Single Photon Emission Computed Tomography and Apolipoprotein E in Alzheimer's Disease: Impact of the ε4 Allele on Regional Cerebral Blood Flow

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ABSTRACT

The aim of this study was to examine the impact of the apolipoprotein E (*APOE*) ε4 allele on semiquantitative regional cerebral blood flow (rCBF) in Alzheimer's disease. Single photon emission computed tomography technetium (SPECT) with (99m)Tc d,l-hexamethyl propylenamine oxine was used to determine rCBF in 41 consecutive patients (18 males/23 females) with probable Alzheimer's disease according to the National Institute of Neurological and Communicative Disorders and Stroke-Alzheimer's Disease and Related Disorders Association criteria (mean age 71.0 years; range 54–85). The mean Mini-Mental State Examination (MMSE) score was 20.4 (range 10–30). After normalization of CBF to mean blood flow in the cerebellum, values for rCBF in several cortical regions of interest, side-to-side asymmetry indices, and anterior-posterior ratios were calculated. Determination of the *APOE* genotype from blood samples was performed using restriction enzyme polymerase chain reaction technique. Multivariate regression analyses and the Wilcoxon rank-sum test for unpaired data (Mann-Whitney) were used for statistical analysis. The patients comprised 27 *APOE* ε4-positive and 14 *APOE* ε4-negative individuals. Five patients were *APOE* ε4 homozygotes. *APOE* ε4-positive patients had significantly reduced rCBF in the right frontal and left occipital lobes. On nonparametric analysis, the most prominent differences between ε4-negative and ε4-positive patients were demonstrated in subregions representing the frontal association cortex (Mann-Whitney, $P < .01$). Age-stratified analysis suggested that these findings could be demonstrated predominantly in the elderly patients. The results of this study suggest that the *APOE* genotype in itself may have an impact on the pattern of rCBF deficits in Alzheimer's disease. The more pronounced reduction of rCBF in frontal association cortex observed in elderly *APOE* ε4-positive patients might predict clinical progression. (*J Geriatr Psychiatry Neurol* 2001; 14:42–51).

Apolipoprotein E (ApoE) is an important transport protein of lipids in the human body. The *APOE* genotype is determined by three different common alleles, ε2, ε3, and ε4, derived from a specific gene locus at chromosome 19. The ε4 allele has been determined as a significant risk factor for Alzheimer's disease (AD) in a dose-dependent manner. Thus, the risk of developing AD will increase threefold with one ε4 allele and eightfold with two ε4 alleles.[1–5] Several studies have confirmed the importance of the *APOE* gene as a major susceptibility gene, and, in most studies, the ε4 allele has been shown to predict early disease onset.[6]

As no fully specific marker for AD has yet been identified, the clinical diagnosis of AD is based on operational clinical criteria (National Institute of Neurological Communicative Disorders and Stroke-Alzheimer's Disease and Related Disorders Association [NINCDS-ADRDA]).[7] In order to fulfill the NINCDS-ADRDA criteria for probable AD, any other possible underlying etiology for dementia must be excluded. The exclusion of other possible etiologies should involve structural imaging of the

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brain by means of computed tomography (CT) or magnetic resonance imaging (MRI). However, the significance of functional imaging methods such as single photon emission computed tomography (SPECT) or positron emission tomography (PET) as diagnostic tools in AD is still controversial. Many studies have addressed the finding of topographically characteristic reductions of relative regional cerebral blood flow (rCBF) and cerebral metabolic rate for glucose (CMR-glc) in AD brains with SPECT and PET (Table 1). Circumscribed reduction of rCBF or CMR-glc in the temporoparietal regions of either one or both hemispheres is a consistent finding.[8–15] Nevertheless, some of these SPECT and PET studies have demonstrated a marked heterogeneity of the functional deficits.[9,13] The question of the involvement of the frontal lobe in AD, which has been found in several SPECT or PET studies, has in particular been a subject of discussion,[9,13,16,17] and it is still undetermined whether the involvement of the frontal lobe in AD is observed in very progressed cases only or if it may also be a feature of the early stages of AD. Other types of dementia such as motor neuron disease with dementia, progressive supranuclear palsy, Pick's disease, and alcoholic dementia, which can be challenging clinical differential diagnoses, may also be associated with deficits in the frontal lobes demonstrated by functional imaging.[9,11,18] Likewise, hypofunction of the frontal lobes has been demonstrated by functional imaging in patients with (severe) depression.[19]

It is uncertain whether the topographic heterogeneity demonstrated by functional brain imaging in AD is due to diagnostic heterogeneity, differences in disease severity between studies, or inherent heterogeneity of patients with AD, for instance, due to genetic factors. Thus, in the present study, we aimed to examine the impact of the $\varepsilon 4$ allele on rCBF measured with SPECT in patients with clinical AD.

PATIENTS AND METHOD

We examined 41 consecutive patients who were all classified as having probable AD according to the NINCDS-ADRDA criteria. The mean age was 71.0 ± 8.8 years (range 54–85) and the mean Mini-Mental Status Examination (MMSE) score[20] was 20.4 ± 4.5 (range 10–30). Estimation of disease duration was performed on the basis of interviews with relatives. The mean disease duration was 2.4 ± 1.4 years. Cranial CT or MRI was obtained in all patients as part of the routine investigation program.

Fifteen healthy volunteers, 11 males and 4 females, were included. The mean age was 71.3 ± 4.7 years (range 64–79) and the mean MMSE score was 29.2 ± 0.8 (range 28–30). The healthy volunteers did not have any subjective impairment of memory or other cognitive skills or depressive symptoms, did not satisfy criteria for a current or previous psychiatric disorder, had no known cardiovascular or cerebrovascular disease, and did not use centrally acting medications. All had normal physical and neurologic examinations; normal blood pressure, electrocardiogram (ECG), and standard blood test screening; normal neuropsychological examination using a standardized evaluation program previously described in detail[21]; and normal MRI scans of the brain.

