ORIGINAL CONTRIBUTION

Absence of an Apolipoprotein E ε4 Allele Is Associated With Increased Parietal Regional Cerebral Blood Flow Asymmetry in Alzheimer Disease

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Background: The apolipoprotein E (*Apo E*) ε4 allele has been associated with parietal metabolic abnormalities and asymmetries in asymptomatic subjects at risk for Alzheimer disease (AD). However, previous research has shown minimal effect of the ε4 allele on regional cerebral blood flow (rCBF) and metabolism in patients with probable AD.

Objective: To determine whether the *Apo E* ε4 allele is associated with parietal rCBF abnormalities and asymmetries in patients with probable AD.

Patients and Methods: Thirty patients with AD with the ε4 allele (ε4+ AD), 22 patients with AD without the ε4 allele (ε4– AD), and 14 healthy control subjects underwent single-photon emission computed tomography (SPECT) scanning with 740 MBq technetium Tc 99m hexamethylpropyleneamine oxime. Ratios of parietal-unaffected regions and a left-right parietal asymmetry index were compared between both patient groups.

Results: The group with ε4– AD was younger ($P = .005$, Student t test) and had an earlier age of onset ($P = .005$) than the group with ε4+ AD. Analysis of covariance revealed no significant difference in the parietal rCBF ratio, controlling for age of onset and Mini–Mental State Examination score ($F_{1,48} = 0.06$; $P = .81$). However, contrary to hypothesis, significantly greater parietal rCBF asymmetry was seen in patients with ε4– AD (mean ± SD, 9.7% ± 5.5%) than those with ε4+ AD (6.3% ± 4.7%; $F_{1,50} = 5.89$; $P = .02$; analysis of variance). When number of ε4 allele copies was considered, this effect appeared to accrue primarily from a difference between patients with 0 and with 2 ε4 allele copies. An exploratory analysis of multiple cortical structures suggested that this asymmetry extended to additional regions (superior temporal) and to combined association cortex.

Conclusions: Greater parietal rCBF asymmetry is involved in ε4– AD than in ε4+ AD. Lack of the ε4 allele may be associated with other (as yet undiscovered) genetic or environmental risk factors, which confer greater neuropathological asymmetry.

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THE ε4 ALLELE of the apolipoprotein E (*Apo E*) locus on chromosome 19 has been identified as a major risk factor for Alzheimer disease (AD).[1] To relate the *Apo E* ε4 allele to the pathophysiological features of AD, investigators have begun to search for phenotypic differences between patients with AD who carry or who lack the ε4 allele. The search for phenotypic correlates of the ε4 allele has included neuropathological studies of the rate of β-amyloid deposition,[2-5] neurofibrillary tangle (NFT) formation,[2-5] and cholinergic markers.[6] Investigators have also sought to elucidate clinical differences among *Apo E* genotypes, eg, in rate of progression,[7-12] responsivity to cholinergic therapies,[6] and behavioral disturbances.[13]

The search for phenotypic correlates of *Apo E* ε4 has also included functional neuroimaging studies. Small et al[14] reported that, in cognitively normal relatives at risk for AD, the *Apo E* ε4 allele was associated with significantly lower parietal metabolism and greater left-right parietal asymmetry. A subsequent study by Reiman et al[15] found that cognitively normal ε4 homozygotes had significantly reduced rates of glucose metabolism in the same brain regions as patients with probable AD. More recently, investigators have examined the effect of the ε4 allele on regional cerebral blood flow (rCBF) or regional cerebral glucose metabolism (rCMRGlu) in patients with AD.[16,17] Lehtovirta et al[16] reported similar rCBF ratios among patients with AD and 0, 1,

PATIENTS AND METHODS

HUMAN SUBJECTS

The study sample consisted of 52 of the 57 patients with probable AD who underwent ^{99m}Tc-HMPAO SPECT scanning in research protocols from July 1, 1992, to December 31, 1995, in the Alzheimer's Disease Research Unit, Yale University, New Haven, Conn. Of the 5 patients not included, 3 had died without available tissue samples for *Apo E* genotyping, and 2 had died and underwent brain autopsy with non-AD histopathological diagnoses (1, atypical Pick disease; 1, dementia of undetermined origin). Six other patients underwent autopsy confirming definite AD.[19] Fourteen healthy controls (all spouses of participating patients with AD) were recruited to undergo ^{99m}Tc-HMPAO SPECT and were selected without regard to genotype. The demographic and clinical characteristics of patients and controls are displayed in **Table 1**. One patient with AD was African American (*Apo E* genotype, *3,3*); all other patients were of European ancestry.

