

## Apolipoprotein E epsilon4 is associated with atrophy of the amygdala in Alzheimer's disease

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### Abstract

Although the ApoE  $\epsilon 4$  allele is well-established as the most important genetic risk factor for Alzheimer's disease (AD), the effects of this allele on regional brain atrophy in AD patients remain controversial. We performed MRI-based volumetric measurements of the hippocampus and amygdala (normalized to intracranial volume) in 32  $\epsilon 4+$  AD patients, 23  $\epsilon 4-$  AD patients, and 42 cognitively normal elderly control subjects. Analysis of covariance revealed that amygdaloid volume was significantly smaller (19.2%) in ApoE  $\epsilon 4+$  than  $\epsilon 4-$  AD patients, controlling for disease severity ( $F = 10.62$ ; d.f. = 1,52;  $p = 0.002$ ; ANCOVA). Alternatively, when ApoE  $\epsilon 4$  dose was considered, this effect appeared to accrue from a difference between the 0 $\epsilon 4$  and each of the other two AD groups, with no significant difference between the 1 $\epsilon 4$  and 2 $\epsilon 4$  AD groups. Hippocampal volumes and asymmetry indices for hippocampus and amygdala did not differ between  $\epsilon 4$  carriers and noncarriers. These results suggest accelerated atrophy of the amygdala in AD in association with ApoE  $\epsilon 4$  and provide further evidence for regionally specific effects of this allele.

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**Keywords:** Apolipoprotein E; Alzheimer's disease; Magnetic resonance imaging; Amygdala; Hippocampus

### 1. Introduction

The  $\epsilon 4$  allele of the apolipoprotein E (ApoE) gene on chromosome 19 has been identified as a major risk factor for Alzheimer's disease (AD) [38]. In order to relate the ApoE  $\epsilon 4$  allele to the pathophysiology of AD, investigators have begun to search for phenotypic differences between AD patients who carry or lack  $\epsilon 4$ . The search for phenotypic correlates of  $\epsilon 4$  has included neuropathological studies of the rate of  $\beta$ -amyloid (A $\beta$ ) deposition [10,30,31,39], neurofibrillary tangle (NFT) formation [10,30,31,39], and cholinergic markers [34]. Investigators have also sought to elucidate clinical differences among subjects with different ApoE genotypes, e.g.,

in rate of progression [10,29,45], responsivity to cholinergic therapies [34], and behavioral disturbances [22].

The search for phenotypic correlates of ApoE  $\epsilon 4$  has also included investigations with structural magnetic resonance imaging (MRI). Since the site of the earliest and most severe pathology in AD is the medial temporal lobe [3,13], MRI studies of AD have focused on medial temporal structures—in particular hippocampus and amygdala. This research has consistently demonstrated the reduction of hippocampal [14,17–19,25,27,32] and/or amygdaloid [17,19,25,27] volumes in AD. MRI studies that examine medial temporal lobe volumetric associations of the ApoE  $\epsilon 4$  allele have been much more conflicting and equivocal. The  $\epsilon 4$  allele has been found to be associated with increased atrophy of hippocampus by most [8,11,21,29] but not all [15] investigators. ApoE  $\epsilon 4$  studies of the amygdala have thus far

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been conducted by only two groups. Lehtovirta et al. [21] found differences in amygdaloid volumes between healthy controls and  $\epsilon 4$  carrier subgroups of AD patients but no significant differences between  $\epsilon 4+$  and  $\epsilon 4-$  AD patients. More recently, Hashimoto et al. [11] reported that amygdaloid volume was inversely correlated with the number of ApoE  $\epsilon 4$  alleles in an AD sample.

Some previous research has also suggested alteration in asymmetry of medial temporal structures in association with ApoE  $\epsilon 4$ . A preponderance of studies have shown a “normal” (right > left) asymmetry of hippocampus [1,9,15,17,18,26,44]. One study [9] reported that the right > left hippocampal asymmetry seen in normals was progressively reduced in AD patients with increasing ApoE  $\epsilon 4$  dose, with reversal of the asymmetry in the  $2\epsilon 4$  group.

In the present study, we performed MRI-based volumetric measurements of the hippocampus and amygdala in AD patients who were also genotyped for ApoE. We sought to resolve conflict and uncertainties among earlier studies in testing the hypothesis that ApoE  $\epsilon 4$  is associated with reduced volumes of the hippocampus and amygdala in patients with AD. We also examined altered asymmetry of these medial temporal lobe structures as a function of  $\epsilon 4$ .