In all subjects, a blood sample was collected for the determination of the *APOE* genotype, and a SPECT perfusion study was performed.

APOE Genotype

The *APOE* genotype was determined in leukocytes by polymerase chain amplification, followed by restriction enzyme digestion and size analysis. Deoxyribonucleic acid (DNA) was isolated from blood leukocytes by simple standard salting out procedures.[22] The polymerase chain amplification method was adapted from that used by Wenham et al,[23] as recently described.[24] AmpliTaq Gold DNA polymerase (Perkin Elmer) was used for the amplification, *Cfo I* (Boehringer) for restriction enzyme digestion of the amplicons, and SYBR-green I (Molecular Probes) for detection of the restriction fragments in Poly (NAT) gels (Elchrom Scientific) in ultraviolet light. The *APOE* genotype was determined from the band pattern of restriction fragments. Besides bands composed of only a few basepairs (bp), the *APOE* $\varepsilon 2$ allele gave clearly visible fragments of 91 and 81 bp, *APOE* $\varepsilon 3$ gave 91 and 48 bp, and *APOE* $\varepsilon 4$ gave 72 and 48 bp.

Single Photon Emission Computed Tomography

With the patient in a dark and quiet room, a dose of 800= 1000 MBq technetium ^{99m}Tc-d,l-hexamethyl propylenamine oxime (Amersham International, London) was injected intravenously. High-resolution SPECT was carried out with Tomomatic 564 (Medimatic, Copenhagen), a rapidly rotating brain-dedicated 9-slice instrument. Twenty minutes after injection of the tracer, the radioactivity distribution in the brain was acquired in a 64 × 64 matrix mode. Nine slices were obtained simultaneously, and by repositioning of the high-resolution collimator, 27 consecutive slices parallel to the orbitomeatal plane were obtained. For regional analysis of the images, the 27 slices were recompressed to 9 slices (adjacent slices were summarized 3 and 3), and, subsequently, 9 predefined region templates were superimposed to the images. After normalization of CBF to mean blood flow in the cerebellum,[25] semiquantitative values for global and rCBF in several cortical regions of interest, side-to-side asymmetry indices (SAIs), and anterior-posterior (AP) ratios were calculated. The methods for regional analysis have previously been described in detail.[9,26]

STATISTICAL ANALYSIS

Initially, an individual image analysis of the SPECT images in the patients with AD was performed. In this

analysis, rCBF values in each patient were compared with mean rCBF values in the control group: to simplify this analysis, the brain was graphically divided into four quadrants, two frontal and two posterior. The frontal quadrants consisted of the following regions: upper frontal, precentral region, superior frontal, middle frontal, inferior frontal, and orbitofrontal. The posterior quadrants consisted of superior temporal gyrus and insula, inferior and middle temporal region, temporal poles, upper parietal region, postcentral gyrus region, supramarginal and angular region, and the occipital region (Fig. 1). The blood perfusion in one of these quadrants was considered abnormally reduced if (1) the AP ratio was more than 2 SD below the mean AP ratio in the control group or (2) the SAI in at least one subregion was more than 2 SD below the mean value of the corresponding region in the control group, or (3) the rCBF in at least one subregion was more than 3 SD below the mean value of the corresponding region in the control group.[14]

Once it was determined which patients had abnormally reduced rCBF compared to normative data, each quadrant was analyzed with multivariate regression analysis using quadrant rCBF as the dependent variable and *APOE* ε4 positivity, age, disease duration, and MMSE score as predictors. These analyses were performed in order to focus the subsequent nonparametric statistical analyses on brain quadrants in which it was likely to find an effect of *APOE* status on rCBF.

Age, MMSE score, disease duration, and the semiquantitative flow values in both summarized and single regions of interest were subsequently statistically compared (Mann-Whitney) after separating the patients into *APOE* ε4-positive and *APOE* ε4-negative groups. As both the initial demographic analysis (see Table 2) and the multivariate regression analyses (Tables 3 and 4) pointed toward a confounding effect of age, at least on the rCBF in the left frontal quadrant, the patients were stratified by age with a cutoff set at age 73 years. The

