SPECT and MRI analysis in Alzheimer's disease: relation to apolipoprotein E $\epsilon 4$ allele

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Abstract

Objectives—The $\epsilon 4$ allele of apolipoprotein E (ApoE) is a risk factor for late onset Alzheimer's disease. ApoE is present in senile plaques, neurofibrillary tangles, and cerebrovascular amyloid, and it is implicated in synaptogenesis. The effect of ApoE polymorphism on the volumes of hippocampus, amygdala, and frontal lobe was studied. The hypothesis was that the patients with Alzheimer's disease carrying the $\epsilon 4$ allele have more pronounced atrophy. The relation of ApoE and cerebral blood flow on cortical areas was also assessed.

Methods—Fifty eight patients with Alzheimer's disease at the early stage of the disease and 34 control subjects were studied. Patients with Alzheimer's disease were divided into subgroups according to the number of the $\epsilon 4$ alleles. Volumes were measured by MRI and regional cerebral blood flow ratios referred to the cerebellum were examined by ^{99m}Tc -HMPAO SPECT. ApoE genotypes were determined by digestion of ApoE polymerase chain reaction products with the restriction enzyme *Hha*I.

Results—patients with Alzheimer's disease had smaller volumes of hippocampi and amygdala compared with control subjects, and the patients with Alzheimer's disease homozygous for the $\epsilon 4$ allele had the most prominent volume loss in the medial temporal lobe structures. The frontal lobe volumes did not differ significantly. All patients with Alzheimer's disease had bilateral temporoparietal hypoperfusion and the subgroups with one or no $\epsilon 4$ alleles also had frontal hypoperfusion compared with control subjects. The occipital perfusion ratios tended to decrease with increasing number of $\epsilon 4$ alleles.

Conclusions—Patients with Alzheimer's disease homozygous for the $\epsilon 4$ allele seem to have severe damage in the medial temporal lobe structures early in the disease process and differ from the patients with Alzheimer's disease with one or no $\epsilon 4$ alleles.

Alzheimer's disease is a devastating disorder and early diagnostic tests for detecting the disease are invaluable. Recent studies with MRI have indicated a pronounced decline in volumes of the hippocampus as an early sign of the disease.¹⁻⁴ Amygdaloid volumes also decrease in the disease process.^{5,6}

Changes in the cortical perfusion and metabolism in Alzheimer's disease can be detected using single photon emission computed tomography (SPECT). Cerebral perfusion is reduced predominantly in the temporal and parietal regions,⁷⁻¹⁰ where the greatest neurochemical abnormalities are seen at post-mortem.¹¹

The frequency of the apolipoprotein E (ApoE) $\epsilon 4$ allele is increased among patients with late onset Alzheimer's disease.¹²⁻¹⁷ In late onset families, the risk of Alzheimer's disease increased from 20% to 90% with increasing number of ApoE $\epsilon 4$ alleles.¹⁸ Immunohistochemical studies have shown the presence of ApoE in senile plaques, neurofibrillary tangles, and cerebrovascular amyloid protein.^{19,20} In addition, data are increasingly suggesting that pathological and neurochemical differences exist in the brains of patients with Alzheimer's disease in relation to the ApoE polymorphism. Distinct binding properties of ApoE isoforms to $\text{A}\beta$ ²¹ and tau proteins²² suggest how ApoE may mediate its action. Two studies have reported that patients with Alzheimer's disease with two $\epsilon 4$ alleles have higher counts of amyloid plaques and cerebrovascular amyloid than those without the $\epsilon 4$ allele.^{23,24} The choline acetyltransferase activity is decreased in the postmortem hippocampus and frontal cortices of patients with Alzheimer's disease with two $\epsilon 4$ alleles compared with patients with one or no $\epsilon 4$ alleles.^{25,26} Studies have suggested that ApoE might also be implicated in the synaptogenesis of the hippocampus.²⁷

This study attempted to approach the role of ApoE $\epsilon 4$ allele in Alzheimer's disease by studying structural changes with MRI volumetry, as well as changes in cerebral perfusion using SPECT. We investigated whether patients with Alzheimer's disease with similar clinical severity of dementia and with distinct ApoE genotypes differ in the degree of volume loss in the hippocampus and amygdala, structures known to be affected early in the disease process. The hypothesis to be tested was that the patients with Alzheimer's disease carrying the $\epsilon 4$ allele have more pronounced atrophy, especially in the hippocampus, where synaptogenesis is strong during memory processing.