## 2. Methods

### 2.1. Human subjects

The study sample comprised 55 patients with probable AD who received MRI scanning in research protocols in the Yale Alzheimer’s Disease Research Unit (ADRU). Patients had been referred to the ADRU from a variety of sources or were self-referred. Five of these patients have subsequently died and had autopsy confirming definite AD [24]. Forty-two healthy elderly control subjects (most spouses of participat-

ing AD patients) were recruited for MRI scanning and were selected without regard to genotype. The demographics and clinical characteristics of patients and controls are displayed in Table 1. Two AD patients were African American (ApoE genotypes 3,4 and 4,4); all other patients and controls were of European ancestry.

All patients and controls underwent a comprehensive evaluation by a research physician and ancillary staff, including cognitive assessment, medical history, physical and neurological examinations, serum chemistries, thyroid function studies, complete blood count, B<sub>12</sub>, folate, VDRL, urinalysis, electrocardiogram, and clinical reading of brain MRI. AD patients were required to meet standard diagnostic criteria for probable AD [23], and controls were required to be in good health for their age. Subjects were excluded for any neurological or medical disorder (other than AD in the patient group) that could produce cognitive deterioration or for significant psychiatric illness, alcohol, or substance abuse. Healthy subjects were also required to have no significant evidence of cognitive impairment, as indicated by a mini-mental state examination (MMSE) [7] score  $\geq 27$  and a normal brain MRI scan.

Neuropsychological testing administered for subject characterization (see Table 1) included the MMSE [7] and the cognitive subscale of the Alzheimer’s Disease Assessment Scale (ADAS-Cog) [37]. Family history of AD was assessed using the Alzheimer Dementia Risk Questionnaire [4] and the Dementia Questionnaire [42] and was considered to be positive if at least one first-degree relative met criteria for primary degenerative dementia. No cases suggestive of autosomal dominant transmission were identified.

### 2.2. Determination of ApoE genotype

All subjects (or their responsible next of kin) provided written informed consent, and were studied under a

Table 1  
Subject characteristics

Variable	Alzheimer’s $\epsilon 4+$ ( $N=32$ ), mean ( $\pm$ S.D.)	Alzheimer’s $\epsilon 4-$ ( $N=23$ ), mean ( $\pm$ S.D.)	Controls ( $N=42$ ), mean ( $\pm$ S.D.)
<b>Demographics</b>			
Age	73.5 $\pm$ 6.9 <sup>a</sup>	68.2 $\pm$ 9.5	73.2 $\pm$ 6.7
Gender	14M, 18F	12M, 11F	22M, 20F
Handedness	32R	21R, 2L	42R
Education (years)	13.9 $\pm$ 3.3	14.1 $\pm$ 3.4	14.3 $\pm$ 3.4
<b>Neuropsychological tests</b>			
MMSE	17.9 $\pm$ 4.5 <sup>b</sup>	18.8 $\pm$ 4.0 <sup>b</sup>	29.0 $\pm$ 1.0
ADAS-Cog	27.9 $\pm$ 10.3 <sup>b</sup>	26.3 $\pm$ 11.1 <sup>b</sup>	5.1 $\pm$ 1.8
<b>Disease characteristics</b>			
Onset age	68.7 $\pm$ 7.0 <sup>a</sup>	63.9 $\pm$ 9.5	
Duration (years)	4.8 $\pm$ 1.6	4.3 $\pm$ 1.2	
Family history	15+, 17–	10+, 13–	
ApoE genotype	1 <sub>2,4</sub> 19 <sub>3,4</sub> 12 <sub>4,4</sub>	21 <sub>3,3</sub> 2 <sub>2,3</sub>	

MMSE, mini-mental state examination; ADAS-Cog, Alzheimer’s disease assessment scale–cognitive subscale; family history is positive if primary degenerative dementia is present in at least one first degree relative.

<sup>a</sup> Differs from Alzheimer’s  $\epsilon 4-$  value,  $p < 0.05$ ,  $t$ -test.

<sup>b</sup> Differs from control value,  $p < 10^{-7}$ ,  $t$ -test.

protocol approved by the Yale Human Investigation Committee. DNA was prepared from whole blood in the laboratory of JG by standard procedures. Genotypes were obtained by the polymerase chain reaction (PCR) – restriction fragment length polymorphism method [12] using a PCR procedure slightly modified from Tsai et al. [48]. The PCR product was digested by HhaI (New England Biolabs) and subjected to electrophoresis in 5% MetaPhor agarose (FMC Corp., Rochland, ME). Gels were stained with ethidium bromide and DNA visualized by UV transillumination. The three alleles were scored as described by Hixson and Vernier [12]. Eight percent of genotypes were repeated as a quality check, with complete concordance.

