

Altered brain white matter integrity in healthy carriers of the *APOE* ϵ 4 allele

A risk for AD?

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Abstract—Background: Previous research has shown that polymorphisms of apolipoprotein E (*APOE*) represent genetic risk factors for dementia and for cognitive impairment in the elderly. The neural mechanisms by which these genetic variations influence behavioral performance or clinical severity are not well understood. **Methods:** The authors used diffusion tensor imaging to investigate ultrastructural properties in brain white matter to detect pathologic processes that modify tissue integrity. Sixty participants were included in the study of which 30 were homozygous for the *APOE* ϵ 3 allele, 10 were homozygous for the *APOE* ϵ 4 allele, and 20 had the *APOE* ϵ 3 ϵ 4 allele combination. All individuals were non-demented, and the groups were matched on demographic variables and cognitive performance. **Results:** The results showed a decline in fractional anisotropy, a marker for white matter integrity, in the posterior corpus callosum of ϵ 4 carriers compared to non-carriers. Additional sites of altered white matter integrity included the medial temporal lobe. **Conclusions:** Although the mechanism underlying vulnerability of white matter tracts in *APOE* ϵ 4 carriers is still unknown, these findings suggest that increased genetic risk for developing Alzheimer disease is associated with changes in microscopic white matter integrity well before the onset of dementia.

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Histopathologic and MRI studies in patients with Alzheimer disease (AD) have demonstrated loss of myelin and axons, and selective changes in lipid components of white matter.^{1–4} Such white matter changes have been seen as one contributing factor to the pathology of AD. White matter changes in aging and AD are revealed as hyperintensities in T2-weighted MR images,⁵ and as reduced water diffusion anisotropy by diffusion tensor imaging (DTI).^{1,6,7}

Changes in white matter integrity in patients with AD seem to be greatest in posterior callosal fiber systems.⁸ This can be contrasted with studies showing that normal aging is mainly associated with changes in frontal regions of the brain, notably structural changes in anterior callosal regions important for functional connectivity within the prefrontal cortex.^{7,9}

In the present study, we applied diffusion tensor imaging in a group of non-demented middle-aged and older subjects to assess possible associations between white matter integrity and *APOE* genotype. An association between polymorphisms of the *APOE* allele and white matter changes has been demonstrated. For example, a recent study found that ϵ 4 carriers had an elevated number of white matter lesions compared to non-carriers.¹⁰ Of main interest

here was to examine whether *APOE* ϵ 4 carriers, similar to patients with AD, would present alterations in posterior white matter. We hypothesized that ϵ 4 carriers compared to ϵ 3 ϵ 3 carriers would show disruption of white matter fibers in posterior corpus callosum, and that homozygous ϵ 4 carriers would show more disruption than heterozygous ϵ 4 carriers (i.e., ϵ 44 < ϵ 34 < ϵ 33).

Methods. Participants. Sixty cognitively intact persons between the ages of 49 and 79 years participated in the study. They were all recruited from The Betula Prospective Cohort Study: Memory, Health, and Aging.¹¹ Thirty subjects were carriers of at least one copy of the *APOE* ϵ 4: 10 were homozygous (ϵ 4/4) and 20 were heterozygous (ϵ 3/4). The remaining 30 subjects carried two copies of *APOE* ϵ 3 and were considered as controls. Three participants were removed due to technical problems. Participants were closely matched according to sex, age, and length of education (table). All subjects were non-demented and scored at or above the standard cut-off point of 24 on the Mini-Mental State Examination (MMSE).¹² To test for dose-dependent effects, individuals carrying one or two copies of the *APOE* ϵ 4 allele (*APOE* ϵ 4/4 and *APOE* ϵ 3/4) were divided into separate groups with 10 subjects in each group. Ten subjects from the *APOE* ϵ 3/3 group served as controls. All subgroups were matched according to sex, age, and length of education (see the table). The selection was done prior to the DTI analyses, which implicate an unbiased selection procedure. Given the possible effects of vascular conditions, such as hypertension, on brain function and anatomy, we compared the two groups on markers for vascular problems. These measure-