Diagnoses of probable AD were made according to standard criteria.[20] Other causes of dementia were excluded through a comprehensive evaluation, including medical history, physical and neurological examinations, serum chemistry studies, thyroid function studies, complete blood cell count, levels of vitamin B_{12} and folate, VDRL, urinalysis, electrocardiography, and magnetic resonance imaging or computed tomography of the brain. At the time of SPECT scanning, no patients or controls were taking medications known to alter rCBF.

Neuropsychological testing administered for subject characterization (Table 1) included the Mini–Mental State Examination (MMSE)[21] and the cognitive subscale of the Alzheimer's Disease Assessment Scale (ADAS-Cog).[22] A neuropsychological asymmetry index (NAI), based on the discrepancy between language and visuospatial construction performance derived from the ADAS-Cog, was used to relate neuropsychological patterns to left-right rCBF asymmetries. Because of different scaling properties for different subtests of the ADAS-Cog, a nonparametric transformation of scores was used. Patients with AD were ranked separately on the language (the sum of the ratings for difficulty making self understood, comprehension of spoken language, word-finding difficulty, commands, and naming)[23] and the constructional praxis subscales, and the absolute difference between ranks was calculated.

Family history of AD was assessed using the Alzheimer Dementia Risk Questionnaire[24] and the Dementia Questionnaire,[25] and results were considered to be positive if at least 1 first-degree relative met criteria for primary degenerative dementia. No cases suggestive of autosomal dominant transmission were identified.

Unless otherwise indicated, data are given as mean ± SD.

DETERMINATION OF *APO E* GENOTYPE

All subjects (or their responsible next of kin) provided informed consent and were studied under a protocol approved by the Yale University Human Investigation Committee. The DNA was prepared from whole blood in one of our laboratories (that of J.G.) using standard procedures. Genotypes were obtained using the polymerase chain reaction (PCR) restriction fragment length polymorphism method,[26] a PCR procedure slightly modified from that of Tsai et al.[27] The PCR product was digested by *Hha*I (New England Biolabs) and subjected to electrophoresis in 5% agarose gel (MetaPhor; FMC Corp, Rockland, Me). Gels were stained with ethidium bromide, and DNA was visualized using UV transillumination. The 3 alleles were scored as described by Hixson and Vernier.[26]

SPECT DATA ACQUISITION

The ^{99m}Tc-HMPAO (20.2 ± 0.7 mCi) was injected intravenously in the NeuroSPECT Laboratory at Yale New Haven Hospital. At the time of injection, subjects were supine with eyes and ears uncovered in a dimly lit room. Quiet was maintained for 5 minutes following injection. Scanning began on average 12.8 ± 3.5 minutes (range, 10-28 minutes) following injection. Acquisition time was 30 minutes. The radiochemical purity of ^{99m}Tc-HMPAO, determined according to the methods outlined in the package insert, was 92.6% ± 3.0%. The SPECT data were acquired as previously described[28,29] using a brain-dedicated camera[30] (CERASPECT, Digital Scintigraphics, Waltham, Mass) with a resolution of 7 to 8 mm full width at half maximum (FWHM) in all 3 axes.

SPECT IMAGE ANALYSIS

The SPECT images were analyzed by one of us (R.A.) who was unaware of subject identity. Reconstructed slices were displayed on a 128 × 128 matrix (1.67 × 1.67-mm pixel size) as a set of 64 transaxial slices (1.67-mm-slice thickness). The SPECT data were rotated and resliced in the canthomeatal plane, which was identified using an algorithm developed using external fiducial markers in a separate sample. The following 5 standard transaxial slices 6.67-mm thick were then created by digitally summing consecutive

Continued on next page

and 2 copies of the ε*4* allele. Corder et al[17] found no distinct patterns of rCMRGlu in patients with AD as a function of *Apo E* genotype. However, these studies of patients with AD have not addressed hemispheric asymmetry in the manner studied by Small et al[14] in cognitively normal relatives.