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This hypothesis was supported by recent findings in a limited number of patients.²⁸ We also investigated the possible association with ApoE polymorphism and cerebral blood flow.

Patients and methods

We studied 58 patients fulfilling the NINCDS-ADRDA criteria of probable Alzheimer’s disease²⁹ and 34 age and sex matched cognitively intact control subjects. Table 1 presents their clinical characteristics. The patients with Alzheimer’s disease were either undergoing diagnostic examination or were recently diagnosed. The Kuopio University Hospital approved the study and all the subjects and caregivers of demented patients gave informed consent for participation in the study.

The patients with Alzheimer’s disease underwent the following examinations: general physical and clinical neurological examination, assessment of clinical severity with the mini mental state examination (MMSE)³⁰ and brief cognitive rating scale (BCRS),³¹ assessment of extrapyramidal signs with the Webster scale,³² assessment of depressive symptoms by the Hamilton scale,³³ neuropsychological tests, laboratory tests to exclude secondary causes of dementia, Brain MRI and SPECT, conventional and quantitative EEG, and event related potentials. All patients with Alzheimer’s disease scored less than 4 on the modified ischaemic scale.³⁴

MAGNETIC RESONANCE IMAGING

The subjects were scanned with a 1.5 T Magnetom (Siemens, Erlangen) using standard head coil and a tilted coronal 3D gradient echo sequence (MP-RAGE: TR 10 ms, TE 4 ms, TI 250 ms, flip angle 12°, FOV 250 mm, matrix 256 × 192, one acquisition). This resulted to 128 T1 weighted partitions with slice thickness of 1.5 to 1.8 mm oriented at right angles to the long axis of the hippocampus. The radiologist who analysed the MRIs was blinded to the subject’s clinical data. The method has been described earlier in detail.³⁵

Determination of volumes of the hippocampus and the amygdala

We used standard anatomical atlases of the human brain^{36,37} with adjustment from previous medical literature³⁸ as guidelines to determine the boundaries of the amygdala and the hippocampus on oblique coronal MRI sec-

tions. The boundaries of the region of interest were outlined by a trackball driven cursor proceeding from anterior to posterior. The number of voxels within the region was integrated with a program developed in house for a standard work console.

Terminology of anatomical regions studied

In this study, the outlines of the amygdala included the deep nuclei, the superficial nuclei, and the remaining nuclei.³⁵ At the most rostral sections of the amygdala, we outlined only the deep amygdaloid nuclei in the MRI image to avoid the overestimation of the amygdaloid volume due to inclusion of the piriform cortex. This, on the other hand, may have resulted in exclusion of the most rostral portions of the periamygdaloid cortex from the total amygdaloid volume. The hippocampus included the dentate gyrus, the hippocampus proper, and the subicular complex. The uncus portion of the rostral hippocampus located ventral to the caudal amygdala was included in the hippocampus. The caudal end of the hippocampus was determined from the section in which the full length of the fornices were still detectable. The fornices were not included.

Determination of volumes of frontal lobe

The gyri were manually outlined on every third slice. The most anterior of the slices was the one with clearly visible gyri. On the most posterior slices, a straight line was drawn from the bottom of the lateral fissure (ventral insular sulcus) to the medially located choroidal fissure to separate the temporal lobe from the frontal lobe. The most caudal slice included in the measurement was the one in which the anterior commissure was present. The volume of the lateral ventricles was also measured and consequently subtracted from the volume of the slice. The volume of each slice was multiplied by three and thereafter, the slice volumes were summed.