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Table Group characteristics

	Test for dose-dependent effects*				
	<i>APOE</i> ε4,† n = 30	<i>APOE</i> ε3/3, n = 30	<i>APOE</i> ε4/4, n = 10	<i>APOE</i> ε3/4, n = 10	<i>APOE</i> ε3/3, n = 10
Female/male	19/11	18/12	9/1	7/3	8/2
Age, y	66.0 (7.2)	66.6 (8.3)	63.1 (8.6)	65.2 (8.1)	64 (11.1)
Range	49–74	49–79	49–74	51–74	50–79
Education, y	10.6 (3.6)	10.2 (3.2)	11.8 (3.2)	10.7 (4.0)	11.8 (3.1)
Range	6–17	6–16	8–16	6–17	9–16
MMSE	28.1 (1.5)	27.9 (1.7)	28.4 (1.5)	28.4 (1.4)	28.1 (2.1)
Range	24–30	24–30	26–30	26–30	24–30
SRB	25.0 (2.6)	22.6 (4.8)	24.9 (1.4)	25.3 (2.4)	23.2 (3.8)
Range	16–29	11–29	23–27	22–29	18–28

Values are mean (SD). For the analyses on dose-effects (right-hand three columns), the *APOE* ε4 group was divided into one group with *APOE* ε4/4 carriers and one group with *APOE* ε3/4 carriers (matched for age, education, and cognitive status). Ten subjects from the *APOE* ε3/3 group were selected to match the *APOE* ε3/4 and *APOE* ε4/4 groups on age, education, and cognitive status.

* Subjects were selected from *APOE* ε4 and *APOE* ε3/3 groups (see also Methods).

† Carriers of at least one copy of the *APOE* ε4 allele: 10 with *APOE* ε4/4; 20 with *APOE* ε3/4.

MMSE = Mini-Mental State Examination (maximum = 30); SRB = word comprehension (maximum = 30).

ments included blood pressure, self-reported use of blood-pressure lowering medication, and self-reported history of vascular conditions. There were no differences between the *APOE* groups on either systolic [$t(55) < 1$] or diastolic [$t(55) = 1.29, p = 0.21$] blood pressure. Also, there were no differences between the groups on self-reported use of blood-pressure lowering medication or history of vascular conditions. They were all right-handed, native Swedish speakers, and had no reported neurologic problems that might cause dementia. Subjects were paid for participation and informed consent was obtained in accordance with the guidelines of the Swedish Council for Research in the Humanities and Social Sciences.

***APOE* genotyping.** A PCR was performed using 200 ng of genomic DNA as template in a 25-mL reaction mixture containing 20 pmol of PCR primers *APOE*-A (5'-TCC-AAG-GAG-CTG-CAG-GCG-GCG-CA-3') and *APOE*-B (5'-ACA-GAA-TTC-GCC-CCG-GCC-TGG-TAC-ACT-GCC-A-3'),¹³ 0.2 U of Taq DNA polymerase (GibcoBRL, Gaithersburg, MD), 1.0 mM MgCl₂, 75 mM Tris-HCl (pH 9.0), 20 mM (NH₄)₂SO₄, and 10% dimethyl sulfoxide. The PCR amplification consisted of 35 cycles of 30 seconds at 94 °C, 30 seconds at 65 °C, and 30 seconds at 72 °C. PCR products were digested using 5 U of *Hha*I (Life Technologies Inc., Rockville, MD) by incubating for 3 hours at 37 °C. Bands were separated on a 5% agarose gel and visualized on an ultraviolet transilluminator after ethidium bromide staining. Alternatively, electrophoresis was performed using ExcellGel gels (Pharmacia, Piscataway, NJ) and the MultiphorII electrophoresis system (Pharmacia), and the bands were visualized by silver staining.