In our study, rCBF was measured using single-photon emission computed tomography (SPECT) and technetium Tc 99m hexamethylpropyleneamine oxime (^{99m}Tc-HMPAO; Ceretec, Amersham, Ltd, Amersham, England)[18] in 30 patients with AD with the ε*4* allele (ε*4*+ AD), 22 patients with AD without the ε*4* allele (ε*4*– AD), and 14 elderly control subjects. We sought to test the hypothesis that the ε*4* allele is associated with greater parietal rCBF reductions and asymmetries in patients with AD. We also conducted an exploratory analysis of the overall pattern of rCBF associated with ε*4*+ AD.

groups of 4 of the 1.67-mm slices at defined anatomical levels as shown previously[28,29]: maximal cerebellar activity (slice 1); maximal thalamic activity (slice 2); lowest central activity (superior to thalamus and basal ganglia and inferior to midline gray matter) (slice 3); and 2 additional high cortical slices approximately 10 and 20 mm above slice 3 (slices 4 and 5). The 5 transaxial slices were thus situated approximately 10, 45, 60, 70, and 80 mm above the canthomeatal plane. In addition, a midtemporal slice (parallel to the long axis of the temporal lobe) was created by rotating the SPECT data −25° from the canthomeatal plane and reslicing and summing 4 slices to obtain a 6.67-mm-thick slice. Attenuation correction was performed using a Chang zero order method ($\mu = 0.15\ cm^{-1}$) within an ellipse drawn around the skull.

A region-of-interest (ROI) template (shown substantially in a previous study[28]; mid-temporal slice available from one of us [C.H.vD.] on request) was constructed, consisting of 147 circular ROIs of preset area (21 pixels or 58.33 mm^2), representing 14 anatomic structures. The ROIs in the template were distributed evenly throughout each structure and centrally in subcortical structures. They were visually adjusted to fit individual anatomy based on landmarks in the SPECT image.

STATISTICAL ANALYSIS

Count densities for anatomic structures were obtained as previously described[28,29] by averaging the values in circular ROIs across all slices in which the structures appeared. For each SPECT scan, a total count density was determined for each of the 14 anatomic structures. The rCBF ratios were then derived for 9 association cortical structures (**Table 2**) by dividing count density for each structure by the mean count density (pixel-weighted) in relatively unaffected structures (defined as cerebellum, thalamus, basal ganglia, occipital cortex, and sensorimotor cortex). The selection of structures as unaffected was based on evidence from neuropathological and positron emission tomography studies and has been validated for ^{99m}Tc-HMPAO SPECT in a previous report.[28]

A hemispheric AI was also derived for cortical structures using the following formula:

$$AI = \frac{|L - R|}{(L + R)/2} \times 100$$

where L and R refer respectively to left and right cortical rCBF ratios.

The scans of 18 patients with AD were also analyzed by one of us (O.O.) to measure interrater reliability. The intraclass correlation coefficient (ρ)[31] was 0.93 for the parietal rCBF ratio and 0.95 for the parietal AI, indicating excellent reliability between both raters. For the other 9 association cortical structures (Table 2 and **Table 3**), ρ ranged from 0.75 to 0.97 (0.89 ± 0.08) for rCBF ratios and from 0.63 to 0.94 (0.81 ± 0.13) for AIs.

All statistical analyses involved comparison of patients with *ε4*+ AD and *ε4*− AD, with the control data presented for visual comparison only. Differences between patients with AD and age-matched controls already have been extensively analyzed[28,29] using methods similar to those used herein.

Based on the report of Small et al,[14] we hypothesized that *Apo E ε4* would be associated with lower rCBF in the parietal cortices and greater left-right parietal asymmetry. The effect of the *ε4* allele on rCBF in the parietal association cortex (combining superior, medial, and inferior parietal cortices with left and right hemispheres averaged) was determined using analysis of covariance (ANCOVA), with MMSE and age of onset as covariates. The MMSE was chosen as a covariate because disease severity is well known to be correlated with parietal rCBF or metabolic abnormalities.[32] Age of onset was chosen as a covariate because the patient groups differed significantly with respect to this variable, and because early-onset AD has been associated with greater parietal rCBF or metabolic abnormalities in several previous studies.[33,34] The effect of the *ε4* allele on parietal asymmetry was then determined using analysis of variance (ANOVA). No covariates were employed for the analysis of AIs, since these have not been related to disease severity or onset age. In the central analyses of this study, *Apo E ε4* heterozygotes and homozygotes were considered together to maintain statistical power, given the small number of homozygotes. However, when significant differences were found between patients with *ε4*+ and *ε4*− AD, the effect of *ε4* number (0, 1, or 2 copies) was also examined using ANOVA with post hoc Scheffé tests.

Further exploratory analyses subsequently were undertaken to examine the effect of the *ε4* allele on rCBF measures in all 9 association cortical structures. This exploratory analysis again examined the effect of the *ε4* allele on rCBF ratios using ANCOVA (adjusting for MMSE and age of onset) and on AIs using ANOVA for each brain structure. No correction for multiple comparisons was used in these exploratory analyses.