### 2.3. MRI methods

All subjects were imaged with a 1.5-T General Electric Signa device (General Electric, Milwaukee, WI) according to a standardized imaging protocol. The protocol began with a T1-weighted sagittal pulse sequence (5-mm contiguous slices, TR = 600 ms, TE = 11 ms, matrix  $256 \times 192$ , number of signal averages = 1, field of view = 24 cm) that was used to measure intracranial volume (ICV) and to localize subsequent image acquisitions. The other pulse sequence germane to this study was a T1-weighted, three-dimensional volume spoiled gradient echo axial sequence through the temporal lobes (60 1.5-mm contiguous slices, TR = 24 ms, TE = 5 ms, matrix  $256 \times 192$ , number of signal averages = 2, field of view = 30 cm, and a 45-degree flip angle). The axial sequence was used to measure volumes of the hippocampus and the amygdala.

Images were transferred to a Sun SPARC 2 workstation (Sun Microsystems, Santa Clara, CA) where volumetric measurements were performed using the ANALYZE version 7.5 image analysis program (CNSoftware, Wilmington, DE) [36] by the same rater (M.B.) who was blinded to all subject information. The axial pulse sequence data were rotated and re-sliced into 1.2-mm isometric voxel coronal sections orthogonal to the long axis of the hippocampus (matrix  $256 \times 256$ ), using ANALYZE software [36]. During this reformatting and realignment step, any head rotation with respect to the orthogonal coronal plane was also corrected. In the resulting orthogonal coronal slices, the borders of the hippocampus and amygdala were then manually traced sequentially on each slice from posterior to anterior using a mouse track ball. The ICV was delineated along the inner border of the skull from all slices in the sagittal pulse sequence. The region-of-interest module of the ANALYZE software package calculated volumes for each slice based on the cross-sectional area measure multiplied by the slice thickness. The final hippocampal and amygdaloid volumes and ICVs were calculated by summing the volumes of the individual slices for each structure. Individual hippocampal and amygdaloid volumes were normalized for intersubject variation in head size by dividing by the ICV of each subject.

### 2.4. Anatomic guidelines

Anatomic guidelines for the hippocampus and amygdala (see Fig. 1) were based on the work of Watson et al. [50] and the atlases of Duvernoy [5,6] and were modified in consultation with a neuroradiologist who is an expert

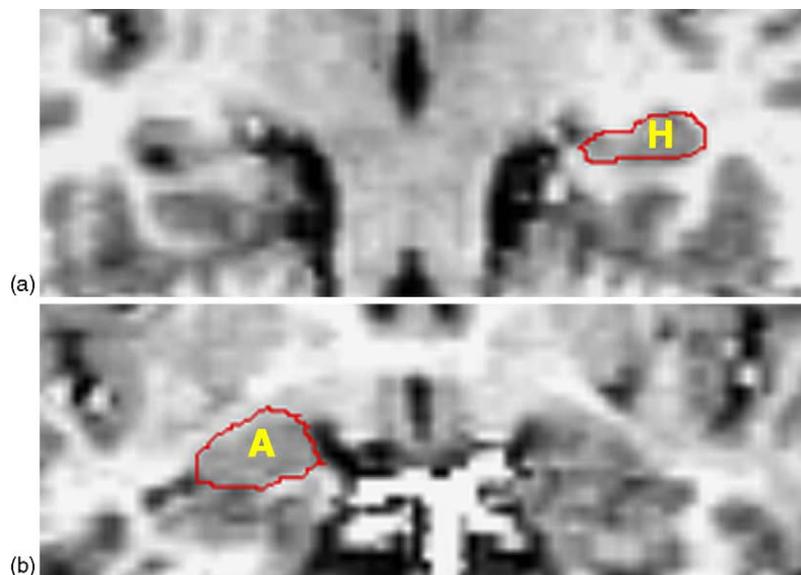


Fig. 1. Neuroanatomic boundaries of hippocampus and amygdala on MRI. Selected images from a 69-year-old female control subject. Panel (a), cropped oblique coronal section through the hippocampus (indicated by H), which was delineated to include the CA1 to CA4 sectors of the hippocampus proper, the dentate gyrus, the subiculum, the alveus, and fimbria. Panel (b), cropped oblique coronal section through the amygdala (indicated by A), which was delineated to include the amygdala proper, the semilunar gyrus and a small portion of the ambient gyrus.

in medial temporal lobe anatomy (R.B.). The hippocampal volume was considered to include the CA1 to CA4 sectors of the hippocampus proper, the dentate gyrus, the subiculum, the alveus, and fimbria. To enhance consistency, the posterior boundary of the hippocampus was arbitrarily determined by the oblique coronal anatomic section on which the thalamus was first clearly seen. At this level the crura of the fornices are also typically observable in profile and used as an additional anatomic landmark. The anterior border of the hippocampus is terminated at the level of the white matter alveus that covers the ventricular surface of the hippocampal head at the uncus recess within the temporal horn. When this was not visible, we used the criteria of Watson et al. [50] to distinguish the hippocampal head from the amygdala. Thus, these measurements included essentially the entire hippocampus from the tail through the head.

