT REPORT

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

C Geroldi, M P Laakso, C DeCarli, A Beltramello, A Bianchetti, H Soininen, M Trabucchi, G B Frisoni

Laboratory of Epidemiology and Neuroimaging, IRCCS San Giovanni di Dio - FBF, via Pilastroni 4, 25125 Brescia, Italy
C Geroldi
A Bianchetti
M Trabucchi
G B Frisoni

Department of Neurology, Kuopio University Hospital, PO Box 1777, 70211 Kuopio, Finland
M P Laakso
H Soininen

Department of Clinical Radiology
M P Laakso

Alzheimer's Disease Center, Kansas University Medical Center, 3901 Rainbow Boulevard, Kansas City, KS, USA
C DeCarli

Institute of Radiology, University of Verona, Ospedale Borgo Trento, Verona, Italy
A Beltramello

Correspondence to: Giovanni B. Frisoni, MD, Laboratory of Epidemiology and Neuroimaging, IRCCS San Giovanni di Dio - FBF, via Pilastroni 4, 25125 Brescia, Italy
email: frisoni@master.cci.unibs.it

Received 22 March 1999 and in revised form 2 August 1999
Accepted 20 August 1999

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 $\epsilon 4$ 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 $-/-$, 9 $\epsilon 4/-$, and 5 $\epsilon 4/4$) 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 $\epsilon 4$ allele, and the asymmetry was reversed in the $\epsilon 4/4$ Alzheimer's disease group. The mean right-left volume differences were: 1.2, 0.7, 0.2, and -1.0 in controls, $-/-$, $\epsilon 4/-$, and $\epsilon 4/4$ patients, respectively (sex adjusted p for trend=0.017). The data indicate a dose dependent effect of the ApoE $\epsilon 4$ allele on hippocampal volume asymmetry in Alzheimer's disease.

(J Neurol Neurosurg Psychiatry 2000;68:93-96)

Keywords: apolipoprotein E; ApoE; Alzheimer's disease; hippocampus; asymmetry; magnetic resonance imaging

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 fetuses 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.³ In addition, certain degenerative conditions such as progressive aphasia and apraxia, Pick's disease, and cortical-basal degeneration are characterised by striking asymmetry.^{7,8} 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 ($\epsilon 2$, $\epsilon 3$, and $\epsilon 4$). 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 al.*¹¹ found a reduction of asymmetry in non-demented older persons heterozygous for the $\epsilon 4$ allele ($\epsilon 4/-$) and inversion of asymmetry in homozygous subjects ($\epsilon 4/4$) when compared with non-carriers ($-/-$). Similar findings have been reported by Tohgi *et al.*¹²

Besides age, the $\epsilon 4$ 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 $\epsilon 4$ 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 $\epsilon 4$ allele dosage.

Methods

Subjects and methods of this study have been previously described in reports on MRI measures of atrophy in the degenerative dementias.^{17,18}

Briefly, the Alzheimer's disease group consisted of 28 outpatients with mild to moderate clinically probable disease.¹⁹ Neuropsychological assessment was carried out following a standard protocol.²⁰ 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.¹⁶ 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).^{18,21} The intracranial area (in mm²) was used for normalisation to cranial size according to the formula: (volume/intracranial area)×100. Hippocampal asymmetry was computed for each subject as the difference between the volumes of the right and the left side.²⁰ 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 *v* 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 $\epsilon 4$ 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 $\epsilon 4$ -, 2=AD $\epsilon 4$ +/-, and 3=AD $\epsilon 4$ /4) and sex were the independent variables. In 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 $\epsilon 4$ 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 $\epsilon 4$ 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 ($F_{2,25}=0.68$; $p=0.51$ on ANOVA).

The figure (A) shows that in controls hippocampal volumes were 8% greater on the right than on the left side ($t=4.42$, $df=29$,

(A) Right and left hippocampal volumes and (B) asymmetry. Error bars around the mean are SD. (A) Volumes are normalised by intracranial area. Asymmetry index = $(2 \times (\text{right} - \text{left}) / (\text{right} + \text{left})) \times 100$. (B) *p* Denotes significance of sex adjusted test for trend.

Table 1 Characteristics of the study groups

	Controls (n = 30)	Alzheimer's disease patients			p Value
		-/- (n = 14)	ϵ 4/- (n = 9)	ϵ 4/4 (n = 5)	
Mean (SD) years of age at observation [range]	69 (8) [53–86]	69 (11) [53–84]	77 (6) [67–86]	74 (4) [67–78]	NS
No (%) of women	20 (67)	9 (64)	8 (89)	5 (100)	NS
Mean (SD) years of education [range]	8 (3) [5–19]	8 (4) [3–18]	7 (5) [2–17]	5 (2) [2–8]	NS
Mean (SD) mini mental state examination [range]	29 (1) [25–30]	20 (4) [12–27]	21 (4) [15–27]	19 (5) [13–25]	<0.00005

p = Significance on one way ANOVA or χ^2 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 ϵ 4 allele, and in ϵ 4/4 patients the effect was reversed (right hippocampus 12% smaller than the left). The effect of the ϵ 4 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), ϵ 4/- (0.2), to ϵ 4/4 (-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 ϵ 4 allele.

The effect of the ϵ 4 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.¹⁸ No effect of the ϵ 4 allele was shown in either region ($p > 0.2$).

