Major decrease in the volume of the entorhinal cortex in patients with Alzheimer's disease carrying the apolipoprotein E ε4 allele

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Abstract

Objective—Recent evidence indicates that the apolipoprotein E (ApoE) ε4 allele is a risk factor for developing Alzheimer's disease. It has also been proposed that it is associated with increased counts of amyloid plaques and neurofibrillary tangles that in turn are neuropathological hallmarks initially appearing in the medial temporal lobe structures in Alzheimer's disease. In this study, the effect of the ApoE ε4 allele on the volume of the entorhinal cortex was evaluated in vivo.

Methods—The volume of the entorhinal cortex was measured on MR images using a recently designed histology based protocol in 16 patients with Alzheimer's disease with ApoE ε4 (mean age 70.4 (SD 9.9)), 11 patients with Alzheimer's disease without ApoE ε4 (mean age 69.1 (SD7.1)), and in 31 healthy age and sex matched normal controls (72.2 (SD 3.9)). The patients met the NINCDS-ADRDA criteria for probable Alzheimer's disease and were in mild to moderate stages of the disease. MRI was performed with a 1.5 Tesla Magnetom and a 3D technique permitting the reconstruction of 2.0 mm thick contiguous slices perpendicular to the axis of the anterior-posterior commissure.

Results—The patients with Alzheimer's disease without the ApoE ε4 allele had atrophy in the entorhinal cortex, the volume was reduced by 27 % compared with control subjects. However, the most prominent shrinkage (45%) in the entorhinal cortex was seen in patients with Alzheimer's disease with the ApoE ε4 allele (p=0.0001). The effect of ε4 on the entorhinal cortex volume was especially prominent in female patients with Alzheimer's disease compared to male patients with Alzheimer's disease (p=0.014). Additionally, patients with the ApoE ε4 allele had inferior performance in verbal and visual memory functions than those without the allele

Conclusions—Volumetric MRI measurements disclose that ApoE ε4 is associated with the degree of atrophy in the entorhinal cortex in early Alzheimer's disease, this effect being especially prominent in female patients with Alzheimer's disease.
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The apolipoprotein E (ApoE) ε4 allele is a risk factor for Alzheimer's disease, in which the ApoE ε4 allele has been reported to be associated with an earlier age of onset, increased counts of amyloid plaques,[1 2] and neurofibrillary tangles[3] in the brain tissue, severe depletion of cholinergic markers in the frontal[4] and temporal cortex and the hippocampus,[5] and reduced capability for plastic response.[6]

Earlier studies have suggested that the ApoE ε4 allele might be particularly detrimental for memory function in patients with Alzheimer's disease and also in non-demented elderly subjects.[7] Moreover, a study using MRI volumetry of the hippocampus proposed that the hippocampal atrophy was related to the number of ApoE ε4 alleles, patients with Alzheimer's disease with the ε44 genotype having the most pronounced shrinkage of the hippocampus.[8] In non-demented elderly subjects, minor changes in the hippocampus were also seen in relation to the ε4 allele.[9]

Hippocampal volumetry has been considered as a sensitive indicator of early Alzheimer's disease.[10–15] As the entorhinal cortex, particularly the transentorhinal area, is one of the very first regions to exhibit neurofibrillary tangles,[16] MRI volumetry of the entorhinal cortex might help in the diagnosis of Alzheimer's disease even before changes are evident in hippocampal volumetry. Using a histology based MRI protocol, we have recently determined that volumetry of the entorhinal cortex is reliable in the diagnosis of Alzheimer's disease, even in mild dementia.[17] Having found a relation between ApoE genotype and hippocampal atrophy, in this study we wanted to investigate whether ApoE ε4 also affects atrophy of other temporal structures involved in Alzheimer's disease, such as the entorhinal cortex. Because the ApoE ε4 allele is a risk factor for developing Alzheimer's disease and the entorhinal cortex is damaged during the very early stages of the disease, we hypothesised that the ApoE genotype might influence the magnitude of atrophy of the entorhinal cortex. To test this hypothesis, we examined whether patients with Alzheimer's disease with different ApoE genotypes differ in the degree of the damage in the entorhinal cortex measured using MRI.