Anatomic boundaries of the amygdala closely followed those of Watson et al. [50] and were defined to include the amygdala proper (the deep/basolateral and superficial/corticomedial nuclei, and other nuclei), the semilunar gyrus (corresponding to the cortical amygdaloid nucleus) and a small portion of the ambient gyrus (corresponding to the olfactory portion of the entorhinal cortex). The posterior, superior, and lateral borders of the amygdala were identified by gray-white matter junctions. The inferior border was defined by either the uncus recess of the temporal horn or the alveus covering the head of the hippocampus. The medial border of the amygdala is covered by part of the entorhinal cortex, which forms the surface of the ambient gyrus in this region. The entorhinal cortex inferior to the tentorial indentation was excluded from the amygdaloid measurement. If this indentation was poorly defined in the anterior amygdaloid region, then it was approximated by a line drawn in direct continuation with the inferior and medial border of the amygdala to the supracellar cistern. This method unavoidably included a small amount of the superior extent of the entorhinal cortex in the amygdaloid volume [50]. The anterior boundary of the amygdala is poorly visualized on MRI and was defined operationally as the anterior most slice prior to the closure of the lateral sulcus to form the endorhinal sulcus. This procedure potentially excludes part of the anterior amygdaloid area but is thought to yield greater consistency of measurement [50].

### 2.5. Inter-rater reliability

A subset of the AD patient scans ( $n=50$  for amygdala,  $n=27$  for hippocampus,  $n=31$  for ICV) were also analyzed by a second investigator (J.Y.) for purposes of measuring inter-rater reliability. The intraclass correlation coefficient ( $\rho$ ) was found to be 0.85 for the left amygdala, 0.80 for the right amygdala, 0.90 for the left hippocampus, 0.84 for the right hippocampus, and 0.95 for the ICV, indicating acceptable reliability between the two raters. No healthy control scans were included in the inter-rater analysis because the

reliability was assumed to be at least as high for the healthy scans.

### 2.6. Statistical analysis

In general, statistical analyses involved direct comparison of  $\epsilon 4+$  AD patients and  $\epsilon 4-$  AD patients, with the control data presented for visual comparison only. Differences between AD patients and age-matched controls have already been extensively analyzed [14,17–19,25,27,32]. Demographic and neuropsychological variables were compared between  $\epsilon 4+$  AD patients and  $\epsilon 4-$  AD patients by chi-square or Student  $t$ -test.

The comparison of normalized hippocampal and amygdaloid volumes (combining left and right hemispheres) between  $\epsilon 4+$  AD patients and  $\epsilon 4-$  AD patients was performed using analysis of covariance (ANCOVA). Covariates for each model were determined using reverse stepwise multiple linear regression and potentially included age, sex, education, disease duration, and disease severity as measured by MMSE. No separate correction was employed for age of onset, which was highly correlated with current age in this sample ( $r=0.99$ ,  $n=55$ ,  $p<0.0001$ ). A significance threshold of  $p<0.10$  was required to retain each covariate in the model. We hypothesized that ApoE  $\epsilon 4$  would be associated with smaller normalized hippocampal and amygdaloid volumes in the AD patients (and thus applied a Bonferroni correction for two planned comparisons  $\alpha=0.05/2=0.025$ ).

In the central analyses of this study, ApoE  $\epsilon 4$  heterozygotes and homozygotes were considered together in order to maintain statistical power, given the small number of homozygotes. However, when significant differences were found between  $\epsilon 4+$  and  $\epsilon 4-$  AD patients, the effect of  $\epsilon 4$  dose (0, 1, or 2 copies) was also examined by ANCOVA using post-hoc Tukey tests.

Some previous investigators have reported that the ApoE  $\epsilon 4$  allele is associated with reduction of the “normal” (right > left) hippocampal asymmetry (including reversal in  $\epsilon 4$  homozygotes) in non-demented elderly subjects [43] or AD patients [9]. We therefore conducted exploratory analyses, comparing  $\epsilon 4+$  and  $\epsilon 4-$  AD patients on an asymmetry index:

$$\text{Asymmetry index (AI)} = \frac{\text{right} - \text{left}}{(\text{right} + \text{left})/2} \times 100$$

for both hippocampus and amygdala, using Student’s  $t$ -test. Since our previous research with SPECT rCBF [49] has suggested that ApoE  $\epsilon 4$  may be associated with a reduction in absolute asymmetry (regardless of direction), we also explored the effect of ApoE  $\epsilon 4$  on the absolute value of the AI for both hippocampus and amygdala. All statistical analyses utilized the SPSS (SPSS Inc., Chicago, IL) or SYSTAT (SYSTAT Inc., Evanston, IL) software packages and employed two-tailed tests of significance.