Diffusion tensor imaging. Subjects were scanned using a single-shot spin echo EPI sequence, and cardiac gating was used to reduce motion artifacts due to pulsation of blood and CSF. The following imaging parameters were used: repetition time: shortest; echo time: 77 msec; field of view: 23 × 23 cm; acquisition matrix: 96 × 96 reconstructed to 128 × 128; and flip angle: 90°. Fifty-four 3.0-mm-thick contiguous axial slices were acquired. The DTI sequence was repeated four times, and the images were averaged using a script implemented in Matlab 6.1 (Mathworks Inc., MA). The DTI sequence included six sets of diffusion gradients placed along non-collinear directions [$b = 1,000$ seconds/mm²; (X, Y, Z) gradient directions = (1,0,0), (0,1,0), (0,0,1) (1/√2, 1/√2, 0), (1/√2, 0, 1/√2), (0, 1/√2, 1/√2)] and one set without diffusion weighting ($b = 0$ seconds/mm²).

The averaged images were processed using a custom toolbox in SPM99 (Wellcome Department of Cognitive Neurology, London, UK) that calculated the diffusion tensor eigenvalues in each voxel. Fractional anisotropy (FA) maps were then calculated. The non-diffusion-weighted image was normalized to a common template

in MNI space, and the resulting affine and non-linear transformation parameters were applied to the anisotropy images. Finally, the FA maps were smoothed with a Gaussian kernel of 8 mm full width at half maximum. The regions of interest (ROI) were outlined on multiple slices on the non-diffusion images acquired along with the DTI images. Each ROI was manually outlined using MRICro (<http://www.psychology.nottingham.ac.uk/staff/cr1/mricro.html>) by the operator (J.P.) who was blind to the *APOE* status of the subjects. The ROIs included the genu, splenium, and body of the corpus callosum. The anatomic regions of the corpus callosum were clearly visible on the non-diffusion weighted images, and standard anatomic landmarks were used to define these regions. The ROIs were then superimposed on the FA maps and mean values for each region and for each subject were calculated.

We also included an exploratory whole-brain analysis, in which we examined the effects of *APOE* status on a map-wise basis by contrasting the FA maps of non-carriers vs carriers. Effects from this analysis were regarded as significant if they reached a threshold of 0.005, uncorrected for multiple comparisons.

Results. ROI analyses. The mean FA values for the three regions (genu, body, and splenium) within the corpus callosum are presented in figure 1. Separate univariate analyses of variance (ANOVAs) were used to examine the difference between carriers and non-carriers of the *APOE* ε4 allele for each of the regions of the corpus callosum. A group difference was apparent in the posterior region with a higher FA value for *APOE* ε3/3 individuals [figure 1C, $F(1,56) = 7.39, p < 0.01$]. We also tested for an allelic dose effect but found no differences between heterozygotic and homozygotic ε4 carriers. Given the rather large age range within each *APOE* group (49 to 79 years), there was a possibility that the observed effects are confounded with age or the inclusion of subjects in the transitional stage between normal aging and dementia. In order to address this issue, we divided the *APOE* ε4 group and the *APOE* ε3/3 groups into groups of older (>65 years) and younger (<65 years) subjects. We then re-analyzed the DTI data for the young subjects and old subjects separately. We found a difference between *APOE* carriers and non-carriers in fractional anisotropy for both younger [$n = 20, 10$ in each group; mean age carriers: 55.90 years (range: 49 to 64),