Materials and methods

SUBJECTS
This study consisted of 27 patients meeting the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders

Association (NINCDS-ADRDA) criteria of probable Alzheimer's disease,[18] and 31 healthy, cognitively normal control subjects. The patients with Alzheimer's disease were recruited at diagnostic examinations or had been recently diagnosed. The controls were randomly selected from participants of the study investigating prevalence of age associated memory impairment.[19] The subjects included as controls in the present study were healthy subjects with intact memory. There were 12 women and 15 men, mean age 69.9 (SD 8.8) (range 50–83) in the Alzheimer's disease group and 20 women and 11 men, mean age 72.2 (SD 3.9) (range 65–79) in the control group. The control subjects and patients with Alzheimer's disease were similar in age (analysis of variance (ANOVA), p=0.3654). The controls had received more education (mean 9.6 (SD 3.7), range 4–16) than the patients with Alzheimer's disease (mean 6.5 (SD 1.8), range, 4–12) (ANOVA, p=0.0007). All subjects underwent a general medical and neurological examination by a neurologist, a battery of laboratory tests to exclude systemic illnesses with adverse effects on cognition, neuropsychological tests, electroencephalography, event related potentials, single photon emission computed tomography (SPECT), and MRI of the brain. All subjects obtained an ischaemic score<4 on the modified ischaemic scale.[20] The clinical severity of Alzheimer's disease was assessed with the mini mental state examination (MMSE)[21] and the clinical dementia rating scale (CDR).[22] The mean score in the MMSE was 20.7 (SD 3.9) (range, 14–28) in the Alzheimer's disease group and 28.3 (SD 1.5) (range, 25–30) in the control group. The cognitive stage in the clinical dementia rating scale indicated mild dementia (CDR=1) in 14 patients with Alzheimer's disease and moderate dementia (CDR=2) in 13 patients with the disease.

The study was approved by the ethics committee of Kuopio University and University Hospital. All subjects provided their informed consent for participation in the study after an explanation of the study protocol.

NEUROPSYCHOLOGICAL ASSESSMENT
We examined verbal memory with the list learning test using shopping items.[23] A "yes" or "no" recognition of the words in the list was asked after a 30 minute delay filled with other psychometric tests. We also used the story recall test with the Boston approach.[24] The recall of the story was tested immediately and after a 30 minute delay. Visual memory was examined with the Heaton visual reproduction test.[25] The recall of the figures was tested both immediately and after a 30 minute delay.

DETERMINATION OF APOE TYPE
DNA was prepared from the venous blood by standard procedures. The ApoE genotypes were analysed using the polymerase chain reaction (PCR) as described earlier[26] with slight modifications. The ApoE genotypes were identified through HhaI digestion. Digested DNA fragments were separated with polyacrylamide gel electrophoresis and separated fragments of DNA were visualised using ethidium bromide staining.

MRI TECHNIQUE
MRI was performed with a 1.5 T Magnetom (Siemens, Erlangen) using a 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). The slice thickness was 1.5–1.8 mm and about 128 contiguous T1 weighted partitions were obtained for each subject. Coronal MRI covering the entire rostrocaudal extent of the entorhinal cortex were obtained perpendicular to the plane which joins the anterior and posterior commissures on the midsagittal section. These angled coronal images were then reconstructed into 2 mm thick contiguous slices.

VOLUMETRIC MEASUREMENTS
The volume of the entorhinal cortex on MRI
The entorhinal cortex (Brodmann's area 28) is defined as the grey matter structure in the ventromedial part of the temporal lobe. It has its superior boundary with the hippocampus and inferior boundary with the collateral sulcus. The lateral boundary for the entorhinal cortex is defined by the perirhinal cortex. Rostrocaudally it continues roughly from the level of the limen insula to the level of the gyrus intralimbicus and the most caudal portion of it is defined by the parahippocampal cortex (areas TH and TF).

The identification and definition of the entorhinal cortex in this study were initially based on a previous study in which the region was identified from histological sections.[27] Next, we designed a protocol for defining the borders of the entorhinal cortex on MRI.[28] The identification of the borders of this brain region on MRI is based on anatomical landmarks, and we determined the location of the entorhinal cortex in the human brain according to the sulci and gyri that would best indicate the boundaries of this cortical area as determined from the histological sections. By using the sulcal and gyral pattern as well as the rostrocaudal level, the outline of the entorhinal cortex was manually outlined by a trackball driven cursor on successive MRI in control subjects and in patients with Alzheimer's disease. Once identified, the number of voxels within the region was calculated by using a program developed in house for a standard work console. The outlining of the boundaries always proceeded in an anterior to posterior direction in a sequential fashion. The measurements were performed by a single rater (KJ) to ensure consistency of the measurements. All measurements were done without any knowledge of the clinical data of the subjects. Within rater and between rater reliabilities for the method have been reported in a previous article.[28] To exclude between subject and between sex variability in the size of the structures studied, the volumes were normalised to the intracranial area measured at the level of the anterior commissure (volume/intracranial area×10⁴).[29]