### 3. Results

The characteristics of the  $\epsilon 4+$  and  $\epsilon 4-$  AD patients and the healthy controls are shown in Table 1. The  $\epsilon 4+$  and  $\epsilon 4-$  AD patient groups did not differ in gender distribution ( $\chi^2 = 0.12$ , d.f. = 1,  $p = 0.73$ ), handedness ( $\chi^2 = 0.94$ , d.f. = 1,  $p = 0.33$ ), years of education ( $t = 0.16$ , d.f. = 53,  $p = 0.87$ ), MMSE ( $t = 0.75$ , d.f. = 53,  $p = 0.45$ ), ADAS-Cog ( $t = 0.54$ , d.f. = 53,  $p = 0.59$ ), family history of AD ( $\chi^2 = 0.00$ , d.f. = 1,  $p = 0.98$ ), or duration of disease ( $t = 1.42$ , d.f. = 53,  $p = 0.16$ ). The  $\epsilon 4-$  AD patient group was younger ( $t = 2.41$ , d.f. = 53,  $p = 0.019$ ) and had an earlier age of onset ( $t = 2.15$ , d.f. = 53,  $p = 0.036$ ) than the  $\epsilon 4+$  AD patient group. In addition, the healthy control group did not differ from the combined patient group in any demographic variable (apart from neuropsychological test scores) in Table 1.

#### 3.1. Effect of ApoE $\epsilon 4$ allele on normalized hippocampal and amygdaloid volumes in AD

Comparisons between the  $\epsilon 4+$  and  $\epsilon 4-$  AD patients revealed no significant differences in the normalized volume of hippocampus, controlling for onset age and disease duration ( $F = 0.51$ ; d.f. = 1,51;  $p = 0.48$ ; ANCOVA; see Fig. 2). The effect of onset age ( $t = -1.70$ , d.f. = 51,  $p = 0.095$ ) and duration ( $t = -1.75$ , d.f. = 51,  $p = 0.086$ ) were significant in the ANCOVA model for hippocampus. However education, gender, and MMSE score were not retained in the model.

Comparisons between the  $\epsilon 4+$  and  $\epsilon 4-$  AD patients revealed significant differences in the normalized volume of amygdala, controlling for disease severity as measured by MMSE score ( $F = 10.62$ ; d.f. = 1,52;  $p = 0.002$ ; ANCOVA; see Fig. 3). The effect of MMSE was significant in the ANCOVA model for amygdala ( $t = 2.69$ , d.f. = 52,  $p = 0.009$ ).

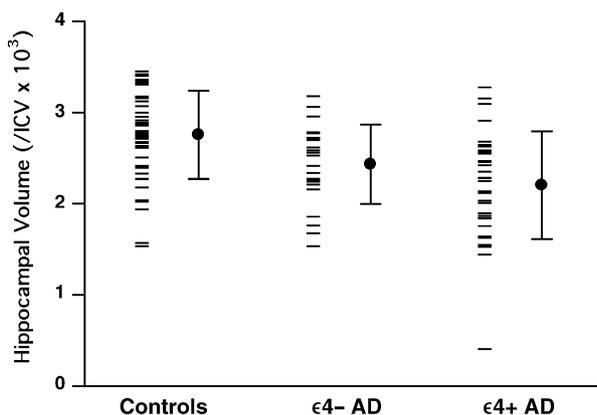


Fig. 2. Scatter plot of normalized hippocampal volume (combined left and right hippocampal volume divided by intracranial volume) in healthy control subjects ( $n = 42$ ) and  $\epsilon 4-$  ( $n = 23$ ) and  $\epsilon 4+$  ( $n = 32$ ) AD patients. Each dash denotes an individual patient; solid circles denote treatment group means ( $\pm$ S.D.). Comparisons between the  $\epsilon 4+$  and  $\epsilon 4-$  AD patients revealed no significant differences in hippocampal volume, controlling for onset age and disease duration ( $F = 0.51$ ; d.f. = 1,51;  $p = 0.48$ ; ANCOVA).