Normalisation of volumes

We used normalised values in all statistical analyses. Normalisation was done by dividing the volumes of the hippocampus, amygdala, or the frontal lobe by brain area. To obtain brain area, we measured the area of both hemispheres in an MR image taken at the level of the anterior commissure.

SINGLE PHOTON EMISSION COMPUTED TOMOGRAPHY

A dose of 370 to 555 MBq ^{99m}Tc-HMPAO (Amersham International, London, UK) was intravenously injected into a vein with the patient in a dark and quiet room. High resolution SPECT was carried out with a three head Siemens MultiSPECT 3 gamma camera equipped with high resolution collimators.³⁹ Ten minutes after injection of the tracer the radioactivity distribution of the brain was acquired in a 128 × 128 matrix mode. Data sets were acquired at 3° intervals for 35 seconds each, to a total of 40 sets (120° per camera head). Transaxial, sagittal, and coronal slices 3.5 mm thick were reconstructed after

Table 1 Demographic data of patients with Alzheimer’s disease (AD) and control subjects

	AD Patients			Control subjects (n = 34)
	2e4 (n = 13)	1e4 (n = 24)	0e4 (n = 21)	
Women/men	6/7	11/13	11/10	20/14
Age (y)	66 (9)*	72 (7)	70 (9)	72 (4)
Age at onset (y)	63 (9)*	69 (7)	68 (9)	—
Duration (y)	3 (2)	3 (2)	3 (1)	—
MMSE	21 (3)	21 (4)	23 (4)	—
BCRS	23 (5)	25 (7)	23 (7)	—

Values are expressed as means (SD).
*P < 0.05 v other groups

Table 2 Normalised volumes of hippocampus, amygdala, and frontal lobe according to the number of the $\epsilon 4$ alleles in patients with Alzheimer's disease (AD) and control subjects

	AD Patients			Control subjects (n = 34)	ANOVA F value
	2 $\epsilon 4$ (n = 13)	1 $\epsilon 4$ (n = 24)	0 $\epsilon 4$ (n = 21)		
Right hippocampus	0.26 (0.07)	0.33 (0.08)†	0.34 (0.09)†	0.43 (0.05)§	18.5***
Left hippocampus	0.24 (0.03)	0.28 (0.06)	0.30 (0.07)†	0.40 (0.06)§	24.9***
Right amygdala	0.17 (0.06)	0.21 (0.06)†	0.23 (0.07)†	0.23 (0.05)†	3.7*
Left amygdala	0.20 (0.07)	0.21 (0.05)	0.23 (0.05)	0.25 (0.05)††	4.2*
Right frontal lobe	14.19 (1.92)	14.14 (1.51)	14.03 (1.67)	14.25 (1.74)	NS
Left frontal lobe	14.06 (1.46)	13.67 (1.43)	12.95 (1.39)	13.84 (1.73)	NS

Values are expressed as means (SD).

Duncan post hoc analysis ($P < 0.05$).

* $P < 0.05$; *** $P < 0.0001$. (ANOVA over the study groups.)

† Differs from 2 $\epsilon 4$ subgroups.

†† Differs from 1 $\epsilon 4$ subgroups.

§ Differs from all other groups.

high resolution Butterworth filtering (a cut off frequency of 0.5/cm) and the uniform attenuation correction of 0.12/cm. The imaging resolution was 8–9 mm.³⁹ Two consecutive slices were summarised (7 mm slice thickness) and visually surveyed on a TV screen. Regional count densities (regional cerebral blood flow) were calculated for the frontal, temporal, parietal, and occipital cortices. The regional counts were related to the cerebellar counts. The rater was not aware of the clinical data of the subjects.