The relationship of the NAI to the parietal rCBF AI was evaluated using the Pearson product moment correlation coefficient (r, using the nonabsolute value of each index). The NAI (absolute value) of the patients with *ε4*+ and *ε4*− AD was compared using the Student t test. All statistical analyses used the SPSS 6.1 (SPSS Inc, Chicago, Ill) or SYSTAT 5.2.1(SYSTAT Inc, Evanston, Ill) software packages and employed 2-tailed tests of significance.

RESULTS

The characteristics of the patients with *ε4*+ and *ε4*− AD and the healthy controls are shown in Table 1. The patient groups did not differ in sex distribution ($\chi^2 = 0.85$; $P = .36$), handedness, years of education, MMSE, ADAS-Cog, or duration of disease. The patients with *ε4*− AD were younger ($P = .005$; t test) and had an earlier age of onset ($P = .005$) than the patients with *ε4*+ AD. In addition, the healthy controls did not differ ($P > .05$) from the combined patient group in any demographic variable in Table 1.

EFFECT OF *APO E ε4* ON PARIETAL rCBF AND ASYMMETRY IN AD

Comparisons between patients with *ε4*+ and *ε4*− AD revealed no significant difference in the parietal rCBF ratio,

Table 1. Subject Characteristics*

Variable	ε4+ AD (n = 30)	ε4– AD (n = 22)	Controls (n = 14)
Demographic			
Age, y	72.7 ± 6.1†	66.8 ± 8.4	68.9 ± 7.8
Sex, No. M/F	10:20	11:11	9:5
Handedness, No. right-left	29:1	21:1	14:0
Education, y	14.0 ± 2.6	13.7 ± 3.4	15.8 ± 3.7
Neuropsychological tests, score			
Mini–Mental State Examination	18.5 ± 4.3‡	18.0 ± 5.0‡	29.1 ± 1.1
Alzheimer's Disease Assessment Scale, cognitive subscale	26.5 ± 9.9‡	28.6 ± 12.0‡	5.0 ± 1.7
Disease characteristics			
Onset age, y	68.3 ± 5.9†	62.4 ± 8.6	. . .
Duration, mo	52.1 ± 23.2	52.8 ± 17.7	. . .
Family history, No. positive-negative§	17:13	11:11	. . .
Apolipoprotein E genotype	*3, 4* (n = 20) *4, 4* (n = 10)	*3, 3* (n = 19) *2, 3* (n = 3)	. . .

**Unless otherwise indicated, all data are presented as mean ± SD by Student* t *test. ε4+ AD indicates Alzheimer disease with the ε4 allele present; ε4– AD, with the ε4 allele absent; and ellipses, not applicable.*

†*Differs from ε4 – AD value,* P = *.005,* t *test.*

‡*Differs from control value,* P<*.001,* t *test.*

§*Family history is positive if primary degenerative dementia is present in at least 1 first-degree relative.*

controlling for age of onset and MMSE score ($F_{1,48} = 0.06$; $P = .81$; ANCOVA) (**Figure 1**). However, contrary to hypothesis, significantly greater parietal rCBF asymmetry was seen in patients with *ε4*– AD (9.7% ± 5.5%) than those with *ε4*+ AD (6.3% ± 4.7%; $F_{1,50} = 5.89$; $P = .02$; ANOVA) (**Figure 2**, left). In the combined patient sample, the parietal rCBF ratio was significantly correlated with MMSE score ($r = 0.35$; $P = .01$) and age of onset ($r = 0.36$; $P = .008$), further supporting the use of these variables as covariates, but not with sex, years of education, or duration of disease. The parietal AI was uncorrelated with age of onset ($r = –0.13$; $P = .36$), years of education, or duration of disease. This index was nearly correlated with MMSE score ($r = –0.25$; $P = .07$) and was discovered unexpectedly to be related to sex, with men having significantly greater asymmetry than women ($t = 2.61$; $P = .03$). These potential confounders on the relationship between *Apo E ε4* status and parietal asymmetry were therefore examined using ANCOVA, with sex, MMSE, or both as covariates. However, when included in the same model with *Apo E ε4* status, these covariates lacked significance; and the effect of *Apo E ε4* status remained significant regardless of model.

To further elucidate the relationship between the *Apo E ε4* allele and parietal rCBF asymmetry, number of *ε4* copies was also examined. There remained a significant effect of ε4 number on the parietal AI ($F_{2,49} = 3.63$; $P = .03$; ANOVA) (Figure 2, right). Post hoc Scheffé test revealed that the patient group with 0 *ε4* allele copies showed significantly greater asymmetry than that with 2 *ε4* allele copies ($P = .04$); whereas the group with 1 *ε4* allele copy had an intermediate value and did not differ significantly from the other 2 groups.