Table 1   Clinical characteristics in patients with Alzheimer's disease (AD) with (ε4+) and without (ε4−) ApoE ε4 allele and in healthy controls

	Controls	AD ε4−	AD ε4+
Number	31	11	16
Age (y)	72.2 (3.9)	69.1 (7.1)	70.4 (9.9)
F/M	20/11	6/5	6/10
Education (y)	9.6 (3.7)	5.8 (0.8)	6.9 (2.2)
MMSE	28.3 (1.5)	22.3 (3.6)	19.6 (3.7)
CDR 1/ CDR 2	—	6/5	8/8
Story recall, immediate	—	9 (6)*	4 (3)
Story recall, delayed	—	8 (7)**	2 (3)
List learning, immediate	45 (6)	22 (8)***	12 (6)
List learning , delayed	10 (0)	7 (3)	5 (3)
Heaton visual, immediate	13 (3)	6 (2)**	4 (2)
Heaton visual, delayed	11 (3)	2 (3)	0 (1)

*p=0.05; **p<0.01; ***p<0.001, AD ε4− cases *v* AD ε4+ cases.
Values are means (SD). The stage of the Alzheimer's disease was determined by the mini-mental status examination (MMSE) and clinical dementia rating scale (CDR). Student's *t* test.

STATISTICAL ANALYSIS OF DATA

Multivariate analysis of variance (MANOVA) for repeated measures was used for comparisons between different groups. Firstly, measured volumes were analysed for controls, patients with Alzheimer's disease with the ε4 (ε4+) allele, and patients without the ε4 (ε4-) allele. Next, only patients with Alzheimer's disease were included in the analyses. The volume of the entorhinal cortex was the dependent measure in MANOVA with hemisphere as a repeated factor, and group and sex as grouping factors. Analysis of variance (ANOVA) with Duncan post hoc analysis was used to compare means over the subgroups. The sex and CDR distribution were analysed by χ^2 test. The means of the clinical data over the study groups were compared by one way ANOVA over three study groups and Student's *t* test over two study groups. The level of significance was set at p<0.05.

Results

The ApoE genotypes for patients with Alzheimer's disease were: ε33, n=11; ε24, n=1; ε34, n=7; and ε44, n=8. The ε4+and ε4− patients with Alzheimer's disease and control subjects did not differ in age (ANOVA; *F*=1.03, p=0.3654) or sex (χ^2=3.12, df=2, p=0.2105) (table 1). The control subjects had undergone more extensive schooling than patients with Alzheimer's disease (ANOVA; *F*=8.31, p=0.0007). As expected, controls had higher MMSE scores than patients with Alzheimer's disease (ANOVA; *F*=59.22, p=0.0001). When dividing patients with Alzheimer's disease according to the ApoE ε4 allele, those patients who were carrying one or two ε4 alleles had slightly lower MMSE scores than patients with Alzheimer's disease without the ε4 allele, but

this difference was not significant (Student's *t* test p=0.07) and these Alzheimer's disease subgroups did not differ in their clinical severity as assessed by CDR. Patients with Alzheimer's disease with the ε4 allele and patients without the ε4 allele did not differ in age, age at onset (ε4−/ε4+, 67 (SD 8) / 67 (SD 10) years, p=0.93), duration of disease (ε4−/ε4+, 35 9(SD 18)/42 (SD 4) months, p=0.45), or education (table 1).

The volume measurements of the right and left entorhinal cortex are presented for control subjects and patients with Alzheimer's disease in table 2. MANOVA over the controls and Alzheimer's disease subgroups showed a significant difference in the mean volume of the entorhinal cortex (*F*=53.32 p=0.0001). The post hoc analysis disclosed that both Alzheimer's disease subgroups had smaller mean volumes of their entorhinal cortex than those of the controls. Moreover, patients with Alzheimer's disease carrying one or two ε4 alleles had 27% smaller volume of the entorhinal cortex on the left side than those patients not possessing any ε4 allele. The mean volume of the entorhinal cortex on the right side was also smaller in the ε4+ subgroup than in the ε4−subgroup, but this difference did not reach significance.