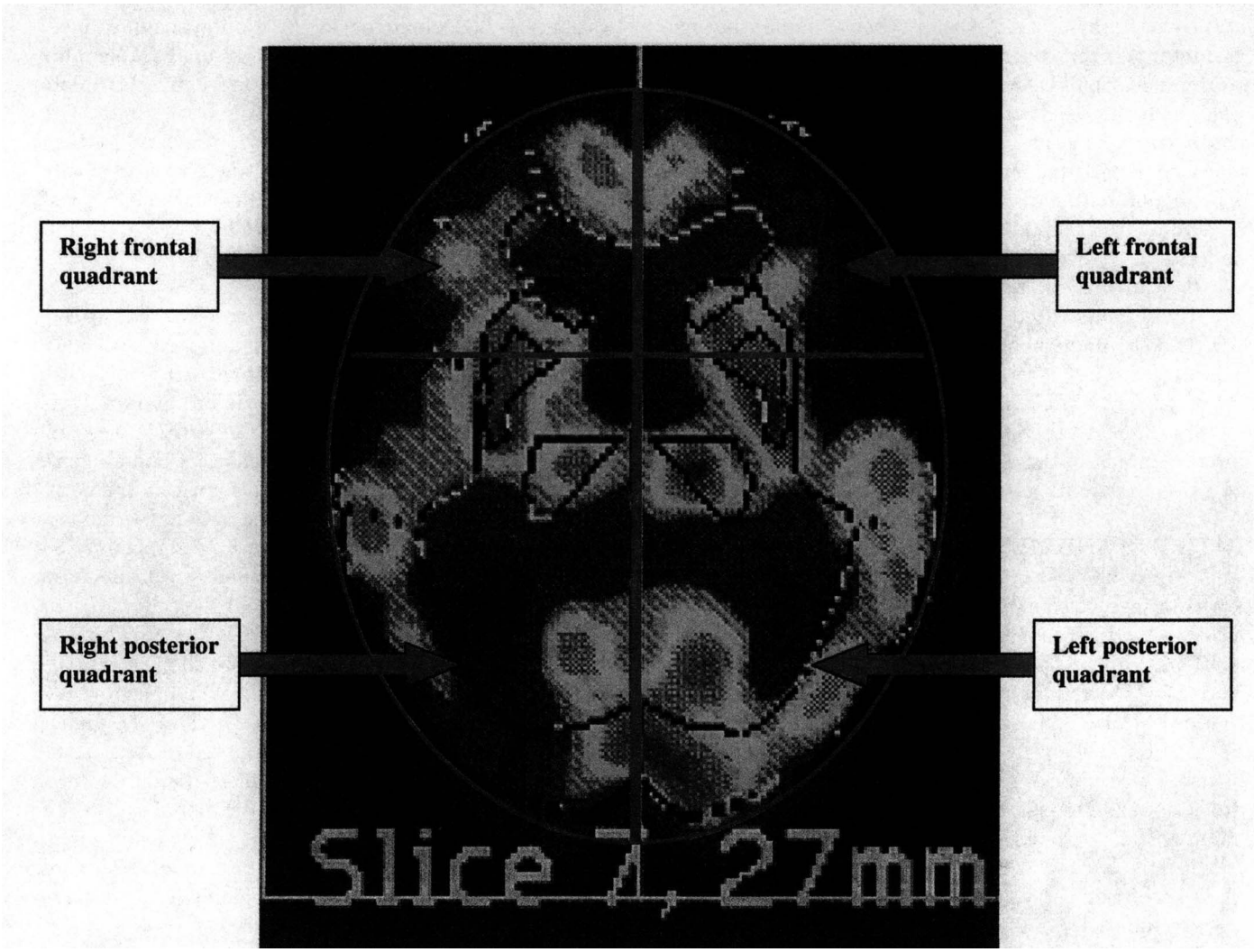

Figure 1. SPECT regions: the four quadrants. The brain regions identified by the superimposed templates are shown at level 27 mm above the orbitomeatal plane. In total, rCBF values from 13 subregions (see Tables 4 and 5, regions F1–F6, T1–T3, P1–P3, and OC1) were calculated from the nine SPECT image slices. For individual image analysis, the SPECT images were split into four quadrants, and the statistical methods described were applied. Regional CBF values from the basal ganglia (regions drawn on figure around thalami, caudate nuclei, and putamen) were not used in the regional analysis.

Table 1. Summary of the Results from Previous Studies on ApoE and Functional Imaging

Authors	*Method*	*Design*	*Conclusions*
Lethovirta et al, 1996[8]	SPECT and MRI (^{99m}Tc-HMPAO)	58 patients with clinically significant or suspected AD—control group of 34 age-matched healthy volunteers	ε4-homozygotes have decreased hippocampal volume (MRI) and left occipital hypoperfusion (SPECT) compared to ε4-heterozygote and ε4–negative patients
Lethovirta et al, 1998[33]	SPECT (^{99m}Tc-HMPAO)	31 AD patients and 8 age-matched healthy controls	ε4-positive AD patients have decreased temporal and occipital blood flow compared to ε4-negative patients
van Dyck et al, 1998[34]	SPECT (^{99m}Tc-HMPAO)	30 ε4-positive AD patients, 22 ε4-negative patients, and 14 healthy volunteers	Greater asymmetry of parietal blood flow in ε4-negative patients compared to ε4-positive patients
Tanaka et al, 1998[35]	SPECT and MRI (^{133}Xenon)	34 patients with clinical AD—22 age-matched control subjects	ε4-positive patients have increased atrophy of the inferior temporal lobe and reduced absolute blood flow in cortex, most pronounced in the inferior temporal lobe
Small et al, 1995[46]	PET	38 volunteers (7 demented and 31 nondemented) with at least 2 family members with AD—no control group	Reduced parietal glucose consumption and increased parietal right-left asymmetry among ε4-positive "at-risk" subjects compared to ε4-negative subjects
Reiman et al, 1996[47]	PET	33 volunteers—11 ε4-homozygote and 22 ε4-negative subjects—images were compared with images from 37 patients with probable AD and 22 healthy controls	Significantly reduced CMR-glc in posterior cingulate, parietal, temporal and prefrontal regions in cognitively normal ε4-homozygote subjects
Corder et al, 1997[37]	PET	31 patients with clinical AD—nonconsecutive—no control group	No difference in regional glucose consumption between ε4-positive and ε4-negative patients
Higuchi et al, 1997[38]	PET	20 patients with clinical AD—nonconsecutive—no control group	Increased CMR-glc in the frontal lobe in ε4-positive patients compared to ε4-negative patients
Mielke et al, 1998[39]	PET	49 patients with clinical AD—no control group	
Hirono et al, 1998[40]	PET	83 AD patients and 26 age-matched healthy volunteers	No relation between *APOE* genotype and regional CMR-glc The ε4-allele is an independent predictor of temporoparietal and frontal hypometabolism in AD

CMR-glc = cerebral metabolic rate for glucose.

cutoff was chosen arbitrarily to leave a fair number of patients in each subgroup. The alpha level for statistical significance was 0.05.