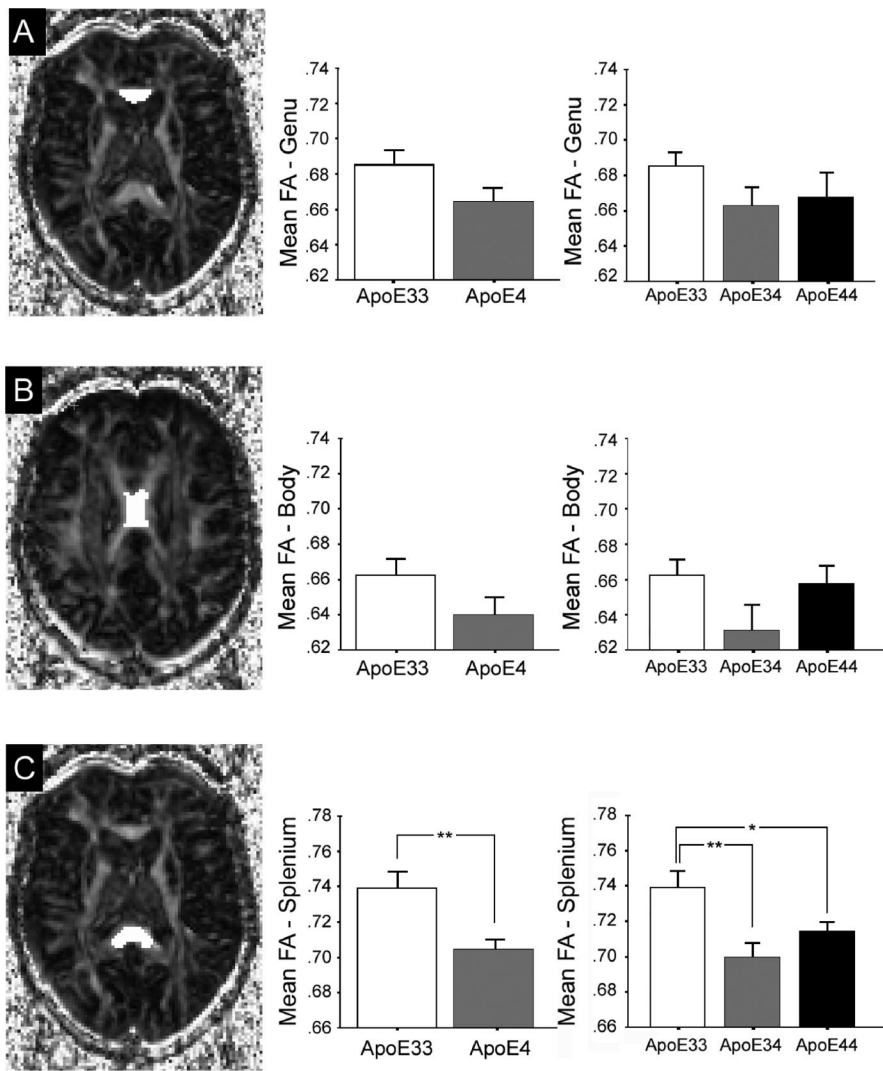


Figure 1. (Left) Regions of interest (A) anterior corpus callosum (genu), (B) body of the corpus callosum, (C) posterior corpus callosum (splenium) outlined on transverse slices of fractional anisotropy images. High signal intensity (brightness) reflects higher fractional anisotropy (FA). (Middle) Mean FA as a function of APOE status (white, APOE 3/3; gray, APOE 4). (Right) Mean FA as a function of APOE status (white, APOE 3/3; gray, APOE 3/4; black, APOE 4/4). **Two-sample *t* test $p < 0.01$; *two-sample *t* test $p < 0.05$ (one-tailed).

mean age non-carriers 57.64 years (range: 49 to 64); $t(18) = 3.16$, $p < 0.01$], and older subjects [n = 37, 19 carriers and 18 non-carriers; mean age carriers: 70.45 years (range: 65 to 74), mean age non-carriers: 71.84 years (range: 65 to 74); $t(35) = 2.106$, $p < 0.05$]. The differences between APOE carriers and non-carriers in relatively young subjects suggest that these effects are truly related to APOE status, and not related to age or confounded by subjects in a transitional stage between normal aging and AD, such as mild cognitive impairment. In line with previous findings,^{1,14} age was correlated with FA in all ROIs, although this finding was only significant for the body of the corpus callosum ($r = -0.354$; $p < 0.01$). This suggests that increasing age is related to a general breakdown in white matter integrity.