EFFECT OF *APO E ε4* ON rCBF AND ASYMMETRY IN MULTIPLE CORTICAL REGIONS IN AD

An exploratory analysis of multiple association cortical regions is displayed in Tables 2 and 3. This analysis re-

Table 2. rCBF Ratios in Association Cortices for Patients With AD According to *Apo E ε4* Status*

Cortical Structure	ε4– AD (n = 22)	ε4+ AD (n = 30)	ANCOVA, F	Controls (n = 14)
Anterior cingulate				
Left	0.94 ± 0.11	0.92 ± 0.09	0.00	1.01 ± 0.05
Right	0.97 ± 0.11	0.92 ± 0.10	0.21	0.99 ± 0.08
Prefrontal				
Left	0.92 ± 0.08	0.89 ± 0.07	1.38	0.95 ± 0.04
Right	0.93 ± 0.07	0.89 ± 0.06	3.74†	0.94 ± 0.04
Premotor				
Left	0.89 ± 0.07	0.88 ± 0.05	0.58	0.96 ± 0.05
Right	0.93 ± 0.07	0.90 ± 0.05	2.09	0.96 ± 0.03
Medial temporal				
Left	0.83 ± 0.09	0.78 ± 0.06	1.36	0.86 ± 0.08
Right	0.83 ± 0.06	0.81 ± 0.07	0.09	0.88 ± 0.07
Lateral temporal				
Left	0.77 ± 0.07	0.78 ± 0.07	0.37	0.87 ± 0.04
Right	0.83 ± 0.05	0.82 ± 0.07	0.38	0.88 ± 0.05
Superior temporal				
Left	0.80 ± 0.05	0.82 ± 0.06	2.25	0.89 ± 0.05
Right	0.85 ± 0.06	0.86 ± 0.07	0.27	0.91 ± 0.03
Inferior parietal				
Left	0.76 ± 0.08	0.80 ± 0.07	1.56	0.88 ± 0.04
Right	0.81 ± 0.08	0.83 ± 0.09	0.12	0.90 ± 0.04
Medial parietal				
Left	0.90 ± 0.10	0.94 ± 0.07	0.57	1.03 ± 0.05
Right	0.91 ± 0.11	0.93 ± 0.09	0.19	1.03 ± 0.05
Superior parietal				
Left	0.79 ± 0.07	0.81 ± 0.07	0.04	0.90 ± 0.04
Right	0.84 ± 0.09	0.84 ± 0.08	1.57	0.93 ± 0.04

**Unless otherwise indicated, all data are presented as mean ± SD. All values are normalized to unaffected regions (cerebellum, thalamus, basal ganglia, occipital cortex, and sensorimotor cortex).* F *values are for comparison of ε4– and ε4+ AD, adjusted for Mini–Mental State Examination score and age at disease onset. No statistically significant differences were observed. Healthy control values are shown for reference only and are not analyzed statistically. rCBF indicates regional cerebral blood flow; AD, Alzheimer disease;* Apo E, *apolipoprotein E; ε4–, ε4 allele absent; ε4+, ε4 allele present; and ANCOVA, analysis of covariance.*

†P = *.06.*

Table 3. Asymmetry Indexes in Association Cortices for Patients With AD According to *Apo E* ε4 Status*

Cortical Structure	ε4– AD, (n = 22)	ε4+ AD, (n = 30)	ANOVA F	ANOVA P	Controls (n = 14)
Anterior cingulate	4.3 ± 3.3	6.0 ± 5.5	1.70	. . .	5.7 ± 5.1
Prefrontal	4.8 ± 2.8	3.5 ± 2.6	2.97	.09	2.8 ± 1.7
Premotor	7.0 ± 4.2	5.2 ± 4.0	2.61	. . .	3.0 ± 1.8
Medial temporal	7.7 ± 5.9	7.2 ± 5.2	0.12	. . .	5.8 ± 3.4
Lateral temporal	9.8 ± 7.2	7.9 ± 5.1	1.12	. . .	4.7 ± 2.6
Superior temporal	10.0 ± 5.3	6.8 ± 4.8	5.24	.03	3.7 ± 2.8
Inferior parietal	12.1 ± 6.9	8.9 ± 6.3	3.03	.09	4.1 ± 2.7
Medial parietal	7.4 ± 4.8	6.3 ± 5.1	0.63	. . .	4.7 ± 2.7
Superior parietal	10.3 ± 6.3	6.0 ± 4.9	7.70	.008	3.5 ± 2.0
Combined association cortex	7.4 ± 3.8	4.9 ± 3.6	5.59	.02	1.9 ± 1.3