DETERMINATION OF APOLIPOPROTEIN E GENOTYPE

DNA was prepared from blood leucocytes of patients with Alzheimer's disease by standard procedures. ApoE genotypes were analysed using the polymerase chain reaction (PCR) described earlier^{40,41} with slight modifications.²⁸ ApoE genotypes were identified through *HhaI* digestion. Digested DNA fragments were analysed through polyacrylamide gel electrophoresis and separated fragments of DNA were visualised by staining with ethidium bromide.

STATISTICAL ANALYSIS OF DATA

The data were analysed using SPSS-PC 4.1 software. We used analysis of variance (ANOVA) to detect differences in means over the study groups and control subjects. The Duncan method was applied in post hoc analysis when needed. Patients with Alzheimer's disease were divided into three subgroups according to the number of ApoE alleles; two $\epsilon 4$ alleles, one $\epsilon 4$ allele, and no $\epsilon 4$

alleles. In the statistical analyses of the volumetric data, we used volumes normalised for brain area. The level of significance was set at $P < 0.05$.

Results

Patients with Alzheimer's disease and control subjects did not differ in age and sex. Alzheimer's disease subgroups had equal clinical severity as assessed by MMSE and BCRS, and had a similar duration of dementia. However, when they were divided according to the number of $\epsilon 4$ alleles, the homozygous $\epsilon 4$ subgroup had an earlier onset of the disease and was younger than the other subgroups (table 1).

Table 2 presents the normalised volumes of hippocampi, amygdala, and frontal lobes. ANOVA over the control subjects and Alzheimer's disease subgroups showed significant differences in the volume ratios of the right and left hippocampus ($P < 0.0001$), and the right and left amygdala ($P < 0.05$), but not in the ratios of frontal lobes. The Duncan post hoc analysis disclosed that the control subjects had larger hippocampi than the Alzheimer's disease subgroups. They also had the largest amygdaloid volumes, but the difference was only significant in the right amygdala compared with the homozygous $\epsilon 4$ subgroup and in the left amygdala compared with the one $\epsilon 4$ and two $\epsilon 4$ subgroups. Duncan post hoc analysis across the Alzheimer's disease subgroups showed that the two $\epsilon 4$ subgroup had the smallest hippocampi; the difference was significant in the right hippocampus compared with the no $\epsilon 4$ and one $\epsilon 4$ subgroups and in the left hippocampus compared with the no $\epsilon 4$ subgroup. The two $\epsilon 4$ subgroup had a significantly smaller right amygdala than the other subgroups. When BCRS was used as a covariate the results did not change, but when we used MMSE the significance for the right hippocampus was $P = 0.06$, for the left hippocampus $P = 0.07$, and for the right amygdala $P = 0.03$. The volumes of the frontal lobe did not differ significantly over the study groups. However, the frontal lobe volumes tended to increase with increasing number of the $\epsilon 4$ alleles.

Perfusion ratios measured on SPECT for all patients with Alzheimer's disease were significantly reduced in all regions other than the occipital cortices. Moreover, some differences were evident in perfusion ratios for Alzheimer's disease subgroups. The perfusion for the two $\epsilon 4$ subgroup did not differ from control subjects in the frontal cortex and in the right temporal cortex, whereas the other subgroups also had hypoperfusion in these regions. By contrast, the homozygous $\epsilon 4$ subgroup had a significantly reduced perfusion ratio in the left occipital region compared with control subjects and the no $\epsilon 4$ subgroup, but in the zero $\epsilon 4$ and one $\epsilon 4$ subgroups occipital perfusion was preserved compared with control subjects. The differences in the right occipital region showed the same tendency, but were not significant (table 3).