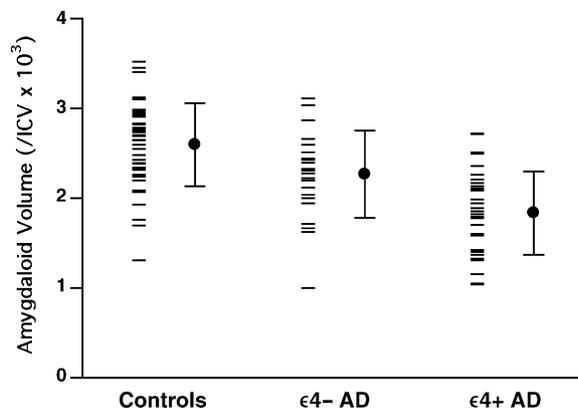


Fig. 3. Scatter plot of normalized amygdaloid volume (combined left and right amygdaloid volume divided by intracranial volume) in healthy control subjects ( $n = 42$ ) and  $\epsilon 4-$  ( $n = 23$ ) and  $\epsilon 4+$  ( $n = 32$ ) AD patients. Each dash denotes an individual patient; solid circles denote treatment group means ( $\pm$ S.D.). Comparisons between the  $\epsilon 4+$  and  $\epsilon 4-$  AD patients revealed significant differences in amygdaloid volume, controlling for MMSE score ( $F = 10.62$ ; d.f. = 1,52;  $p = 0.002$ ; ANCOVA). The effect of MMSE was significant in the ANCOVA model for amygdala ( $t = 2.69$ , d.f. = 52,  $p = 0.009$ ).

However, onset age, gender, education, and disease duration were not retained in the model.

In order to further elucidate the relationship between the ApoE  $\epsilon 4$  allele and normalized amygdaloid volume,  $\epsilon 4$  dose (0, 1, or 2 copies) was also examined. There remained a significant effect of  $\epsilon 4$  dose on normalized amygdaloid volume, controlling for MMSE score ( $F = 5.53$ ; d.f. = 2,51;  $p = 0.007$ ; ANCOVA; see Fig. 4). Post-hoc Tukey test revealed that the 0 $\epsilon 4$  group showed significantly larger volumes than both the 1 $\epsilon 4$  ( $p = 0.038$ ) and 2 $\epsilon 4$  ( $p = 0.013$ ) groups. However, the 1 $\epsilon 4$  and 2 $\epsilon 4$  groups did not differ from each other ( $p = 0.75$ ).

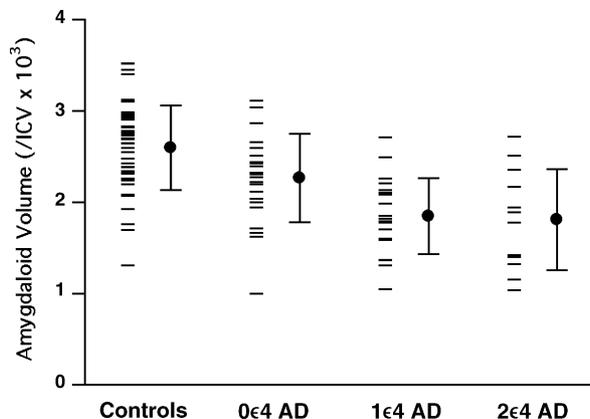


Fig. 4. Scatter plot of normalized amygdaloid volume (combined left and right amygdaloid volume divided by intracranial volume) in healthy control subjects ( $n = 42$ ) and AD patients with 0 ( $n = 23$ ), 1 ( $n = 20$ ), and 2 ( $n = 12$ )  $\epsilon 4$  alleles. Each dash denotes an individual patient; solid circles denote treatment group means ( $\pm$ S.D.). There was a significant effect of  $\epsilon 4$  dose on amygdaloid volume, controlling for MMSE score ( $F = 5.53$ ; d.f. = 2,51;  $p = 0.007$ ; ANCOVA). Post-hoc Tukey test revealed that the 0 $\epsilon 4$  group showed significantly larger volumes than both the 1 $\epsilon 4$  ( $p = 0.038$ ) and 2 $\epsilon 4$  ( $p = 0.013$ ) groups. However, the 1 $\epsilon 4$  and 2 $\epsilon 4$  groups did not differ from each other ( $p = 0.75$ ).

### 3.2. Effect of ApoE $\epsilon 4$ carrier status on hippocampal and amygdaloid asymmetry in AD

No significant differences in the asymmetry index (AI) for hippocampus were observed between the  $\epsilon 4+$  (AI =  $0.07 \pm 0.16$ ) and  $\epsilon 4-$  (AI =  $0.03 \pm 0.11$ ) AD patients ( $t = 1.15$ , d.f. = 53,  $p = 0.25$ ). Nor were significant differences seen in the AI for amygdala between the  $\epsilon 4+$  (AI =  $0.01 \pm 0.18$ ) and  $\epsilon 4-$  (AI =  $0.03 \pm 0.22$ ) AD patients ( $t = 0.48$ , d.f. = 53,  $p = 0.48$ ). The absolute value of the AI for both hippocampus and amygdala was also unrelated to ApoE  $\epsilon 4$  carrier status by  $t$ -test. If more complex models were considered (ANCOVA controlling for age, sex, education, disease duration, MMSE score), the AI (and absolute value of AI) remained unrelated to ApoE  $\epsilon 4$  carrier status for hippocampus and amygdala (data not shown).