Table 2. Demography and *APOE* Genotype Frequency

Variable	*Control (n = 15)*	*AD (n = 41)*	*APOE ε4 Positive*	*APOE ε4 Negative*
N	15	41	27	14
Male	11	18	12	6
Female	4	23	15	8
Age	71.3 ± 4.7	71.0 ± 8.8	69.1 ± 9.0	74.8 ± 7.3*
Disease duration (yr)	—	2.4 ± 1.4	2.5 ± 1.6	2.4 ± 0.8
MMSE	29.2 ± 0.8	20.4 ± 4.5	19.8 ± 4.7	21.5 ± 4.1
ε4/ε4	13 % (2)	12 % (5)	—	—
ε4/ε3	13 % (2)	51 % (21)	—	—
ε4/ε2	0	2 % (1)	—	—
ε3/ε3	53 % (8)	30 % (12)	—	—
ε3/ε2	21 % (3)	5 % (2)	—	—
ε2/ε2	0	0	—	—

*$P < .05$ (Mann-Whitney), n = number of patients or controls. Values given as mean ± 1 SD.

RESULTS

The distribution of *APOE* genotypes in the control subjects and patients is given in Table 2. As expected, the *APOE* ε4-allele frequency in patients with AD was considerably higher than in the control group. Sixty-five percent of the patients were *APOE* ε4 positive, compared with 26% in the control group (see Table 2). Mean age, MMSE, and disease duration in the patients with AD and control subjects are also given in Table 2. There was no statistically significant difference in mean age between patients and control subjects. There were no statistically significant differences between ε4-positive and ε4-negative patients with AD when MMSE and disease duration were compared, whereas the ε4-negative patients with AD were slightly but significantly older than ε4-positive patients with AD (Mann-Whitney, $P < .05$).

The initial analysis of the individual patterns of rCBF deficits defined 93% of the SPECT studies as abnormal, with equal proportions in *APOE* ε4-positive (25/27) and *APOE* ε4-negative (13/14) patients. However,

Table 3. Individual SPECT Image Analysis

	Frontal Quadrant (Right or Left)	*Left Frontal Quadrant*	*Right Frontal Quadrant*	*Posterior Quadrant (Right or Left)*	*Left Posterior Quadrant*	*Right Posterior Quadrant*
ε4 positive (n = 27)	22*	14	14	23	21	15
AP ratio (>2 SD)		3	1		11	11
SAI (> 2 SD)		8	11		9	4
rCBF (<3 SD)		3	2		1	0
ε4 negative (n = 14)	7	6	3	13	11	10
AP ratio (>2 SD)		2	0		9	10
SAI (>2 SD)		4	2		2	0
rCBF (<3 SD)		0	1		0	0

*The values given indicate the number of patients having abnormally reduced regional blood flow in each quadrant and by which criteria rCBF was classified as abnormal. The proportion of patients (22/27 vs 7/14) with abnormally reduced frontal rCBF was significantly higher in the subgroup of *APOE* ε4-positive patients (proportion test: $P < .05$). Analysis of right frontal quadrant selectively (3/14 vs 14/22) gave *P* value just above the 5% level (proportion test: $P = .06$).

focus on the frontal quadrants in the two subgroups of patients revealed that 81% (22/27) of the patients in the group of *APOE* ε4-positive patients had significantly reduced blood flow (one or both hemispheres) in the frontal quadrants, compared to 50% (7/14) of the patients in the group of ε4-negative patients (see Table 3; $P < .05$). Converging with these findings, the initial multivariate regression analyses predicted that significant effects could be found between the *APOE* genotype and frontal quadrant rCBF at least in the right hemisphere, whereas there appeared to be a confounding effect of age in the left frontal quadrant (see Table 4).

Table 4. Multivariate Regression Analyses

lm(formula = Left Frontal Quadrant ~ APOE + Age + DD + MMSE)

Residuals	*Minimum*	*1Q*	*Median*	*3Q*	*Maximum*
	−0.83	−0.39	0.03	0.34	0.71
Coefficients		*Estimate*	*Standard Error*	*t Value*	*P*
(Intercept)		−1.58	0.94	−1.68	.10
*APOE**		0.26	0.16	1.59	.12
Age		0.02	0.01	2.36	.02
DD**		−0.17	0.05	−3.18	.01
MMSE		0.03	0.02	1.84	.07

lm(formula = Right Frontal Quadrant ~ APOE + Age + DD + MMSE)

Residuals	*Minimum*	*1Q*	*Median*	*3Q*	*Maximum*
	−0.67	−0.40	−0.20	0.46	0.78
Coefficients		*Estimate*	*Standard Error*	*t Value*	*P*
(Intercept)		−0.81	1.04	−0.78	.44
APOE		0.36	0.18	1.99	.05
Age		0.01	0.01	1.06	.30
DD		−0.01	0.06	−0.06	.95
MMSE		0.01	0.02	0.45	.66

lm(formula = Left Posterior Quadrant ~ APOE + Age + DD + MMSE)

Residuals	*Minimum*	*1Q*	*Median*	*3Q*	*Maximum*
	−0.87	0.01	0.13	0.27	0.42
Coefficients		*Estimate*	*Standard Error*	*t Value*	*P*
(Intercept)		1.49	0.88	1.69	.10
APOE		−0.09	0.15	−0.58	.57
Age		−0.01	0.01	−0.49	.63
DD		0.07	0.05	1.48	.15
MMSE		−0.03	0.02	−1.53	.13

lm(formula = Right Posterior Quadrant ~ APOE + Age + DD + MMSE)

Residuals	*Minimum*	*1Q*	*Median*	*3Q*	*Maximum*
	−0.87	0.01	0.13	0.27	0.42
Coefficients		*Estimate*	*Standard Error*	*t Value*	*P*
(Intercept)		1.49	0.88	1.69	.10
APOE		−0.09	0.15	−0.58	.57
Age		−0.01	0.01	−0.49	.63
DD		0.07	0.05	1.48	.15
MMSE		−0.03	0.02	−1.53	.13

APOE = APOE-ε4 positivity; DD = disease duration.