Discussion

These data indicate a dose dependent effect of the ApoE ϵ 4 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, Lehtovirta *et al* found that in 16 controls the right hippocampal volume was 11% greater than the left, whereas in five ϵ 4/4 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 ϵ 4/- and an inversion of the asymmetry in the ϵ 4/4 subjects. The difference between right and left hippocampal volumes was 401 (SD 181) mm³ in 18 -/-, 132 (SD 209) in 10 ϵ 4/-, and -258 (SD 181) in four ϵ 4/4

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 ϵ 4 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 ϵ 4 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 development²³ or senescence. Further studies on the effect of ApoE on brain function and behaviour in health and disease are warranted.

This study was supported by the Research Council for Health of the Academy of Finland (MPL), and by grants from the IRCCS San Giovanni di Dio and NIH (grant AG10182) (CD). We gratefully acknowledge the contribution of: Dr Naftali Raz, Department of Psychology, University of Memphis, for his useful comments on the manuscript; Dr Giuliano Binetti, IRCCS San Giovanni di Dio - FBF, Brescia, for providing the neuropsychological data; Drs Giovanni Puppini and Ghassan El-Dhalati, Institute of Radiology, University of Verona, for management of the image files; and Drs Tiziana Metitieri and Alessandra Pezzini for help with the data collection.

- Geschwind N, Galaburda AM. Cerebral lateralization. Biological mechanisms, associations, and pathology: a hypothesis and a program for research. *Arch Neurol* 1985;42:428–59;521–52;634–54.
- Bigler ED, Blatter DD, Anderson CV, *et al*. Hippocampal volume in normal aging and traumatic brain injury. *AJNR Am J Neuroradiol* 1997;18:11–23.
- Weinberger DR, Luchins DJ, Moriguchi J, *et al*. Asymmetrical volumes of the right and left frontal and occipital regions of the human brain. *Ann Neurol* 1982;11:97–100.
- Jack CR Jr, Theodore WH, Cook M, *et al*. MRI-based hippocampal volumetrics: data acquisition, normal ranges, and optimal protocol. *Magn Reson Imaging* 1995;13:1057–64.
- Crow TJ. Sexual selection, machiavellian intelligence, and the origins of psychosis. *Lancet* 1993;342:594–8.
- LeMay M. Asymmetries of the skull and handedness. *Phrenology revisited*. *J Neurol Sci* 1977;32:243–53.
- Kitagaki H, Mori E, Yamaji S, *et al*. Frontotemporal dementia and Alzheimer disease: evaluation of cortical atrophy with automated hemispheric surface display generated with MR images. *Radiology* 1998;208:431–9.
- Matochik JA, Molchan SE, Zametkin AJ, *et al*. Regional cerebral glucose metabolism in autopsy-confirmed Creutzfeldt-Jakob disease. *Acta Neurol Scand* 1995;91:153–7.
- Raz N, Raz S, Yeo RA, *et al*. Relationship between cognitive and morphological asymmetry in dementia of the Alzheimer type: a CT scan study. *Int J Neurosci* 1987;35:225–32.
- Weisgraber KH, Mahley RW. Human apolipoprotein E: the Alzheimer's disease connection. *FASEB J* 1996;10:1485–94.
- Soininen H, Partanen K, Pitkanen A, *et al*. Decreased hippocampal volume asymmetry on MRIs in non-demented elderly subjects carrying the apolipoprotein E ϵ 4 allele. *Neurology* 1995;45:391–2.
- Tohgi H, Takahashi S, Kato E, *et al*. Reduced size of right hippocampus in 39–80 year old normal subjects carrying the apolipoprotein E ϵ 4 allele. *Neurosci Lett* 1997;236:21–4.
- Frisoni GB, Trabucchi M. Clinical rationale of genetic testing in dementia. *J Neurol Neurosurg Psychiatry* 1997;62:217–21.

- 14 Meyer MR, Tschanz JT, Norton MC, et al. APOE genotype predicts when—not whether—one is predisposed to develop Alzheimer disease. *Nat Genet* 1998;19:321–2.
- 15 Lehtovirta M, Soininen H, Helisalmi S, et al. Clinical and neuropsychological characteristics in familial and sporadic Alzheimer's disease: relation to apolipoprotein E polymorphism. *Neurology* 1996;46:413–9.
- 16 Frisoni GB, Govoni S, Geroldi C, et al. Gene dose of the $\epsilon 4$ allele of apolipoprotein E and disease progression in sporadic late-onset Alzheimer's disease. *Ann Neurol* 1995;37:596–604.
- 17 Frisoni GB, Beltramello A, Weiss C, et al. Linear measures of atrophy in mild Alzheimer's disease. *AJNR Am J Neuroradiol* 1996;17:913–23.
- 18 Frisoni GB, Laakso MP, Beltramello A, et al. Hippocampal and entorhinal cortex atrophy in frontotemporal dementia and Alzheimer's disease. *Neurology* 1999;52:91–100.
- 19 McKhann G, Drachman D, Folstein M, et al. Clinical diagnosis of Alzheimer's disease: report of NINCDS/ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer's disease. *Neurology* 1984;34:939–44.
- 20 Binetti G, Magni E, Padovani A, et al. Neuropsychological heterogeneity in mild Alzheimer's disease. *Dementia* 1993;4:321–6.
- 21 Laakso MP, Partanen K, Riekkinen P Jr, et al. Hippocampal volumes in Alzheimer's disease, Parkinson's disease with and without dementia, and in vascular dementia: an MRI study. *Neurology* 1996;46:678–81.
- 22 Lehtovirta M, Laakso MP, Soininen H, et al. Volumes of hippocampus, amygdala and frontal lobe in Alzheimer patients with different apolipoprotein E genotypes. *Neuroscience* 1995;67:65–72.
- 23 Chapman S, Michaelson DM. Specific neurochemical derangements of brain projecting neurons in apolipoprotein E-deficient mice. *J Neurochem* 1998;70:708–14.