## 4. Discussion

This study compared MRI-based volumetric measurements of the amygdala and hippocampus in patients with probable AD according to the presence or absence of the ApoE  $\epsilon 4$  allele. Normalized volumes of amygdala were significantly smaller in ApoE  $\epsilon 4+$  than  $\epsilon 4-$  AD patients. When ApoE  $\epsilon 4$  dose was considered, this effect appeared to accrue from a difference between the  $0\epsilon 4$  and each of the other two AD groups, with no significant difference between the  $1\epsilon 4$  and  $2\epsilon 4$  AD groups. Hippocampal volumes did not differ between the two AD genotypic groups, and no significant effect of ApoE  $\epsilon 4$  was observed on any of several asymmetry indices.

### 4.1. Previous studies of ApoE $\epsilon 4$ effects on volumetry of amygdala and hippocampus

#### 4.1.1. Amygdala

Our finding of reduced amygdaloid volumes in ApoE  $\epsilon 4+$  patients is generally consistent with previous results. Lehtovirta et al. [20] initially reported that a small sample ( $N = 5$ ) of  $2\epsilon 4$  AD patients had reduced right amygdaloid volumes compared to  $0\epsilon 4$  ( $N = 12$ ) and  $1\epsilon 4$  ( $N = 9$ ) patients; no difference was seen in left amygdaloid volumes. A follow-up report from the same group in a larger sample found significant differences in amygdaloid volumes only between healthy controls and  $\epsilon 4$  carrier subgroups of AD patients but no significant differences between  $\epsilon 4+$  and  $\epsilon 4-$  AD patients [21].

A more recent study by Hashimoto et al. [11] reported that (averaged right and left) amygdaloid volume was inversely correlated (Spearman rank correlation coefficient,  $r_s$ ) with the number of ApoE  $\epsilon 4$  alleles in an AD sample. Although the authors employed this analysis to examine the dose dependency of ApoE  $\epsilon 4$ , this particular test is not designed to detect a true dose effect, as it may yield statistical significance without differences existing between each dose level (amygdaloid

volume for  $0\epsilon 4 > 1\epsilon 4 > 2\epsilon 4$ ). For example, if we alternatively analyzed our results using Spearman's  $r_s$ , we would also report a significant correlation between  $\epsilon 4$  dose and amygdaloid volume ( $r_s = -0.38$ ,  $p = 0.004$ ), although ANCOVA with post-hoc Tukey test fails to demonstrate a difference between the  $1\epsilon 4$  and  $2\epsilon 4$  groups. Therefore, a true dose effect of ApoE  $\epsilon 4$  on amygdaloid volume in AD remains to be established. The magnitude of the amygdaloid volume reduction that we observed in the  $\epsilon 4+$  patients compared to  $\epsilon 4-$  patients (19.2% reduction in  $\epsilon 4+$ , comprising 18.5% reduction in  $1\epsilon 4$ , 20.2% reduction in  $2\epsilon 4$ ) is substantially larger than that observed by Hashimoto et al. [11] (6.7% reduction in  $\epsilon 4+$ , comprising 4.5% reduction in  $1\epsilon 4$ , 8.9% in  $2\epsilon 4$ ). However, in general our findings pertaining to the amygdala are consistent with their report.

#### 4.1.2. Hippocampus

Like Jack et al. [15], we found no difference in normalized hippocampal volumes in AD patients on the basis of ApoE genotype. Nonetheless, several previous investigators have reported reductions in hippocampal volume in association with ApoE  $\epsilon 4$ . Lehtovirta et al. [21] observed that  $2\epsilon 4$  AD patients had significantly smaller right hippocampus compared to  $0\epsilon 4$  and  $1\epsilon 4$  patients; and  $2\epsilon 4$  AD patients had significantly smaller left hippocampus than  $0\epsilon 4$  patients. Geroldi et al. [8] have reported reduced hippocampal volumes in AD in association with increasing dose of  $\epsilon 4$ . The above-referenced study of Hashimoto et al. [11] reported that hippocampal volume was also inversely correlated (Spearman's rank order) with the number of ApoE  $\epsilon 4$  alleles. In a longitudinal study from the same group, Mori et al. [29] reported that the annual rate of hippocampal atrophy was significantly greater in AD ApoE  $\epsilon 4$  carriers ( $9.76 \pm 4.27\%$ ) compared to noncarriers ( $6.99 \pm 4.24\%$ ). In addition to studies in AD patients, studies in nondemented subjects have reported significant [33,47] or trend-level [35] reductions in hippocampal volume in  $\epsilon 4+$  compared to  $\epsilon 4-$  subjects, and a longitudinal study [26] has demonstrated an increase in the rate of hippocampal atrophy in nondemented  $\epsilon 4$  carriers.