The results from comparing the semiquantitative rCBF values in *APOE* ε4-positive and *APOE* ε4-negative patients with AD are given in Tables 5 and 6. Relative rCBF values were lower in the ε4-positive patients than in the ε4-negative patients in all regions measured, but the differences only attained statistical significance in the right frontal cortex, left occipital cortex, superior frontal region (both hemispheres), midfrontal region

Table 5. Results from Regional SPECT Image Analysis—Global Values

Region	*Control (n = 15)*	*APOE ε4 (n = 27)*	*APOE Non-ε4 (n = 14)*	*P (ε4 vs Non-ε4)*
Hemisphere				
$F_{i(L)}$	70.9 ± 5.3	65.4 ± 7.1	70.2 ± 9.7	NS
$F_{i(R)}$	71.3 ± 5.7	65.7 ± 7.4	71.1 ± 10.5	NS
SAI	1.6 ± 0.8	3.5 ± 2.4	4.1 ± 2.2	NS
Frontal cortex				
$F_{i(L)}$	67.5 ± 5.5	62.9 ± 7.7	68.3 ± 9.8	NS
$F_{i(R)}$	67.3 ± 5.3	62.6 ± 7.5	68.9 ± 9.9	< .05*
SAI	1.7 ± 1.3	3.5 ± 2.7	3.1 ± 2.5	NS
Temporal cortex				
$F_{i(L)}$	76.5 ± 5.9	67.3 ± 7.8	69.9 ± 10.4	NS
$F_{i(R)}$	77.5 ± 5.2	68.0 ± 8.4	72.1 ± 9.9	NS
SAI	3.4 ± 2.2	6.2 ± 4.6	6.4 ± 4.9	NS
Parietal cortex				
$F_{i(L)}$	64.6 ± 8.1	61.1 ± 7.7	68.6 ± 13.4	NS
$F_{i(R)}$	65.5 ± 8.6	61.2 ± 8.7	67.4 ± 13.8	NS
SAI	2.5 ± 1.7	5.3 ± 3.4	4.4 ± 3.0	NS
Occipital cortex				
$F_{i(L)}$	84.4 ± 6.6	81.4 ± 8.9	89.4 ± 10.6	< .01**
$F_{i(R)}$	84.5 ± 10.9	82.5 ± 9.3	87.1 ± 12.0	NS
SAI	3.8 ± 3.0	4.6 ± 3.5	5.0 ± 4.5	NS

$F_{i(L)}$ = left hemispheric rCBF given in percent relative to mean rCBF in the cerebellum; $F_{i(R)}$ = right hemispheric rCBF given in percent relative to mean rCBF in the cerebellum; SAI = numerical value of SAI given in percent by $[(F_{i(H)} - F_{i(V)}) : F_{i,MAX}] \times 100$.
All values are given as mean ± 1 SD.
NS = not significant.
*$P < .05$ (Mann-Whitney); **$P < .01$ (Mann-Whitney).