**Calculations for asymmetry indexes are given in the "Statistical Analysis" subsection of the "Patients and Methods" section. Unless otherwise indicated, all data are presented as mean ± SD in percents. F values are for comparison of ε4– and ε4+ AD. Healthy control values are shown for reference only and are not analyzed statistically. AD indicates Alzheimer disease;* Apo E*, apolipoprotein E; ε4–, ε4 allele absent; ε4+, ε4 present; and ellipses, not significant.*

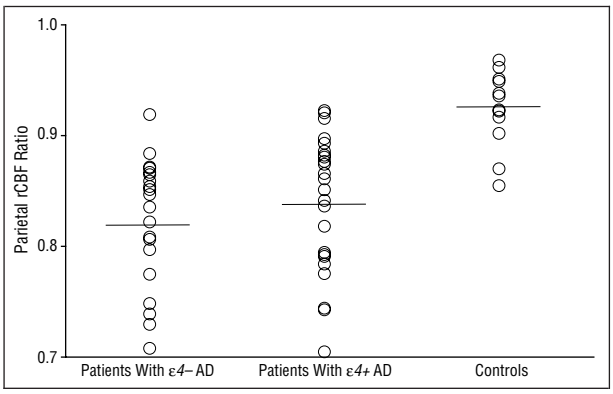

Figure 1. *Regional cerebral blood flow (rCBF) ratios for parietal association cortex for patients with Alzheimer disease without (ε4– AD) (n = 22) and with the ε4 allele (ε4+ AD) (n = 30) and for healthy control subjects (n = 14). Values represent count densities normalized to regions relatively unaffected in AD (cerebellum, thalamus, basal ganglia, occipital cortex, and sensorimotor cortex) obtained using technetium Tc 99m hexamethylpropyleneamine oxime and single-photon emission computed tomography. Horizontal bars indicate group means. Comparisons between both patient groups with AD showed no significant difference in the parietal rCBF ratio, controlling for age of onset and Mini–Mental State Examination score ($F_{1,48} = 0.06$;* P *= .81; analysis of covariance).*

vealed no significant differences in rCBF ratios between patients with ε4+ and ε4– AD, controlling for age of onset and MMSE score. However, patients with ε4– AD demonstrated significantly greater asymmetry in the superior parietal ($F_{1,50} = 7.70$; $P = .008$) and superior temporal ($F_{1,50} = 5.24$; $P = .03$) regions and trends toward greater asymmetry in the inferior parietal ($F_{1,50} = 3.03$; $P = .09$) and prefrontal ($F_{1,50} = 2.97$; $P = .09$) regions. Finally, patients with ε4– AD showed significantly greater asymmetry in the combined association cortex ($F_{1,50} = 5.59$; $P = .02$).

When effects of number of ε4 allele copies were analyzed, there remained a significant effect for the superior parietal AI ($F_{2,49} = 4.33$; $P = .02$, ANOVA; patients with 0 ε4 allele copies were significantly more asymmetric than those with 2 ε4 allele copies [$P = .03$], Scheffé test) and the combined association cortex AI ($F_{2,49} = 3.85$; $P = .03$, ANOVA; the patients with 0 ε4 allele copies were significantly more asymmetric than those with 2 ε4 allele copies; $P = .03$, Scheffé test).

NEUROPSYCHOLOGICAL ASYMMETRY

No significant difference was seen between patients with ε4+ and ε4– AD in the NAI ($P = .75$; *t* test). This index was modestly correlated with the parietal rCBF AI in the combined AD sample ($r = 0.31$; $P = .03$; using the nonabsolute value of each index). The ^{99m}Tc-HMPAO SPECT images are shown in **Figure 3** for 2 patients with ε4– AD who evidenced large parietal rCBF asymmetries with clinical correlates.

COMMENT

We compared SPECT rCBF measures in patients with AD according to the presence or absence of the *Apo E* ε4 allele. No significant differences emerged between patients with ε4+ and ε4– AD in average parietal rCBF. However, contrary to hypothesis, the absence of an ε4 allele was associated with increased parietal rCBF asymmetry. When effects of number of ε4 allele copies were considered, this effect appeared to accrue primarily from a difference between the groups with 0 and with 2 ε4 allele copies, although the sample size may have been insufficient to detect differences among all 3 groups. An exploratory analysis of multiple association cortices suggested that this asymmetry extended to additional regions (superior temporal) and to combined association cortex.