#### 4.1.3. Asymmetry

Our results do not corroborate previous reports of an alteration in asymmetry of medial temporal structures in association with ApoE  $\epsilon 4$ . A preponderance of studies have shown a "normal" (right > left) asymmetry of hippocampus [1,9,15,17,18,26,44], although this has not been uniformly observed [33]. Soininen et al. [43] found that "normal" (right > left) hippocampal asymmetry was diminished in *nondemented* elderly subjects carrying the ApoE  $\epsilon 4$  allele. This observation was corroborated by Tohgi et al. [47] but not by other investigators [15,33]. Geroldi et al. [9] reported that the right > left hippocampal asymmetry seen in normals was progressively reduced in AD patients with increasing ApoE  $\epsilon 4$  dose, with reversal of the asymmetry in the  $2\epsilon 4$  group. However, Bigler et al. [2] observed no asymmetry of hippocampal volume in association with the presence of the  $\epsilon 4$  allele. To

our knowledge, no previous study has examined amygdaloid asymmetry in association with ApoE  $\epsilon$ 4 in AD.

#### 4.2. Pathological and functional correlates of amygdaloid atrophy in ApoE $\epsilon$ 4+ AD

If ApoE  $\epsilon$ 4+ AD involves accelerated amygdaloid atrophy compared to ApoE  $\epsilon$ 4– AD, the pathophysiology underlying this difference is unclear. Post-mortem studies of advanced AD have demonstrated that atrophy of the amygdala preferentially involves the magnocellular regions of the basal and accessory basal nuclei [40]. This atrophy is related to loss of neurons (with medium and large neurons preferentially affected) and glia rather than loss of neuropil [41]. Volumetric pathology studies of the amygdala have not analyzed the effects of ApoE genotype.

Several studies have examined the histopathological correlates of ApoE  $\epsilon$ 4 in AD. The ApoE  $\epsilon$ 4 allele has generally been associated with increased accumulation of A $\beta$  in AD brains [10,30,31,39]. The  $\epsilon$ 4 allele association with NFTs has been less clear, with mild increases in some studies [30,31] but no increase in others [10,39]. Those studies that have examined hippocampus have found no increase in either accumulation of A $\beta$  [31,39] or NFTs [31] in association with  $\epsilon$ 4. To our knowledge, no study of histopathological correlates of  $\epsilon$ 4 has examined the amygdala, although A $\beta$  deposition and neurofibrillary pathology in the amygdala and cerebral cortex are generally well correlated [51]. In light of the present results, an investigation of amygdala neuropathology in relation to ApoE genotype would be interesting.

The functional correlates of greater amygdaloid damage in the ApoE  $\epsilon$ 4+ form of AD are also unknown. Some previous studies have suggested that MRI-based amygdaloid volumes in AD may be related to memory [25,27] or emotional memory [28] performance. In conjunction with the present results, these studies raise the possibility that the ApoE  $\epsilon$ 4+ form of AD might involve subtle alterations in cognition or behavior on the basis of differential amygdaloid damage and point to an important direction for future research.

Some limitations of the present study are inherent in the MRI-based delineation of anatomy. Because the amygdaloid volume we measured necessarily includes some adjacent cortical regions—the semilunar gyrus (corresponds to the cortical amygdaloid nucleus) and a small portion of the ambient gyrus (which corresponds to the olfactory portion of the entorhinal cortex)—its reduction in ApoE  $\epsilon$ 4+ AD patients may accrue in part from an effect of ApoE  $\epsilon$ 4 on these other structures. Certainly, we cannot exclude the possibility that a reduced volume of entorhinal cortex in  $\epsilon$ 4+ AD patients—as reported by Juottonen et al. [16]—could influence our results. A second limitation stems from the decision to operationalize the posterior boundary of the hippocampus at the level of the thalamus and the crura of the fornices. This method is commonly employed in MRI studies of hippocampus to enhance reliability but overlooks an area of the hippocampus that has been relatively neglected in AD research and may show spe-

cific effects of ApoE  $\epsilon$ 4. Finally, our ROI-based methods may have lacked sensitivity to detect a difference in hippocampal volume between ApoE  $\epsilon$ 4 carriers and noncarriers. A newer method, voxel-based morphometry (VBM) has been reported in one study to have superiority over ROI-based volumetry for detection of hippocampal atrophy [46]. To our knowledge, VBM has not yet been used to study the effects of ApoE  $\epsilon$ 4 on brain morphometry in AD.

In summary, our results are consistent with some previous reports that the ApoE  $\epsilon$ 4 allele is associated with increased atrophy of the amygdala in patients with probable AD. However, we were unable to replicate previous findings of a relationship between  $\epsilon$ 4+ AD and increased hippocampal atrophy or altered hippocampal asymmetry. These results suggest accelerated atrophy of the amygdala in AD in association with ApoE  $\epsilon$ 4 and provide further evidence for regionally specific effects of this allele.

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