Table 6. Results from Regional SPECT Image Analysis—Regional Values

Subregion	N	Control (n = 15)	N	APOE ε4 (n = 27)	N	APOE Non-ε4 (n = 14)	P (ε4 vs Non-ε4)
Upper frontal region (F1)							
$F_{i(L)}$	14	53.4 ± 7.4	22	50.5 ± 8.0	13	59.5 ± 14.5	NS
$F_{i(R)}$	14	52.3 ± 7.2	22	50.2 ± 7.6	13	57.6 ± 14.0	NS
SAI	14	4.5 ± 3.4	22	5.2 ± 5.0	13	4.8 ± 4.2	NS
Precentral region (F2)							
$F_{i(L)}$	15	69.9 ± 4.8	27	66.5 ± 8.2	14	71.5 ± 10.5	NS
$F_{i(R)}$	15	70.3 ± 6.5	27	65.4 ± 8.3	14	70.9 ± 10.3	NS
SAI	15	3.5 ± 2.2	27	4.9 ± 2.9	14	4.6 ± 3.1	NS
Superior region (F3)							
$F_{i(L)}$	15	66.9 ± 5.4	27	62.6 ± 8.2	14	68.6 ± 9.8	< .05*
$F_{i(R)}$	15	66.7 ± 5.6	27	63.0 ± 7.9	14	70.1 ± 10.2	< .05*
SAI	15	4.0 ± 3.3	27	3.7 ± 2.4	14	4.2 ± 2.5	NS
Midfrontal region (F4)							
$F_{i(L)}$	15	69.2 ± 4.9	27	63.0 ± 8.2	14	69.5 ± 9.7	< .05*
$F_{i(R)}$	15	68.6 ± 4.7	27	62.0 ± 9.0	14	70.1 ± 10.2	< .01**
SAI	15	2.9 ± 2.5	27	5.2 ± 4.3	14	3.8 ± 2.9	NS
Inferior frontal region (F5)							
$F_{i(L)}$	15	76.5 ± 6.3	27	69.0 ± 9.8	14	72.5 ± 11.7	NS
$F_{i(R)}$	15	76.5 ± 5.0	27	68.4 ± 9.3	14	75.2 ± 10.8	< .05*
SAI	15	4.1 ± 4.2	27	5.4 ± 4.6	14	6.1 ± 4.3	NS
Orbitofrontal region (F6)							
$F_{i(L)}$	15	69.4 ± 10.3	27	66.1 ± 8.9	14	66.1 ± 12.1	NS
$F_{i(R)}$	15	69.8 ± 9.6	27	65.4 ± 8.5	14	68.4 ± 12.0	NS
SAI	15	3.8 ± 3.3	27	5.9 ± 4.6	14	6.1 ± 4.6	NS
Superior gyrus and insula (T1)							
$F_{i(L)}$	15	78.4 ± 6.5	27	69.6 ± 8.4	14	70.9 ± 11.8	NS
$F_{i(R)}$	15	78.2 ± 6.5	27	69.9 ± 9.0	14	73.1 ± 12.8	NS
SAI	15	3.3 ± 2.6	27	6.0 ± 4.3	14	7.5 ± 7.1	NS
Inferior and mid region (T2)							
$F_{i(L)}$	15	77.7 ± 6.8	27	68.7 ± 8.8	14	73.2 ± 12.5	NS
$F_{i(R)}$	15	79.7 ± 5.5	27	69.5 ± 9.7	14	74.8 ± 11.3	NS
SAI	15	3.9 ± 4.0	27	7.4 ± 5.4	14	6.4 ± 5.8	NS
Temporal poles (T3)							
$F_{i(L)}$	15	68.6 ± 6.9	26	60.3 ± 8.0	13	59.0 ± 9.0	NS
$F_{i(R)}$	15	69.3 ± 6.0	26	61.4 ± 7.9	13	63.2 ± 8.2	NS
SAI	15	4.7 ± 3.3	26	7.9 ± 6.8	13	9.4 ± 5.9	NS
Upper parietal region (P1)							
$F_{i(L)}$	14	55.7 ± 9.4	23	54.0 ± 7.7	13	62.2 ± 15.5	NS
$F_{i(R)}$	14	56.4 ± 9.7	23	54.2 ± 8.8	13	60.5 ± 15.8	NS
SAI	14	3.5 ± 3.7	23	5.4 ± 4.4	13	4.5 ± 2.6	NS
Postcentral gyrus region (P2)							
$F_{i(L)}$	15	70.5 ± 5.6	27	65.4 ± 8.0	14	70.3 ± 11.6	NS
$F_{i(R)}$	15	70.9 ± 7.2	27	63.6 ± 10.2	14	70.7 ± 13.0	NS
SAI	15	3.1 ± 2.4	27	7.3 ± 7.4	14	5.1 ± 3.9	NS
Supramarginal and angular region (P3)							
$F_{i(L)}$	15	66.9 ± 8.5	27	62.7 ± 9.3	14	70.2 ± 14.4	NS
$F_{i(R)}$	15	68.5 ± 8.8	27	63.3 ± 9.6	14	68.7 ± 14.9	NS
SAI	15	2.7 ± 1.9	27	6.3 ± 4.5	14	5.7 ± 4.4	NS

$F_{i(L)}$: = left hemispheric rCBF given in percent relative to mean rCBF in the cerebellum. $F_{i(R)}$ = right hemispheric rCBF given in percent relative to mean rCBF in the cerebellum. SAI = numerical value of SAI given in percent by $[(F_{i(H)} - F_{i(V)})\ Fi,max] \times 100$.
All values given as mean ± 1 SD. NS = not significant. N = number of subjects in whom the region was drawn.
*$P < .05$ (Mann-Whitney); **$P < .01$ (Mann-Whitney).

(both hemispheres), and inferior frontal region (right hemisphere). The AP and side-to-side asymmetry ratios were not statistically different in the two groups. After stratification of the data by age in age groups younger or older than 73 years, the difference in frontal rCBF between *APOE* ε4-positive and *APOE* ε4-negative patients with AD was statistically significant in the group of elderly patients only (see Tables 7 and 8).

DISCUSSION

The significance of the *APOE* ε4 allele as an important risk factor for AD is now well established. However, the specific mechanism by which ApoE influences the pathogenesis in AD remains unclear. Previous studies have stressed that the *APOE* ε4 allele may influence the precipitation of β-amyloid in the brain[27] or the processing of the microtubulus-associated protein tau[28] (hyperphosphorylation). Considering the physiologic function of ApoE as a compound of major importance in transport and distribution of lipids, ApoE is likely to be involved in repair mechanisms and membrane turnover at the cellular level. There have been previous indications that within the group of patients with clinical AD, there could be important physiologic differences depending on the

Table 7. Regional and Subregional Analyses of Frontal Quadrants Stratified by Age—Age Group ≤ 73 Years