Two other studies have examined the effect of the ε4 allele on rCBF or rCMRGlu in patients with AD.[16,17] Lehtovirta et al[16] reported similar rCBF ratios among patients with 0, 1, and 2 ε4 allele copies, although occipital rCBF ratios tended to decrease with increasing number of ε4 allele copies. They did not report any hemispheric asymmetry data. Corder et al[17] reported no distinct patterns of rCMRGlu in patients with AD as a function of genotype. With regard to asymmetry, they reported limited data and did not use an absolute AI as we did. Our data thus support an emerging literature, suggesting no overall effect of ε4 allele copies on the average pattern of rCBF or rCMRGlu in AD. However, our finding of in-

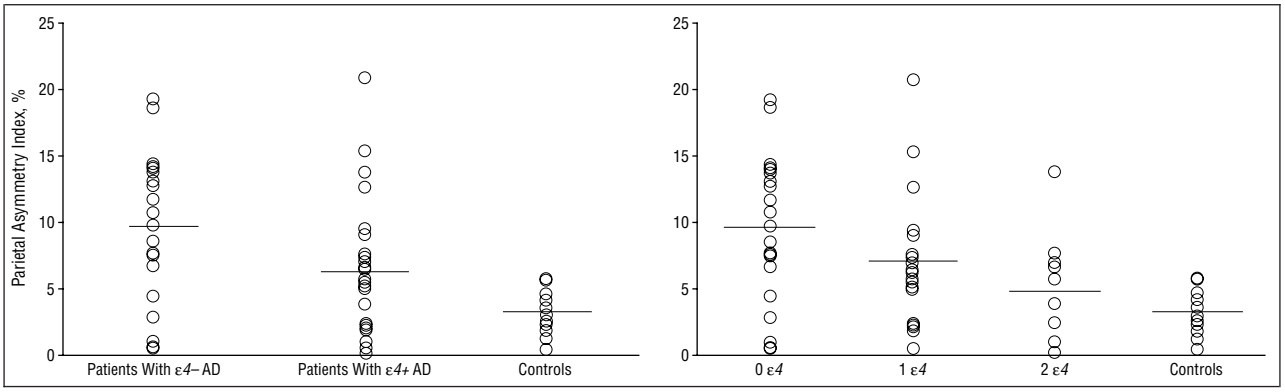

Figure 2. *Parietal asymmetry indexes in patients with ϵ4– and ϵ4+ AD and healthy control subjects (left) and patients with 0, 1, and 2 apolipoprotein E ϵ4 allele copies and healthy controls (right). Calculations for asymmetry indexes are given in the "Statistical Analysis" subsection of the "Patients and Methods" section. Horizontal bars indicate group means. Significantly greater parietal rCBF asymmetry was seen in the patients with ϵ4– AD (9.7% ± 5.5%) than in those with ϵ4+ AD (6.3% ± 4.7%; $F_{1,50}$ = 5.89; P = .02; analysis of covariance). The effect of number of ϵ4 allele copies remained significant ($F_{2,49}$ = 3.63; P = .034; analysis of variance). Post hoc Scheffé test revealed that the group with 0 ϵ4 allele copies showed significantly lower asymmetry than those with 2 (P = .04); whereas the group with 1 ϵ4 allele copy did not differ significantly from the other 2 groups. Abbreviations are explained in the legend to Figure 1.*

creased parietal rCBF asymmetry in ϵ4– AD has not been previously reported and awaits replication.

Our data suggest that the effect of *Apo E ϵ4* on rCBF or rCMRGlu may be very different in patients with AD than in asymptomatic or presymptomatic controls. Small et al[14] found that in nondemented relatives at risk for AD, the presence of an ϵ4 allele was associated with significantly lower parietal rCMRGlu and greater parietal asymmetry. Reiman et al[15] compared rCMRGlu between cognitively normal ϵ4 homozygotes and subjects without the *ϵ4* allele, all of whom reported a family history of probable AD. The ϵ4 homozygotes had significantly reduced rCMRGlu in the same brain regions as patients with probable AD. In contrast to controls without the *ϵ4* allele, patients with *ϵ4*– AD are likely enriched in other, presently undiscovered, genetic or environmental risk factors for AD. Such factors may be associated with a form of AD conferring equally severe parietal rCBF abnormalities and still greater parietal rCBF asymmetry.

NEUROPATHOLOGICAL CORRELATES OF rCBF ASYMMETRIES?