Table 3 Regional cerebral blood flow related to cerebellar blood flow: control subjects and subgroups with Alzheimer's disease (AD) according to the number of the $\epsilon 4$ alleles

	AD Patients			Control subjects (n = 13)	ANOVA F value
	2 $\epsilon 4$ (n = 13)	1 $\epsilon 4$ (n = 24)	0 $\epsilon 4$ (n = 21)		
Right frontal	0.82 (0.11)	0.77 (0.09)*	0.75 (0.11)*	0.85 (0.10)	3.5*
Left frontal	0.78 (0.09)	0.75 (0.11)*	0.71 (0.12)*	0.84 (0.12)	4.0*
Right temporal	0.80 (0.12)	0.75 (0.10)*	0.76 (0.11)*	0.88 (0.11)	4.6**
Left temporal	0.73 (0.13)*	0.72 (0.11)*	0.67 (0.13)*	0.86 (0.11)	6.2**
Right parietal	0.70 (0.70)*	0.72 (0.10)*	0.72 (0.07)*	0.79 (0.09)	3.2*
Left parietal	0.64 (0.84)*	0.67 (0.11)*	0.65 (0.11)*	0.77 (0.10)	4.5**
Right occipital	0.88 (0.11)	0.95 (0.10)	0.98 (0.15)	0.99 (0.12)	NS
Left occipital	0.86 (0.12)*	0.92 (0.10)	0.96 (0.14)†	1.00 (0.12)	3.3*

Values are expressed as means (SD).

* Differs from control subjects.

† Differs from 2 $\epsilon 4$ subgroup.

** $P < 0.01$ (ANOVA); ANOVA/Duncan over the subgroups ($P < 0.05$).

Discussion

It is essential for various treatment strategies to find diagnostic ways to detect early Alzheimer's disease and to identify Alzheimer's disease subtypes. Earlier data have shown that patients with Alzheimer's disease have smaller volumes of hippocampi and amygdala than control subjects,^{1,2,5} which is in accordance with the severe histological changes seen in the hippocampus⁴² and amygdala⁶ of patients with Alzheimer's disease. The frequency of the ApoE $\epsilon 4$ allele has been found to be increased in patients with late onset Alzheimer's disease.¹²⁻¹⁷ Two recent studies have reported that the patients with the $\epsilon 4$ allele are prone to more severe neuropathological changes, particularly increased counts of amyloid plaques and increased degree of cerebrovascular amyloid.^{23,24} Our results confirmed the earlier results showing that patients with Alzheimer's disease have smaller volumes of hippocampi and amygdala than control subjects.^{1,2,5} The major finding of this study was, however, that the patients with Alzheimer's disease homozygous for the $\epsilon 4$ allele had the most prominent volume loss in the medial temporal lobe structures, hippocampus, and amygdala. By contrast, the frontal lobe volumes did not differ significantly in the study groups, although an opposite trend of volume loss with decreasing number of the $\epsilon 4$ alleles was seen. These findings suggest that the ApoE $\epsilon 4$ allele may predominantly affect medial temporal lobe structures for reasons as yet unknown.

Experimental studies have found that induction of ApoE gene expression coincided with reactive synaptogenesis and terminal proliferation in the hippocampus after lesioning the entorhinal cortex in rats.²⁷ In addition, during memory processing strong synaptogenesis and reorganisation take place in the hippocampus.⁴³ Therefore, it is possible that ApoE interferes with synaptogenesis contributing to differences in volume loss in patients with Alzheimer's disease with different ApoE genotypes. Earlier in the same study group, we found that the patients with Alzheimer's disease with two $\epsilon 4$ alleles had the lowest scores on immediate and delayed tests assessing verbal memory. Verbal memory was examined with a list learning test using shopping items⁴⁴ and the Wechsler logical memory test using one story.⁴⁵ The delayed recall of the story and a yes/no recognition of the words in the list were asked after a 30 minute delay filled with other psychometric tests.⁴⁶ Even though the severity of global disease was equal, the patients with Alzheimer's disease with two $\epsilon 4$ alleles had more severe memory dysfunction. This could indicate that the $\epsilon 4$ allele interferes with the course of Alzheimer's disease and particularly affects the structures involved in the memory processing.