	N	Control (n = 15)	N	APOE ε4 (n = 17)	N	APOE Non-ε4 (n = 5)	P (ε4 vs Non-ε4)
Region							
Hemisphere (H)							
$F_{i(L)}$		70.9 ± 5.3		66.6 ± 7.9		69.2 ± 4.1	NS
$F_{i(R)}$		71.3 ± 5.7		66.2 ± 8.3		70.6 ± 2.3	NS
SAI		1.6 ± 0.8		3.4 ± 2.5		4.6 ± 3.4	NS
Frontal cortex (F)							
$F_{i(L)}$		67.5 ± 5.5		64.1 ± 8.9		65.6 ± 8.4	NS
$F_{i(R)}$		67.3 ± 5.3		63.5 ± 8.5		65.0 ± 6.0	NS
SAI		1.7 ± 1.3		3.5 ± 3.0		2.9 ± 2.8	NS
Subregion							
Upper frontal region (F1)							
$F_{i(L)}$	14	53.4 ± 7.4	14	51.0 ± 8.8	5	59.0 ± 17.6	NS
$F_{i(R)}$	14	52.3 ± 7.2	14	49.9 ± 8.3	5	53.4 ± 14.4	NS
SAI	14	4.5 ± 3.4	14	3.8 ± 2.7	5	7.9 ± 3.3	NS
Precentral region (F2)							
$F_{i(L)}$	15	69.9 ± 4.8	17	67.7 ± 9.1	5	69.8 ± 9.4	NS
$F_{i(R)}$	15	70.3 ± 6.5	17	66.2 ± 9.4	5	66.6 ± 6.2	NS
SAI	15	3.5 ± 2.2	17	4.4 ± 2.8	5	4.4 ± 3.2	NS
Superior region (F3)							
$F_{i(L)}$	15	66.9 ± 5.4	17	64.1 ± 8.8	5	63.4 ± 10.0	NS
$F_{i(R)}$	15	66.7 ± 5.6	17	64.1 ± 8.6	5	64.8 ± 9.1	NS
SAI	15	4.0 ± 3.3	17	3.8 ± 2.4	5	3.9 ± 2.2	NS
Midfrontal region (F4)							
$F_{i(L)}$	15	69.2 ± 4.9	17	63.8 ± 9.5	5	67.8 ± 8.2	NS
$F_{i(R)}$	15	68.6 ± 4.7	17	63.0 ± 9.6	5	67.2 ± 4.3	NS
SAI	15	2.9 ± 2.5	17	4.8 ± 2.9	5	4.8 ± 4.0	NS
Inferior frontal region (F5)							
$F_{i(L)}$	15	76.5 ± 6.3	17	69.8 ± 11.4	5	69.2 ± 7.3	NS
$F_{i(R)}$	15	76.5 ± 5.0	17	68.6 ± 10.7	5	71.0 ± 4.8	NS
SAI	15	4.1 ± 4.2	17	5.0 ± 4.0	5	7.5 ± 5.0	NS
Orbitofrontal region (F6)							
$F_{i(L)}$	15	69.4 ± 10.3	17	67.1 ± 10.9	5	63.6 ± 8.8	NS
$F_{i(R)}$	15	69.8 ± 9.6	17	66.2 ± 9.2	5	66.8 ± 9.3	NS
SAI	15	3.8 ± 3.3	17	5.2 ± 4.7	5	4.9 ± 2.9	NS

APOE genotype alone. Thus, it has been shown that the degree of neuropathologic changes and the decrease in choline acetyltransferase activity is more severe in *APOE* ε4-homozygote patients[29–31] and that the clinical effect of treatment with cholinesterase inhibitors is better in the *APOE* ε4-negative than in the *APOE* ε4-positive patients.[32]

The conclusions from previous studies on the subject of functional imaging and *APOE* genotype in AD are summarized in Table 1. Results have been inconsistent, and the significance of the *APOE* ε4 allele from these studies remains unclear. However, the majority of the studies show that there are significant differences between otherwise comparable *APOE* ε4-positive and *APOE* ε4-negative patients with AD, although the exact topography of these differences varies between studies. As shown in the present study, the initial multivariate regression analyses suggested an a priori focus on the right frontal quadrant as an area that might be significantly different in *APOE* ε4-positive and *APOE* ε4-negative patients with respect to abnormally reduced rCBF. This suggestion was confirmed by the subsequent nonparametric analysis in which the difference between the groups was statistically significant in the frontal cortex predominantly in the right hemisphere and in the left occipital lobe. When the subregions were considered (see Table 6), the rCBF was reduced in several regions, primarily in regions representing the frontal association cortex (superior frontal region) (both hemispheres), middle frontal region (both hemispheres), and inferior frontal region (right hemisphere). The AP ratios were similar in the two groups. Thus, the most striking difference between the two groups was seen in the frontal association cortex. This finding was finally confirmed by the age-stratified analysis, in which the difference between *APOE* ε4-positive and *APOE* ε4-negative patients was found in the age group above 73 years (see Tables 7 and 8).

It may be argued that the level for statistical significance (0.05) should be lower in a study, such as in the present study, where no conservative (i.e., Bonferroni) correction for multiple comparisons was carried out. Nevertheless, initial multivariate regression analysis was sufficient to identify the right frontal cortex as being significantly abnormal in *APOE* ε4-positive patients. Moreover, it is generally accepted not to apply conservative corrections on data sets that are clearly individually dependent.

As shown, the differences in rCBF between *APOE* ε4-positive and *APOE* ε4-negative patients could not be explained by differences in disease severity (MMSE) or

Table 8. Regional and Subregional Analyses of Frontal Quadrants Stratified by Age—Age Group > 73 Years