Exploratory whole-brain analysis. Differences in fractional anisotropy between carriers and non-carriers of the APOE4 allele are shown in figure 2. Regions where carriers showed reduced FA compared to controls were located in the occipito-frontal fasciculus (x, y, z = 14, 12, 28; figure 2A), and the body/posterior corpus callosum (-4, -22, 26; -4, -44, 16; figure 2A). We also found a difference between carriers and controls in the left hippocampus (x, y, z = -26, -38, -4; figure 2B). The reduced FA in posterior corpus callosum was located in a region that was overlap-

ping with the region that was anatomically defined as the posterior corpus callosum in the ROI analysis.

Discussion. Our results provide a demonstration of changes in white matter integrity in healthy APOE $\epsilon 4$ carriers using diffusion-tensor imaging. We found converging evidence for a decline in FA in posterior corpus callosum in individuals carrying a copy of the APOE $\epsilon 4$ allele. This difference was found for both young and old individuals, suggesting that the effects of APOE may influence brain structure in early ages, and are not confounded by early onset dementia. We also observed decreased FA in occipito-frontal fasciculus, and the hippocampus in carriers of the APOE $\epsilon 4$ allele compared to controls. We did not, however, find support for dose-dependent effects in white matter integrity.

The finding of decreased fractional anisotropy in the corpus callosum in carriers of the APOE $\epsilon 4$ allele is supported by observations from previous pathologic¹⁵ and MRI studies^{9,16,17} showing macroscopic white matter lesions in patients with AD. Also, a study on non-demented aging found that $\epsilon 4$ carriers had an elevated number of white matter lesions compared to