When the effect of the number of ε4 alleles was examined, it was evident that the patients with Alzheimer's disease with the ε44 genotype had a 22% smaller right entorhinal cortex than patients with Alzheimer's disease with one ε4 allel; this difference did not, however, reach significance. On the left side patients with the ε44 allele had 11% larger volumes than patients with one ε4.

MANOVA over the study groups showed that the right entorhinal cortex was significantly larger than the left side (main effect of side, *F*=12.96, p=0.001). The hemispheric asymmetry was seen in control subjects and in both Alzheimer's disease subgroups (group×side interaction, *F*=0.56, p=0.576) and also in men and women (sex×side interaction, *F*=0.78, p=0.382). Although the volumes of the entorhinal cortex did not differ between men and women (main effect of sex, *F*=2.16, p=0.148), there was a significant sex×group interaction (*F*=3.34, p=0.043) (table 2). On the left side, the volume of the entorhinal cortex was almost the same between control men and women, whereas in both Alzheimer's disease subgroups, especially in the Alzheimer's disease ε4+ subgroup, men had larger mean volumes of the entorhinal cortex than women (figure A). On the right side, control male subjects had roughly as large a volume in the entorhinal cortex as control female subjects. There was no sex difference in Alzheimer's disease ε4- patients but female patients with Alzheimer's disease with the ε4 allele had smaller volumes in the right entorhinal cortex than male patients with Alzheimer's disease with the ε4 allele (figure B). The mean volume of the entorhinal cortex was roughly 33% smaller in women than men with Alzheimer's disease carrying one or two ε4 alleles. The sex difference found was not explained by age,

Table 2   Normalised entorhinal cortical volumes in patients with Alzheimer's disease (AD) with (ε4+) and without (ε4−) the ApoE ε4–allele and in control subjects

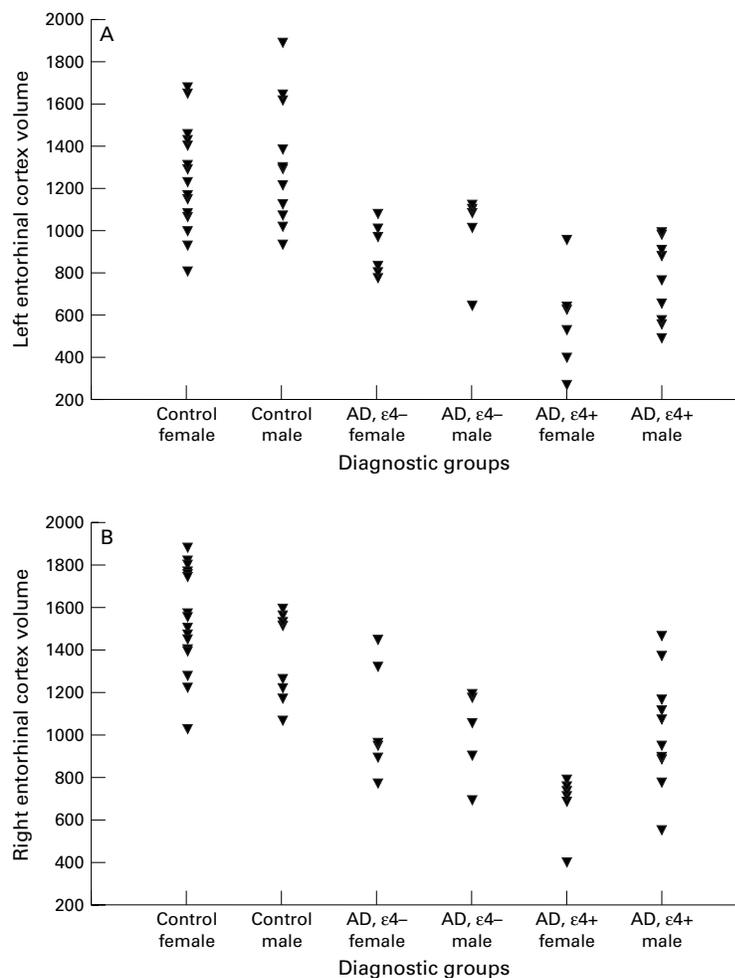|	Controls	AD ε4−	AD ε4+
Entorhinal cortex, left	1281 (251)	954 (160)	695 (221)
Female	1259 (229)	916 (125)	572 (236)
Male	1323 (294)	999 (199)	769 (185)
Entorhinal cortex, right	1461 (231)	1037 (232)	898 (284)
Female	1511 (232)	1064 (267)	684 (143)
Male	1371 (209)	1005 (208)	1026 (273)

Volumes are presented as mean (SD). All volumes are normalised to the individual intracranial area (ICA) at the level of the anterior commissura. MANOVA for repeated measures showed significant main effect of group (*F*=53.32, p=0.0001), side (*F*=12.96, p=0.001) and interaction between group and sex (*F*=3.34, p=0.043) for the entorhinal cortex.