	N	Control (n = 15)	N	APOE ε4 (n = 10)	N	APOE Non-ε4 (n = 9)	P (ε4 vs Non-ε4)
Region							
Hemisphere (H)							
$F_{i(L)}$		70.9 ± 5.3		63.3 ± 5.5		70.8 ± 12.0	NS
$F_{i(R)}$		71.3 ± 5.7		64.8 ± 5.9		71.4 ± 13.2	NS
SAI		1.6 ± 0.8		3.5 ± 2.4		3.8 ± 1.2	NS
Frontal cortex (F)							
$F_{i(L)}$		67.5 ± 5.5		60.9 ± 4.7		69.8 ± 10.7	<.05*
$F_{i(R)}$		67.3 ± 5.3		61.2 ± 5.4		71.0 ± 11.3	<.05*
SAI		1.7 ± 1.3		3.5 ± 2.4		3.2 ± 2.4	NS
Subregion							
Upper frontal region (F1)							
$F_{i(L)}$	14	53.4 ± 7.4	9	49.8 ± 7.0	9	59.9 ± 13.5	NS
$F_{i(R)}$	14	52.3 ± 7.2	9	50.6 ± 6.9	9	60.3 ± 14.1	NS
SAI	14	4.5 ± 3.4	9	7.3 ± 6.8	9	2.3 ± 2.8	NS
Precentral region (F2)							
$F_{i(L)}$	15	69.9 ± 4.8	10	64.5 ± 6.3	9	72.4 ± 11.6	NS
$F_{i(R)}$	15	70.3 ± 6.5	10	64.1 ± 6.2	9	73.2 ± 11.6	NS
SAI	15	3.5 ± 2.2	10	5.8 ± 3.0	9	4.7 ± 3.3	NS
Superior region (F3)							
$F_{i(L)}$	15	66.9 ± 5.4	10	60.1 ± 6.7	9	71.4 ± 9.0	<.01**
$F_{i(R)}$	15	66.7 ± 5.6	10	61.2 ± 6.6	9	73.0 ± 11.0	<.05*
SAI	15	4.0 ± 3.3	10	3.5 ± 2.5	9	4.4 ± 2.8	NS
Midfrontal region (F4)							
$F_{i(L)}$	15	69.2 ± 4.9	10	61.6 ± 5.4	9	70.4 ± 10.8	<.05*
$F_{i(R)}$	15	68.6 ± 4.7	10	60.3 ± 8.0	9	71.7 ± 12.4	<.05*
SAI	15	2.9 ± 2.5	10	5.8 ± 6.2	9	3.3 ± 2.3	NS
Inferior frontal region (F5)							
$F_{i(L)}$	15	76.5 ± 6.3	10	67.8 ± 6.7	9	74.3 ± 13.3	NS
$F_{i(R)}$	15	76.5 ± 5.0	10	68.1 ± 6.6	9	77.6 ± 12.7	NS
SAI	15	4.1 ± 4.2	10	6.1 ± 5.6	9	5.2 ± 3.8	NS
Orbitofrontal region (F6)							
$F_{i(L)}$	15	69.4 ± 10.3	10	64.3 ± 3.2	9	67.6 ± 13.9	NS
$F_{i(R)}$	15	69.8 ± 9.6	10	64.1 ± 7.3	9	69.2 ± 13.8	NS
SAI	15	3.8 ± 3.3	10	7.2 ± 4.4	9	6.7 ± 5.4	NS

disease duration (see Table 2), nor was it due to selection of patients with extensive leukoencephalopathy into the group of *APOE* ε4-positive patients; only two of the patients with AD (one *APOE* ε4 positive and one *APOE* ε4 negative) had moderate degrees of leukoencephalopathy, whereas the remaining patients had no significant white-matter changes on cranial CT or MRI. The demographic analysis (see Table 2) and multivariate regression analyses (see Table 4) were suggestive of a confounding effect of age, but the subsequent age-stratified analyses revealed that the differences in rCBF between *APOE* ε4-positive and *APOE* ε4-negative patients could not be explained by confounding of age. Moreover, the rCBF differences between *APOE* ε4-positive and *APOE* ε4-negative patients were predominantly found in the group of elderly patients with AD.

As also demonstrated by Lethovirta et al,[8] we found a significant flow reduction in the left occipital lobe in *APOE* ε4-positive patients. There is no obvious explanation for the reduced blood flow in the occipital lobes found in the present study. No patients had clinical symptoms related to occipital cortical regions. Usually, the occipital regions are relatively spared in AD until the very late stages, a finding that was also confirmed in the present study, as there was no significant difference between patients and controls in the occipital regions (see Table 5). It should be mentioned that although none of the included patients had clinical features of Lewy body disease, there could be a confounding effect of patients with Lewy body disease in which both a high frequency of the *APOE* ε4 allele and occipital hypometabolism has been demonstrated.[36]

Assuming that the demonstrated reduction in rCBF in *APOE* ε4-positive patients is an expression of a true difference in the pathophysiology of AD depending on the *APOE* genotype, it could be a finding of relevance to the diagnostic evaluation and treatment. Nevertheless, previous studies have not found any certain correlation between *APOE* genotype and disease progression.[37–45] The finding of one or two *APOE* ε4 alleles and frontal hypoperfusion may improve diagnostic certainty in early stages of AD or may characterize a certain subgroup of AD patients who need specific considerations. Small et al and Reiman et al have recently demonstrated that in *APOE* ε4-positive patients with familial AD, regional reductions in cerebral glucose consumption could be demonstrated even in the preclinical stage.[46,47] As in the present study, the study by Reiman et al also suggested that the ε4 allele

could be related to a form of AD that preferentially affects the frontal lobes.

CONCLUSION

We have demonstrated that elderly patients fulfilling the NINCDS-ADRDA criteria for AD, and who have one or two *APOE* ε4 alleles, have significantly reduced rCBF in several cortical regions of interest compared with *APOE* ε4-negative patients. The flow deficits are most pronounced in the frontal association cortex, where they are demonstrated in both hemispheres. The study supports most previous studies in that the *APOE* genotype, in otherwise comparable AD patients, has an influence on cerebral neurophysiology as demonstrated by functional imaging. However, there are still inconsistencies in the exact topography of abnormal brain function in *APOE* ε4-positive patients. The findings in this study add further weight to the significance of frontal hypoperfusion in AD, even in the early stages of the disease. The SPECT method, which is the most widely available method for functional imaging, may be an important instrument in evaluation of this group of patients and may be useful in the evaluation of drug treatment efficacy.

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