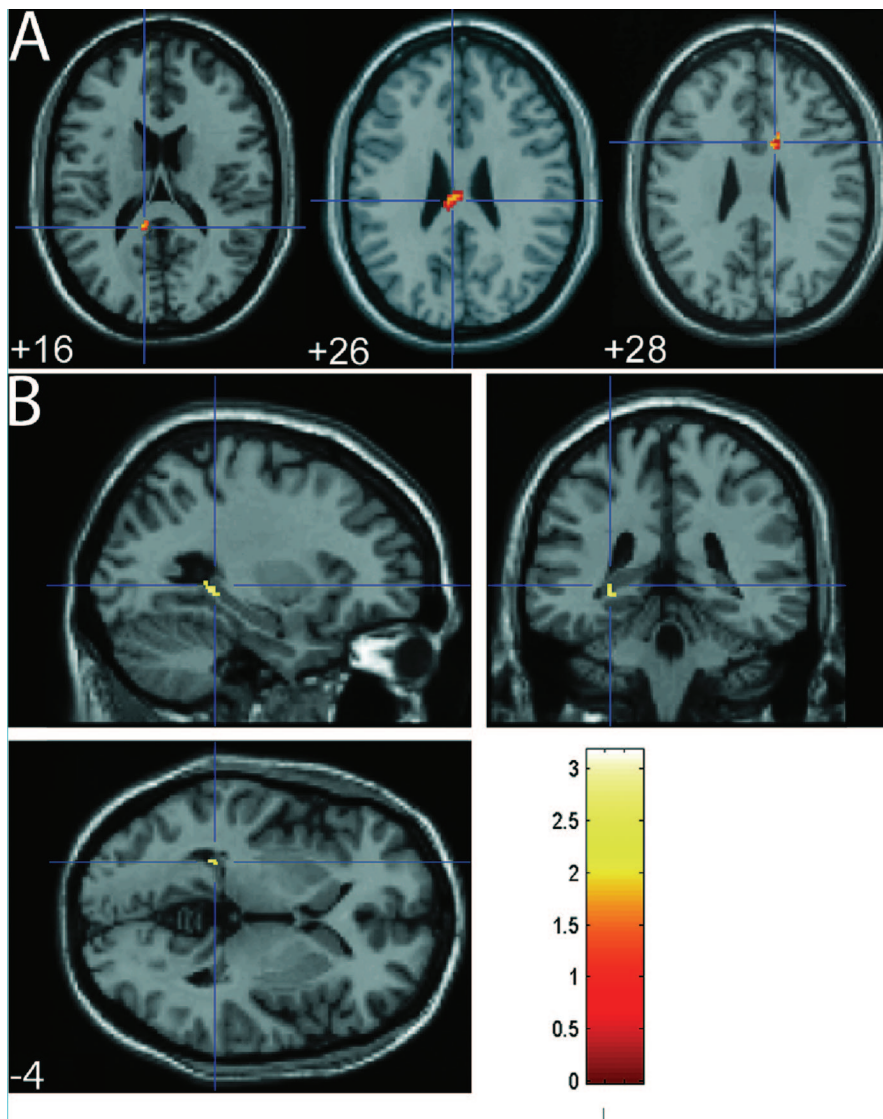


Figure 2. (A) Transverse sections of an anatomic template on which are superimposed loci where white matter integrity was significantly reduced in carriers of the *APOE4* allele compared to non-carriers. Left = posterior corpus callosum, center = posterior/body of the corpus callosum, right = occipito-frontal fasciculus/anterior cingulum. (B) Sagittal (top left), coronal (top right), and transverse (bottom left) sections of an anatomic template showing reduced white matter integrity in the hippocampus for carriers of the *APOE4* allele compared to non-carriers.

non-carriers.¹⁰ Studies using DTI have shown that there is a relatively large amount of microscopic white matter pathology in patients with AD.^{1,7,18,19} Although the differences in FA between carriers and non-carriers may seem small, the magnitude of the decrease in posterior corpus callosum is comparable to the difference observed between old and young adults^{7,20} and between patients with AD and controls.⁷ We did not find support for a dose-dependent effect in white matter integrity, and *APOE* ϵ 34 carriers actually had somewhat lower FA overall compared to *APOE* ϵ 44 carriers. Clearly more studies are needed to address the issue of dose-dependent effects. Together, our results suggest that the presence of the *APOE* ϵ 4 allele may influence microscopic white matter integrity of the corpus callosum before the onset of AD.

Atrophy in the corpus callosum is considered to indicate neocortical dysfunction, and the topography of atrophic changes in callosal regions may correspond to regional neocortical atrophy. White matter tracts of the splenium originate from temporo-parietal regions of the brain,²¹ and these regions are

characteristically affected in AD.²² It has been argued that while normal aging is associated with changes in anterior callosal regions facilitating the functions of the prefrontal cortex, AD is expected to relate to changes in posterior parts of the corpus callosum.⁷ Indeed, hippocampal and entorhinal atrophy in AD has been found to produce metabolic decline in the posterior neocortices,²³ and such posterior neocortical decline is related to callosal atrophy, especially in the posterior portion of the corpus callosum.⁸

The observation of group-related differences in the hippocampal region, with reduced FA in *APOE* ϵ 4 carriers compared to controls, likely relates to changes in white matter integrity within the medial temporal lobe (e.g., hippocampal–parahippocampal connectivity). Indeed, a recent study using DTI found evidence for such connectivity.²⁴ The entorhinal cortex, a region highly susceptible to AD-related deterioration, is the major source of projections to the hippocampus proper, and also the target of hippocampal efferent projections. One possibility is that carriers of the *APOE* ϵ 4 allele display alterations in

hippocampal gray matter, which affect surrounding white matter integrity through Wallerian degeneration.²⁵ The limited spatial resolution of DTI and the fact that the connections within the medial temporal lobe predominantly consist of small fiber tracts make it difficult to precisely localize these projections. In the current study, *APOE* ϵ 4-related atrophy was found in the left hippocampus, although at least one previous study on demented subjects has suggested that atrophy in *APOE* ϵ 4 carriers may be more pronounced in the right hippocampus.²⁶ In nondemented elderly subjects, however, such a laterality effect on hippocampal atrophy has not been firmly established.

Diffusion tensor imaging seems to be a helpful tool for detecting ultrastructural white matter alterations in carriers of the *APOE* ϵ 4 allele. Continued longitudinal follow-up measurements of this sample can be used to determine whether changes in white matter integrity provide useful information for pre-clinical identification of individuals susceptible to memory problems and AD.

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