The neuropathological counterpart of rCBF asymmetries in AD is unknown. However, rCBF and metabolism have been shown in a variety of experimental paradigms to reflect regional neuronal activity, and areas of focal reduction are thought to reflect decreased synaptic activity caused by loss or dysfunction of synapses.[35] Previous clinicopathological studies correlating rCMRGlu with histopathological studies at autopsy in patients with definite AD have found that rCMRGlu is correlated with neuronal density[36] or with NFT density,[37] but not with β-amyloid–containing senile plaques.

Several studies have examined the neuropathological correlates of *Apo E ϵ4* in AD. The *Apo E ϵ4* allele has generally been associated with increased accumulation of β-amyloid in brains with AD.[2-5] The *ϵ4* allele association with NFTs has been less clear, with mild increases in some studies[3,5] but no increase in others.[2,4] No study of pathological correlates of the *ϵ4* allele has examined hemispheric asymmetries. Indeed, neuropathological studies broadly have ignored issues of left-right asymmetry.[38] However, in light of our results, an investigation of neuronal or synaptic loss or NFTs in both hemispheres in relation to *Apo E* genotype would be interesting.

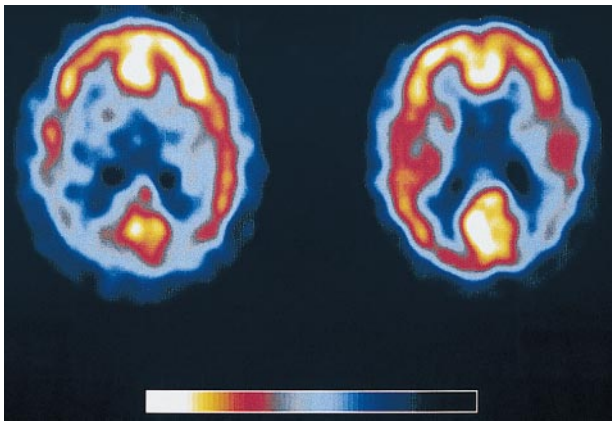

Figure 3. *Cerebral transaxial images 70 mm above the canthomeatal plane obtained using technetium Tc 99m hexamethylpropyleneamine oxime and single-photon emission computed tomography (SPECT) for 2 subjects (apolipoprotein E genotype,* 3,3*) demonstrating significant parietal rCBF asymmetry. The patients' left sides appear on the right in these images. Left, A 60-year-old left-handed woman who evidenced profound visuospatial deficits with relatively preserved language abilities, including a neuropsychological asymmetry index (NAI) of 28 (language performance higher than visuospatial construction) derived from the cognitive subscale of the Alzheimer's Disease Assessment Scale. The SPECT reveals relative right parietal rCBF reduction (AI = 19.6%). Right, A 76-year-old right-handed woman who demonstrated disproportionate language deficits, including an NAI of 23 (visuospatial construction performance higher than language). The SPECT reveals relative left parietal rCBF reduction (AI = 18.6%). Magnetic resonance imaging scans in both subjects revealed only diffuse cortical atrophy. In the entire sample of patients with Alzheimer disease, the NAI was modestly correlated with the parietal rCBF AI (r = .31,* P = .025*; using the nonabsolute value of each index). All other abbreviations are explained in the legend to Figure 1.*

CLINICAL CORRELATES OF rCBF ASYMMETRIES?

Previous research has established a relationship between rCMRGlu and rCBF asymmetries and neuropsychological function.[39] However, the degree of lateral asymmetry has not been well-correlated with disease severity,

a finding confirmed in our study. Thus, the greater rCBF asymmetries seen in the patients with *ε4*– AD may not indicate a more severe form of the disease. An emerging literature examining the relationship between the *ε4* allele and disease severity has yielded contradictory results, including a more benign course[7,9,12] or no difference in course[8,10,11] in association with the *ε4* allele.

Greater rCBF hemispheric asymmetry in *Apo E ε4*– AD may be associated with greater hemispheric asymmetry of neuropsychological function. In our sample, there was no difference between the patients with *ε4*+ and *ε4*– AD in a crude AI of language and visuospatial construction, although this index was modestly correlated with parietal rCBF asymmetry in the combined AD sample. More sensitive AIs[39] derived from more extensive neuropsychological batteries may be necessary to address this question.

Our results confirm previous reports that the *Apo E ε4* allele is unrelated to the average pattern of rCBF or metabolism in patients with AD. However, the absence of an *Apo E ε4* allele is associated with significantly greater parietal rCBF asymmetry. This finding suggests that the *ε4*– form of AD may be associated with other (as yet undiscovered) genetic or environmental risk factors that confer greater neuropathological asymmetry.

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