Scatterplots displaying the effect of sex on the volume of the left (A) and right (B) entorhinal cortex in female and male control subjects and in patients with Alzheimer's disease (AD) with (e4+) and without (e4−) the ApoE ε4 allele. There was a significant sex×group interaction (p=0.043) in the entorhinal cortex.

duration of the disease, or MMSE scores. The bilateral reduction in the volume of the entorhinal cortex was markedly more prominent in female ε4+ patients with Alzheimer's disease than female ε4− patients with Alzheimer's disease than the corresponding reduction in male ε4+ patients compared with male ε4− patients with Alzheimer's disease. On the right side, male ε4+ and ε4− patients with Alzheimer's disease had similar entorhinal cortical volumes, whereas on the left side, ε4+ males with Alzheimer's disease had slightly smaller volumes than ε4− males with Alzheimer's disease. This sex difference between Alzheimer's disease subgroups could not be explained by differences in age, duration, or MMSE scores. In repeated MANOVA for Alzheimer's disease subgroups, no interactions other than the sex×Alzheimer's disease subgroup were detected.

The cognitive performance of both Alzheimer's disease subgoups was significantly impaired compared with control subjects (ANOVA, p<0.0001) (table 1). Those patients with Alzheimer's disease with one or two ε4 alleles had significantly lower scores in an immediate test of list learning (Student's *t* test, p<0.001) and immediate (p<0.05) and de-

layed (p<0.01) tests of story recall than patients with Alzheimer's disease without any ε4 allele. In Heaton's visual reproduction test, the two Alzheimer's disease subgroups also differed in immediate testing (p<0.01). Although Alzheimer's disease subgroups had equal clinical severity assessed by CDR scores, there was a slight but non-significant difference in their MMSE scores. The influence of the ApoE ε4 allele on the volume of the entorhinal cortex independently of MMSE scores was tested further within the Alzheimer's disease subgroups. The main effect of the ApoE type on the volume of the entorhinal cortex was significant (p=0.025) and there was also a significant main effect of MMSE (p=0.008).

Discussion

Braak and Braak have shown that the earliest pathological changes of Alzheimer's disease are the appearance of neurofibrillary tangles in the transentorhinal region; later these changes spread to the entorhinal cortex and hippocampus and other limbic structures and subsequently to the isocortex.[16] Recent data from a stereological study indicated that the counts of neurons and volume of the entorhinal cortex were preserved in aging from 60 to 90, but significantly reduced even in the very early stages of Alzheimer's disease.[30] These data emphasise that the entorhinal cortex is one of the first structures affected in Alzheimer's disease. Therefore, the measurement of the entorhinal volume is of interest as a diagnostic tool for detecting early Alzheimer's disease. The major finding of the present study was that the shrinkage of the entorhinal cortex measured using MRI volumetry was more pronounced in patients with Alzheimer's disease carrying the ApoE ε4 allele than in those patients without the allele. The reduction compared to controls was 27% for ε4− patients and 45% for ε4+ patients with Alzheimer's disease. The age or duration of disease are unlikely to explain the more pronounced atrophy in the entorhinal cortex because these variables did not differ between ε4+ and ε4− patients. Interestingly, the effect of the ApoE ε4 was more prominent in female patients with Alzheimer's disease. Female ε4+ patients with Alzheimer's disease had 55% smaller volume of the entorhinal cortex than their sex matched controls, whereas male ε4+ patients with Alzheimer's disease had 42% smaller volumes than their controls. This sex effect was not explained by age or severity of dementia.