T2 relaxometry is a reliable and sensitive method for identifying hippocampal sclerosis,⁴⁷ but in Alzheimer's disease its role is more complex. Kirsch and coworkers reported hippocampal T2 prolongation in 13 patients with Alzheimer's disease and suggested that T2

relaxation time is a sensitive marker for Alzheimer's disease.⁴⁸ Some pathological conditions are known to cause prolongation of T2 time. The most important reason is the presence of CSF; the others are gliosis, glioma, chronic inflammation, and oedema. We also studied T2 relaxometry to find out whether the patients with different ApoE genotypes have different T2 times in the medial temporal lobe structures as well as in the neocortical white matter. The only significant difference was between the control subjects and patients with Alzheimer's disease in T2 relaxation time of the right hippocampus calculated as the mean from anterior, middle, and posterior sections. However, there was a significant overlap in the study groups and only three patients with Alzheimer's disease exceeded the limit of 110 ms. Our patients were at the early stage of Alzheimer's disease and it is possible that the prolongation of the hippocampal T2 relaxation time is seen only at the moderate or severe dementia.

Changes in cortical perfusion and metabolism in Alzheimer's disease can be detected using SPECT and PET methods. Decreased metabolism and reduced cerebral perfusion are known to occur predominantly in temporal and parietal areas.^{9,10} However, some studies have reported greatly reduced perfusion in frontal areas⁴⁹⁻⁵¹ and it is considered that 15% to 20% of patients with Alzheimer's disease have frontal hypoperfusion. Similarly, occipital areas have been used as a reference region for normalising perfusion values, although PET studies have shown occipital changes in patients with Alzheimer's disease⁸ and a few SPECT studies have confirmed this finding.^{10,52} Our patients with mild dementia had decreased perfusion ratios in the parietal and temporal regions compared with the control subjects. In the frontal regions the subgroups of no and one $\epsilon 4$ allele differed significantly from the control subjects; however the two $\epsilon 4$ subgroup had higher perfusion ratios in these regions than the other subgroups (not significant), which is in accordance with the larger volumes of frontal lobes seen on MRI. However, we want to emphasise the small number of patients and a possibility of a chance finding in frontal changes. The occipital perfusion ratios tended to decrease with increasing number of the $\epsilon 4$ alleles. The regional cerebral blood flow is strongly regulated by the ascending neurotransmitter system such as the cholinergic, noradrenergic, and serotonergic pathways.⁵³ Recent data suggest that the magnitude of the cholinergic depletion is greater in patients with Alzheimer's disease with two $\epsilon 4$ alleles.^{25,26} Because the density of the cholinergic innervation is lower in the primary sensory areas—for example, in the occipital cortex than in the associative cortical areas and the limbic structures⁵⁴—it is possible that the stronger cholinergic depletion in the $\epsilon 4$ homozygotes may be earlier reflected as a hypoperfusion in the regions where the cholinergic innervation is less dense. Our study confirms the finding that in the occipital regions there are changes in

Alzheimer's disease and this area should not be used as a reference region.

In conclusion, we found that the patients with Alzheimer's disease had smaller volumes of medial temporal lobe structures, hippocampus, and amygdala, than control subjects and the homozygous $\epsilon 4$ patients had the smallest volumes. The frontal lobe volumes of $\epsilon 4$ homozygotes tended to be larger than for patients with one or no $\epsilon 4$ alleles. Besides hypoperfusion in the temporoparietal regions that was evident for all patients with Alzheimer's disease, the $\epsilon 4$ homozygotes also had hypoperfusion in the occipital cortices, but their frontal perfusion was preserved. This study adds to the body of medical literature suggesting that the ApoE $\epsilon 4$ allele is involved in the pathogenesis of Alzheimer's disease. It will be of interest to determine whether the volume loss in the hippocampus and amygdala is related to greater loss of synapses or increased accumulation of the amyloid and paired helical filaments. Moreover, studying the regional distribution of neurotransmitter deficits may throw light on the mechanisms underlying differences in regional cerebral blood flow related to ApoE genotypes.

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