The pronounced volume loss in the entorhinal cortex in ε4+ patients with Alzheimer's disease found in this study is comparable with previous volumetric MRI investigations of the hippocampus. An earlier study indicated that the hippocampal volume loss was greater in ε4+ patients with Alzheimer's disease and the shrinkage was most pronounced in patients with the ε44 genotype and significant particularly on the right side.[8] Accordingly, the ApoE ε4 allele seems to contribute to the atrophy especially in several medial temporal lobe structures, but does not seem to have any major effect on volume loss in the isocortical area.[8]

Interestingly, we found that the effect of the ApoE ε4 allele on the entorhinal cortical volume loss was particularly prominent in women. There is also additional evidence to indicate that the contribution of ε4 to the risk of having Alzheimer's disease is higher in women.[31 32] The reason for this finding remains unclear. However, it is possible that there is a synergistic negative effect of decreased oestrogen concentrations in postmenopausal women and the ApoE ε4 allele on the plasticity of neurons as oestrogen influences cellular plasticity as evidenced by experimental studies.[33] Oestradiol concentrating cells are present in the CA1 region and oestrogen prevents neuronal atrophy in the CA1 region of the rat hippocampus.[34] Oestrogen has been reported to promote survival of neurons, acting in concert with growth factors.[35 36 37]

Consistent with other studies, the present results detected a more severe impairment in verbal and visual memory tests in those patients with Alzheimer's disease with the ε4 allele than in those without the ε4 allele. The evaluation of the profile of cognitive deficits in patients with Alzheimer's disease with different ApoE genotypes has shown more severe memory impairment in patients carrying the ε4 allele, whereas performance in tests assessing other cognitive domains and scores in scales indicating global clinical severity have not disclosed ε4 related differences.[38] Despite the fact that ε4+ patients and ε4−patients with Alzheimer's disease were matched for clinical severity as assessed by CDR in this study, patients had a slight non-significant difference in their MMSE scores. However, these data clearly show that there was a significant main effect of ApoE genotype on the volumes of the entorhinal cortex independently of global cognitive status. By contrast, there are data suggesting that the ApoE ε2 allele may be protective for cognitive and particularly memory functions.[38] In view of this type of data, the association between the volume loss of the entorhinal cortex and the ApoE ε4 allele is not unexpected as damage to the entorhinal cortex causes memory impairment[40 41] and the ApoE ε4 allele is related to more conspicuous atrophy in the entorhinal cortex.

Although ApoE is known to modify the incidence and age of onset of Alzheimer's disease,[42 43] the mechanism by which it modulates the pathogenesis is still unknown. Immunohistochemical studies have disclosed the presence of ApoE in amyloid plaques and in neurofibrillary tangles,[44 45] which are the major pathological hallmarks of Alzheimer's disease, these being apparent in the early stage in the entorhinal cortex.[16 46] The ApoE ε4 allele is also associated with an increased amount and density of amyloid plagues and neurofibrillary tangles.[45 47–49] However, the mechanism underlying the adverse effect of the ApoE ε4 allele on the entorhinal cortex, hippocampus, and memory functions remains elusive. It is possible that there is more severe accumulation of amyloid and formation of neurofibrillary tangles in ε4 carriers and this could contribute to the degree of entorhinal atrophy. Alternatively, the volume loss in the entorhinal cortex may be due to impaired neuronal regeneration process in patients with Alzheimer's disease with the ApoE ε4 allele.[6 50–53] Arendt *et al*[6] recently showed that plastic dendrite remodelling is impaired in patients carrying the ε4 allele. The effect of the ApoE ε4 allele on the regenerative capacity of brain tissue is perhaps of particular importance in the medial temporal lobe structures such as the entorhinal cortex where intense synaptogenesis and synaptic remodelling commonly takes place. The reduced ability to compensate for the damage may prevent neuronal remodelling leading to neurodegeneration, this accounting for the differences in the entorhinal cortex volume in patients with Alzheimer's disease carrying the ε4 allele.

As the ApoE ε4 allele is considered to be a strong predictor of Alzheimer's disease, determination of the ApoE genotype could be of value. However, not all subjects carrying the ε4 allele develop dementia[54] and ApoE type is not a single causative factor but rather a risk factor for Alzheimer's disease. By combining the ApoE type with the volume of the entorhinal cortex, the significance of the ApoE type as a risk factor for the ultimate clinical outcome could be improved.

In conclusion, this study showed that the ApoE ε4 allele is associated with severe volume loss of the entorhinal cortex in patients with Alzheimer's disease and this effect of the ε4 allele is more prominent in women. These results suggest that low entorhinal volumes, especially in a female subject carrying the ε4 allele and with memory impairment, may indicate incipient Alzheimer's disease. However, in subjects without the ε4 allele but with early Alzheimer's disease, loss of volume in the entorhinal cortex may be more modest.

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